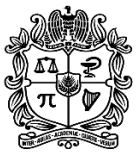


REVISTA COLOMBIANA DE CIENCIAS QUÍMICO-FARMACÉUTICAS

Volumen 55, Número 1, enero-abril 2026

Universidad Nacional de Colombia, Sede Bogotá, Facultad de Ciencias, Departamento de Farmacia

ISSN: 0034-7418, ISSN electrónico: 1909-6356

Fax: 3165060, Cra. 30 No. 45-03, Bogotá, D. C., Colombia

Correo electrónico: rcciquifa_fcbog@unal.edu.co

http://www.revistas.unal.edu.co/index.php/rccquifa
http://www.scielo.org.co/scielo.php?script=sci_serial&pid=0034-7418&rep=
https://scholar.google.com/citations?user=TvBT68sAAAAJ&hl=es
https://portal.issn.org/resource/issn/1909-6356
https://dialnet.unirioja.es/servlet/revista?codigo=27336
https://www.scimagojr.com/journalsearch.php?q=21827&tip=sid#google_vignette

Evaluadores:

Antonio Peña, Ph. D., Departamento de Cirugía, Ciencias Médicas y Sociales, Facultad de Medicina y Ciencias de la Salud, Universidad de Alcalá, Alcalá de Henares, Madrid, España.
Carlos A. Calderón, Ph. D., Unidad de Farmacología, Facultad de Medicina, Universidad del Rosario, Bogotá, D. c., Colombia.
Cristian Buendía-Atencio, Ph. D., Facultad de Ciencias, Universidad Antonio Nariño, Bogotá D. C., Colombia.
Daniel R. Delgado, Ph. D., Facultad de Ingeniería, Universidad Cooperativa de Colombia, Neiva, Colombia.
Darío A. Tinjacá, Ph. D., Facultad de Ciencias, Universidad El Bosque, Bogotá D. C., Colombia.
Diego I. Caviedes, Ph. D., Facultad de Ingeniería, Universidad Cooperativa de Colombia, Neiva, Huila, Colombia.
Fleming Martínez, Ph. D., Departamento de Farmacia, Facultad de Ciencias, Universidad +– Nacional de Colombia, Sede Bogotá, D. C., Colombia.
Jennifer K. Castro, Ph. D., Facultad de Ingeniería, Universidad Surcolombiana, Neiva, Huila, Colombia.
Jorge E. Hernández, Ph. D., Facultad de Ciencias Básicas, Universidad Santiago de Cali, Cali, Colombia.
Jorge A. Díaz, Ph. D., Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional de Colombia, Sede Bogotá, D. C., Colombia.
José J. López, Ph. D., Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional de Colombia, Sede Bogotá, D. C., Colombia.
María A. Peña, Ph. D., Facultad de Farmacia, Universidad de Alcalá, Alcalá de Henares, Madrid, España.
Máximo A. Rodríguez, M. Sc., Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional de Colombia, Sede Bogotá, D. C., Colombia.
Reinaldo G. Sotomayor, M. Sc., Programa de Farmacia, Facultad de Química y Farmacia, Universidad del Atlántico, Puerto Colombia, Atlántico, Colombia.
Rossember E. Cárdenas, M. Sc., Facultad de Ciencias y Humanidades, Universidad de América, Bogotá D. C., Colombia.

MISIÓN

La *Revista Colombiana de Ciencias Químico-Farmacéuticas* es un órgano de difusión en el cual se publican investigaciones científicas, comunicaciones técnicas y revisiones temáticas originales en las áreas de las ciencias farmacéuticas (véanse Normas para publicación). La revista está destinada principalmente a químicos farmacéuticos, químicos, ingenieros químicos, médicos cirujanos, médicos veterinarios, y a otros profesionales de las ciencias físicas y naturales, de la ingeniería y de las profesiones sanitarias relacionadas con el uso de medicamentos.

VISIÓN

En pro de la difusión de las investigaciones, los contenidos de la *Revista Colombiana de Ciencias Químico-Farmacéuticas* son de acceso libre, con ello se espera llegar a un número mayor de lectores, propiciando la consolidación de comunidades académicas. Además, se proyecta que los contenidos publicados contribuyan al desarrollo e innovación de las ciencias farmacéuticas.

ÉTICA

La responsabilidad de lo expresado en cada artículo es exclusiva de los autores y no representa ni compromete posiciones de la revista ni del cuerpo editorial. Los contenidos publicados pueden reproducirse siempre que se cite la fuente respectiva. Esta revista sigue los lineamientos del COPE (Committee on Publication Ethics), que pueden ser consultados en *http://publicationethics.org/*

LICENCIA CREATIVE COMMONS

La totalidad de los números anteriores se encuentran disponibles en el sitio web:
http://www.revistas.unal.edu.co/index.php/rccquifa

Contenido

Scientific research article

Evaluation of antifungal and antibiofilm activity of commercial mouthwashes sold in Türkiye against oral biofilm-forming yeasts: an *in vitro* microbiological study

Gülçin Özcan Ateş[1]* & Müşerref Otkun[2]

[1] Department of Medical Services and Techniques, Vocational School of Health Services, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye. gulcinozcan@comu.edu.tr https://orcid.org/0000-0002-8467-2378

[2] Department of Medical Microbiology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye. otkun2000@yahoo.com https://orcid.org/0000-0001-8977-114

Correspondence: gulcinozcan@comu.edu.tr

Received: March 13, 2025
Corrected: August 23, 2025
Accepted: August 26, 2025

https://doi.org/10.15446/rcciquifa.v55n1.119332

SUMMARY

Introduction: In recent years, the use of mouthwashes has become quite common in various situations, from bad breath to treating minor infections. Although oral care products have a broad spectrum of antibacterial activity, little is known about their antifungal properties, and studies on antifungal and antibiofilm activity on oral-isolated *Candida* isolates are limited. **Objective:** This study aimed to assess the antifungal and antibiofilm activities of the best-selling mouthwashes in Türkiye against yeast strains isolated from the mouth, known for their strong biofilm production. **Methods**: This study investigated the antifungal and antibiofilm activity of twenty-five commercially available types of mouthwash against 19 *Candida* sp. and 1 *Pichia manshurica* strains isolated from the oral cavity of 18- 25-year-olds. **Results**: It was determined that twelve mouthwashes containing cetylpyridinium chloride had antifungal activity. It was determined that MIC values of mouthwashes varied between 0.20 and 1.56 μL/mL. Also, the effect of mouthwashes on mature biofilm and biofilm formation was evaluated according to MIC values. It was determined that mouthwashes are remarkably effective during biofilm formation but have little effect on mature biofilm structure. **Conclusion**: This study demonstrates that mouthwashes may possess not only antibacterial but also antifungal properties. Mouthwashes containing cetylpyridinium chloride have been shown to exhibit antifungal activity against oral *Candida* species, as confirmed by MIC values. Furthermore, these mouthwashes strongly inhibit biofilm formation but have a limited impact on the structure of mature biofilms. These findings suggest that mouthwashes should undergo evaluation for their antifungal effects and may play a significant role in clinical applications, particularly due to their biofilm-inhibiting characteristics.

Keywords: oral hygiene; mouthwash; antifungal activity; antibiofilm activity.

RESUMEN

Evaluación de la actividad antifúngica y antibiofilm de enjuagues bucales comerciales vendidos en Turquía contra levaduras formadoras de biofilm oral: un estudio microbiológico *in vitro*

Introducción: En los últimos años, el uso de enjuagues bucales se ha generalizado en diversas situaciones, desde el mal aliento hasta el tratamiento de infecciones leves. Si bien los productos de higiene bucal

poseen un amplio espectro de actividad antibacteriana, se conoce poco sobre sus propiedades antifúngicas, y los estudios sobre la actividad antifúngica y antibiofilm en cepas de *Candida* aisladas de la cavidad oral son limitados. **Objetivo:** Este estudio tuvo como objetivo evaluar la actividad antifúngica y antibiofilm de los enjuagues bucales más vendidos en Turquía contra cepas de levaduras aisladas de la cavidad oral, conocidas por su alta capacidad de formación de biofilm. Métodos: Se investigó la actividad antifúngica y antibiofilm de veinticinco enjuagues bucales comerciales contra 19 cepas de *Candida* sp. y 1 cepa de *Pichia manshurica* aisladas de la cavidad oral de personas de entre 18 y 25 años. **Resultados:** Se determinó que doce enjuagues bucales que contenían cloruro de cetilpiridinio presentaban actividad antifúngica. Se determinó que los valores de la concentración inhibitoria mínima (CIM) de los enjuagues bucales variaban entre 0,20 y 1,56 μL/mL. Asimismo, se evaluó el efecto de los enjuagues bucales sobre la biopelícula madura y su formación en función de los valores de la CIM. Se determinó que los enjuagues bucales son notablemente eficaces durante la formación de la biopelícula, pero tienen poco efecto sobre la estructura de la biopelícula madura. **Conclusión:** Este estudio demuestra que los enjuagues bucales pueden poseer propiedades tanto antibacterianas como antifúngicas. Se ha demostrado que los enjuagues bucales que contienen cloruro de cetilpiridinio presentan actividad antifúngica contra especies de *Candida* oral, como lo confirman los valores de la CIM. Además, estos enjuagues bucales inhiben fuertemente la formación de biopelícula, pero tienen un impacto limitado en la estructura de las biopelículas maduras. Estos hallazgos sugieren que los enjuagues bucales deben someterse a una evaluación por sus efectos antifúngicos y podrían desempeñar un papel importante en aplicaciones clínicas, en particular debido a sus características inhibidoras de la biopelícula.

Palabras clave: higiene bucal; enjuague bucal; actividad antifúngica; actividad antibiofilm.

RESUMO

Avaliação da atividade antifúngica e antibiofilme de enxaguantes bucais comerciais vendidos na Turquia contra leveduras formadoras de biofilme oral: um estudo microbiológico *in vitro*

Introdução: Nos últimos anos, o uso de enxaguantes bucais tornou-se bastante comum em diversas situações, desde mau hálito até o tratamento de infecções menores. Embora os produtos para higiene bucal apresentem um amplo espectro de atividade antibacteriana, pouco se sabe sobre suas propriedades antifúngicas, e os estudos sobre a atividade antifúngica e antibiofilme em isolados de *Candida* da cavidade oral são limitados. **Objetivo:** Este estudo teve como objetivo avaliar as atividades antifúngica e antibiofilme dos enxaguantes bucais mais vendidos na Turquia contra cepas de leveduras isoladas da cavidade oral, conhecidas por sua forte produção de biofilme. Métodos: Este estudo investigou a atividade antifúngica e antibiofilme de 25 tipos de enxaguantes bucais disponíveis comercialmente contra 19 cepas de *Candida* sp. e 1 cepa de *Pichia manshurica* isoladas da cavidade oral de indivíduos de 18 a 25 anos. **Resultados:** Foi determinado que doze enxaguantes bucais contendo cloreto de cetilpiridínio apresentaram atividade antifúngica. Os valores da CIM (Concentração Inibitória Mínima) dos enxaguantes bucais variaram entre 0,20 e 1,56 μL/mL. Além disso, o efeito dos enxaguantes bucais sobre o biofilme maduro e a formação do biofilme foi avaliado de acordo com os valores da CIM. Constatou-se que os enxaguantes bucais são notavelmente eficazes durante a formação do biofilme, mas têm pouco efeito sobre a estrutura do biofilme maduro. **Conclusão:** Este estudo demonstra que os enxaguantes bucais podem possuir propriedades não apenas antibacterianas, mas também antifúngicas. Os enxaguantes bucais contendo cloreto de cetilpiridínio demonstraram atividade antifúngica contra espécies de *Candida* oral, conforme confirmado pelos valores da CIM. Além disso, esses enxaguantes bucais inibem fortemente a formação do biofilme, mas têm um impacto limitado na estrutura dos biofilmes maduros. Esses achados sugerem que os enxaguantes bucais devem ser avaliados quanto aos seus efeitos antifúngicos e podem desempenhar um papel significativo em aplicações clínicas, principalmente devido às suas características inibidoras de biofilme.

Palavras-chave: higiene bucal; enxaguante bucal; atividade antifúngica; atividade antibiofilme.

1. INTRODUCTION

The oral microbiota is the most complex microbial community in the body, second only to the colon, consisting of approximately 1,000 species of microorganisms residing in various areas such as the teeth, tongue, cheeks, palate, gums, and tonsils [1, 2]. *Candida* species, naturally present in around 75% of healthy individuals as part of the oral microbiota, can become opportunistic pathogens and cause acute or chronic infections. This is particularly likely due to factors like denture use, smoking, a weakened immune system, xerostomia, and broad-spectrum antibiotic treatment. *Candida albicans* is the most prevalent and pathogenic yeast linked to oral candidiasis; however, non-albicans Candida (NCAC) species can also lead to disease [3].

Dental plaque constitutes a structurally and functionally organized multi-species microbial biofilm, playing a significant role in the etiology of oral diseases. The primary factor for maintaining good oral health is the routine management of dental plaque that forms on tooth surfaces and adheres to the gingival margins [4]. Traditional strategies for preventing and controlling dental plaque include mechanical removal and the use of broad-spectrum antibiotics such as chlorhexidine. The effectiveness of mechanical cleaning methods like brushing and flossing relies on personal knowledge, skill, and motivation. Although meticulous mechanical hygiene can mitigate diseases, many individuals struggle to uphold good oral health [5, 6]. Excessive use of chlorhexidine, beneficial for oral health, has been shown to increase the risk of drug resistance among oral bacteria and cause cellular damage [4].

Using toothbrushes, toothpaste, and mouthwash daily is an effective oral hygiene practice [7]. Mouthwash frequently prevent and manage *C. albicans* infections, particularly in dentistry. These mouthwashes include active ingredients such as water, chlorhexidine, ethanol, essential oils, and anti-inflammatory compounds [7, 8]. Mouthwash with antibacterial agents help eliminate bacteria left in the mouth after brushing. Additionally, clinical, and *in vitro* studies have demonstrated that cetylpyridinium chloride (CPC), a cationic quaternary ammonium compound, inactivates oral bacteria and diminishes plaque and gingivitis [7]. However, Ardizzoni *et al.* and Paulone *et al.* indicated that mouthwash containing chlorhexidine digluconate, CPC, and essential oils in their formulations can impact on the hyphal development of *C. albicans* and its biofilm formation and persistence [9, 10].

In recent years, various oral care brands with diverse formulations have claimed that their products enhance oral hygiene, decrease plaque, and reduce gingivitis or tooth decay. Different oral care products contain antimicrobial and antiplaque agents within their formulations. Recently, the use of mouthwash has gained popularity in numerous situations, from combating bad breath to addressing minor infections [6, 11]. Consequently, this study aimed to investigate the antifungal and antibiofilm properties of twenty-five commercial types of mouthwash against 20 yeast isolates sourced from the oral cavities of young individuals.

2. METHODS

2.1. Cultures

Four *Candida albicans* isolates (1, 2, 3, 4), five *Candida dubliniensis* isolates (5, 6, 7, 8, 9), ten *Candida parapsilosis* isolates (11, 12, 13, 14, 15, 16, 17, 18, 19), and one *Pichia manshurica* isolate (20) known to be strong biofilm producer from the researchers' culture collection were used in the study, all isolated from the oral cavity in 18-25-year-olds [12].

2.2. Mouthwashes

Mouthwashes were collected from beauty shops in Çanakkale, Türkiye in 2021. Mouthwashes and their contents (whether they contained alcohol, sodium fluoride, plant extract, or CPC) are detailed in Table 1.

Table 1. Contents of the mouthwashes used in the study.

No	**901**	**902**	**903**	**904**	**905**
Brand	A	A	A	A	A
Product name	Advanced white mild taste	For enhanced protection sensitivity	Total care	Total care mild taste	Fresh brust
Alcohol	-	-	+	-	+
Sodium fluoride	220 ppm	220 ppm	220 ppm	220 ppm	-
Plant extract	-	-	-	-	-
CPC	-	-	-	-	-
Other ingredients	Aqua, Aroma, Caprylyl Glycol, Critic Acid, Eucalyptol, Menthol, Sorbitol, Sodium Saccharin, Sucralose, Sodium Methyl Cocoyl Taurate, Pentasodium Triphosphate, Poloxamer 407, Propylene Glycol, Tetrapotassium Pyrophosphate, Thymol,	Aqua, Aroma, Sorbitol, Sodium Saccharin, Sodium Benzoate, Sucralose, Sodium Lauryl Sulfate, Sodium Methyl Cocoyl Taurate, Phosphoric Acid, Propylene Glycol, 1.4% Dipotassium Oxalate	Aqua, Aroma, Benzoic Acid, Cl 16035, Cl 42090, Eucalyptol, Methyl Salicylate, Menthol, Sorbitol, Sodium Saccharin, Sodium Benzoate, Sucralose, Propylene Glycol, Thymol, Zinc Chloride	Aqua, Aroma, Benzoic Acid, Cl 16035, Cl 42090, Eucalyptol, Methyl Salicylate, Menthol, Sorbitol, Sodium Saccharin, Sodium Benzoate, Sucralose, Sodium Lauryl Sulfate, Poloxamer 407, Propylene Glycol, Thymol, Zinc Chloride	Aqua, Aroma, Benzoic Acid, Cl 42053, Cl 47005, Eucalyptol, Methyl Salicylate, Menthol, Sorbitol, Sodium Saccharin, Sodium Benzoate, Poloxamer 407, Thymol

912	**911**	**910**	**909**	**908**	**907**	**906**
D	C	C	B	A	A	A
Cool mint mouthwash	Promine	Cool mint for sensitive teeth	Gum x Enamel Care	Stay white	Cool mint mild taste	Cool mint
-	-	-	-	+	-	+
*	450 ppm	217 ppm	450 ppm	220 ppm	220 ppm	-
-	-	-	-	-	-	-
+	+	-	+		-	-
Aqua, Critic Acid, Cl 42053, Eucalyptol, Flavor, Menthol, Sodium Saccharin, Sodium Benzoate, Polysorbate 20, Poloxamer 407, Thymol	Aqua, Aroma, Cellulose Gum, Cl 42090, Disodium Phosphate, Glycerin, Methylparaben, Sorbitol, Sodium Saccharin, Sodium Benzoate, Sodium Phosphate, Potassium Nitrate, Peg-60 Hydrogenated Castor Oil, Propylparaben, VP/VA Copolymer, Xanthan Gum	Aqua, Aroma, Cl 42090, Disodium Phosphate, Glycerin, Sorbitol, Sodium Saccharin, Sodium Benzoate, Potassium Nitrate 3% w/w, Potassium Nitrate, Peg-60 Hydrogenated Castor Oil, Poloxamer 407	Aqua, Aroma, Benzoic Acid, Cinnamal, Cl 15985, Cl 42053, Glycerin, Sodium Benzoate, Sucralose, Poloxamer 407, Propylene Glycol	Aqua, Aroma, Benzoic Acid, Cl 42090, Eucalyptol, Methyl Salicylate, Menthol, Sorbitol, Sodium Saccharin, Sodium Benzoate, Sucralose, Sodium Lauryl Sulfate, Poloxamer 407, Thymol, Zinc Chloride	Aqua, Aroma, Benzoic Acid, Cl 42053, Eucalyptol, Methyl Salicylate, Menthol, Sorbitol, Sodium Saccharin, Sodium Benzoate, Sucralose, Sodium Lauryl Sulfate, Poloxamer 407, Propylene Glycol, Thymol	Aqua, Aroma, Benzoic Acid, Cl 42053, Eucalyptol, Methyl Salicylate, Sorbitol, Sodium Saccharin, Sodium Benzoate, Poloxamer 407, Thymol

919	**918**	**917**	**916**	**915**	**914**	**913**
E	E	E	E	D	D	D
Mint refreshment	Plax tea-lemon	Total 12	Plax complete care	Tartar control	Shining white	Mint bamboo charcoal herbal mouthwash
-	-	-	-	+	+	-
225 ppm	225 ppm	225 ppm	225 ppm	-	-	*
-	*Camellia sinensis* Leaf Extract *Citrus limon* Peel	-	-	-	-	*Camellia sinensis* Leaf Extract *Commiphora myrrha*
+	+	+	+	+	+	+
Aqua, Aroma, Cl 42051, Glycerin, Menthol, Sorbitol, Sodium Saccharin, Potassium Sorbate, Poloxamer 407, Propylene Glycol	Aqua, Aroma, Cl 19140, Cl 42051, Glycerin, Menthol, Sorbitol, Sodium Saccharin, Potassium Sorbate, Poloxamer 407, Propylene Glycol	Aqua, Aroma, Cl 17200, Cl 42051, Glycerin, Lactic Acid, Menthol, Sorbitol, Sodium Saccharin, Potassium Sorbate, Poloxamer 407, Propylene Glycol, Zic Lactate	Aqua, Aroma, Cl 42051, Glycerin, Menthol, Sorbitol, Sodium Saccharin, Potassium Sorbate, Poloxamer 407, Propylene Glycol	Aqua, Critic Acid, Cl 42090, Eucalyptol, Flavor, Menthol, Sorbitol, Sodium Saccharin, Sodium Benzoate, Sodium Hydroxide, Polysorbate 20, Poloxamer 407, Thymol, Zinc Chloride	Aqua, Critic Acid, Cl 42090, D-limonene, Eucalyptol, Flavor, Menthol, Sorbitol, Sodium Saccharin, Sodium Benzoate, Sodium Hydroxide, Polysorbate 20, Poloxamer 407, Thymol, Zinc Chloride	Aqua, Critic Acid, Cl 42053, D-limonene, Eugenal, Flavor, Menthol, Sorbitol, Sodium Benzoate, Potassium Sorbate, Polysorbate 20, Poloxamer 407, Propylene Glycol,

925	**924**	**923**	**922**	**921**	**920**
G	G	F	F	F	E
Active oral care flouride protection	Active flouride protection mounthrince green	Bad breath	Klorhex gum care	Full protection	Optic white
-	-	-	-	-	-
225 ppm	225 ppm	-	500 ppm	*	225 ppm
-	-	*Mentha arvensis* plant oil	*Mentha arvensis* plant oil	*Cistus* species Resin Extract *Mentha arven-*	-
+	+	-	+	-	-
Aqua, Aroma, Cl 42051, Glycerin, Menthol, Sodium Saccharin, Polysorbate 20, 2-Bromo-2-Nitropropane-1,3-diol	Aqua, Aroma, Cl 42090, Cl 47005, Flavor, Glycerin, Menthol, Sodium Saccharin, Polysorbate 20, 2-Bromo-2-Nitropropane-1,3-diol	Aqua, Aroma, Citric acid, Cl 42051, Sorbitol, Sodium Benzoate, Sodium Hydroxide, Sucralose, Potassium Sorbate, Peg-40 Hydrogenated Castor Oil, Propylene Glycol, Zinc Citrate	Aqua, Aroma, Ascorbic Acid, Chlorhexidine Digluconate, Cl 42051, Cl 47005, Sorbitol, Sodium Metabisulfite, Sodium Phosphate, Sodium Hydroxide, Sucralose, Peg-40 Hydrogenated Castor Oil, Propylene Glycol	Aqua, Aroma, Citric acid, Cl 16035, Glycerin, Sucralose, Potassium Sorbate, Potassium Sorbate, Peg-40 Hydrogenated Castor Oil, Propylene Glycol,	Aqua, Aroma, Benzyl Alcohol, Cl 42051, Glycerin, Sorbitol, Sodium Saccharin, Potassium Sorbate, Propylene Glycol, PVM/MA Copolymer, Tetrasodium Pyrophosphate, Tetrapotassium Pyrophosphate, Zinc Citrate

"-" = absent, "+" = present, *unknown quantify

2.3. Determination of Antifungal Activity of Mouthwashes

First, the agar well diffusion method was used to determine the antimicrobial activity of mouthwashes. Then, the agar well diffusion method determined the minimum inhibitory concentrations of five types of mouthwashes that were effective. The *in vitro* antifungal activity of mouthwashes was performed by modifying the method outlined in CLSI M44-A [13]. Stock cultures were inoculated on Sabouraud 2% Dextrose Agar (SDA, Neogen), and plates were incubated at 37 °C for 24 hours. After incubation, the isolates were adjusted to 0.5 McFarland density (1-5x10^6 cells/mL) with physiological saline (PS, 0.85% w/v NaCl). The cell suspension

was inoculated on the dried surface of Mueller-Hinton Agar + 2% Glucose, 0.5 μg/mL Methylene Blue Agar (MHA+ GMB) (Himedia, India) plate with the help of a cotton swab. Then, 6 mm diameter wells were opened on the plate, and 20 μL of mouthwash was added. Plates were then incubated at 37 °C for 24 hours. Zone diameters were measured after incubation. The analysis was done in three parallels.

The antifungal activity of mouthwashes was performed *in vitro* as in CLSI M27-A2 [14]. The cell suspension was adjusted to the density of 0.5 McFarland with PS from a fresh culture grown on an SDA medium for 24 hours at 37 °C. Then cell suspension was diluted at 1:100 (1-5x10^3 cell/mL), and the final cell concentration was 0.5-2.5x10^3 cells/mL by inoculating 1:1 into RPMI 1640 medium containing mouthwash.

RPMI 1640 medium (Himedia, India) containing 0.165 M MOPS containing ten different mouthwash concentrations was prepared. Mouthwash concentration was prepared using a two-fold dilution from 1000 μL/mL to 2 μL/mL. 100 μL of the prepared medium was dispensed into the wells of the microdilution plates. 100 μL of the cell suspension was added to the microplates. The final mouthwash concentration was between 1 μL/mL and 500 μL/mL. RPMI 1640 broth medium containing 500 μL/mL mouthwash was used as a negative control, and RPMI 1640 + culture was used as the positive control. Microplates were evaluated at 660 nm in a microplate reader (Thermo Multiscan FC) after 24 hours and 48 hours of incubation at 37 °C. The study was carried out in 3 parallels.

2.4. Effect of Mouthwash on Biofilm Formation

Yeast isolates were resuscitated overnight at 37 °C in 5 mL of Sabouraud 2% Dextrose Broth (SDB) (NCM0147, Neogen, USA/Canada) medium. Revived cultures were adjusted to OD600 = 1.0 (10^7 cells/mL) in an SDB+8% glucose medium containing mouthwash in line with the determined MIC value. Then, 200 μL of inoculated SDB + 8% glucose medium was added to the wells of 96-well flat-bottom microplates. Microplates were incubated at 37°C for 48 hours. After incubation, the microplates were washed three times with sterile PS. After washing, 200 μL of 99% methanol (Merck, Germany) was added for fixation and incubated for 15 minutes. The plates were then emptied and dried at room temperature. Afterwards, 200 μL of 1% (w/v in distilled water) crystal violet (Himedia, India) was added to each well and incubated for 15 minutes. After incubation, the microplates were washed twice with sterile distilled water, and the plates were dried at room temperature. Then, 200 μL of 33% acetic acid (Merck, Germany) was added to the plates and evaluated in a microplate reader (Thermo Multiscan FC) at 570 nm. Only medium + culture was used as the control group, and the results were compared with the control group. The study was conducted in two parallels and three repetitions [12].

2.5. Determination of the Effect of Mouthwash on Mature Biofilm

Evaluation of the effect of mouthwash by MIC values on mature biofilms was done according to Özcan Ateş and Otkun [12]. In the microplate method, we used to evaluate biofilm formation before. After 48 hours of incubation at 37 °C, the plates were emptied, and 200 μL of SDB medium containing mouthwash was added to the wells, then incubated at 37 °C for 24 hours. After incubation, the effect of mouthwash on mature biofilm was evaluated as in the biofilm formation method. The study was carried out in two parallels and three repetitions.

2.6. Statistical analysis

All results are given as mean (M) ± standard deviation (SD) and were evaluated at a 0.05 significance using SPSS Package Program (v23.0, IBM Corp).

3. RESULTS

The agar well diffusion method was initially used to evaluate the antifungal activity of twenty-five commercial mouthwashes against yeasts isolated from the oral cavity. Twelve mouthwashes that contain CPC displayed antifungal activity against these yeasts. The inhibition zone diameters for mouthwashes with antifungal activity are presented in Table 2. However, 13 mouthwashes that did not contain CPC (901, 902, 903, 904, 905, 906, 907, 908, 910, 920, 921, 922, and 923) were found to have no antifungal activity against the tested isolates. Among the tested mouthwashes, only 922 contained chlorhexidine digluconate but did not show antifungal activity against the tested yeasts. It was concluded that, among the mouthwashes identified as having antifungal activity, 909 exhibited the highest antifungal effect against yeasts isolated from the mouth, while 913 exhibited the lowest antifungal effect.

Table 2. Agar well diffusion inhibition zone diameters (in mm) (M ± sd)

Isolate	909	911	912	913	914	915	916	917	918	919	924	925
1	17.15±1.34	11.73±0.65	11.97±0.74	10.97±0.53	11.48±0.56	13.34±0.75	20.44±3.23	17.47±1.56	16.90±1.97	20.12±1.76	15.77±0.99	15.82±1.46
2	18.04±1.13	11.59±0.69	11.52±0.26	10.67±0.58	11.13±0.34	13.20±0.37	18.23±1.97	16.76±1.55	16.24±1.37	17.98±2.11	16.04±0.68	15.66±0.81
3	27.63±4.21	11.74±0.55	12.35±0.88	10.47±0.59	12.36±0.44	16.47±1.58	22.23±1.80	25.76±4.13	19.58±2.58	26.35±3.62	20.31±1.60	21.11±2.64
4	17.88±1.24	11.49±0.75	11.52±0.75	10.38±0.73	11.12±0.92	13.07±1.15	16.92±0.80	17.82±1.57	15.50±0.91	16.20±1.66	15.67±0.70	15.84±1.39
5	19.98±1.94	12.31±0.33	12.65±0.46	12.10±0.82	12.29±0.68	14.57±0.89	18.79±1.16	17.08±2.11	17.90±2.51	20.04±2.28	18.30±1.29	15.78±1.86
6	24.12±3.70	12.36±0.57	13.93±1.87	11.18±0.25	12.56±0.48	16.10±0.83	23.84±3.34	20.71±3.39	17.95±2.03	21.67±2.09	19.94±2.51	21.66±2.89
7	23.74±3.67	13.19±1.43	12.65±0.73	10.73±0.77	12.56±0.55	16.13±1.07	24.23±2.01	22.01±3.90	21.42±1.38	19.23±1.91	21.33±1.75	20.02±2.02

19	18	17	16	15	14	13	12	11	10	9	8
24.53±2.10	27.15±3.62	22.31±1.75	24.42±4.95	24.00±2.49	22.18±2.02	23.77±3.42	29.33±2.76	25.46±2.66	26.69±5.26	23.38±1.96	24.91±2.66
12.12±0.68	11.56±0.54	11.13±0.68	10.25±1.28	12.10±0.54	11.50±1.88	12.32±0.47	10.99±0.81	13.03±0.45	13.48±0.86	10.83±0.72	11.57±0.43
12.60±0.99	12.87±1.45	11.58±0.62	12.18±0.67	12.65±1.02	12.94±0.63	12.06±0.28	11.97±0.71	12.38±0.35	12.95±0.71	9.98±0.89	11.77±0.95
9.72±0.45	8.29±0.39	7.90±0.31	7.91±0.72	9.75±0.52	10.19±0.86	10.29±0.50	9.08±0.97	10.79±0.70	11.80±0.58	8.03±0.83	8.58±0.35
12.27±1.58	10.94±1.40	10.53±0.48	11.02±0.46	11.78±0.34	12.66±0.99	12.23±0.24	12.10±1.23	12.24±1.06	13.35±0.44	10.05±0.73	10.76±0.52
14.95±1.77	15.50±2.76	14.88±1.55	15.51±1.84	15.33±1.59	15.23±1.47	15.29±1.54	15.52±1.52	14.65±0.82	15.47±1.12	14.16±0.83	14.62±0.87
25.55±2.78	23.02±2.96	23.00±3.89	23.11±3.40	21.35±2.62	23.60±4.10	26.11±3.46	20.41±2.28	19.35±4.25	25.00±3.13	20.63±2.21	21.76±1.58
24.57±2.19	22.20±1.93	19.69±3.32	20.79±2.49	20.27±2.84	19.66±2.69	22.75±3.21	24.44±4.51	20.47±3.85	19.31±4.05	22.06±2.31	17.20±0.95
21.45±3.40	18.68±0.26	19.47±1.98	19.54±1.60	17.85±3.00	18.32±1.31	23.54±2.90	20.27±2.16	18.81±3.11	20.71±4.01	20.22±2.34	17.82±1.92
25.89±2.62	19.36±0.90	21.39±2.06	22.06±3.61	18.66±3.20	19.34±2.19	26.37±3.39	23.74±3.91	21.41±5.52	24.14±4.38	20.42±2.12	19.19±1.75
22.30±1.62	16.43±1.63	18.18±1.25	17.42±2.16	18.59±2.30	18.35±1.69	18.49±1.39	18.27±1.45	17.85±1.10	19.25±1.69	17.02±1.27	16.97±1.72
18.88±1.38	17.78±0.88	18.26±0.74	18.01±1.16	18.058±1.30	18.62±1.80	19.16±1.20	20.74±2.59	18.00±2.02	17.65±1.71	16.93±1.33	17.58±2.66

20	35.52±8.37	14.03±0.71	19.79±2.44	16.02±0.81	17.32±1.42	23.96±1.56	31.84±4.57	26.29±6.77	23.36±2.84	32.46±4.16	28.33±3.36	27.76±2.66

The antifungal activity of the mouthwashes was found to be statistically significant, depending on the isolate and the type of mouthwash (P=0.000). Furthermore, both the alcohol and plant extract content of the mouthwashes, along with the amount of sodium fluoride, demonstrated statistically significant antifungal activity (P=0.000). Five mouthwashes exhibiting antifungal activity (909, 911, 915, 919, and 924), each sourced from different manufacturers, were selected, and their MIC values were determined and presented in Table 3.

Table 3. MIC values of mouthwashes (μL/mL)

Isolates	Microorganisms	909	911	915	919	924
1	*C. albicans*	3.90	7.81	15.62	2.00	3.90
2	*C. albicans*	2.00	7.81	15.62	2.00	3.90
3	*C. albicans*	3.90	7.81	7.81	2.00	3.90
4	*C. albicans*	3.90	7.81	15.62	3.90	3.90
5	*C. dublinensis*	2.00	7.81	15.62	2.00	3.90
6	*C. dublinensis*	3.90	7.81	15.62	2.00	3.90
7	*C. dublinensis*	3.90	7.81	15.62	2.00	3.90
8	*C. dublinensis*	3.90	7.81	15.62	3.90	3.90
9	*C. dublinensis*	2.00	7.81	15.62	2.00	3.90
10	*C. parapsilosis*	3.90	7.81	15.62	2.00	3.90
11	*C. parapsilosis*	2.00	7.81	7.81	3.90	3.90
12	*C. parapsilosis*	2.00	7.81	7.81	2.00	3.90
13	*C. parapsilosis*	2.00	7.81	7.81	3.90	7.81
14	*C. parapsilosis*	3.90	7.81	15.62	3.90	3.90
15	*C. parapsilosis*	3.90	7.81	15.62	3.90	3.90
16	*C. parapsilosis*	3.90	7.81	15.62	2.00	7.81
17	*C. parapsilosis*	3.90	7.81	7.81	2.00	3.90
18	*C. parapsilosis*	3.90	15.62	7.81	3.90	3.90
19	*C. parapsilosis*	3.90	7.81	15.62	2.00	3.90
20	*P. manshurica*	2.00	3.90	15.62	2.00	3.90

It was found that the MIC values of 909 and 919 against planktonic yeast isolates with biofilm formation potential ranged from 2.0 to 3.90 μL/mL. The MIC values of 911 and 924 ranged from 3.90 to 7.81 μL/mL. The highest MIC value was 7.81 and 15.62 μL/mL for 915. The MIC values of 909, 915, 919, and 924 were similar across all tested isolates and were not influenced by species differences. However, the MIC value for 911 was influenced by the species. The lowest MIC value (3.90 μL/mL) was observed in the *P. manshurica*. In contrast, the MIC value was 7.81 μL/mL for all tested *Candida* isolates, except for one *C. parapsilosis* isolate. The effects of the mouthwashes on the biofilm formation capacity of yeasts and mature biofilms were also assessed based on the determined MIC values.

The effects of the mouthwashes on biofilm formation are presented in Table 4. In contrast, the impact on mature biofilms is outlined in Table 5. When evaluating the effects of the mouth-

washes on biofilm formation according to the MIC values, it was found that the highest inhibition rate (95.05%) was noted in the 915 against the strong biofilm producer *C. parapsilosis* (16). In comparison, the lowest inhibition rate (1.83%) was seen in 909 against *C. parapsilosis* (18). 911 inhibited biofilm formation in all tested isolates by 2.83% to 91.12%. Conversely, 909 enhanced the biofilm formation potential of six isolates by 11.76% to 53.36%.

Table 4. Effect of mouthwashes on biofilm formation potential of yeasts evaluated at MIC values (in %)

Isolates	Microorganisms	909	911	915	919	924
1	*C.albicans*	37.24	77.49	82.48	72.86	17.62
2	*C. albicans*	**-16.45***	58.69	55.43	32.24	36.71
3	*C. albicans*	73.79	89.57	88.32	65.34	85.14
4	*C. albicans*	11.71	2.83	**-9.92**	1.84	29.19
5	*C. dublinensis*	**-11.76**	19.74	47.39	39.25	**-9.10**
6	*C. dublinensis*	58.10	67.01	74.20	**-5.79**	61.43
7	*C. dublinensis*	**-16.93**	50.50	50.50	61.63	51.16
8	*C. dublinensis*	55.77	89.88	93.65	69.82	89.51
9	*C. dublinensis*	10.80	80.83	90.33	**-12.68**	76.32
10	*C. parapsilosis*	38.45	60.32	65.88	7.53	62.75
11	*C. parapsilosis*	**-16.18**	74.92	72.41	61.87	64.91
12	*C. parapsilosis*	71.71	8.70	**-46.35**	72.38	61.91
13	*C. parapsilosis*	18.15	87.73	88.21	87.14	88.55
14	*C. parapsilosis*	45.44	71.44	82.38	79.20	82.35
15	*C. parapsilosis*	64.13	76.51	76.88	78.26	73.80
16	*C. parapsilosis*	26.85	91.12	95.05	15.37	93.11
17	*C. parapsilosis*	**-53.36**	85.49	89.55	6.94	56.19
18	*C. parapsilosis*	1.83	89.60	85.57	64.25	74.58
19	*C. parapsilosis*	**-24.00**	62.46	70.77	10.88	57.62
20	*P. manshurica*	34.26	72.04	77.92	38.10	66.51

*** ineffective against biofilm formation**

According to the MIC values of the mouthwashes, the highest inhibition rate (52.59%) on the mature biofilm structure formed by the tested yeasts was observed with sample 915, while the lowest inhibition rate (0.49%) was noted with the *C. parapsilosis*, mouthwash of 924. The 909 specifically inhibited the mature biofilm of *P. manshurica* and had no effect on the biofilm structure formed by the tested *Candida* isolates; on the contrary, it increased biofilm formation by up to 217.08%. 911 affected seven isolates, 915 affected eight isolates, 919 affected nine isolates, and 924 affected the mature biofilms of six isolates. Additionally, when the tested mouthwashes were applied at MIC values, they formed a more robust biofilm structure, resulting in considerably increased biofilm production compared to the control group. All mouthwashes impacted the biofilm structure formed by the *P. manshurica* isolate, providing an inhibition rate in the mature biofilm structure of 2.26% and 34.79%.

Table 5. Effect of on mature biofilm structure formed by yeasts evaluated at MIC values (in %).

Isolates	Microorganisms	909	911	915	919	924

1	*C.albicans*	-25.98	**8.13***	-57.70	-71.73	-44.29
2	*C. albicans*	-217.08	-22.25	-57.91	-22.67	-129.54
3	*C. albicans*	-90.55	-22.85	-4.50	-13.70	**5.77**
4	*C. albicans*	-42.06	-40.05	**38.80**	-1.20	-6.31
5	*C. dublinensis*	-80.44	-40.71	-50.44	**51.38**	-21.91
6	*C. dublinensis*	-83.27	**15.61**	**7.33**	**12.82**	-36.17
7	*C. dublinensis*	-33.29	-4.94	**48.68**	**27.01**	**4.90**
8	*C. dublinensis*	-73.64	-19.37	-114.96	-88.92	-105.22
9	*C. dublinensis*	-60.82	-58.95	-65.72	-80.00	-72.81
10	*C. parapsilosis*	-69.48	**6.04**	-8.00	-26.70	-12.80
11	*C. parapsilosis*	-115.68	**4.91**	-40.22	-39.18	-35.35
12	*C. parapsilosis*	-0.76	**31.99**	**50.00**	**11.06**	**0.49**
13	*C. parapsilosis*	-43.86	-12.54	**52.59**	**28.15**	**49.38**
14	*C. parapsilosis*	-30.33	**23.63**	-14.97	30.83	-28.59
15	*C. parapsilosis*	-19.41	**45.12**	**9.42**	**36.07**	-21.52
16	*C. parapsilosis*	-65.51	-50.30	-112.31	-142.93	-37.97
17	*C. parapsilosis*	-156.33	-126.05	-121.84	-118.22	-129.01
18	*C. parapsilosis*	-119.11	-103.12	-57.70	-149.10	-70.48
19	*C. parapsilosis*	-70.16	-4.85	**48.92**	**34.58**	**42.90**
20	*P. manshurica*	**2.29**	**34.79**	**27.54**	**14.77**	**26.77**

*** effective on mature biofilm structure**

When evaluating the effects of mouthwashes on both biofilm formation and mature biofilm, it was found that their effectiveness during biofilm formation was high. In contrast, their impact on the structure of existing biofilm was low.

4. DISCUSSION

The health status of the oral region is closely related to numerous factors, including personal and professional hygiene, regular care, daily dental care, and toothpaste use [15]. Mouthwashes can be mechanical irrigation methods for removing organisms from teeth or supporting integration [16]. Mouthwashes serve various purposes, including teeth whitening, combating bad breath, and treating minor infections. As a result, they contain a range of compounds such as water, antimicrobial agents, and anti-inflammatory substances [8, 17-19]. The antimicrobial agents in mouthwashes specifically help control microorganisms. Using antimicrobial mouthwashes is advisable to reduce the presence of *Streptococcus mutans*, which is known to be the primary cause of dental caries due to its ability to form biofilms on teeth [18, 20]. Literature also indicates that mouthwashes influence microorganisms linked to dental plaque and the oral cavity, exhibiting antimicrobial properties [20-23]. Particularly, mouthwashes that contain alcohol, chlorhexidine, or plant extracts have demonstrated high antimicrobial effectiveness [21-25].

When examining the antifungal effects of mouthwashes, recent studies have shown that those containing CPC are effective against both planktonic and biofilm-embedded fungal cells [7, 26, 27]. CPC is a quaternary ammonium compound categorized within the cationic surfactant group, which interacts with the cell walls of microorganisms, leading to leakage of cytoplasmic material and disruption of their metabolism, ultimately resulting in cell death [18, 20]. CPC possesses plaque and tartar inhibitory properties and has a broad antimicrobial spectrum

that targets gram-positive bacteria and yeasts [18]. You *et al.* found that the MIC values of two different mouthwashes containing CPC were 0.97 and 1.95 μL/mL against the *C. albicans* KCTC 727, while five different mouthwashes without CPC also yielded MIC values ranging from 125 to 250 μL/mL [22]. Korbecka-Paczkowska and Karpiński investigated the anticandidal activity of fifteen mouthwashes available in the European market against ten clinical strains of *C. albicans* obtained from patients diagnosed with candidiasis and two standard cultures (*C. albicans* ATCC 10231 and ATCC 14053). They found that the mouthwashes containing CPC at 0.13% concentration exhibited good activity against *C. albicans* [28]. The study indicated that only mouthwashes containing CPC displayed antifungal activity against yeasts capable of forming biofilms isolated from the mouth, with the MIC values varying between 2.00 and 15.62 μL/mL, consistent with previous literature.

Chlorhexidine is a cationic biguanide with a broad antimicrobial spectrum. It is particularly effective against dental biofilm and gingivitis. Chlorhexidine is highly persistent, capable of binding to tissues in the mouth, which allows it to have a long-term effect after application [20]. Di Lodovico *et al.* found that the MIC value of mouthwashes containing chlorhexidine digluconate at concentrations of 0.05-0.12% against *C. albicans* ranged from 0.02% to 0.09% [29]. Korbecka-Paczkowska and Karpiński reported that the MIC value of mouthwashes containing chlorhexidine digluconate was determined to be 0.12% [28]. While several other studies have also shown that chlorhexidine-containing mouthwashes exhibit antifungal activity against yeast cells [10, 27, 30, 31], only one mouthwash contained chlorhexidine, which was ineffective.

Unlike other yeast species, *Candida* species can exist as either a single cell, a budding cell, or a filamentous yeast form depending on environmental conditions. This phenomenon, known as dimorphic transition, is crucial for adhesion, invasion, tissue damage, dissemination, immune evasion, and virulence related to biofilm formation. Biofilm formation is a significant event in the pathogenesis of many infections, including oral candidiasis [32]. Therefore, the effects of mouthwashes on biofilm formation and mature biofilm were also evaluated in the study. In the antibiofilm activity study, it was determined that CPC-containing mouthwashes affected biofilm formation, while their impact on mature biofilm was seen to be limited. The existing literature on the effects of mouthwashes on biofilms formed by yeasts is quite scarce. Various methods were employed in these limited studies. Using a method like this study, Nikseresht *et al.* evaluated the effects of herbal mouthwashes on the biofilm of *Streptococcus mutans* [33]. Other studies in literature also employed different methods [28, 34, 35]. Therefore, comparing our study results with the available data is quite challenging.

This study has several limitations. It examined mouthwashes sold in beauty salons and markets in Türkiye, but the exact concentrations of the substances in these mouthwashes are not specified in the ingredient list. Because this information is unavailable, comparing and interpreting the study results is challenging. Additionally, due to budget constraints, not all products on the market could be tested. Moreover, these budget limitations restricted the number of cultures studied, making it impossible to increase this number, particularly for antifungal-resistant isolates. These shortcomings hinder the findings of our study.

5. CONCLUSION

Consequently, it was determined that the impact of the five selected mouthwashes on biofilm formation was significant, but their effect on mature biofilm was minimal. Thus, the mouthwashes can be utilized in daily oral hygiene routines. However, the effectiveness of these

mouthwashes against bacterial and fungal microorganisms in both patients and healthy individuals should be further investigated. Their effects on complex biofilms and antifungal resistance isolates must be examined, and the results should be presented.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit with project number: TSA-2020-3341. This study was conducted at Çanakkale Onsekiz Mart University Experimental Research Center (ÇOMÜDAM) and Çanakkale Onsekiz Mart University Faculty of Medicine Medical Microbiology Laboratory.

REFERENCES

1. W.G. Wade. The oral microbiome in health and disease. *Pharmacological Research*, **69**(1), 137–143 (2013). https://doi.org/10.1016/j.phrs.2012.11.006
2. N. Garg, T. Luzzatto-Knaan, A.V. Melnik, A.M. Caraballo-Rodríguez, D.J. Floros, D. Petras, R. Gregor, P.C. Dorrestein & V.V. Phelan. Natural products as mediators of disease. *Natural Products Reports*, **34**, 194–219 (2017). https://doi.org/10.1039/c6np00063k
3. T. Kuriyama, D.W. Williams, J. Bagg, W.A. Coulter, D. Ready & M.A.O. Lewis. *In vitro* susceptibility of oral *Candida* to seven antifungal agents. *Oral Microbiology and Immunology*, 20(6), 349–353 (2005). https://doi.org/10.1111/j.1399-302x.2005.00236.x
4. Y. Liu, Y. Xu, Q. Song, F. Wang, L. Sun, L. Liu, *et al.* Anti-biofilm activities from *Bergenia crassifolia* leaves against *Streptococcus mutans*. *Frontiers in Microbiology*, **8**, 1738 (2017). https://doi.org/10.3389/fmicb.2017.01738
5. A. Ardizzoni, G. Boaretto, E. Pericolini, D. Pinetti, A. Capezzone de Joannon, L. Durando, L. Ragni & E. Blasi. Effects of benzydamine and mouthwashes containing benzydamine on *Candida albicans* adhesion, biofilm formation, regrowth, and persistence. *Clinical Oral Investigations*, **26**(4), 3613–3625 (2022). https://doi.org/10.1007/s00784-021-04330-8
6. E.M. Costa, S. Silva, A.R. Madureira, A. Cardelle-Cobas, F.K. Tavaria & M.M. Pintado. A comprehensive study into the impact of a chitosan mouthwash upon oral microorganism's biofilm formation *in vitro*. *Carbohydrate Polymers*, **101**, 1081–1086 (2014). https://doi.org/10.1016/j.carbpol.2013.09.041
7. J. Latimer, J.L. Munday, K.M. Buzza, S. Forbes, P.K. Sreenivasan & A.J. McBain. Antibacterial and anti-biofilm activity of mouthrinses containing cetylpyridinium chloride and sodium fluoride. *BMC Microbiology*, **15**(1), 169 (2015). Doi: https://doi.org/10.1186/s12866-015-0501-x
8. M. Amin, F. Babadi, N. Baghipour & B. Sadeghi-Nejad. Evaluation of the effect of Jaftex herbal mouthwash on the growth of *Candida albicans* and *Candida tropicalis*. *Journal of Family Medicine and Primary Care*, **10**(10), 3815–3819 (2021). https://doi.org/10.4103%2Fjfmpc.jfmpc_1067_21
9. A. Ardizzoni, E. Pericolini, S, Paulone, C.F. Orsi, A. Castagnoli, I. Oliva, E. Strozzi & E. Blasi. *In vitro* effects of commercial mouthwashes on several virulence traits of *Candida albicans*, viridans streptococci and *Enterococcus faecalis* colonizing the oral cavity, *PLoS One*, **13**(11), e0207262 (2018). https://doi.org/10.1371/journal.pone.0207262
10. S. Paulone, G. Malavasi, A. Ardizzoni, C.F. Orsi, S. Peppoloni, R.G. Neglia & E. Blasi. *Candida albicans* survival, growth and biofilm formation are differently affected by mouthwashes: an *in vitro* study. *New Microbiologica*, **40**(1), 45–52 (2017). URL: https://www.newmicrobiologica.org/pub/allegati_pdf/2017/1/45.pdf

11. A.N. Ellepola, Z.U. Khan, R. Chandy & L. Philip. A comparison of the antifungal activity of herbal toothpastes against other brands of toothpastes on clinical isolates of *Candida albicans* and *Candida dubliniensis*. *Medical Principles and Practice*, **20**(2), 112–117 (2011). https://doi.org/10.1159/000321199
12. G. Özcan-Ateş & M. Otkun. *In vitro* evaluation of biofilm forming capacity and antifungal resistance of yeast isolated from individuals aged 18-25 years. *Sinop Üniversitesi Fen Bilimleri Dergisi*, **8**(1), 1–18 (2023). https://doi.org/10.33484/sinopfbd.1310745
13. Clinical and Laboratory Standards Institute. *Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts: Approved Guideline.* NCCLS document M44-A [ISBN 1-56238-532-1]. NCCLS, Wayne (PA), USA, 2004. URL: https://clsi.org/shop/standards/m44/
14. Clinical and Laboratory Standards Institute. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard—Second Edition*. NCCLS document M27-A2 [ISBN 1-56238-469-4]. NCCLS, Wayne (PA), USA, 2002. URL: https://clsi.org/shop/standards/m27/
15. S. Stepanović, D. Vuković, I. Dakić, B. Savić & M. Švabić-Vlahović. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *Journal of Microbiological Methods*, **40**(2), 175–179 (2000). https://doi.org/10.1016/s0167-7012(00)00122-6
16. P. Axelsson & J. Lindhe. Effect of controlled oral hygiene procedures on caries and periodontal disease in adults: Results after 6 years. *Journal of Clinical Periodontology*, **8**(3), 239–248 (1981). https://doi.org/10.1111/j.1600-051x.1981.tb02035.x
17. A. Haerian-Ardakani, M. Rezaei, M. Talebi-Ardakani, N.K. Valian, R. Amid, M. Meimandi, A. Esmailnejad & A. Ariankia. Comparison of antimicrobial effects of three different mouthwashes. *Iranian Journal of Public Health*, **44**(7), 997–1003 (2015). URL: https://pmc.ncbi.nlm.nih.gov/articles/PMC4645771/
18. S. Haps, D.E. Slot, C.E. Berchier & G.A. Van der Weijden. The effect of cetylpyridinium chloride-containing mouth rinses as adjuncts to toothbrushing on plaque and parameters of gingival inflammation: a systematic review. *International Journal of Dental Hygiene*, **6**(4), 290–303 (2008). https://doi.org/10.1111/j.1601-5037.2008.00344.x
19. Y. Khanchemehr, H. Hoseynrezaei, G. Sepehri, M. Torabi, Y. Jahani & A. Khanchemehr. A comparison of the effects of mouthwash obtained from green tea and chlorhexidine mouthwash on the oral health of patients admitted in the intensive care unit. *Journal of Oral Health Oral Epidemiology*, **8**(1), 9–16 (2019). https://doi.org/10.22122/johoe.v8i1.410
20. E. Watanabe, A.P. Nascimento, J.M. Guerreiro-Tanomaru, A.M. Razaboni, D. de Andrade & M. Tanomaru-Filho. Antiseptic mouthwashes: *in vitro* antibacterial activity. *Acta Odontológica Latinoamericana*, **28**(2), 180–184 (2015). URL: https://actaodontologicalat.com/wp-content/uploads/2017/07/aol_2015_28_2_180.pdf
21. A. Sharma, N. Agarwal, A. Anand & Z. Jabin. To compare the effectiveness of different mouthrinses on *Streptococcus mutans* count in caries active children. *Journal of Oral Biology and Craniofacial Research*, **8**(2), 113–117 (2018). https://doi.org/10.1016/j.jobcr.2018.05.002
22. M.S. You, S.Y. Lee & D.-S. Ma. *In vitro* antimicrobial activity of different mouthwashes available in Korea. *Journal of Korean Academy of Oral Health*, **41**(3), 188–193 (2017). http://doi.org/10.11149/jkaoh.2017.41.3.188
23. B. Serbiak, T. Fourre, A.R. Geonnotti & R.J. Gambogi. *In vitro* efficacy of essential oil mouthrinse versus dentifrices. *Journal of Dentistry*, **69**, 49–54 (2018). https://doi.org/10.1016/j.jdent.2017.08.016
24. R. Singhal, M. Siddibhavi, R. Sankeshwari, P. Patil, S. Jalihal & A. Ankola. Effectiveness of three mouthwashes–Manuka honey, Raw honey, and Chlorhexidine on plaque and gingival scores of 12–15-year-old school children: A randomized controlled field trial. *Journal of Indian Society of Periodontology*, **22**(1), 34–39, (2018). https://doi.org/10.4103/jisp.jisp_356_17
25. L.S. Bafti, M. Rad, M.S. Soormaghi & M. Rezaei. An *in vivo* evaluation of antimicrobial effects of Persica herbal mouthwash on *Candida albicans* and *Enterococcus faecalis*. *Journal of Oral Health Oral Epidemiology*, **2**(2), 64–69 (2013). URL: https://johoe.kmu.ac.ir/article_84783.html
26. P.K. Sreenivasan, V.I. Haraszthy & J.J. Zambon. Antimicrobial efficacy of 0.05% cetylpyridinium chloride mouthrinses. *Letters in Applied Microbiology*, **56**(1), 14–20 (2013). https://doi.org/10.1111/lam.12008

27. J. Fu, P. Wei, C. Zhao, C. He, Z. Yan & H. Hua. *In vitro* antifungal effect and inhibitory activity on biofilm formation of seven commercial mouthwashes. *Oral Diseases*, **20**, 815–820 (2014). https://doi.org/10.1111/odi.12242
28. M. Korbecka-Paczkowska & T.M. Karpiński. *In vitro* assessment of antifungal and antibiofilm efficacy of commercial mouthwashes against *Candida albicans*. *Antibiotics*, **13**(2), 117 (2024). https://doi.org/10.3390/antibiotics13020117
29. S. Di Lodovico, T.C. Dotta, L. Cellini, G. Iezzi, S. D'Ercole & M. Petrini. The antibacterial and antifungal capacity of eight commercially available types of mouthwash against oral microorganisms: an *in vitro* study. *Antibiotics*, **12**(4), 675 (2023). https://doi.org/10.3390/antibiotics12040675
30. P. Agarwal & L. Nagesh. Comparative evaluation of efficacy of 0.2% Chlorhexidine. Listerine and Tulsi extract mouth rinses on salivary *Streptococcus mutans* count of high school children—RCT. *Contemporary Clinical Trials*, **32**(6), 802–808 (2011). https://doi.org/10.1016/j.cct.2011.06.007
31. M.M. Pathan, K.G. Bhat & V.M. Joshi. Comparative evaluation of the efficacy of a herbal mouthwash and chlorhexidine mouthwash on select periodontal pathogens: An *in vitro* and *ex vivo* study. *Journal of Indian Society of Periodontology*, **21**(4), 270–275 (2017). https://doi.org/10.4103/jisp.jisp_382_16
32. I.D. Jacobsen, D. Wilson, B. Wächtler, S. Brunke, J.R. Naglik & B. Hube. *Candida albicans* dimorphism as a therapeutic target. *Expert Review of Anti-infective Therapy*, **10**(1), 85–93 (2012). Doi: https://doi.org/10.1586/eri.11.152
33. R. Nikseresht, S. Shams, A. Mehdipour, S. Kermani, A. Hashemi & A. Hamta. Evaluation of the antibacterial effects of three natural formulated mouthwashes against *Streptococcus mutans*: An *in vitro* study. *Journal of Oral Health Oral Epidemiology*, **8**(2), 97–103 (2019). https://doi.org/10.22122/johoe.v8i2.1003
34. G. Krasowski, A. Junka, J. Paleczny, J. Czajkowska, E. Makomaska-Szaroszyk, G. Chodaczek, M. Majkowski, P. Migdał, K. Fijałkowski, B. Kowalska-Krochmal & M. Bartoszewicz. *In vitro* evaluation of polihexanide, octenidine and NaClO/HClO-based antiseptics against biofilm formed by wound pathogens. *Membranes*, **11**(1), 62 (2021). https://doi.org/10.3390/membranes11010062
35. M. Kollmuss, K. Tolksdorf, S.N. Wuersching, R. Hickel & K.C. Huth. Effect of polyhexanide as antiseptic mouth rinse against oral pathogens in an *in vitro* biofilm model. *Acta Odontologica Scandinavica*, **79**(7), 506–513 (2021). https://doi.org/10.1080/00016357.2021.1899280

HOW TO CITE THIS ARTICLE

G. Özcan-Ateş & M. Otkun. Evaluation of antifungal and antibiofilm activity of commercial mouthwashes sold in Türkiye against oral biofilm-forming yeasts: an *in vitro* microbiological study. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 7–23 (2026). https://doi.org/10.15446/rcciquifa.v55n1.119332

Artículo de investigación científica

Estudio de la capacidad de crecimiento capilar de productos derivados del romero (*Rosmarinus officinalis*), en ratones (*Mus musculus*) C57BL6//BIOU

Robert J. Aljorna-Molero*, Freddy Carrillo-Rodríguez & Juan M. Amaro-Luis

Laboratorio de Productos Naturales, Departamento de Química, Facultad de Ciencias, Universidad de Los Andes (ULA), 5115, Mérida, Venezuela.
*Correo electrónico: robertaljorna98@gmail.com

Recibido: 14 de julio de 2025
Corregido: 30 de agosto de 2025
Aceptado: 3 de septiembre de 2025

https://doi.org/10.15446/rcciquifa.v55n1.121587

RESUMEN

Introducción: el romero (*Rosmarinus officinalis*) ha sido tradicionalmente reconocido por sus propiedades beneficiosas, incluyendo su potencial para estimular el crecimiento capilar. La búsqueda de alternativas naturales y seguras al minoxidil, el fármaco de referencia, ha impulsado la investigación de extractos vegetales. **Objetivo:** evaluar el efecto sobre el crecimiento capilar de extractos derivados de la planta del romero (*Rosmarinus officinalis*) de manera individual (aceite esencial, hidrolato y extracto alcohólico) y en conjunto (en forma de champú y tónico capilar) en ratones macho de la línea C57BL6//BIOU. **Métodos:** Se utilizaron ratones macho de la línea C57BL6//BIOU, distribuidos en 7 grupos de tratamiento: agua destilada (AD), aceite esencial (AE), extracto alcohólico (ER), hidrolato (HR), tónico capilar (TC), champú (CH) y minoxidil al 5% (MXD). El crecimiento capilar fue evaluado durante 5 semanas mediante dos métodos: una escala de crecimiento con registro fotográfico y la medición de la longitud de los pelos tomados por tracción. Los resultados fueron contrastados con un grupo control negativo (agua destilada) y un control positivo (minoxidil al 5%). **Resultados:** la escala de crecimiento demostró que los ratones de los grupos AE, ER, HR, TC y CH exhibieron un crecimiento capilar considerable, aunque esta escala presentó limitaciones para evaluar ratones con nevo melanocítico. Por su parte, la medición de la longitud de los pelos confirmó que los grupos AE, ER, HR, TC y CH presentaron un crecimiento estadísticamente superior que el de los grupos control (p-valor < 0,05). **Conclusiones:** los extractos de romero empleados en esta investigación (aceite esencial, hidrolato y extracto alcohólico), tanto de forma individual como combinada en champú y tónico capilar, estimulan el crecimiento capilar en ratones macho C57BL6//BIOU, incluso en mayor medida que el fármaco comercial minoxidil al 5%. Estos hallazgos sugieren el gran potencial del romero como una alternativa natural para el tratamiento de la alopecia.

Palabras clave: *Rosmarinus officinalis*; alopecia; crecimiento capilar; ratones C57BL/6; extracto.

SUMMARY

Study of the hair growth capacity of rosemary (*Rosmarinus officinalis*) derived products in C57BL6//BIOU mice (*Mus musculus*)

Introduction: rosemary (*Rosmarinus officinalis*) has been traditionally recognized for its beneficial properties, including its potential to stimulate hair growth. The search for natural and safe alternatives to minoxidil, the reference drug, has driven research into plant extracts. **Objective:** to evaluate the effect

on hair growth of extracts derived from the rosemary plant (*Rosmarinus officinalis*) individually (essential oil, hydrolate, and alcoholic extract) and in combination (as shampoo and hair tonic) in male C57BL6//BIOU mice. **Methods:** male C57BL6//BIOU mice were used, divided into 7 treatment groups: distilled water (DW), essential oil (EO), alcoholic extract (AE), hydrolate (HR), hair tonic (HT), shampoo (SH), and 5% minoxidil (MXD). Hair growth was evaluated for 5 weeks using two methods: a growth scale with photographic records and the measurement of hair length obtained by traction. The results were contrasted with a negative control group (distilled water) and a positive control (5% minoxidil). **Results:** the growth scale showed that mice in the EO, AE, HR, HT, and SH groups exhibited considerable hair growth, although this scale had limitations when evaluating mice with melanocytic nevi. Furthermore, the measurement of hair length confirmed that the EO, AE, HR, HT, and SH groups presented statistically superior growth compared to the control groups (p-value < 0.05). **Conclusions:** The rosemary extracts used in this research (essential oil, hydrolate, and alcoholic extract), both individually and combined in shampoo and hair tonic, stimulate hair growth in male C57BL6//BIOU mice, even to a greater extent than the commercial drug 5% minoxidil. These findings suggest the great potential of rosemary as a natural alternative for the treatment of alopecia.

Keywords: *Rosmarinus officinalis*; alopecia; hair growth; C57BL/6 mice; extract.

RESUMO

Estudo da capacidade de crescimento capilar de produtos derivados do alecrim (*Rosmarinus officinalis*) em camundongos (*Mus musculus*) C57BL6//BIOU

Introdução: o alecrim (*Rosmarinus officinalis*) tem sido tradicionalmente reconhecido por suas propriedades benéficas, incluindo seu potencial para estimular o crescimento capilar. A busca por alternativas naturais e seguras ao minoxidil, o fármaco de referência, tem impulsionado a pesquisa de extratos vegetais. **Objetivo:** avaliar o efeito no crescimento capilar de extratos derivados da planta do alecrim (*Rosmarinus officinalis*) de forma individual (óleo essencial, hidrolato e extrato alcoólico) e em conjunto (na forma de shampoo e tônico capilar) em camundongos machos da linhagem C57BL6//BIOU. **Métodos:** foram utilizados camundongos machos da linhagem C57BL6//BIOU, distribuídos em 7 grupos de tratamento: água destilada (AD), óleo essencial (OE), extrato alcoólico (EA), hidrolato (HL), tônico capilar (TC), shampoo (SH) e minoxidil a 5% (MXD). O crescimento capilar foi avaliado durante 5 semanas por meio de dois métodos: uma escala de crescimento com registro fotográfico e a medição do comprimento dos pelos retirados por tração. Os resultados foram contrastados com um grupo controle negativo (água destilada) e um controle positivo (minoxidil a 5%). **Resultados**: a escala de crescimento demonstrou que os camundongos dos grupos OE, EA, HL, TC e SH exibiram um crescimento capilar considerável, embora essa escala tenha apresentado limitações para avaliar camundongos com nevo melanocítico. Por sua vez, a medição do comprimento dos pelos confirmou que os grupos OE, EA, HL, TC e SH apresentaram um crescimento estatisticamente superior ao dos grupos controle (p-valor < 0,05). **Conclusões:** os extratos de alecrim utilizados nesta pesquisa (óleo essencial, hidrolato e extrato alcoólico), tanto de forma individual quanto combinada em shampoo e tônico capilar, estimulam o crescimento capilar em camundongos machos C57BL6//BIOU, inclusive em maior medida que o fármaco comercial minoxidil a 5%. Esses achados sugerem o grande potencial do alecrim como uma alternativa natural para o tratamento da alopecia.

Palavras-chave: *Rosmarinus officinalis*; alopecia; crescimento capilar; camundongos C57BL/6; extrato.

1. INTRODUCCIÓN

El hombre, desde sus inicios, ha tratado sus enfermedades con el uso de los recursos que tenía a su alcance, siendo el Reino Vegetal su principal proveedor de fuentes terapéuticas; hecho reportado en todas las civilizaciones antiguas. Con el avance de la ciencia, se han comprobado

las propiedades de las plantas medicinales, haciendo con estas plantas tratamientos más eficaces y seguros [1-3].

La Organización Mundial de la Salud (OMS) reconoce muchas plantas medicinales y tóxicas en su manual ''*Estrategia de la OMS sobre la medicina tradicional 2014-2023*''. Este manual engloba los usos de las plantas medicinales, en tratamientos más naturales, inocuos, efectivos, de bajo costo, además de estar al alcance de la población general y ser ampliamente aceptadas por la misma [4-6].

Con el gran potencial terapéutico de las plantas medicinales, no es de extrañarse que desde tiempos muy tempranos el hombre estuviera interesado en emplearlas con fines estéticos, como el cuidado del cabello [7, 8]. En la gran mayoría de culturas antiguas, el cabello es una representación de estatus social y del atractivo personal, en donde la ausencia del mismo causaba, la mayoría de las veces, un gran impacto negativo tanto en hombres como mujeres [1, 9].

Muchas plantas se han utilizado en el cabello por diversas circunstancias, siendo bastante razonable la afirmación, de que la mayoría de las conocidas por el hombre, hayan sido probadas en el cabello en algún momento de la historia [9].

Una de las plantas que ha despertado el interés de los investigadores es el *Rosmarinus officinalis*, comúnmente conocida como romero, esta es una planta perteneciente a la familia *Lamiaceae* [10, 11], siendo autóctona de los países ribereños al Mar Mediterráneo [12]. Su uso está ampliamente ligado a la gastronomía [13, 14].

Existen diversos estudios que demuestran que, el extracto de *Rosmarinus officinalis* presenta una capacidad antiandrogénica (evitan la caída del cabello) [11, 12, 15]. Bajo este contexto se justifica el objetivo de la presente investigación; consiste en estudiar la capacidad de crecimiento capilar de productos derivados del romero (*Rosmarinus officinalis*) como el extracto etanólico, aceite esencial e hidrolato; en ratones (*Mus musculus*) C57BL6//BIOU.

La presente investigación es un derivado directo del trabajo de grado titulado ''Estudio de la capacidad de crecimiento capilar de productos derivados del romero (*Rosmarinus officinalis)* en ratones (*Mus musculus*) C57BL6/BIOU'', disponible en el repositorio digital de la Universidad de Los Andes [16].

2. MATERIALES Y MÉTODOS

2.1. Preparación del extracto etanólico de romero (*Rosmarinus officinalis*)

Se recolectaron 10 kg de material vegetal en la localidad de Mucuchíes, municipio Rangel, del estado Mérida-Venezuela, este fue identificado por el PhD. Juan Amaro consultando bibliografía especializada y comprándola con muestras del herbario de la Facultad de Ciencias de La Universidad de Los Andes, correspondiendo este a la especie *Rosmarinus officinalis*. Todo el material fue lavado con abundante agua para retirar la suciedad presente.

Se procedió a separar las hojas del resto de la planta (tallos, flores y semillas). Las hojas fueron secadas en una estufa a una temperatura de 40 °C, durante un período de 24 horas, obteniéndose 2,330 kg de hojas secas.

Las hojas secas fueron sometidas a una extracción continua en Soxhlet, en una proporción de 250 g de material vegetal con 600 mL de etanol (Merk al 98%) al 70%, a una temperatura de 70 °C durante 4 horas continuas. Se realizaron 15 extracciones en total, con un gasto de 10,36 L de etanol al 70%.

El extracto obtenido fue concentrado hasta una tercera parte de su volumen inicial (3,2 L), en un rotavapor a una temperatura de 70 °C por 2 horas. Se obtuvo un líquido ligeramente

espeso, de coloración verde-marrón, olor agradable y de pH=5; el cual fue resguardado en un envase de vidrio color ámbar hasta su posterior uso.

Para determinar el rendimiento del extracto obtenido, se procedió a pesar 6 matraces, luego se añadió a cada uno 10 mL del extracto (medidos con una pipeta volumétrica) y se pesaron nuevamente. Posteriormente, se secó el extracto dentro de la estufa a una temperatura de 40 °C por 48 horas y se pesaron los matraces de nuevo.

2.2. Preparación del champú y tónico capilar

Para la formulación del champú y tónico capilar emplearon las proporciones mostradas en las Tablas 1 y 2.

Tabla 1. Formulación del champú.

Componente	% m/m	Marca y pureza
Disolvente	40%	N/A
Tensoactivos	11,6%	N/A
Controlador de dureza del agua	0,08%	N/A
Hidratante	3%	N/A
Regulador de acidez	0,08%	N/A
Conservantes	0,2%	N/A
Espesante	1,9%	N/A
Hidrolato de romero	40%	Laboratorio Industrial de Productos Químicos Mérida C.A, No especifica.
Extracto de romero	3,1%	Fabricación propia, 2,7%.
Aceite esencial de romero	0,04%	QuimicHouse, 98% v/v.

Leyenda: N/A: no aplica.

Tabla 2. Formulación del champú.

Componente	% m/m	Marca y pureza
Disolvente	27%	N/A
Vehículo	30%	N/A
Humectantes	6%	N/A
Hidrolato de romero	27%	Laboratorio Industrial de Productos Químicos Mérida C.A, No especifica.
Extracto de romero	5%	Fabricación propia, 2,7% m/v.
Aceite esencial de romero	5%	QuimicHouse, 98% v/v

Leyenda: N/A: no aplica.

2.3. Elaboración del champú

Inicialmente, se midieron los volúmenes adecuados de disolvente e hidrolato de romero, a estos se les adicionaron las cantidades necesarias de los tensoactivos; y se agitó hasta su total dilución.

Se procedió a pesar las cantidades necesarias de hidratante, regulador de acidez y conservantes. Posteriormente, se incorporaron estos componentes a la mezcla líquida y, seguidamente, se mezclaron hasta lograr su total integración.

La mezcla se dejó reposar hasta la desaparición de la espuma, y se procedió a medir el pH de la mezcla. La acidez del champú se corrigió agregando pequeñas cantidades del regulador de acidez hasta obtener un pH deseado de 6,10.

Luego, se agregó pequeños volúmenes de del espesante hasta obtener la densidad deseada en la mezcla.

Finalmente, se añadió la cantidad adecuada de extracto y aceite esencial de romero. El preparado formulado fue resguardado en botellas de vidrio color ámbar debidamente rotuladas.

2.4. Elaboración del tónico capilar

Se midieron y mezclaron las cantidades necesarias de disolvente, extracto e hidrolato de romero y humectantes, hasta lograr su completa integración. En un recipiente aparte, se mezclaron el vehículo con la cantidad establecida de aceite esencial de romero y se agitó hasta homogenizar la solución.

Finalmente, ambas soluciones se mezclaron vigorosamente hasta su total integración. El preparado elaborado fue resguardado en una botella de vidrio color ámbar debidamente rotulada.

2.5. Elaboración del aceite esencial de romero al 4,8%

Se mezclaron 6 ml de aceite esencial de romero (al 98% v/v) con 114 mL de aceite mineral. El preparado fue guardado en un recipiente de vidrio color ámbar debidamente rotulado.

2.6. Elaboración del extracto de romero al 0,135%

Se mezclaron 6 ml de aceite extracto de romero (al 2,7% m/v) con 114 mL de agua destilada. El preparado fue guardado en un recipiente de vidrio color ámbar debidamente rotulado.

2.7. Ensayo en ratones C57BL6//BIOU

Todos los procedimientos aplicados sobres los animales fueron evaluados y aceptados por el ''Comete de Bioética del Bioterio de La Universidad de los Andes'' (CEBIOULA) con la identificación de protocolo ''CEBIOULA/131''.

Se emplearon 30 ratones machos de la línea C57BL6//BIOU, de aproximadamente 8 semanas de vida y un peso promedio de 25,7 g, a los cuales se les proporciono un tiempo de adaptación de 1 semana y se mantuvieron bajo las siguientes condiciones de alojamiento:

- Tipo de Jaula: T1.
- Individuos por Jaula: 4.
- Temperatura Ambietal: (22±1) °C.
- Periodo de Adaptación: 1 semana.
- Alimento y Agua: *at libitum* (a demanda).
- Ciclo de Luz/Oscuridad: 12 horas.

Terminado el periodo de adaptación, los animales fueron anestesiados con una mezcla de ketamina y xilacina, con una dosis de 90 mg/Kg de ketamina y 10 mg/Kg de xilacina, por vía intraperitoneal, obteniéndose un tiempo de anestesia promedio de 7-10 minutos, luego se procedió a afeitar un área de 4 cm^2 (2 x 2 cm) de la región dorsal de su cuerpo (**Figura 1**), y cada animal fue identificado mediante pequeños cortes en sus orejas (**Figura 2** y **Tabla 3**).

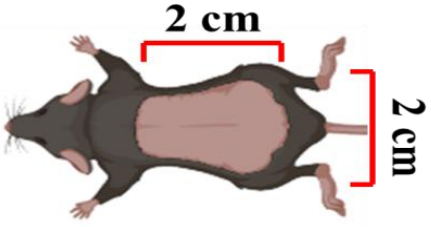


Figura 1. Representación del área afeitada en la región dorsal de los ratones.

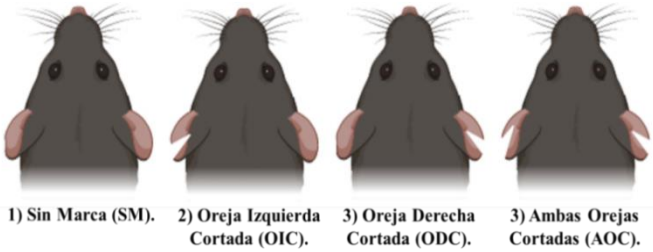


Figura 2. Representación del marcado de los ratones.

Pasado el tiempo de anestesia, los ratones fueron distribuidos, de manera aleatoria, en 7 grupos distintos, conformados por 4 individuos cada uno, en función de la aplicación tópica que se les proporcionó: agua destilada (AD), aceite esencial de romero al 4,9% (AR), extracto de romero al 0,135% (ER), hidrolato de romero (HR), tónico capilar (TC), champú (CH) y minoxidil al 5% (MDX).

Adicionalmente, se añadió un octavo grupo, llamado grupo control-control (CC), el cual estaba constituido por 2 ratones. A dicho grupo no se le sometió a ninguno de los procedimientos mencionados (anestesia, rapado, evaluación capilar y aplicación de sustancias). Este tiene la finalidad de servir como una referencia de un crecimiento capilar normal.

La aplicación de los productos se realizó de manera diaria, propiciándole a cada individuo 0,1 mL (medidos con una micropipeta) del producto correspondiente y este se distribuyó adecuadamente con ayuda de los dedos. En el caso del champú, luego de su aplicación este se retiró con ayuda de un pañuelo húmedo.

2.8. Evaluación del crecimiento capilar

La evaluación del crecimiento capilar de los ratones se realizó empleando dos escalas:

2.8.1. Método 1: Escala de crecimiento y registro fotográfico

Una evaluación fotográfica al final de las semanas 1, 2, 3, 4 y 5. Para la cual se armó una caja de observación, que consistía en una caja sin tapa con las siguientes dimensiones: 15 cm de alto, 5 cm de ancho y 7 cm de profundidad.

Dicha caja presentaba una abertura pequeña en su cara superior, la cual permitía el paso de la cola del animal. Esta caja tiene como función suspender al ratón en el aire, limitando su movimiento, facilitando así la toma de las fotografías sin necesidad de anestesiar al individuo (**Figura 3**).

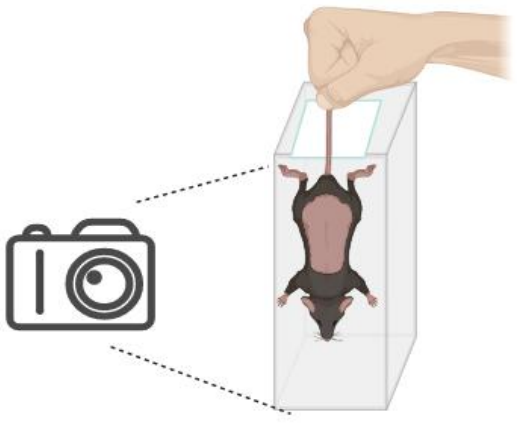


Figura 3. Representación de la caja de observación fabricada.

En este método, se empleó la siguiente escala de puntaje (**Tabla 3** y **Figura 4**):

Tabla 3. Escala de crecimiento capilar.

Puntaje	**% de Crecimiento**
1	(0-20) %
2	(20-40) %
3	(40-60) %
4	(60-80) %
5	(80-100) %

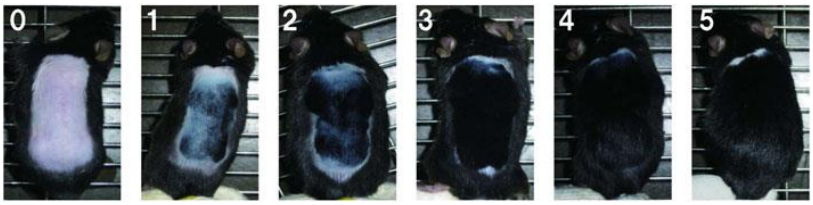


Figura 4. Puntuaciones para la evaluación del crecimiento capilar: 0= sin crecimiento; 1= menos del 20% de crecimiento, 2= 20% al 40% de crecimiento, 3= 40% al 60% de crecimiento, 4= 60% al 80% de crecimiento, 5= 80% al 100% de crecimiento **[**12**]**.

2.8.2. Método 2: Medición de muestras de pelos

Al final de las semanas 2, 3, 4 y 5; se tomó un mechón de pelos, por tracción, dentro de la zona afeitada, procurando que estos sean extraídos con sus respectivos folículos.

En la semana 5, tomo un mechón de pelos, por tracción, dentro y fuera de la zona afeitada, para ser comparados. Dicho procedimiento también se realizó con los ratones del grupo control-control.

La medición de longitud de estos pelos se realizó con la ayuda de una lupa de 6 dioptrías (aumento x6 de la imagen) y un vernier digital (marca DIGITAL CAPILPER) de una apreciación de 0,02 mm.

2.9. Análisis Estadístico

Los datos obtenidos en el estudio fueron procesados en el paquete estadístico GraphPrism versión 8. Se utilizó la prueba de Shapiro-Wilk para observar la distribución normal de los datos.

Los resultados de las variables cuantitativas se presentaron con medidas de tendencia central en medidas de frecuencias, porcentajes y valores absolutos mediante gráficos. Para las diferencias de los valores medios, se utilizó la prueba t de Student, aceptando valores significativos inferiores a $p<0,050$.

En este sentido, para observar las diferencias entre los valores medios entre los distintos grupos se realizó una prueba de ANOVA de 1 vía para obtener el valor F de Fisher.

Para obtener las diferencias y valores comparativos entre cada grupo se realizó una prueba de ANOVA de 2 vías, considerando el factor de columna como el tratamiento y el factor de filas como los valores obtenidos entre las semanas del estudio.

A este análisis se le hizo una prueba Pos-Hoc bajo el método de Tukey para verificar las relaciones entre medias, considerando que cada medida fue tomada en tiempos diferentes. Los valores F significativos fueron aceptados por encima de 1, así como diferencias estadísticas con valores de $p<0,050$.

3. RESULTADOS Y DISCUSIÓN

Es necesario acotar que, durante el procedimiento inicial de afeitado de los ratones, se observó que 3 individuos del grupo AD (agua destilada), presentaban un nevo melanocítico (lunar), que cubría toda la región dorsal del animal **(Figura 5**), el cual mostraba la misma coloración del pelo, dicho rasgo no se presentó en ningún otro ratón de los demás grupos de estudio (CC, AE, ER, HR, TC, CH y MXD).

Inicialmente, se pensó que dicha particularidad no traería ningún impacto en la evaluación capilar de estos individuos y estos no se descartaron debido que no se contaban con más ratones de la misma línea y edad. Sin embargo, los lunares demostraron tener un efecto negativo en la escala de crecimiento capilar y registro fotográfico al momento de obtener resultados. Por otro lado, no pareció afectar la metodología en la que se mide la longitud de los pelos arrancados.

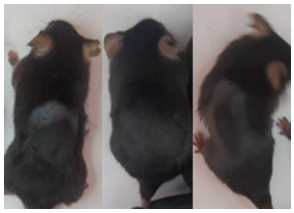


Figura 5. Ratones AD1, AD2 y AD3 que presentaban nevo melanocítico (lunar), en la región dorsal de su cuerpo.

3.1. Escala de crecimiento y registro fotográfico

Los resultados mostrados en la **Figura 6**, ilustran la progresión del crecimiento capilar, con respecto al tiempo, desde la semana 0 hasta la semana 5. Después del afeitado (semana 0), se percibe que todos los ratones C57BL6//BIOU presentan una coloración rosada en su piel (a excepción de 3 individuos del grupo AD).

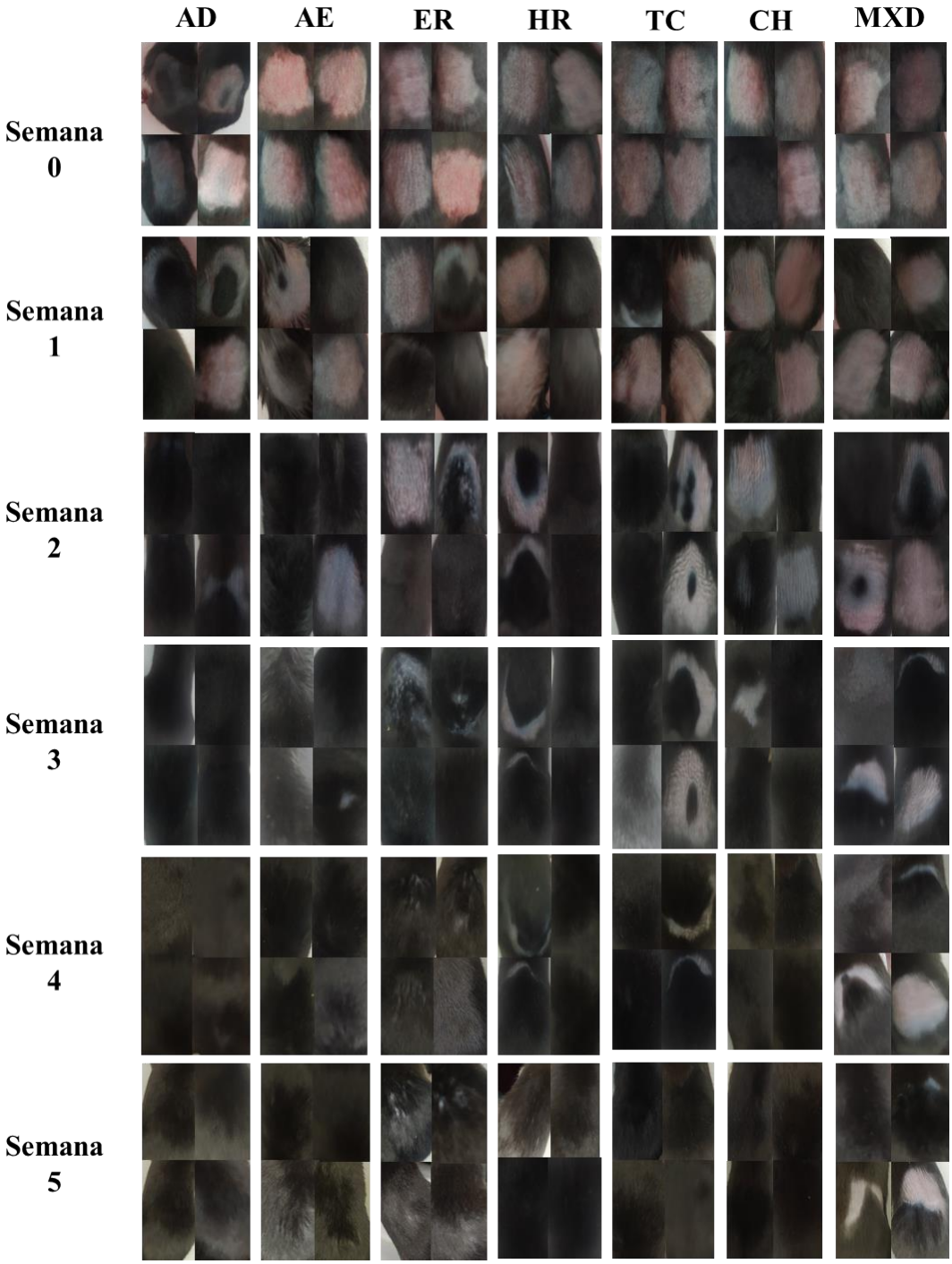


Figura 6. Observación macroscópica de la región dorsal de los ratones C57BL6//BIOU. AD (agua destilada), AE (aceite esencial de romero al 4,8%), ER (extracto de romero al 0,135%), HR (hidrolato de romero), TC (tónico capilar), CH (champú) y MXD (minoxidil al 5%).

La estimulación del crecimiento capilar se evaluó observando el oscurecimiento en el color de la piel, de un rosa vivo a un color gris/negro, los cuales son indicativos del paso de la fase telógena (descanso) a fase anágena (crecimiento activo) de los folículos pilosos de la zona afeitada [16].

Durante todo el estudio, no se observaron efectos adversos debidos a la aplicación de los productos por vía tópica como: irritación, descamación o señales conductuales que indiquen

malestar en los ratones (barbering, acicalamiento excesivo o inapetencia).

A partir de la semana 2, se puede observar que la piel de la mayoría de los individuos de los grupos AD, AE, ER, HR, TC y CH pasó de un color rosado a una tonalidad gris/negro, lo cual se traduce en un crecimiento considerable del pelo en estos grupos. Por otro lado, solo la mitad de los individuos del grupo MXD presentaban dicho fenómeno.

En la semana 4, todos los ratones de los grupos AD, AE, HR, TC y CH, mostraron tener un crecimiento capilar considerable (puntaje entre 3,5 y 5 en la escala), mientras que el grupo MXD aún presentaban grandes zonas de piel sin pelo (un puntaje de 2), siendo este el grupo que presentó menor crecimiento capilar.

Al final de la semana 5, se puede observar que todos los ratones de los grupos AD, AE, HR, TC y CH, recuperaron prácticamente todo el pelo de la región afeitada (puntaje entre 4,75 y 5), mientras que en el grupo MXD solo dos individuos lograron recuperar todo su pelaje, resultando en un puntaje promedio de 3,75.

La Tabla 4 y Figura 7 muestran, la media grupal, del puntaje en el crecimiento capilar de todos los grupos, a lo largo de las 5 semanas del ensayo.

Tabla 4. Escala de crecimiento capilar.

Grupo	Semana 1	Semana 2	Semana 3	Semana 4	Semana 5
AD	1	2,5	3,75	4,75	5
AE	0,5	2,25	3,75	5	5
ER	1	1,5	2,5	4,25	5
HR	1,25	1,5	3,25	3,75	4,75
TC	0,5	1,75	2,75	3,5	4,5
CH	0,5	1,25	3,75	5	5
MXD	0,25	1	2	2,75	3,75

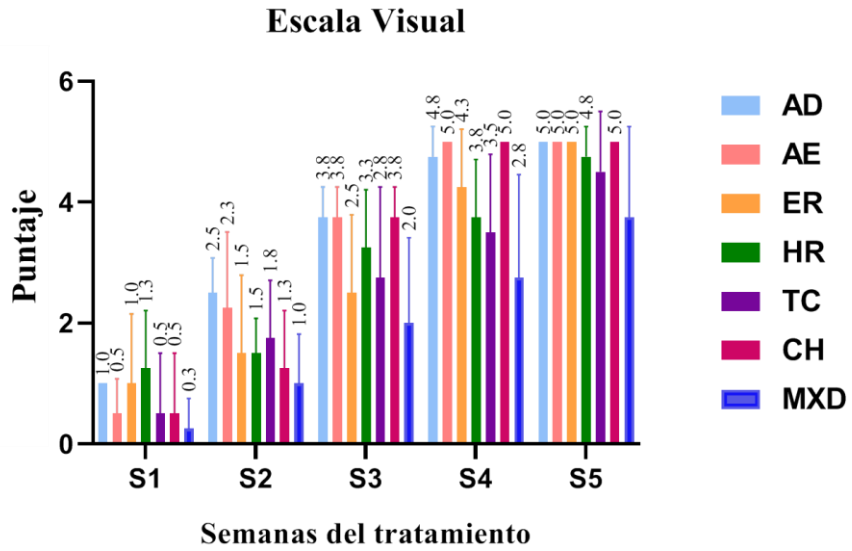


Figura 7. Gráfica de la comparación del efecto del crecimiento capilar, mediante la escala de puntaje, en ratones C57BL6//BIOU luego de la aplicación tópica de agua destilada (AD), aceite esencial al 5% (AE), extracto de romero al 5% (ER), hidrolato de romero (HR), tónico capilar (TC), champú (CH) y minoxidil al 5% (MXD).

3.2. Medición de muestras de pelo

A continuación, en la **Figura 8**, se muestra la comparación del crecimiento capilar entre la semana 2 y 5, en forma de gráficos de columna y cajas.

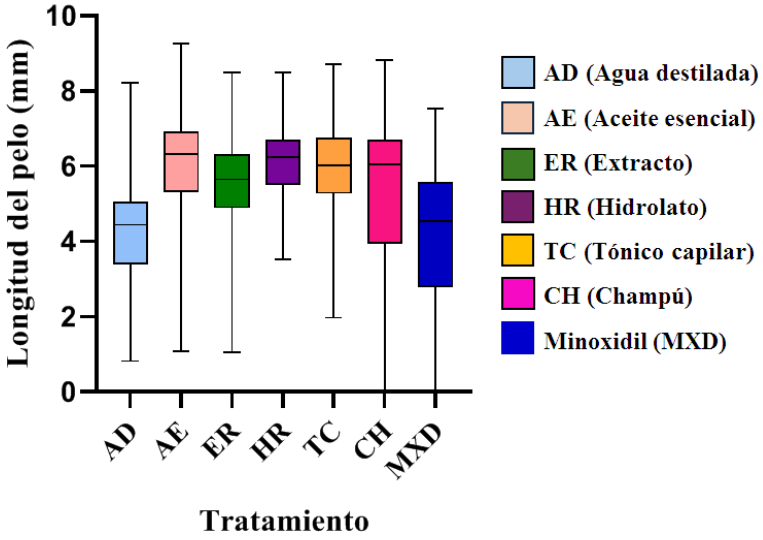


Figura 8. Comparación en el crecimiento capilar (medido en mm) en forma de cajas, de los grupos AD, AE, ER, HR, TC, CH y MXD; entre las semanas 2 y 5.

Los resulatos del p-valor para la prueba de Shapiro-Wilk realizada (Tabla 5) , demuestran que los valores de longitud del pelo a lo largo del estudio se ajustan al modelo de normalidad en todos los grupos de estudio.

Tabla 5. Prueba de normalidad de Shapiro-Wilk para los datos de los grupos AD, AE, ER, HR, TC, CH y MXD entre las semanas 2 y 5.

	AD	AE	ER	HR	TC	CH	MXD
	Prueba Shapiro-Wilk						
W	0,9893	0,8245	0,9304	0,8423	0,7596	0,8654	0,9561
p-valor	0,9538	0,1539	0,5970	0,2022	0,0673	0,2800	0,7545
Normalidad	Si	Si	Si	Si	Si	Si	Si

Nota: se comprueba la normalidad de los datos sí p-valor>0,05.

Por su parte, los resultados de la prueba ANOVA realizada considerando a todos los grupos de estudios, demostró que existen diferencias estadisticamentes significativas (p<0,05) en el crecimiento capilar de estos (Tabla 6 y Figura 9).

Tabla 6. Resultados de la prueba ANOVA de una vía, de la comparación del largo del pelo de todos los grupos de estudio.

	SS	DF	MS	Valor de F	Valor de P
Entre grupos	17.14	6	2,587	17,11	0,0026
Dentro de los grupos	27,14	3	9,246	55,37	
Residual	3,006	18	0,1670		
Total	47,88	27			

SS: suma de cuadrados. DF: grados de libertad. MS: media de los cuadrados. Nota: existen diferencias estadísticamente significativas entre las medias de los grupos sí p<0,05.

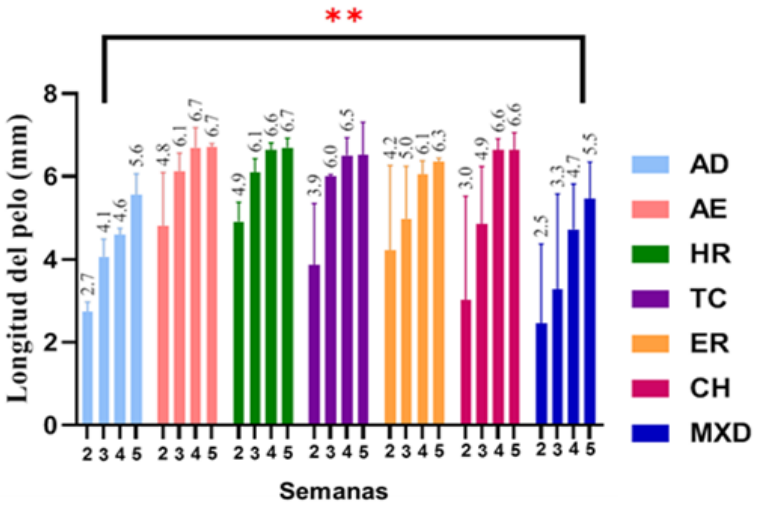


Figura 8. Resultados de la prueba ANOVA de la longitud del pelo de los grupos AD, AE, ER, HR, TC, CH y MXD, en las semanas 2 y 5. El resultado de la prueba se indica con (**), el cual expresa que $p \leq 0,05$.

Sabiendo esto, se procedió a comparar cada una de las medias de los grupos de tratamiento (AE, ER, HR, TC y CH) con respecto a los grupos AD y MXD, empleando la prueba de Tukey. Con esta prueba, se determinó que el largo de pelo de los grupos de tratamiento era estadísticamente diferente (más largo) que el de los grupos AD y MXD (Tabla 7 y Figura 9).

Tabla 7. Prueba de ANOVA con análisis *post-Hoc* bajo la prueba de Tukey de los grupos AE, ER, HR, TC y CH, asumiendo como HDS a los grupos AD y MXD.

Estímulo Evaluado	Valor de p	
	Agua Destilada (AD)	Minoxidil al 5% (MXD)
AE	0,0039*	0,0079*
ER	0,0075*	0,0057*
HR	0,0044*	0,0086*
TC	0,0015*	0,0036*
CH	0,0047*	0,0203*

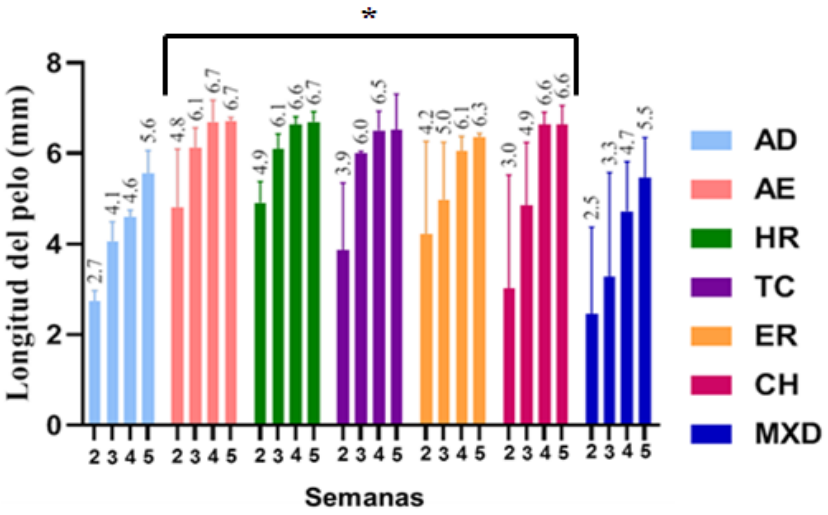


Figura 9 Prueba de ANOVA con análisis *post-Hoc* bajo la prueba de Tukey de los grupos AE, ER, HR, TC y CH, asumiendo como HDS a los grupos AD y MXD. El símbolo (*) indica que la media del grupo presenta diferencias estadísticamente significativas con los grupos AD y MXD ($p<0,05$).

Ahora bien, sabiendo que todos los tratamientos estimulan el crecimiento capilar en mayor medida que el agua destilada y el minoxidil, se procederá a determinar cuál de estos tratamientos es el mejor. Para esto, se procedió a realizar nuevamente un test de ANOVA, pero comparando únicamente a los grupos AE, ER, HR TC y CH (Tabla 8 y Figura 10).

Tabla 8. Resultados de la prueba ANOVA de una vía, de la comparación del largo del pelo de los grupos AE, ER, HR, TC y CH.

	SS	DF	MS	Valor de F	Valor de P
Entre grupos	88,78	4	22,19	9,02	0,32
Dentro de los grupos	1954,67	795	2,45	2,38	
Residual	5,26	20	0,1670		
Total	2043,4	799			

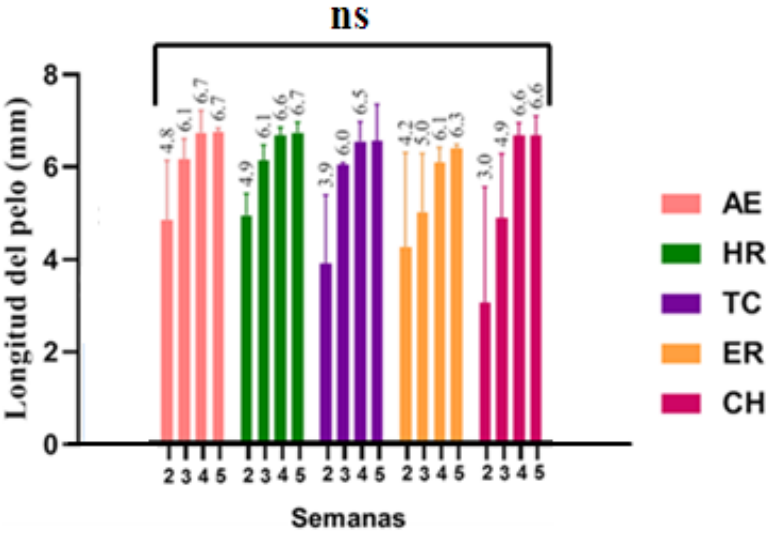


Figura 10. Resultados de la prueba ANOVA de la longitud del pelo de los grupos AE, ER, HR, TC y CH, en las semanas 2 y 5. El resultado de la prueba se indica con (ns), el cual expresa que $p > 0,05$.

Al final de la semana 5, la prueba de ANOVA realizada considerando el largo del pelo dentro y fuera del área afeitada de todos los grupos de tratamiento (Tabla 9 y Figura 11), demostró que no existen diferencias estadísticamente significativas entre estos (p-valor >0,05), es decir, que los individuos recuperaron la longitud normal de su pelo al culminar el ensayo.

Tabla 9. Resultados de la prueba ANOVA de una vía, en la comparación de la longitud del pelo dentro y fuera del área afeitada de los ratones de los grupos AE, ER, HR, TC y CH.

	SS	DF	MS	Valor de F	Valor de P
Entre grupos	0,8506	4	0,1418	0,9240	0,5126
Dentro de los grupos	0,3974	1	0,3974	2,590	-------
Residual	0,9205	4	0,1670	-------	-------
Total	2,168	13	-------	-------	-------

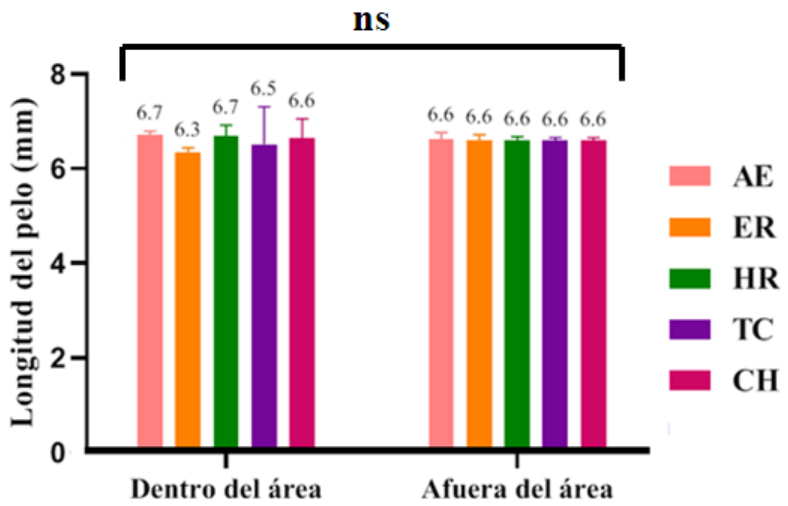


Figura 11. Comparación de la longitud del pelo dentro y fuera del área afeitada, de los grupos AE, ER, HR, TC y CH, al final de la semana 5.

Finalmente, al comparar la longitud del pelo de los ratones de los grupos de tratamiento (AE, HR, ER, TC Y CH) con los ratones del grupo control-control (CC), la prueba de ANOVA relizada demostro que no existian diferencias estadisticamente significativas (p-valor >0,05) entre estos grupos (Tabla 10 y Figura 12).

Tabla 10. Prueba de ANOVA con análisis *post-Hoc* bajo la prueba de Tukey de los grupos AE, ER, HR, TC y CH, asumiendo como HDS al grupo CC.

Estímulo Evaluado	Valor de p
	CC
AE	0,164
ER	0,489
HR	0,539
TC	0,835
CH	0,866

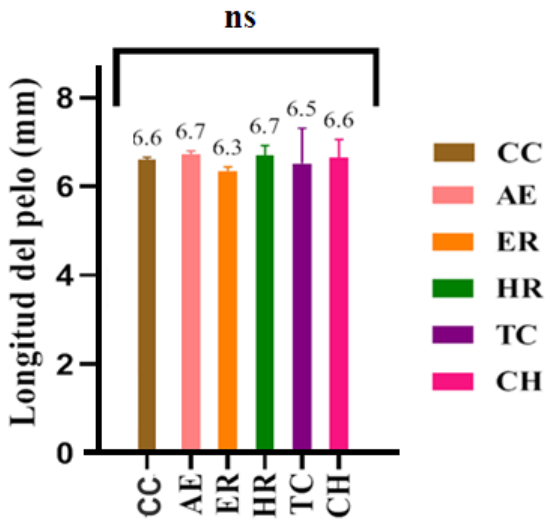


Figura 12. Comparación de la longitud del pelo (en mm) de los grupos tratados (AE, ER, HR, TC y CH) con respecto al grupo CC. El símbolo (**ns**) señala que no existen diferencias estadísticamente significativas entre los grupos.

4. CONCLUSIONES

Se realizó un estudio de crecimiento capilar comparando el efecto de compuestos derivados del romero, en forma individual (aceite, extracto e hidrolato) y combinada (tónico capilar y champú). Los resultados obtenidos demostraron que el método de la escala capilar (método 1), presenta serias limitaciones a la hora de evaluar el crecimiento capilar de ratones con nevo melanocítico. Por otro lado, el método 2 demostró que los grupos que los grupos tratados con productos derivados del romero (AE, HR, ER, CH y TC) presentaron un crecimiento capilar estadísticamente superior (p-valor<0,05) en comparación al grupo control negativo (agua destilada) y control positivo (minoxidil al 5%). Además, de que el largo del pelo de estos grupos, al final de la semana 5, fue estadísticamente similar al de los pelos fuera de la zona afeitada y del grupo control-control.

Los resultados de esta investigación demuestran que los tratamientos empleados estimulan el crecimiento capilar en mayor medida que el fármaco comercial minoxidil, y que estos podrían ser una posible alternativa terapéutica.

CONFLICTO DE INTERES

Los autores manifestamos no presentar ningún conflicto de interés.

REFERENCIAS

1. R.A. Caro, J.P. Carrera, M.M. Cabello, B.V. Guerrero, C. Ovelar, I. Ennme & M.D. Jiménez. Uso de plantas medicinales en la provincia de Sevilla. RESCIFAR *Revista Española de Ciencias Farmacéuticas*, **1**(2), 138–147 (2020). URL: https://dialnet.unirioja.es/servlet/articulo?codigo=8084413
2. A.A. Barquero. Plantas sanadoras: pasado, presente y futuro. *Revista QuímicaViva*, **6**(2), 53–69 (2007). URL: http://www.quimicaviva.qb.fcen.uba.ar/v6n2/barquero.pdf
3. M. Marrelli. Medicinal plants. *Plants* (Basel), **10**(7), 1355 (2021). https://doi.org/10.3390/plants10071355
4. Organización Mundial de la Salud. *Estrategia de la OMS sobre medicina tradicional 2014-2023*. Ginebra, 2013; 75 p. URL: https://www.who.int/es/publications/i/item/9789241506096
5. E. Salmerón-Manzano, J.A. Garrido-Cardenas & F. Manzano-Agugliaro. Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health*, **17**(10), 3376 (2020). https://doi.org/10.3390/ijerph17103376
6. S. Hosseinzadeh, A. Jafarikukhdan, A. Hosseini & R. Armand. The application of medicinal plants in traditional and modern medicine: A review of *Thymus vulgaris*. *International Journal of Clinical Medicine*, **6**(9), 635–642 (2015). doi: https://doi.org/10.4236/ijcm.2015.69084
7. S. Patel, V. Sharma, N.S. Chauhan, M. Thakur & V.K. Dixit. Hair growth: Focus on herbal therapeutic agent. *Current Drug Discovery Technologies*, **12**(1), 21–42 (2015). https://doi.org/10.2174/1570163812666150610115055
8. U. Sanches-Abelan, A. Costa de Oliveira, E.S. Pinto-Cacoci, T.E. Azevedo-Martins, V. Mansanares-Giacon, M.V. Robles-Velasco & C.R.R. de Castro-Lima. Potential use of essential oils in cosmetic and dermatological hair products: A review. *Journal of Cosmetic Dermatology*, **21**(4), 1407–1418 (2022). https://doi.org/10.1111/jocd.14286
9. K. Barve & A. Dighe. *The Chemistry and Applications of Sustainable Natural Hair Products.* Springer Cham, 2016. URL: http://link.springer.com/10.1007/978-3-319-29419-3
10. J.M. Andrade, C. Faustino, C. Garcia, D. Ladeiras, C.P. Reis & P. Rijo. *Rosmarinus officinalis* L.: an

update review of its phytochemistry and biological activity. *Future Science OA*, **4**(4), FSO283 (2018). https://doi.org/10.4155/fsoa-2017-0124

11. L. Malvezzi de Macedo, É. Mendes dos Santos, L. Militão, L. Lacalendola-Tundisi, J. Artem-Ataide, E. Barbosa-Souto & P. Gava-Mazzola. Rosemary (*Rosmarinus officinalis* L., syn *Salvia rosmarinus* Spenn.) and its topical applications: A review. *Plants* (Basel), **9**(5), 651 (2020). https://doi.org/10.3390/plants9050651
12. K. Murata, K. Noguchi, M. Kondo, M. Onishi, N. Watanabe, K. Okamura & H. Matsuda. Promotion of hair growth by *Rosmarinus officinalis* leaf extract. *Phytotherapy Research*, **27**(2), 212–217 (2013). https://doi.org/10.1002/ptr.4712
13. J. Moore, M. Yousef & E. Tsiani. Anticancer effects of rosemary (*Rosmarinus officinalis* L.) extract and rosemary extract polyphenols. *Nutrients*, **8**(11), 731 (2016). https://doi.org/10.3390/nu8110731
14. G. Nieto, G. Ros & J. Castillo. Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis*, L.): A review. *Medicines*, **5**(3), 98 (2018). https://doi.org/10.3390/medicines5030098
15. F. Masoud, H.A. Alamdari, S. Asnaashari, J. Shokri & Y. Javadzadeh. Efficacy and safety of a novel herbal solution for the treatment of androgenetic alopecia and comparison with 5% minoxidil: A double-blind, randomized controlled trial study. *Dermatologic Therapy*, **33**(6), e14467 (2020). https://doi.org/10.1111/dth.14467
16. R.J. Aljorna-Molero. *Estudio de la capacidad de crecimiento capilar de productos derivados del romero (Rosmarinus officinalis) en ratones (Mus musculus) C57BL6/BIOU*. Trabajo de Grado. Universidad de Los Andes, Mérida, Venezuela, 2004; 103 p. URL: http://bdigital2.ula.ve:8080/xmlui/handle/654321/16139

CÓMO CITAR ESTE ARTÍCULO

R.J. Aljorna-Molero, F. Carrillo-Rodríguez & J.M. Amaro-Luis. Estudio de la capacidad de crecimiento capilar de productos derivados del romero (*Rosmarinus officinalis*), en ratones (*Mus musculus*) C57BL6//BIOU. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 24–39 (2026). https://doi.org/10.15446/rcciquifa.v55n1.121587

Artículo de investigación clínica

Medición de la adherencia y cumplimiento de la medicación en pacientes con abuso y dependencia a sustancias psicoactivas, atendidos en una institución de prestación de servicios de salud en Bogotá D.C., Colombia

José Ricardo Urrego Novoa[1]*, Karol Andrea Méndez Leguizamón[1], Katherin Quintero Parra[1], Ulises Cruz Granados[1], Danilo Duarte Cadena[1], Elkin Crisóstomo Higuera Dagovett[2], Jaime Tomas Rojas Valencia[2] & Jimmy Torres Galeano[3]

[1] Programa Química Farmacéutica [2] Programa Medicina, [3] Programa Enfermería; Universidad de Ciencias Aplicadas y Ambientales U.D.C.A., Campus Universitario Calle 222 No 55-37, Bogotá D.C., Colombia.

*Autor para correspondencia: Correo electrónico: jurrego@udca.edu.co,
ORCID ID: https://orcid.org/0000-0002-1496-6610
Teléfono: +57 (1) 3106097121

Recibido: 6 de mayo de 2025
Corregido: 3 de septiembre de 2025
Aceptado: 9 de septiembre de 2025

https://doi.org/10.15446/rcciquifa.v55n1.125020

RESUMEN

Objetivo: Medir el grado de adherencia a tratamientos farmacoterapéuticos prescritos en el tratamiento del abuso y dependencia de Sustancias Psicoactivas (SPA) por pacientes con intervenciones que requirieron medicamentos. **Métodos:** Estudio descriptivo de tipo transversal para medición de adherencia a tratamientos farmacoterapéuticos por parte de pacientes con abuso y dependencia de SPA mediante la utilización de la escala de Morisky (MMAS-8). **Resultados:** Se encontró un nivel de baja adherencia (80%) del total de pacientes que fueron prescritos con medicamentos en el tratamiento de abuso o dependencia de SPA. **Conclusion:** La baja adherencia al tratamiento farmacoterapéutico representó un comportamiento superior al 80% de los pacientes participantes en el estudio.

Palabras-clave: Trastornos relacionados con sustancias; adherencia y cumplimiento del tratamiento; adherencia a la medicación; evaluación de la adherencia a la medicación.

SUMMARY

Measuring medication adherence and compliance in patients with psychoactive substance abuse and dependence treated at a healthcare institution in Bogotá, Colombia

Objective: To measure the degree of adherence to prescribed pharmacotherapeutic treatments for Substance-related disorders (SRD) by patients with interventions requiring medication. **Methods:** Descriptive cross-sectional study to measure adherence to pharmacotherapeutic treatments by patients with PAS abuse and dependence using the Morisky scale (MMAS-8). **Results:** A low level of adherence was found (80%) in the total number of patients who were prescribed medications for the treatment of Substance-related disorders. **Conclusion:** Low adherence to pharmacotherapy treatment was observed in more than 80% of patients participating in the study.

Keywords: Substance-related disorders; treatment adherence and compliance; medication adherence; medication adherence assessment.

RESUMO

Mensuração da adesão e do cumprimento da medicação em pacientes com abuso e dependência de substâncias psicoativas tratados em uma instituição de saúde em Bogotá, Colômbia

Objetivo: Mensurar o grau de adesão aos tratamentos farmacoterapêuticos prescritos para transtornos relacionados ao uso de substâncias (TRS) por pacientes com intervenções que necessitam de medicação. **Métodos:** Estudo transversal descritivo para mensurar a adesão aos tratamentos farmacoterapêuticos por pacientes com abuso e dependência de SPAs utilizando a escala de Morisky (MMAS-8). **Resultados:** Foi encontrado um baixo nível de adesão (80%) no total de pacientes que receberam medicamentos prescritos para o tratamento de transtornos relacionados ao uso de substâncias. **Conclusão:** Baixa adesão ao tratamento farmacoterapêutico foi observada em mais de 80% dos pacientes participantes do estudo.

Palavras-chave: Transtornos relacionados ao uso de substâncias; adesão e cumprimento do tratamento; adesão à medicação; avaliação da adesão à medicação.

1. INTRODUCCIÓN

En el estudio más reciente de consumo de Sustancias Psicoactivas (SPA) realizado en Bogotá D.C. Colombia, se estimó que 161,800 (2,49%) personas presentan consumo problemático de SPA, con relación a la población total representada equivalente a 6,498,374 personas. El abuso de alcohol es más prevalente en los estratos 1 y 2, mientras que el consumo de marihuana afecta al 2,29% de la población, siendo 1.7 veces más común en hombres. La cocaína presenta un 0,11% de abuso, afectando a más de 7,000 personas, con una mayor incidencia en hombres (0,22%) frente a mujeres (0,01%). El basuco (pasta base de cocaína que se consume fumada), aunque con datos limitados, muestra un 97,8% de casos en hombres, especialmente en el grupo de 35 a 44 años [1].

En Colombia se reconoce que "el consumo (utilización de SPA que actúan sobre el sistema nervioso central, alternado la percepción o el comportamiento), abuso (uso indebido o excesivo de SPA que genera riesgos para la salud, la vida social y laboral del individuo) y adicción (enfermedad caracterizada por la dependencia física y psicológica que lleva al individuo a un consumo compulsivo de SPA, a pesar de las consecuencias negativas), siendo las SPAs, lícitas o ilícitas un asunto de salud pública y bienestar de la familia, la comunidad y los individuos. Por lo tanto, el abuso y la adicción deberán ser tratados como una enfermedad que requiere atención integral por parte del Estado, conforme a la normatividad vigente y las Políticas Públicas Nacionales en Salud Mental y para la Reducción del Consumo de Sustancias Psicoactivas y su Impacto, adoptadas por el Ministerio de Salud y Protección Social" [2]. Lo anterior conlleva la realización intervenciones, procedimientos clínico-asistenciales y terapéuticos y medicamentos para la atención integral de las personas con trastornos mentales o cualquier otra patología derivada del consumo, abuso y adicción a sustancias psicoactivas lícitas e ilícitas, que permitan la plena rehabilitación y recuperación de la salud como está promulgado en la Ley 1566 de 2012 para Colombia. La adherencia a tratamientos con medicamentos es un problema significativo para las personas con consumo de SPA, debido a que la falta de compromiso con el régimen de tratamiento puede resultar en recaídas y un empeoramiento de la

adicción. Este incumplimiento está relacionado con condiciones psicológicas como la desesperanza, que exacerban la resistencia al tratamiento y dificultan la recuperación. Así, el control de la adherencia a medicamentos es fundamental para mejorar los resultados en estos pacientes [3].

En Colombia la información sobre caracterización de adherencia a farmacoterapia para el tratamiento de la adicción a SPA es escasa. El presente trabajo pretende brindar información a partir de la identificación de los medicamentos prescritos en tratamientos farmacológicos de adicción a SPA y el grado de adherencia por parte de los pacientes a este tipo de intervención en particular.

2. METODOLOGIA

Se trata de un estudio descriptivo de corte transversal para medir el grado de adherencia a tratamientos farmacoterapéuticos prescritos por parte de pacientes que reciben intervenciones por adicción a SPA. El universo de estudio fue conformado por pacientes con adicción a SPA que recibieron intervención con tratamiento farmacoterapéutico en una institución en Bogotá D.C., entre los meses de marzo y noviembre de 2024.

La evaluación del grado de adherencia por los pacientes a los tratamientos farmacoterapéuticos instaurados se llevó a cabo teniendo en cuenta la aplicación de la secuencia de trabajo presentada en la Figura 1.

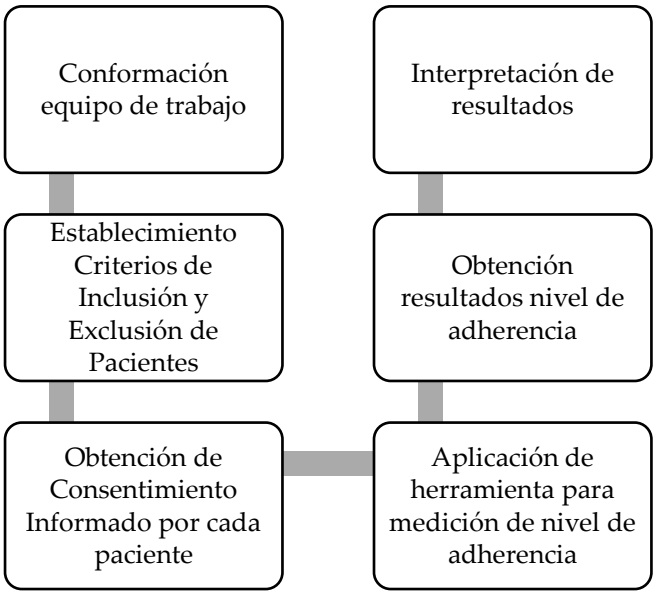


Figura 1. Secuencia de trabajo para medición grado de adherencia a tratamientos farmacoterapéuticos prescritos en intervenciones en pacientes con adicción a SPA.

El equipo de trabajo fue conformado por personal de salud encargado de procesos de atención: Especialista en Psiquiatría, Enfermería, Pedagogo con Especialización en Farmacodependencia y Química Farmacéutica respectivamente. Los criterios de inclusión fueron: paciente en tratamiento farmacoterapéutico prescrito con anterioridad a internación en centro de atención, al momento de la realización del estudio; aprobación de consentimiento informado por parte del paciente al inicio del estudio, aplicación de la herramienta consistente en la Escala de Morisky Green de forma completa a partir de la entrevista realizada con cada paciente en particular.

Para llevar a cabo el estudio, se obtuvo la aprobación del comité ético de investigación de la Universidad de Ciencias Aplicadas y Ambientales U.D.C.A y los permisos necesarios de la administración del centro de tratamiento por abuso de sustancias. Tras informar a los participantes sobre el objetivo y el protocolo del estudio, se obtuvo la aprobación oral de quienes cumplieron con los criterios de inclusión y dieron su consentimiento escrito para el estudio.

La medición del grado de adherencia fue realizada a partir de la utilización de la Escala de Morisky Medication Adherence Scale (MMAS-8) conocido también como Escala de Morisky Green [4]. La escala consta de 8 preguntas. Las primeras 7 preguntas se responden con "Sí" o "No" y la última pregunta se responde con una escala Likert de 5 puntos (probablemente la frecuencia con la que se olvida la utilización del medicamento). La puntuación total se obtiene sumando las respuestas a todas las preguntas. La aplicación de la escala fue desarrollada mediante la realización de una entrevista con cada paciente que cumplió con los criterios de inclusión en el estudio. El nivel de adherencia fue basado obtenido a partir de la obtención del puntaje obtenido a partir de ocho preguntas contempladas en la escala anteriormente mencionada. Una puntuación menor a 6 indica baja adherencia, entre 6 y 8 indica adherencia media, y 8 indica alta adherencia [4-6].

3. RESULTADOS Y DISCUSIÓN

3.1. Características pacientes con abuso o dependencia a SPA

La figura 2 contempla la composición del grupo de pacientes con tratamiento farmacológico como intervención en adicción por SPA. Se encontró una relación aproximada de hombre: mujer de 7:1. El anterior resultado refleja el comportamiento de abuso y dependencia de SPA en el estudio de consumo de SPA para Bogotá D.C. Esta población de pacientes es caracterizada por presentar afectaciones de salud y comportamientos que conllevan la atención en instituciones prestadoras de servicios de Salud (IPS). La composición mayoritaria en hombres representa un comportamiento constante en el consumo, adicción y abstinencia a SPA en estudios de consumo realizados en Colombia y en Bogotá D.C. [1].

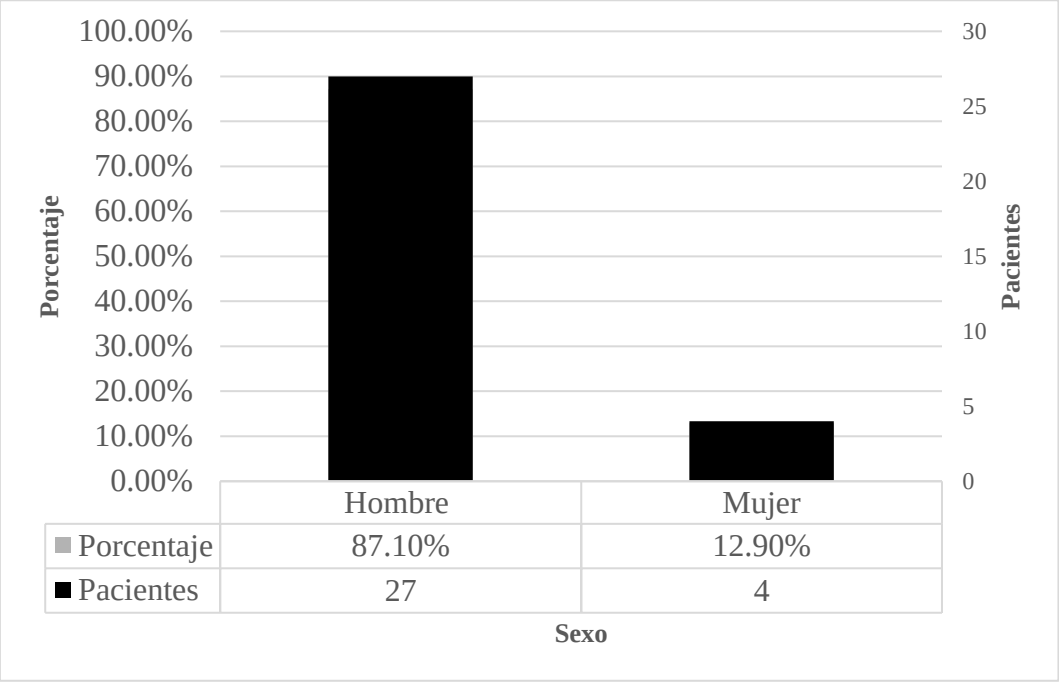


Figura 2. Composición-pacientes con tratamiento farmacoterapéutico por abuso o dependencia a SPA.

El comportamiento etario de los pacientes objeto del presente estudio es presentado en la Figura 3. Se observa una distribución del conjunto de datos de edad con una fuerte cola a la derecha y con una mayor frecuencia de pacientes en un intervalo comprendido entre 17 y 47 años, siendo el intervalo con la mayor frecuencia el comprendido entre 22 y 27 años. El promedio de edad es de 32 años IC95% (28,2-35,6). Este comportamiento refleja caracterización de estudios de consumo, adicción y abstinencia de SPA, en los cuales la población con mayor porcentaje de abuso a cualquier sustancia ilícita se encuentra entre los 25 y 34 años de edad [1].

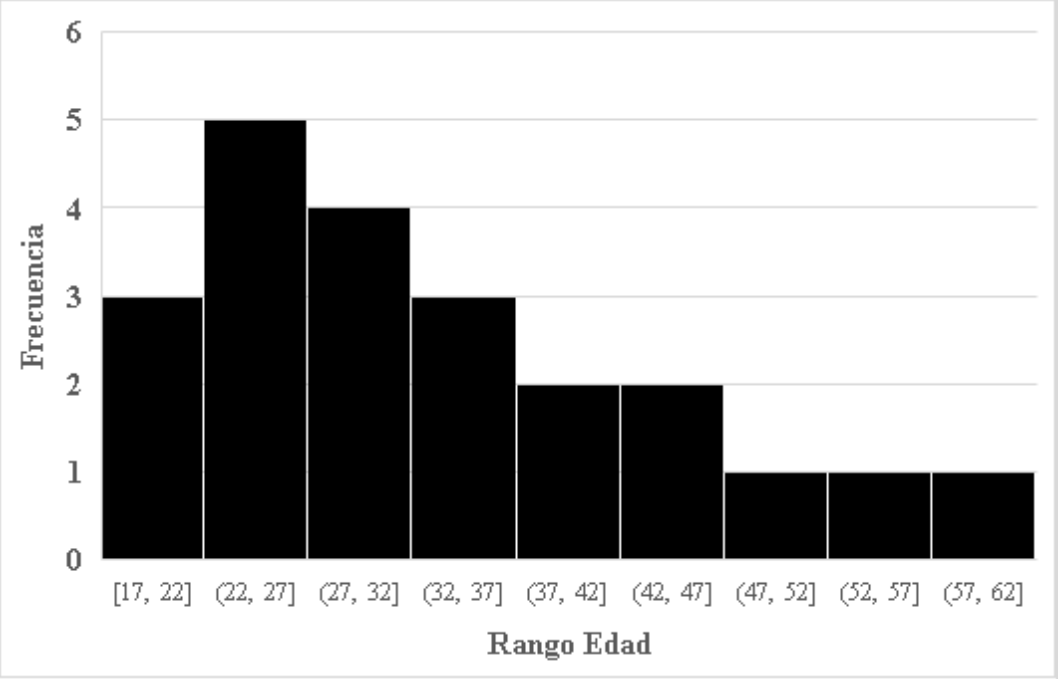


Figura 3. Composición grupo etario pacientes con tratamiento farmacoterapéutico por abuso o dependencia a SPA.

3.2. Características SPA por abuso o dependencia

La tabla 1 evidencia el comportamiento de las SPA que presentan abuso o dependencia en hombres y mujeres que son intervenidas con tratamiento farmacoterapéutico. Se encuentra que en aproximadamente el 90% de los casos, la marihuana seguida por el basuco son las dos SPA ilegales de mayor abuso y dependencia. Llama la atención en el caso de la marihuana, la cual ha sido reportada de mayor abuso en mujeres, con relación a los hombres que manifestaron consumir esta sustancia en el último año [1]. Sin embargo, en el presente trabajo el comportamiento es inverso. Lo anterior debido a posibilidad de realización de intervenciones diferentes a la farmacoterapéutica en el caso de mujeres y a una proporción inferior para este sexo que ha logrado acceder a tratamiento por abuso de SPA. De otra parte, se ha evidenciado en diversos estudios, la mujer afronta obstáculos considerables de acceso al tratamiento farmacoterapéutico, por lo que se cree que está subrepresentada en todo contexto terapéutico. Los tabúes y estigmas culturales determinan que sus problemas de consumo de sustancias no sean frecuentemente reconocidos por ellas mismas, ni por sus familiares o por los profesionales de los servicios que podrían ayudarlas a recibir tratamiento de esta índole [7].

Tabla 1. Sustancias Psicoactivas con abuso o dependencia en hombres y mujeres.

SPA Abuso	Número Hombres	Porcentaje Hombres	Número Mujeres	Porcentaje Mujeres	Total, Pacientes	Porcentaje Total
Marihuana	18	58,06	1	3,23	19	61,29
Basuco	8	25,81	1	3,23	9	29,03
Alcohol	0	0,00	1	3,23	1	3,23
Cocaína	1	3,23	0	0,00	1	3,23
Tabaco	0	0,00	1	3,23	1	3,23
Total	27	87,10	4	12,90	31	100,00

La Tabla 2 muestra la caracterización para hombres y mujeres con abuso y dependencia de SPA.; en la cual se observa que el mayor porcentaje de pacientes que son intervenidos con tratamiento farmacoterapéutico (32,26%), se caracterizan por un policonsumo de cuatro SPA diferentes. De igual manera, en este comportamiento la mayoría de los pacientes fueron hombres en una relación hombre: mujer (9:1) aproximadamente. En el trabajo de Alba Maldonado *et al.* [8] se encontró que la pelea entre padres, consumo de sustancias por parte de un familiar, vivir en zona urbana, estar desempleado, núcleo familiar disfuncional son factores de riesgo, mientras que ser del sexo femenino y tener hijos son factores que disminuyen la probabilidad de consumir sustancias psicoactivas, lo anterior involucra la utilización de dos o más SPA de manera concomitante llegando a generarse abuso o dependencia a SPA.

Tabla 2. Policonsumo en abuso y dependencia de SPA en hombres y mujeres

No. SPA Consumidas	Hombres	Porcentaje Hombres	Mujeres	Porcentaje mujeres	Total, Pacientes	Porcentaje Total
4	9	29,03	1	3,23	10	32,26
1	4	12,90	2	6,45	6	19,35
2	5	16,13	1	3,23	6	19,35
5	5	16,13	0	0,00	5	16,13
3	4	12,90	0	0,00	4	12,90
Total	27	87,10	4	12,90	31	100,00

3.3. Características tratamiento farmacoterapéutico por abuso o dependencia a SPA

Los medicamentos prescritos en el tratamiento de abuso y dependencia por SPA en hombres y mujeres, hallados en el presente estudio se muestran en la Tabla 3. Se encontró que la Clozapina perteneciente a las benzodiazepinas, la Levomepromazina perteneciente al grupo de fenotiazinas y el Clonazepam perteneciente al grupo de las benzodiazepinas, representaron aproximadamente el 51% de los casos de tratamiento farmacoterapéutico de pacientes con abuso y dependencia de SPA. En el caso de la Clozapina, se utiliza en el tratamiento de la patología dual, en la cual se presenta la existencia de una conducta adictiva junto con un trastorno psiquiátrico no relacionado con sustancias. De otra parte, el tratamiento con Clozapina ha sido asociado con una mayor probabilidad de mantener abstinencia del consumo de SPA y una menor probabilidad de hospitalización psiquiátrica, en comparación con el tratamiento continuo con otros antipsicóticos [9, 10]. La levomepromazina es utilizada por su uso como "neuroregulador" en el síndrome de abstinencia opioide debido a sus propiedades agonistas

dopaminérgicas, serotoninérgicas, noradrenérgicas, y adrenérgicas [11]. El clonazepam representa una benzodiazepina de acción prolongada, utilizada con precaución en pacientes con antecedentes de abuso o dependencia de sustancias. Aunque su perfil reduce el riesgo de tolerancia y abuso en comparación con benzodiazepinas de acción corta, su uso debe ser cuidadosamente evaluado, priorizando alternativas terapéuticas y monitoreando posibles riesgos de dependencia farmacológica. De otra parte, se evidenció la utilización de medicamentos como Acetaminofén, Desloratadina y Omeprazol los cuales, aunque no son directamente relacionados con guías clínicas de tratamiento farmacológico para abuso y dependencia de SPA, representan antiinflamatorios no esteroideos AINE, antihistamínicos o inhibidores de la bomba de protones respectivamente para aquellos casos en los cuales fueron identificados signos y síntomas de situaciones relacionadas con indicaciones propias de cada caso particular que conllevaron a su prescripción [12].

Tabla 3. Medicamentos prescritos en hombres y mujeres con abuso y dependencia de SPA

Nombre Medicamento	Hombres	Porcentaje Hombres	Mujeres	Porcentaje Mujeres	Total, Pacientes	Porcentaje Total
Clozapina	6	19,35	0	0,00	6	19,35
Levomepromazina	6	19,35	0	0,00	6	19,35
Clonazepam	3	9,68	1	3,23	4	12,90
Quetiapina	3	9,68	0	0,00	3	9,68
Risperidona	2	6,45	1	3,23	3	9,68
Acetaminofén	1	3,23	1	3,23	2	6,45
Sertralina	2	6,45	0	0,00	2	6,45
Desloratadina	1	3,23	0	0,00	1	3,23
Fluoxetina	1	3,23	0	0,00	1	3,23
Olanzapina	0	0,00	1	3,23	1	3,23
Omeprazol	1	3,23	0	0,00	1	3,23
Trazodona	1	3,23	0	0,00	1	3,23
Total, general	27	87,10	4	12,90	31	100,00

La intervención farmacoterapéutica en relación con el número de medicamentos prescritos de forma concomitante, son presentados en la Tabla 4. Como se observa, existe una variabilidad en el número de medicamentos reportados con una oscilación entre 1 y 5 medicamentos prescritos de forma concomitante; presentándose un comportamiento con relación a la prescripción del 71% de dos o más medicamentos, 29% tres o más medicamentos, 7% cuatro o más medicamentos y 3% cinco medicamentos, respectivamente. La polifarmacia carece de una definición universalmente aceptada debido a su enfoque heterogéneo, que incluye definiciones numéricas y descriptivas. La más común es el uso simultáneo de cinco o más medicamentos [13]. Bajo la anterior premisa se encuentra que en aquellos pacientes a los cuales les fueron prescritos medicamentos por abuso y dependencia de SPA, se encontró un 3% con polifarmacia. Un ejemplo de una definición de polifarmacia que reconoce el uso de medicamentos apropiados e inapropiados es: "La polifarmacia abarca desde el uso de un gran número de medicamentos hasta el uso de medicamentos potencialmente inapropiados, la infrautilización y la duplicación de medicamentos" y "medicamentos potencialmente inapropiados", lo cual puede conllevar la presencia de interacciones farmacocinéticas y farmacodinámicas [14].

Tabla 4. Cantidad medicamentos prescritos en hombres y mujeres con abuso y dependencia de SPA

No. Medicamentos Prescritos	Hombres	Porcentaje Hombres	Mujeres	Porcentaje Mujeres	Total, Pacientes	Porcentaje Total
2	12	38,71	1	3,23	13	41,94
1	8	25,81	1	3,23	9	29,03
3	5	16,13	2	6,45	7	22,58
4	1	3,23	0	0,00	1	3,23
5	1	3,23	0	0,00	1	3,23
Total, general	27	87,10	4	12,90	31	100,00

El grado de adherencia por hombres y mujeres con abuso y dependencia de SPA a intervención con medicamentos, se presenta globalmente en la Tabla 5. Se observa un valor del grado de baja adherencia aproximadamente del 81% de los pacientes prescritos. En el trabajo de Colizzi *et al.* se encontró que en el 44% (N=100) de los pacientes se asoció significativamente con una baja adherencia a la medicación, aumentando el riesgo de no remisión en pacientes, especialmente aquellos con dependencia de nicotina y cannabis que desarrollaron psicosis [15]. En otro estudio se encontró que la adherencia a la medicación en pacientes con trastorno bipolar y trastornos por consumo de sustancias es problemática, debido a factores como las malas creencias sobre el tratamiento, abuso o dependencia de sustancias y falta de alineación con valores personales dificultan seguir de forma adecuada el esquema terapéutico. En este estudio se reportó que 43,9% (N=205) de los pacientes una adherencia deficiente a su tratamiento [16].

Las personas que padecen trastornos por consumo de sustancias tienden a tener un estilo de vida inestable, lo que puede dificultar su capacidad para seguir un plan de medicación prescrito [17].

Tabla 5. Adherencia global a medicamentos prescritos en hombres y mujeres con abuso y dependencia de SPA

Grado Adherencia	Porcentaje Adherencia
Baja Adherencia	80,65
Moderada Adherencia	16,13
Alta Adherencia	3,23

La tabla 6 muestra el comportamiento de la adherencia a medicamentos prescritos en pacientes con abuso y dependencia de SPA en una IPS. Esta descripción detallada evidencia el grado de adherencia teniendo en cuenta que los resultados obtenidos hacen alusión en su mayoría a una baja adherencia en la mayor parte de los medicamentos prescritos. Llama la atención de presentarse un único caso de alta adherencia a Clozapina, evidenciándose un tratamiento en monoterapia por ingreso reciente a la institución por primera vez. La información obtenida posee limitación en relación con ausencia de registros de seguimiento en el cumplimiento de la farmacoterapia en escenarios extramurales o ambulatorios. Lo anterior a partir del presente trabajo se considera una actividad a considerar dentro del diseño de intervenciones en la institución. De otra parte, el presente estudio involucró pacientes que cumplieron criterios de inclusión y aceptaron participar en el mismo.

Aunque los casos presentados no pueden considerarse una muestra representativa de la población que es prescrita con medicamentos para el tratamiento del abuso y dependencia de SPA para Colombia; el presente estudio brinda información detallada del comportamiento del grado de adherencia en este tipo de población para la cual la información es reducida en el caso colombiano.

Las condiciones de baja adherencia se consideran un factor que conlleva a la recaída de un paciente en la utilización de SPA. En este caso particular se evidenció en promedio dos reingresos por recaída de los pacientes que participaron en el estudio.

Tabla 6. Adherencia a medicamentos prescritos en hombres y mujeres con abuso y dependencia de SPA

Medicamento	Moderada Adherencia		Alta Adherencia		Baja Adherencia		Total, Pacientes	Porcentaje
	No Pacientes	%	No Pacientes	%	No Pacientes	%		%
Clozapina	0	0	1	3,23	5	16,13	6	19,35
Levomepromazina	1	3,23	0	0	5	16,1	6	19,35
Clonazepam	0	0	0	0	4	12,9	4	12,9
Quetiapina	0	0	0	0	3	9,68	3	9,68
Risperidona	0	0	0	0	3	9,68	3	9,68
Acetaminofén	0	0	0	0	2	6,45	2	6,45
Sertralina	2	6,45	0	0	0	0	2	6,45
Desloratadina	1	3,23	0	0	0	0	1	3,23
Fluoxetina	0	0	0	0	1	3,23	1	3,23
Olanzapina	0	0	0	0	1	3,23	1	3,23
Omeprazol	1	3,23	0	0	0	0	1	3,23
Trazodona	0	0	0	0	1	3,23	1	3,23
Total, general	5	16,13	1	3,23	25	80,65	31	100

4. CONCLUSIONES

A partir del presente estudio se encontró que más del 80% del total de casos presentaron abuso y dependencia a más de dos SPAs, siendo el 32% de la totalidad de población objeto de estudio, situaciones de cuatro o más SPAs, todos los cuales, recibieron tratamiento farmacoterapéutico. Este comportamiento fue más frecuente en el sexo masculino en una relación hombre: mujer (9:1). Más del 90% de los casos de abuso o dependencia de SPA con tratamiento farmacoterapéutico, correspondieron a marihuana y basuco respectivamente, siendo SPAs de carácter ilícito. Los medicamentos utilizados en el tratamiento de abuso y dependencia de SPA de mayor prescripción fueron Clozapina, Levomepromazina y Clonazepam respectivamente, representando más del 50% de los pacientes que recibieron tratamiento farmacológico. En el grupo de pacientes que recibieron tratamiento farmacoterapéutico más del 40% de los casos fueron prescritos con dos medicamentos de forma simultánea. Con relación al grado de adherencia al tratamiento con medicamentos por parte de los pacientes con abuso y dependencia de SPA, se encontró una baja adherencia en más del 80% de los casos, seguido por un 16% con una moderada adherencia. A partir de la información reportada en el presenta estudio, se requiere el

diseño de intervenciones en pacientes con tratamiento farmacoterapéutico, tendientes a mejorar el grado de adherencia en pacientes con abuso y dependencia a SPA. Lo anterior a partir de la interacción del grupo de profesionales de la salud en donde el Químico Farmacéutico puede aportar estrategias para tal fin a partir de su formación.

AGRADECIMIENTOS

Los autores agradecen a la Fundación La Luz I.P.S por permitir el desarrollo de la presente investigación.

CONFLICTO DE INTERES

Los autores manifestamos no presentar ningún conflicto de interés.

REFERENCIAS

1. Alcaldía Mayor de Bogotá, Secretaría Distrital de Salud de Bogotá & Oficina de las Naciones Unidas contra la Droga y el Delito (UNODC). *Estudio de consumo de sustancias psicoactivas en Bogotá D.C., 2022*. Bogotá D.C., 2022, p. 47-93. URL: https://saludata.saludcapital.gov.co/osb/wp-content/uploads/2024/05/Estudio-SPA-2022_compressed.pdf
2. Congreso de la República de Colombia. Ley 1566 de 2012 (Julio 31). Por la cual se dictan normas para garantizar la atención integral a personas que consumen sustancias psicoactivas y se crea el premio nacional "entidad comprometida con la prevención del consumo, abuso y adicción a sustancias" psicoactivas. Bogotá D. C., 2012. URL: http://www.secretariasenado.gov.co/senado/basedoc/ley_1566_2012.html, consultado en enero de 2025.
3. N.D. Volkow (director). *Principles of Drug Addiction Treatment: A Research-Based Guide.* National Institute on Drug Abuse, NIH, North Bethesda (MD), 2014; 44 p. URL: https://archive.org/details/podat-3rd-ed/page/n1/mode/2up
4. A. Intilangelo, S. Majic, V. Palchik & M.L. Traverso. Cuestionarios validados de adherencia a la medicación y factores asociados en pacientes crónicos: revisión sistemática. *Farmacia Hospitalaria*, **48**(4), 185–192 (2024). https://doi.org/10.1016/j.farma.2024.01.001
5. D.E. Morisky, A. Ang, M. Krousel-Wood & H. Ward. Predictive validity of a medication adherence measure in an outpatient setting (Retracted Article). *The Journal of Clinical Hipertension*, **10**(5), 348–354 (2008). https://doi.org/10.1111/j.1751-7176.2008.07572.x
6. N.P. Puigdemont & M.I. Valverde-Merino. Métodos para medir la adherencia terapéutica. *Ars Pharmaceutica*, **59**(3), 163–172 (2018). https://doi.org/10.30827/ars.v59i3.7387
7. Naciones Unidas: Oficina contra la Droga y el Delito. *Tratamiento del abuso de sustancias y atención para la mujer: Estudios monográficos y experiencia adquirida.* Nueva York, 2005, p. 7. URL: https://www.unodc.org/docs/treatment/04-55686_ebook%20Spanish.pdf
8. J.C. Alba-Maldonado, L.J. Vargas-R/guez, P.A. Bolívar, S.A. Granados-López, M.A. Fonseca-Leal, G.V. Ayala-Cuervo & A.F. Barajas-Santos. Factores asociados al consumo de sustancias psicoactivas en una población atendida en un hospital de Tunja: Estudio de casos y controles. *Ciencia e Innovación en Salud*, **e139**, 311–322 (2021). https://doi.org/10.17081/innosa.139
9. B. Arranz, M. Garriga, C. García-Rizo & L. San. Clozapine use in patients with schizophrenia and a comorbid substance use disorder: A systematic review. *European Neuropsychopharmacology*, **28**(2), 227–242 (2018). https://doi.org/10.1016/jeuroneuro.217.12.006
10. R. Rafizadeh, M. Danilewitz, C.A. Bousman, N. Mathew, R.F. White, A. Bahji, W.G. Honer & C.G. Schütz. Effects of clozapine treatment on the improvement of substance use disorders other tan nicotine in individuals with schizophrenia spectrum disorders: A systematic review and meta-

analysis. *Journal of Psychopharmacology*, **37**(2), 135–143 (2023). https://doi.org/10.1177/02698811221142575

11. S.B. Serra-Rojas. Levomepromazina: una nueva opción terapéutica ante la abstinencia opioide. *Revista Electrónica de PortalesMedicos.com*, **18**(23), 1100 (2023). URL: https://www.revista-portalesmedicos.com/revista-medica/levomepromazina-una-nueva-opcion-terapeutica-ante-la-abstinencia-opioide/
12. V. Starcevic. The reappraisal of benzodiazepines in the treatment of anxiety and related disorders. *Expert Review of Neurotherapeutics*, **14**(11), 1275–1286 (2014). https://doi.org/10.1586/14737175.2014.963057
13. N. Masnoon, S. Shakib, L. Kalisch-Ellet & G.E. Cauguey, What is polypharmacy? A systematic review of definitions. *BMC Geriatrics*, **17**(1), 230 (2017). https://doi.org/10.1186/s12877-017-0621-2
14. R.J. Maggiore, C.P. Gross & A. Hurria. Polypharmacy in older adults with cancer. *The Oncologist*, **15**(5), 507–522 (2010). doi: https://doi.org/10.1634/theoncologist.2009-0290
15. M. Collizi, E. Carra, S. Fraietta, J. Lally, D. Quattrone, S. Bonaccorso, *et al.* Substance use, medication adherence and outcome one year following a first episode of psychosis. *Schizophrenia Research*, **170**(2-3)**,** 311–317 (2016). https://doi.org/10.1016/j.schres.2015.11.016
16. B.A. Gaudiano, S. J. Wenze, L M. Weinstock, K. M. Tezanos & I.W. Miller. Valued living and its relationship to medication adherence in patients with polar and comorbid substance use disorders. *The Journal of Nervous and Mental Disease*, **205**(3), 178–181 (2017). https://doi.org/10.1097/nmd.0000000000000533
17. A. Kamayab, M. Bijani, H. Jormand, P.A. Harsini & A.K. Jeihooni. Education based on the theory of planned behavior to improve medication adherence, hope and marital quality in Patients with substance use disorder. *BMC Public Health*, **24**, 3173 (2017). https://doi.org/10.1186/s12889-024-20651-1

CÓMO CITAR ESTE ARTÍCULO

J.R. Urrego-Novoa, K.A. Méndez-Leguizamón, K. Quintero-Parra, U. Cruz-Granados, D. Duarte-Cadena, E.C. Higuera-Dagovett, J.T. Rojas-Valencia & J. Torres-Galeano. Medición de la adherencia y cumplimiento de la medicación en pacientes con abuso y dependencia a sustancias psicoactivas, atendidos en una institución de prestación de servicios de salud en Bogotá D.C., Colombia. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 40–50 (2026). https://doi.org/10.15446/rcciquifa.v55n1.125020

Artículo de revisión

Puntos cuánticos de carbono: Un enfoque nanotecnológico para la encapsulación de fármacos

Johana Maldonado-Villamizar

Facultad Experimental de Ciencia y Tecnología, Universidad de Carabobo, Ciudad Universitaria Bárbula, Municipio Naguanagua, Venezuela. Práctica privada: Carrera 39 A # 18B Sur 154, El Poblado-Medellín, Colombia. Correo electrónico: johanamaria.mv@gmail.com

Recibido: 16 de junio de 2025
Corregido: 5 de septiembre de 2025
Aceptado: 9 de septiembre de 2025

https://doi.org/10.15446/rcciquifa.v55n1.120972

RESUMEN

Introducción: A la vanguardia de la nueva tendencia de las nanopartículas emergentes en la familia de nanomateriales carbónicos, los "Puntos cuánticos de carbono" se proyectan como una alternativa para múltiples aplicaciones biomédicas. Estos biomateriales a base de carbono con dimensiones inferiores a 10 nm se caracterizan por su emisión fluorescente. **Objetivo:** En el presente artículo se analizan los conceptos más relevantes de las nanopartículas tipo "Puntos cuánticos de carbono" como portadores de fármacos para aplicaciones terapéuticas. **Métodos:** La búsqueda incluyó las siguientes bases de datos especializadas PubMed/Medline, Cochrane Library, SciELO y el motor de búsqueda Google Scholar. Para la búsqueda se utilizó vocabulario controlado (MeSH y DeCS), empleando los términos definidos para esta revisión como "Nanoparticles", "Nanotechnology", "Nanomaterials", "Quantum Dots", "Carbon nanoparticles", "Drug delivery systems". **Hallazgos:** Los Puntos cuánticos de carbono al ser pequeñas nanopartículas fluorescentes pueden sintetizarse rápidamente mediante diversas rutas económicas y sencillas, lo que los convierte en una alternativa viable frente a otros nanomateriales fluorescentes. El uso de estas nanopartículas en la nanomedicina abarca el empleo de tecnologías de soporte o plantillas mediante la funcionalización o encapsulación de fármacos. Estas nanopartículas pueden imitar o modificar procesos biológicos, permitiendo abordar problemas asociados con la solubilidad, biodisponibilidad, inmunocompatibilidad y citotoxicidad de muchos de los medicamentos de uso tradicional. **Conclusión:** La aplicación de los "Puntos cuánticos de carbono" en futuras innovaciones en el campo de la nanomedicina podría generar entidades multifuncionales capaces de diagnosticar, tratar y monitorear el tratamiento simultáneamente. De igual manera, el estudio de la citotoxicidad debe ser abordado para lograr un andamio altamente efectivo en la liberación controlada de fármacos.

Palabras clave: Nanopartículas; nanotecnología; nanomateriales; puntos cuánticos; nanopartículas de carbono; liberación controlada.

SUMMARY

Carbon Quantum Dots: A nanotechnological approach to drug encapsulation

Introduction: At the forefront of the emerging trend in carbon-based nanomaterials, Carbon Quantum Dots (CQDs) are positioned as a promising alternative for a wide range of biomedical applications. These carbon-derived biomaterials, with dimensions below 10 nm, are distinguished by their fluorescent emission. Objective: This article analyzes the most relevant concepts surrounding Carbon Quantum Dots as drug carriers for therapeutic applications. Methods: The literature search included the following specialized databases: PubMed/Medline, Cochrane Library, SciELO, and the search engine Google

Scholar. Controlled vocabulary (MeSH and DeCS) was employed, using the terms defined for this review: "Nanoparticles," "Nanotechnology," "Nanomaterials," "Quantum Dots," "Carbon nanoparticles," and "Drug delivery systems." **Findings:** Due to their small size and fluorescent properties, CQDs can be synthesized rapidly through various cost-effective and straightforward methods, making them a viable alternative to other fluorescent nanomaterials. Their use in nanomedicine encompasses support technologies or scaffolds for drug functionalization or encapsulation. These nanoparticles can mimic or modulate biological processes, offering solutions to longstanding challenges related to solubility, bioavailability, immunocompatibility, and cytotoxicity of many conventional drugs. **Conclusion:** The application of Carbon Quantum Dots in future innovations within nanomedicine could lead to the development of multifunctional platforms capable of diagnosing, treating, and monitoring therapy simultaneously. Likewise, the issue of cytotoxicity must be addressed in depth to achieve highly effective scaffolds for controlled drug release.

Keywords: Nanoparticles; nanotechnology; nanomaterials; quantum dots; carbon nanoparticles; drug delivery systems.

RESUMO

Pontos quânticos de carbono: uma abordagem nanotecnológica para encapsulamento de medicamentos

Introdução: Na vanguarda da nova tendência das nanopartículas emergentes na família dos nanomateriais carbônicos, os "pontos quânticos de carbono" são projetados como uma alternativa para múltiplas aplicações biomédicas. Esses biomateriais à base de carbono com dimensões inferiores a 10 nm são caracterizados por sua emissão fluorescente. **Objetivo:** No presente artigo são analisados os conceitos mais relevantes das nanopartículas como "Pontos Quânticos de Carbono" como portadores de medicamentos para aplicações terapêuticas. **Métodos:** A pesquisa incluiu as seguintes bases de dados especializadas PubMed/Medline, Cochrane Library, SciELO e o motor de pesquisa Google Scholar. Para a pesquisa foi utilizado um vocabulário controlado (MeSH e DeCS), empregando os termos definidos para esta revisão como "Nanopartículas", "Nanotecnologia", "Nanomateriais", "Pontos Quânticos", "Nanopartículas de carbono", "Sistemas de distribuição de medicamentos". **Hallazgos:** Os pontos quânticos de carbono, como pequenas nanopartículas fluorescentes, podem ser sintetizados rapidamente em diversas rotas econômicas e sencilais, o que os converte em uma alternativa viável diante de outros nanomateriais fluorescentes. O uso dessas nanopartículas na nanomedicina abandonou o emprego de tecnologias de suporte ou plantas por meio da funcionalização ou encapsulamento de medicamentos. Estas nanopartículas podem imitar ou modificar processos biológicos, permitindo abordar problemas associados à solubilidade, biodisponibilidade, imunocompatibilidade e citotoxicidade de muitos dos medicamentos de uso tradicional. **Conclusão:** A aplicação dos "Pontos Quânticos de Carbono" em futuras inovações no campo da nanomedicina poderá gerar entidades multifuncionais capazes de diagnosticar, tratar e monitorar o tratamento simultaneamente. Da mesma forma, o estudo de citotoxicidade deve ser abordado para lograr um estudo altamente eficaz na liberação controlada de medicamentos.

Palavras-chave: Nanopartículas; nanotecnologia; nanomateriais; pontos cuánticos; nanopartículas de carbono; libertação controlada.

1. INTRODUCCION

En los últimos tiempos, la nanotecnología ha surgido como un campo innovador con un alto potencial de avances en múltiples dominios científicos [1]. El concepto de nanotecnología se introdujo por primera vez en 1959 cuando el físico Richard Feynman realizó una presentación sobre cómo hacer cosas a nivel atómico y molecular. A nivel atómico, las propiedades de un

elemento están determinadas por su configuración electrónica, pero los fenómenos cuánticos emergen cuando la materia se reduce a dimensiones nanométricas [2, 3].

Al reducir un material a esta escala, sus propiedades fisicoquímicas pueden modificarse significativamente debido al efecto de confinamiento cuántico. Este efecto de confinamiento crea una cuantización de la energía en niveles discretos en las bandas de conducción y valencia, por lo que el material puede considerarse implícitamente un "átomo virtual" [4, 5]. Actualmente, este efecto producido en diversos sistemas es llamado "Quantum Dots", definidos como diminutas nanopartículas semiconductoras de tan solo unos pocos nanómetros de tamaño (2-10 nm) que presentan propiedades electrónicas y ópticas únicas. Estos materiales generalmente consisten en carbono, silicio, seleniuro de cadmio, sulfuro de cadmio o arseniuro de indio y emiten fluorescencia cuando se excitan con una fuente de luz [6].

En el auge de los alótropos de carbono nanoestructurados como el fullereno, los nanotubos de carbono y el grafeno se ha allanado el camino para una nueva era en la ciencia de los materiales [7]. El carbono por estar omnipresente en la naturaleza como una sustancia de color oscuro con baja solubilidad en agua y fluorescencia débil, ha atraído un gran interés gracias a su facilidad de funcionalización superficial, métodos de síntesis fáciles, biodegradabilidad y nula toxicidad [8, 9].

Al reducir este compuesto a escala nanométrica se crean los "Puntos cuánticos de carbono" (CQDs) o puntos cuánticos basados en carbono, también conocidos como nanoluces de carbono, esta es una nueva clase de nanomateriales de carbono cuasiesféricos y fotoluminiscentes [10-12]. Los CQDs debido a su tamaño poseen características magnéticas, eléctricas, ópticas y físicas que los proyecta hacer un nanomaterial altamente versátil para todas las industrias [13, 14].

A nivel de la biomedicina específicamente en la biofarmacología, estas nanopartículas pueden utilizarse como posibles nanotransportadores de bioactivos y fármacos. La implementación de estos nuevos nanosistemas de administración de fármacos ha demostrado ser prometedora al mejorar el índice terapéutico del fármaco y la eficacia en el objetivo diana. La eficacia de los nanosistemas basados en CQDs radica en su pequeño tamaño y capacidad de penetración en la membrana celular, esta ventaja reduce los efectos adversos ocasionados por los sistemas de administración convencionales. Este artículo tiene como objetivo analizar los conceptos más relevantes de las nanopartículas tipo "Puntos cuánticos de carbono" como portadores de fármacos para aplicaciones terapéuticas.

2. MÉTODOS

Esta revisión no utilizó inteligencia artificial (IA). La búsqueda incluyó las siguientes bases de datos especializadas Pubmed/ Medline, Scielo y el motor de búsqueda Google Scholar. Para la búsqueda se utilizó vocabulario controlado (MeSH y DeCS), definidos para esta revisión como "Nanoparticles", "Nanotechnology" "Nanomaterials", "Quantum Dots" "Carbon nanoparticles" "Drug delivery systems". Se evitó homónimos y variaciones ortográficas. Para la estrategia aplicada a Google Scholar se planteó un límite artificial de 70 resultados no replicables. Como estrategia complementaria se utilizó el escaneo de referencias de citas de artículos relacionados al tema. Se utilizó el operador booleano – AND–, cuando la primera opción no estaba disponible se utilizó el operador booleano – OR. Solo se buscaron artículos en inglés y español. El filtro de temporalidad se registró 2019 al 2024, este filtro permitió encontrar la literatura actualizada.

Los criterios de inclusión aplicados fueron de acuerdo con el rigor metodológico de la literatura y con un tipo de fuente primaria y secundaria. Según el formato del material se incluyeron artículos originales, revisiones de literatura, comunicaciones cortas, actas de congreso, informes técnicos, cartas al editor. Los artículos seleccionados para la fase de tamizado cumplieron con los siguientes criterios de elegibilidad: a) el artículo que reportara resultados investigativos relacionados con "Puntos cuánticos de carbono", b) artículos que mencionen "Quantum Dots" y "fármacos". La extracción de los resultados (n=14) fue realizada por el autor principal.

3. HALLAZGOS Y SINTESIS

3.1. "Carbon quatum dots" (CQDs)

Los puntos cuánticos (QDs) son cristales coloidales semiconductores, compuestos por elementos de los grupos II-VI o III-V, constituidos por cientos o miles de átomos ordenados en una estructura cristalina de forma habitualmente esférica y de dimensiones inferiores a 10 nm [15]. En la década de 1980, los puntos cuánticos fueron observados experimentalmente por Alexey Ekimov, lo que lo hizo merecedor del Premio Nobel 2023, posteriormente fue Reed *et al.* (1988) quienes acuñaron el término "puntos cuánticos" para demostrar nanoestructuras fotoluminiscentes con estados de energía completamente cuantificados [16].

Una de las características de los QDs es que los electrones están obligados a mantenerse confinados entre las tres dimensiones, lo que genera diversos fenómenos cuánticos y lo ubica en cero dimensiones. Para que dicho proceso tenga lugar, el tamaño de los puntos cuánticos ha de ser similar al radio del excitón de Bohr. Los QDs convencionales suelen constar de núcleos de metales pesados y carcasas semiconductoras de banda ancha, lo que genera problemas de toxicidad [17].

En comparación con los puntos cuánticos (QDs) tradicionales, los CQDs ofrecen una gama más amplia de soluciones y menor toxicidad, lo que los hace preferibles para aplicaciones biomédicas [18-20]. Estas nanopartículas hemisféricas, compuestas de carbono amorfo o cristalino con dimensiones de 1 a 10 nm, son objeto de una amplia investigación debido a sus propiedades ventajosas: alta luminiscencia, estabilidad química, solubilidad en agua, bajo fotoblanqueo, biocompatibilidad, bajo costo y baja toxicidad.

Fue en el 2004 cuando Xu *et al.* descubrieron accidentalmente los CQDs al trabajar con nanotubos de pared simple. Sun y sus colaboradores demostraron una ruta sintética y los denominaron "Puntos cuánticos de carbono" [21]. Los CQDs tienen un parámetro de red cristalina aproximadamente de 0,34 nm lo que corresponde a una separación entre capas de grafito (002) [19]. Tanto los QDs como los CQDs tienen la propiedad de emitir fluorescencia, esto se debe al control del tamaño de nanopartícula que permite ajustar las longitudes de onda (correspondiente al color) de los fotones emitidos por los electrones.

Este ajuste produce diversas longitudes de ondas, es decir, mientras más grandes sean las nanopartículas, producirán longitudes de ondas más largas y frecuencia más bajas y así a la inversa. Esto indica que los CQDs más grandes emiten luz cercana al rojo y las más pequeñas emiten luz azul. Este efecto se debe al tamaño de banda prohibida, dado que la frecuencia de luz emitida es proporcional a la energía. Los puntos más grandes tienen niveles de energía más espaciados, por lo que emiten frecuencias más bajas y, por lo tanto, longitudes de onda más largas [22, 23].

Los CQDs poseen una estructura "núcleo-capa" con un núcleo de carbono (2–3 nm con un espaciado reticular de 0,2 nm) y una capa autopasivada (que comprende grupos funcionales). El núcleo (estados intrínsecos), según los estudios, puede ser cristalino grafítico (sp^2) o amorfo (sp^2 /sp^3 mixto). Dependiendo de si el carbono sp^2 está presente, las arquitecturas de núcleo-capa de los puntos cuánticos de carbono (CQDs) pueden tener una estructura cristalina amorfa o grafítica [24].

La naturaleza soluble en agua de los CQDs se atribuye a la presencia de grupos funcionales que contiene oxígeno, a saber, carboxilo (-COOH) e hidroxilo (-OH) en su superficie. Los grupos funcionales de los CQDs también contribuyen a su solubilidad, formando coloides estables en disolventes acuosos u orgánicos polares, lo cual resulta ventajoso frente a los puntos cuánticos de grafeno (GQDs), que presentan una baja solubilidad en disolventes comunes [25, 26].

3.2. Propiedades de los "Puntos cuánticos de carbono"

Entre las propiedades de CQDs se encuentran la baja toxicidad, superficie hidrófila, fluorescencia sin parpadeo, resistencia al fotoblanqueo, fácil pasivación, estabilidad química y buena compatibilidad celular. Estas características son fundamentales para convertirlos en nanomateriales altamente prometedores para aplicaciones biomédicas, ópticas y terapéuticas, ya que permiten su integración en sistemas biológicos sin generar efectos adversos significativos. Además, su superficie funcionalizable facilita la conjugación con biomoléculas específicas, potenciando su selectividad en procesos de diagnóstico y tratamiento. A continuación, se desglosarán las propiedades más importantes de los CQDs:

3.2.1. Absorción de luz

Los picos de absorción UV- visibles podría revelar la información de los estados de emisión y excitación del material. El pico de absorción en el rango de 280 a 350 nm se atribuye a transiciones electrónicas de enlaces C–O o C=O a orbitales π^*. Está claro que los electrones pueden moverse a los enlaces C=N desde la órbita π. La transición electrónica de los enlaces C–O o C=O al orbital π^* es responsable del espectro de absorción en el rango de 280 a 350 nm [27].

Shaabir *et al.* (2023) [28] mencionan que los grupos funcionales como C=O y C–O inducen transiciones electrónicas $n \rightarrow \pi^*$ y $\pi \rightarrow \pi^*$, responsables de los picos de absorción UV-visible, además, la oxidación de los precursores y la composición química determinan la naturaleza de los grupos funcionales, lo que a su vez modula el comportamiento óptico de los CQDs. Estos hallazgos sugieren que la superficie funcionalizada de los CQDs desempeña un papel clave en la modulación de sus propiedades ópticas.

3.2.2. Fluorescencia

Los fenómenos de fluorescencia se producen principalmente por dos mecanismos: (a) defectos superficiales, pasivación/funcionalización superficial, estado del núcleo de carbono, efecto del tamaño cuántico y (b) brechas de banda de dominios π-conjugados (hibridados sp^2) que se asemejan a moléculas aromáticas que incorporan ciertas brechas de banda de energía con respecto a la absorción y emisión [29].

La energía de banda prohibida de un CQDs es la diferencia en el nivel de energía entre el estado de energía excitado del punto y su estado de reposo. El punto cuántico puede absorber luz fluorescente a la frecuencia de su banda prohibida para excitarse o emitir la misma frecuencia de luz para volver a su estado de reposo. El tamaño de un CQDs es inversamente proporcional al nivel de energía de la banda prohibida y, por lo tanto, altera la frecuencia de la luz emitida y tiene un efecto en el color [28].

3.2.3. Transferencia de electrones fotoinducida

En soluciones, los CQDs serán aceptores de electrones o donantes de electrones que pueden extinguirse eficazmente, lo que indica que los CQDs fotoinducidos también son mayores donantes y aceptores de electrones [20]. La transferencia de electrones en CQDs está directamente relacionada con su estructura electrónica y la naturaleza de sus orbitales superficiales.

En estas soluciones, los CQDs excitados pueden donar electrones a especies oxidantes o aceptar electrones de moléculas reductoras, lo que los convierte en plataformas versátiles para sensores electroquímicos y fotocatalizadores (11). Esta capacidad de transferencia electrónica también permite modular la fluorescencia de los CQDs en presencia de biomoléculas, lo que los convierte en herramientas sensibles para bioimagen y diagnóstico [12].

3.2.4. Efecto de confinamiento cuántico

El efecto de confinamiento por tamaño se produce por el confinamiento espacial de los iones excitados en una dimensión adicional dentro de un material. La energía de emisión depende del radio de la partícula, de modo que a medida que esta se hace más pequeña, tanto el espectro de excitación como el de emisión se desplazan a longitudes de onda más cortas [30].

En una distribución de CQDs con tamaños variables, diferentes subconjuntos se excitan favorablemente a medida que varía la longitud de onda de excitación. A medida que se excitan CQDs según el tamaño, el espectro de emisión se desplaza con la longitud de onda [8]. El confinamiento espacial en CQDs genera una separación discreta de los niveles de energía, lo que modifica directamente sus propiedades ópticas. Esta relación inversa entre el tamaño y la longitud de onda emitida permite ajustar la fluorescencia de los CQDs mediante control sintético, lo que resulta clave para aplicaciones en bioimagen y sensores ópticos [14].

3.3. Síntesis, purificación y caracterización de "Puntos cuánticos de carbono"

En el enfoque descendente se utilizan métodos físicos o químicos para destruir o dispersar la fuente de carbono a granel en CQDs de tamaño pequeño. Este método generalmente emplea estructuras de carbono con hibridación sp^2 que no presentan brechas de energía ni brechas de banda efectivas como materiales precursores [31]. Entre los enfoques descendentes se encuentran la ablación por láser, oxidación acida, ablación química, carbonización electroquímica, descarga de arco y síntesis ultrasónica (Tabla 1) [32, 33].

Tabla 1. Síntesis con enfoque descendente

Síntesis	Descripción
Ablación por laser	El enfoque ideal es utilizar un pulso láser con una energía muy alta para irradiar la superficie de las moléculas objetivo y transferirla al estado termodinámico donde se crean altas temperaturas y presiones. Esto evapora rápidamente las moléculas objetivo y las transfiere a un estado de plasma donde cristalizan para formar nanopartículas.
Oxidación acida	Se trata de exfoliar y desintegrar carbono a granel en nanopartículas mientras se insertan simultáneamente grupos funcionales hidrófilos en la superficie, como grupos hidroxilo o carboxilo.
Ablación química	La ablación química es un proceso en el que pequeñas moléculas orgánicas se carbonizan en materiales nanocarbonosos con ácidos oxidantes fuertes, que luego se convierten en pequeñas láminas mediante una oxidación controlada.
Carbonización electroquímica	Los CQDs se producen utilizando una configuración de tres electrodos con un electrodo de trabajo (WE) como electrodo de grafito, un contraelectrodo (CE) como

	una lámina de platino o alambre de Pt y un electrodo de referencia (RE) como un electrodo de Ag/AgCl.
Descarga de arco	Este proceso utiliza plasma de gas generado en un reactor sellado dentro de un electrodo anódico para reorganizar las moléculas de carbono a medida que se rompen en segmentos más pequeños a partir de fuentes de carbono a granel.
Síntesis Ultrasónica	El proceso de preparación de CQDs es fácil y económico con el uso de tecnología ultrasónica. Crea ondas de alta y baja presión alternas que causan que pequeñas burbujas en el líquido evolucionen y revienten.

El enfoque ascendente se realiza mediante reacciones térmicas o químicas, los CQDs se ensamblan a partir de precursores de carbono más pequeños en enfoques ascendentes [34, 35]. Entre los enfoques ascendentes se desglosan en la Tabla 2.

Tabla 2. Síntesis de CQD con enfoque ascendente

Método hidrotermal	Para hacer el precursor de la reacción, se suspenden compuestos orgánicos de pequeño tamaño en agua o un disolvente orgánico. Luego, se transfiere al autoclave de acero inoxidable revestido con teflón, que se utiliza comúnmente en el laboratorio para el método hidrotermal. A una temperatura relativamente alta, las moléculas orgánicas se fusionaron para producir núcleos de siembra de carbono, que crecen en CQDs con tamaños inferiores a 10 nm.
Combustión	Este proceso utiliza tratamientos con ácido oxidativo para ajustar las características de fluorescencia, mejorar la solubilidad en agua y agregar pequeños recursos de carbono en CQDs.
Pilorosis	La técnica de pirólisis es una descomposición térmica de un precursor, típicamente por encima de 430 °C, para crear partículas coloidales a escala nanométrica (1). El proceso implica carbonizar y priorizar pequeñas moléculas orgánicas a una temperatura específica para producir puntos de carbono luminosos.
Solvotermal	El método solvotérmico se desarrolló con base en el método hidrotérmico. Se diferencia del método hidrotérmico en que los solventes utilizados son orgánicos en lugar de agua. Bajo fase líquida o condiciones supercríticas, los reactantes dispersos en la solución se vuelven más activos y los productos se forman lentamente.
Síntesis asistida por microondas	Este método utiliza radiación electromagnética en el rango de microondas (con longitudes de onda entre 1 mm y 1 m) para proporcionar un calentamiento volumétrico uniforme directamente al medio de reacción. El rápido calentamiento interno rompe los enlaces precursores y acelera la carbonización, produciendo CQDs con propiedades ópticas deseables en cuestión de minutos.

La separación y purificación del CQDs es fundamental para el control de calidad de los sistemas biológicos. Los métodos de purificación incluyen la electroforesis, la centrifugación, la diálisis y la cromatografía en columna [36]. La caracterización para los CQDs se puede realizar a través de Difracción de Rayos X (DRX) que proporciona la información de las dimensiones de la celda unitaria y el espaciado de red presente en los núcleos de carbono cristalino [21].

La resonancia magnética nuclear (RMN), la espectroscopia Raman, la microscopia electrónica de transmisión (TEM) y de barrido (SEM), la espectroscopia de fotoelectrones de rayos X (XPS), el análisis elemental (EA), el infrarrojo por transformada de Fourier (FT-IR) y microcopia de Fuerza atómica (AFM) son métodos adicionales (Tabla 3). El análisis de absorción de

nitrógeno se utiliza para determinar el área superficial de las nanopartículas de carbono. Utilizando la potencial zeta, se verifica la existencia de grupos funcionales en la capa externa [37].

Tabla 3. Métodos para caracterización de CQDs [37]

Método	**Resonancia magnética nuclear (RMN)**	**Espectroscopía Raman**	**Microscopía electrónica de transmisión (TEM)**
Definición	Basado en el fenómeno RMN de núcleos con espín no cero en un campo magnético, probando transiciones con ondas de radio; analiza coeficientes de difusión vía ecuación de Stokes-Einstein, estudia interacciones ligando-superficie y difusión de partículas.	Utiliza la dispersión inelástica de luz, analizando cambios de longitud de onda para vibraciones moleculares.	Usa un haz de electrones (60-150 keV) transmitido a través de una muestra delgada, formando imágenes basadas en interacciones electrón-muestra, proporcionando visualización de alta resolución.
Función	Analiza la formación y arquitectura de NPs	Mide vibraciones moleculares, características estructurales, interacciones superficiales, estados químicos, modos vibracionales/rotacionales, expansión de red y defectos.	Mide tamaño de nanopartículas, monodispersidad de tamaño, forma, estado de agregación, cinética de crecimiento, morfología, estructura interna, configuraciones core-shell, homogeneidad, capacidad para detectar/localizar/cuantificar en matrices.
Ventajas	No destructivo, altamente reproducible, sensible a detalles estructurales, permite monitoreo in situ, alta resolución espacial y química para mecanismos de reacción, proporciona información química y estructural detallada.	Altamente sensible a la superficie, no destructivo, efectivo para detectar moléculas superficiales, revela grupos orgánicos inespecíficos, proporciona información molecular y estructural, útil para análisis in situ.	Alta resolución (escala atómica), visualización directa, precisa para homogeneidad, estudia partículas individuales, insights en transformaciones dinámicas, interacciones celulares.

Microscopía de fuerza atómica (AFM)	**Infrarrojo por transformada de Fourier (FT-IR)**	**Espectroscopía de fotoelectrones de rayos X (XPS)**	**Microscopía electrónica de barrido (SEM)**
Usa un cantilever con una sonda afilada para escanear la superficie, midiendo la fuerza para mapear la topografía con resolución nanométrica, a menudo en modo tapping para NPs, creando imágenes 3D.	Mide la absorción de radiación infrarroja media (4000–400 cm^{-1}) para identificar transiciones vibracionales, proporcionando información sobre estructuras moleculares, fuerza de enlaces y grupos funcionales por vía de absorción de luz infrarroja.	Irradia la muestra con rayos X, analiza fotoelectrones emitidos de energía cinética para determinar estado elemental/químico.	Escanea por medio de un haz de electrones enfocado a través de la muestra, detectando electrones secundarios/retrodispersados para generar una imagen superficial de alta resolución.
Topografía superficial, tamaño, forma y morfología de nanopartículas en modo 3D; grado de cobertura superficial, dispersión en matrices, precisión en dimensiones laterales.	Composición molecular, grupos funcionales, unión química e interacciones de ligandos en superficies de nanopartículas.	Composición elemental, estado químico, estado electrónico de átomos superficiales, química superficial de las nanopartículas.	Morfología superficial, tamaño, forma, dispersión en células/matrices/soportes, precisión en dimensiones laterales, composición elemental, información topográfica, arreglo espacial.
No destructivo, proporciona visualización 3D, alta resolución espacial (escala nanométrica), alta precisión en dimensiones laterales, examen rápido, no requiere modificación o coating superficial, resolución similar a SEM/TEM a menor costo y espacio; caracteriza altura de NPs.	No destructivo, proporciona información química detallada sobre grupos funcionales superficiales e interacciones moleculares.	Proporciona información química y electrónica superficial detallada, no destructivo para análisis superficial.	Alta resolución (hasta 1 nm), preparación de muestra rápida/fácil.

Potencial zeta	Mide la movilidad electroforética en campo eléctrico para determinar la carga superficial.	Carga superficial y estabilidad de dispersiones coloidales de nanopartículas.	Proporciona insight en estado de aglomeración y carga superficial, útil para análisis de estabilidad; indica estabilidad coloidal, no destructivo.

La distribución de tamaño de los CQDs se analiza estadísticamente mediante TEM y AFM. La composición básica del CQDs consiste en elementos C, H, O y N y se confirma mediante XPS. Los grupos funcionales presentes en los CQDs son COC, COOH, C-OH, CH y CC, confirmados por FTIR. Se utiliza espectroscopia UV-vis y espectroscopia de fluorescencia para caracterizar las propiedades ópticas de los CQDs [36].

3.4. Aplicaciones de los "Puntos cuánticos de carbono"

3.4.1. Bioimagen y biosensores

La aplicación de puntos cuánticos de carbono (CQDs) en bioimagen ha cobrado relevancia por su alta biocompatibilidad, baja toxicidad y capacidad de emisión fluorescente multicolor. Estudios recientes han demostrado que los CQDs pueden diseñarse para emitir en rangos específicos del espectro, lo que permite su uso en técnicas de imagenología de tejidos profundos y seguimiento celular en tiempo real.

Das *et al.* (2024) [38] destacan el potencial de los CQDs como agentes de contraste en bioimagen, subrayando su versatilidad para combinar fluorescencia con funcionalización molecular dirigida. Por su parte, Lesani *et al.* (2020) [39] desarrollaron una sonda basada en CQDs con emisión dual para imagenología mediante excitación bifotónica, logrando una visualización eficiente en tejidos con alta densidad óptica.

La versatilidad de los puntos cuánticos de carbono se ha ampliado mediante su funcionalización con elementos magnéticos y biológicos, como lo demuestra el estudio de Yao *et al.* (2017) [40], quienes desarrollaron CQDs magnetofluorescentes. Estos nanomateriales fueron dopados con iones metálicos (Gd^{3+}, Mn^{2+} y Eu^{3+}), lo que les confirió propiedades contrastantes para imagenología por resonancia magnética (MRI), además de una intensa fluorescencia para bioimagen óptica. Además, al incorporar doxorrubicina como fármaco modelo, los CQDs mostraron una citotoxicidad significativamente superior frente a células tumorales en comparación con el fármaco libre, sin evidenciar toxicidad en modelos *in vivo*.

Aunque la bioimagen y los biosensores comparten el uso de puntos cuánticos de carbono (CQDs) por sus propiedades ópticas excepcionales, sus aplicaciones biomédicas se distinguen por su propósito y enfoque. Mientras que la bioimagen permite visualizar estructuras biológicas en tiempo real, los biosensores están diseñados para detectar y cuantificar biomoléculas específicas mediante cambios en la señal óptica del nanomaterial.

Esta capacidad de respuesta ante estímulos moleculares convierte a los CQDs en transductores altamente sensibles para el monitoreo clínico. Un ejemplo destacado es el estudio de Vyas *et al.* (2024) [41], donde se desarrollaron biosensores de película delgada, basados en

CQDs conjugados con enzimas para la detección específica de contaminantes organofosforados como etil paraoxón y metil paraoxón. El sistema mostró alta sensibilidad, selectividad y estabilidad en condiciones ambientales reales, lo que demuestra el potencial de los CQDs en aplicaciones de diagnóstico molecular y control toxicológico.

3.4.2. Fototérmica

La terapia fototérmica (PTT) es una estrategia terapéutica mínimamente invasiva que utiliza nanomateriales capaces de convertir la energía lumínica, generalmente en el espectro del infrarrojo cercano (NIR), en calor localizado. Este aumento de temperatura inducido por irradiación láser induce la destrucción térmica de células patológicas, como las tumorales, sin afectar significativamente los tejidos sanos circundantes.

La integración de propiedades ópticas y terapéuticas en nanomateriales ha impulsado el desarrollo de plataformas teragnóstica para oncología. En este contexto, Bao *et al.* (2018) [42] diseñaron puntos cuánticos de carbono emisores en el infrarrojo cercano (NIR-CDs), dopados con azufre y nitrógeno, capaces de combinar bioimagen y terapia fototérmica de alta eficiencia. Tras su administración intravenosa en modelos, los NIR-CDs se acumularon pasivamente en tejido tumoral sin necesidad de ligandos específicos, lo que permitió una visualización precisa mediante fluorescencia y fotoacústica.

De igual manera, Zhao *et al.* (2023) [43] lograron sintetizar CQDs con estados excitados localizados y de transferencia de carga, lo que les confirió una doble capacidad de emisión en NIR-I/NIR-II y una alta eficiencia de absorción multifotonica. Estos CQDs mostraron una conversión fototérmica superior al 60%, lo que permitió realizar imagenología profunda con resolución subcelular y ablación tumoral localizada tras irradiación láser. La combinación de diagnóstico y tratamiento en una sola plataforma nanométrica ha impulsado el desarrollo de sistemas teragnóstica basados en puntos cuánticos de carbono emisores en el infrarrojo cercano (NIR-CDs) para tratamientos localizados.

3.4.3. Fotodinámica

La terapia fotodinámica (PDT) es una modalidad terapéutica no invasiva que combina un agente fotosensibilizador, luz de longitud de onda específica y oxígeno molecular para generar especies reactivas de oxígeno (ROS) capaces de inducir daño celular selectivo. Al ser activados por irradiación lumínica, los fotosensibilizadores producen ROS como el oxígeno singlete, que desencadena procesos de apoptosis, necrosis o autofagia en células patológicas, sin afectar significativamente el tejido sano circundante.

La optimización de sistemas fotodinámicos para el tratamiento de tumores ha llevado al desarrollo de nanocompuestos activables por luz. En este contexto, Negi *et al.* (2024) [44] diseñaron puntos cuánticos de carbono sensibles a dos fotones, incorporados en estructuras metal-orgánicas nanoestructuradas (NMOFs), funcionalizadas con ácido fólico para direccionamiento específico hacia células tumorales que sobreexpresan el receptor de folato. Bajo irradiación con láser de 980 nm, el sistema demostró una generación eficiente de especies reactivas de oxígeno (ROS), incluyendo oxígeno singlete, lo que permitió una inhibición celular del 73% en líneas cancerosas. La fluorescencia cuántica del sistema alcanzó un rendimiento del 13%, lo que además habilita su uso en imagenología simultánea.

La terapia fotodinámica también ha demostrado ser eficaz en el tratamiento de infecciones bacterianas resistentes, especialmente cuando se emplean nanocompuestos funcionalizados con capacidad fotosensibilizadora. En el estudio de Marković *et al.* (2019) [45], se desarrollaron nanocompuestos de puntos cuánticos de carbono (CQDs) encapsulados en polidimetilsiloxano (PDMS), diseñados para generar especies reactivas de oxígeno (ROS) bajo irradiación con luz

azul (470 nm). Estos CQDs mostraron una potente actividad antibacteriana frente a cepas resistentes como *Staphylococcus aureus, Escherichia coli* y *Klebsiella pneumoniae*, logrando una erradicación significativa sin inducir citotoxicidad en células NIH/3T3.

3.5. "Puntos cuánticos de carbono" para la encapsulación de fármacos

La evolución de los materiales nanocompuestos ha mejorado significativamente la capacidad de administración de fármacos gracias a los diversos vehículos de administración. Estos sistemas presentan características únicas que facilitan la administración dirigida de fármacos a través de diversas barreras fisiológicas. La integración de mecanismos de administración inteligentes con enfoques terapéuticos convencionales ha mejorado la eficacia de los fármacos y reducido la toxicidad sistémica.

La búsqueda de sistemas no invasivos para la administración de péptidos terapéuticos ha impulsado el desarrollo de formulaciones basadas en nanomateriales funcionalizados. En este contexto, Çamlik *et al.* (2022) [46] diseñaron una formulación oral de insulina utilizando puntos cuánticos de carbono compuestos (CQDs), dopados con nitrógeno y recubiertos con polímeros biocompatibles como PEG 3350 y metilcelulosa. Esta arquitectura permitió proteger la insulina frente a la degradación gastrointestinal y facilitar su absorción sistémica, logrando una reducción significativa de la glucemia en modelos animales diabéticos y demostrando efectos antihiperglucémicos independientes, atribuibles a su capacidad para modular la actividad mitocondrial y estimular la glucólisis celular.

Los CQDs, gracias a su química de superficie ajustable, permiten modificaciones precisas para mejorar la carga del fármaco y la liberación controlada, mientras que su pequeño tamaño facilita la penetración profunda en los tejidos. La integración de CQDs en matrices de hidrogel amplía aún más sus aplicaciones biomédicas, en particular en el cuidado de heridas y la ingeniería de tejidos. Al incorporar CQDs, estos hidrogeles obtienen capacidades mejoradas de administración de fármacos y propiedades antibacterianas, a la vez que mantienen la biocompatibilidad, lo que facilita la monitorización no invasiva del fármaco [37].

Además de su aplicación en hidrogeles, los CQDs han sido explorados en otros sistemas de liberación controlada. En el estudio de Samimi *et al.* (2021) [47], se desarrolló una formulación que combina CQDs con ácido quínico para facilitar la entrega dirigida de gemcitabina a células de cáncer de mama, logrando un prometedor nanotransporte para dicho fármaco. Por su parte, la resistencia bacteriana y la formación de biopelículas representan desafíos críticos en el tratamiento de infecciones persistentes. En este contexto, Wang *et al.* (2019) [48] desarrollaron puntos cuánticos de carbono dopados con nitrógeno (N-CQDs) como una estrategia antimicrobiana innovadora. Estos nanomateriales fueron sintetizados a partir de sales biscuaternarias, lo que les confirió una carga positiva capaz de interactuar electrostáticamente con las membranas bacterianas, logrando una actividad antimicrobiana del 99% frente a cepas resistentes como *Staphylococcus aureus* resistente a meticilina (MRSA) y *Escherichia coli* resistente a ampicilina, superando incluso a antibióticos clínicos como vancomicina y gentamicina.

Los ensayos *in vitro* evidenciaron una mejora significativa en la citotoxicidad frente a células tumorales, en comparación con el fármaco libre, lo que sugiere una mayor eficacia terapéutica y menor toxicidad sistémica. Este enfoque representa un avance relevante en la nanotecnología aplicada a la oncología, al integrar funcionalización bioactiva y liberación controlada en un solo sistema nanométrico [47].

Al igual que otros nanomateriales, la administración de fármacos basada en CQDs implica la unión de fármacos a CQDs o adsorción para una administración en un sitio específico con efectos secundarios mínimos. El enlace entre CQDs y el fármaco se escinde en el complejo

CQDs-fármaco en un entorno ácido del sitio enfermo u otros factores estimulantes, permitiendo así una liberación controlada del fármaco en sitios específicos [28].

En el caso de los CQDs dopados con material electromagnético o inducido magnéticamente, el fármaco se administrará a la ubicación objetivo con la ayuda del campo magnético o electromagnético externo mediante el portador de fármaco controlado [20]. Los CQDs pueden volverse magnéticos para su uso en resonancia magnética (RM), a la vez que ofrecen la ventaja de la administración de fármacos y la obtención de imágenes de fluorescencia. La RM combinada con la fluorescencia proporciona una penetración tisular superior, una resolución espacial superior y un examen microscópico del tejido mediante imágenes de fluorescencia [36].

Los CQDs son un buen sustituto de las nanoparticulas de oro (AuNP), y la funcionalización variada podría llevar a mejores posibilidades de conjugación de fármacos en combinación con agentes diana, lo que resulta en un aumento de la eficiencia de administración de fármaco. La modificación controlable de la superficie para variar funciones, el tamaño pequeño, el bajo coste, la biocompatibilidad y la casi ausencia de efectos secundarios hacen de los CQDs una elección fácil como diana farmacológica [49].

La integración de nanomateriales funcionalizados con componentes biológicos ha abierto nuevas posibilidades en la terapia dirigida contra el cáncer. Un ejemplo destacado es el estudio de Tiwari *et al.* (2023) [50], quienes desarrollaron un sistema híbrido de liberación controlada que combina puntos cuánticos de carbono cargados con dacarbazina y recubiertos con exosomas derivados de células de cáncer de mama. Esta formulación, denominada Ex-DC@CQDs, demostró una alta especificidad tumoral, mejor penetración celular y una liberación sostenida del fármaco, lo que se tradujo en una eficacia antitumoral significativamente superior en modelos in vivo, con menor toxicidad sistémica en comparación con la dacarbazina libre.

4. CONSIDERACIONES FINALES

Los CQDs son ejemplos de nanomateriales artificiales fabricados mediante procedimientos mecánicos y precisos. Debido a sus efectos cuánticos, elevada relación superficie-volumen y la emisión de luz visible, pueden ser utilizados como nanotransportadores y sondas moleculares para el seguimiento y la monitorización simultáneos de la administración de fármacos y la eficacia terapéutica. Muchas proteínas y biomoléculas comunes existen en una escala de tamaño similar, y estas pequeñas escalas de longitud permiten que los materiales inorgánicos interactúen estrechamente con entidades biológicas a nivel molecular.

El tamaño nanométrico les permite una mayor sensibilidad y una dosis efectiva menor, sin sacrificar la funcionalidad. Esta relación superficie-volumen, sumada a la amplia gama de productos químicos disponibles, facilita la modificación de la superficie para mejorar la biocompatibilidad, la solubilidad y la reactividad. Los CQDs se preparan mediante la funcionalización superficial de nanopartículas de carbono con moléculas orgánicas y poliméricas.

La mayoría de los métodos de preparación se basan en la carbonización de precursores que contienen carbono. La carbonización de diversos zumos de frutas, cáscaras de sandía o pomelo, numerosos alimentos, hierbas y hojas de plantas ha permitido la obtención de CQDs. La encapsulación de fármacos con CQDs puede ser una ventana hacia nuevas tecnologías que permitan entender como es el funcionamiento de los bioactivos con el elemento diana.

CONFLICTO DE INTERÉS

La autora declara que no tiene ningún conflicto de interés, financiero o de otro tipo, que pudieran influir en los resultados de esta revisión.

REFERENCIAS

1. A. Haleem, M. Javaid, R.P. Singh, S. Rab & R. Suman. Applications of nanotechnology in medical field: a brief review. *Global Health Journal*, **7**(2), 70–77 (2023). https://doi.org/10.1016/j.glohj.2023.02.008
2. R.P. Feynman. There's plenty of room at the bottom. *Resonance*, **16**(9), 890–898 (2011). https://doi.org/10.1007/s12045-011-0109-x
3. M.C. Roco. Nanotechnology: convergence with modern biology and medicine. *Current Opinion in Biotechnology*, **14**(3), 337–346 (2003). https://doi.org/10.1016/S0958-1669(03)00068-5
4. Z. Xie, X. Li, R. Li, S. Lu, W. Zheng, D. Tu, Y. Feng & X. Chen. *In situ* confined growth of ultrasmall perovskite quantum dots in metal–organic frameworks and their quantum confinement effect. *Nanoscale*, **12**(32), 17113–17120 (2020). https://doi.org/10.1039/d0nr04741d
5. B. Rooj & U. Mandal. A review on characterization of carbon quantum dots. *Vietnam Journal of Chemistry*, **61**(6), 693–718 (2023). https://doi.org/10.1002/vjch.202300022
6. P. Singh, V. Bhankar, S. Kumar & K. Kumar. Biomass-derived carbon dots as significant biological tools in the medicinal field: A review. *Advances in Colloid and Interface Science*, **328**, 103182 (2024). https://doi.org/10.1016/j.cis.2024.103182
7. Z. Li, Z. Liu, H. Sun & C. Gao. Superstructured assembly of nanocarbons: fullerenes, nanotubes, and graphene. *Chemical Reviews*, **115**(15), 7046–7117 (2015). https://doi.org/10.1021/acs.chemrev.5b00102
8. M. Gaur, C. Misra, A.B. Yadav, S. Swaroop, F.Ó. Maolmhuaidh, M. Bechelany & A. Barhoum. Biomedical applications of carbon nanomaterials: fullerenes, quantum dots, nanotubes, nanofibers, and graphene. *Materials*, **14**(20), 5978 (2021). https://doi.org/10.3390/ma14205978
9. V. Harish, D. Tewari, M. Gaur, A.B. Yadav, S. Swaroop, M. Bechelany & A. Barhoum. Review on nanoparticles and nanostructured materials: bioimaging, biosensing, drug delivery, tissue engineering, antimicrobial, and agro-food applications. *Nanomaterials*, **12**(3), 457 (2022). https://doi.org/10.3390/nano12030457
10. Y. Wang & A. Hu. Carbon quantum dots: synthesis, properties and applications. *Journal of Materials Chemistry C*, **2**(34), 6921–6939 (2014). https://doi.org/10.1039/c4tc00988f
11. S.E. Elugoke, G.E. Uwaya, T.W. Quadri & E.E. Ebenso. Carbon quantum dots: Basics, properties, and fundamentals. En: *Carbon Dots: Recent Developments and Future Perspectives*. ACS Symposium Series, 1465, 2024, pp. 3–42. https://doi.org/10.1021/bk-2024-1465.ch001
12. H.-L. Yang, L.-F. Bai, Z.-R. Geng, H. Chen, L.-T. Xu, Y.-C. Xie, D.-J. Wang, H.-W. Gu & X.-M. Wang. Carbon quantum dots: Preparation, optical properties, and biomedical applications. *Materials Today Advances*, **18**, 100376 (2023). https://doi.org/10.1016/j.mtadv.2023.100376
13. A. Ben-Amor, H. Hemmami, I. Ben-Amor, S. Zeghoud, A.A. Alhamad, M. Belkacem, N.S. Nair & A.B. Sruthimol. Advances in carbon quantum dot applications: Catalysis, sensing, and biomedical innovations. *Materials Science in Semiconductor Processing*, **185**, 108945 (2025). https://doi.org/10.1016/j.mssp.2024.108945
14. B. Acharya, A. Behera, S. Behera & S. Moharana. Carbon quantum dots: A systematic overview of recent developments in synthesis, properties, and novel therapeutic applications. *Inorganic Chemistry Communications*, **165**, 112492 (2024). https://doi.org/10.1016/j.inoche.2024.112492
15. G.-W. Deng, N. Xu & W.-J. Li. Gate-defined quantum dots: Fundamentals and applications. En: P. Yu & Z. Wang (editores). *Quantum Dot Optoelectronic Devices.* Springer Cham, 2020, pp. 107–133. https://doi.org/10.1007/978-3-030-35813-6_4

16. L. Manna. The bright and enlightening science of quantum dots. *Nano Letters*, **23**(21), 9673–9676 (2023). https://doi.org/10.1021/acs.nanolett.3c03904
17. J. Cassidy & M. Zamkov. Nanoshell quantum dots: Quantum confinement beyond the exciton Bohr radius. *The Journal of Chemical Physics*, **152**(11), 110902 (2020). https://doi.org/10.1063/1.5126423
18. N. Azam, M.N. Ali & T.J. Khan. Carbon quantum dots for biomedical applications: Review and analysis. *Frontiers in Materials*, **8**, 700403 (2021). https://doi.org/10.3389/fmats.2021.700403
19. M.J. Molaei. Carbon quantum dots and their biomedical and therapeutic applications: a review. *RSC Advances*, **9**, 6460–6481 (2019). https://doi.org/10.1039/c8ra08088g
20. N.A. Pechnikova, K. Domvri, K. Porpodis, M.S. Istomina, A.V. Iaremenko & A.V. Yaremenko. Carbon quantum dots in biomedical applications: Advances, challenges, and future prospects. *Aggregate*, **6**(3), e707 (2025) https://doi.org/10.1002/agt2.707
21. X. Xu, R. Ray, Y. Gu, H.J. Ploehn, L. Gearheart, K. Raker & W.A. Scrivens. Electrophoretic analysis and purification of fluorescent single-walled carbon nanotube fragments. *Journal of the American Chemical Society*, **126**(40), 12736–12737 (2004). https://doi.org/10.1021/ja040082h
22. J. Kong, Y. Wei, F. Zhou, L. Shi, S. Zhao, M. Wan & X. Zhang. Carbon quantum dots: Properties, preparation, and applications. *Molecules*, **29**(9), 2002 (2024). https://doi.org/10.3390/molecules29092002
23. X. Guan, Z. Li, X. Geng, Z. Lei, A. Karakoti, T. Wu, P. Kumar, J. Yi & A. Vinu. Emerging trends of carbon-based quantum dots: Nanoarchitectonics and applications. *Small*, 19(17), 2207181 (2023). https://doi.org/10.1002/smll.202207181
24. A.M. Díez-Pascual. Carbon-based nanomaterials. *International Journal of Molecular Sciences*, **22**(14), 7726 (2021). https://doi.org/10.3390/ijms22147726
25. L. Cui, X. Ren, M. Sun, H. Liu & L. Xia. Carbon dots: Synthesis, properties and applications. *Nanomaterials*, **11**(12), 3419 (2021). https://doi.org/10.3390/nano11123419
26. B. Wang, H. Cai, G.I.N. Waterhouse, X. Qu, B. Yang & S. Lu. Carbon dots in bioimaging, biosensing and therapeutics: A comprehensive review. *Small Science*, **2**(6), 2200012 (2022). https://doi.org/10.1002/smsc.202200012
27. Z.A. Qureshi, H. Dabash, D. Ponnamma & M.K.G. Abbas. Carbon dots as versatile nanomaterials in sensing and imaging: Efficiency and beyond. *Heliyon*, **10**(11), e31634 (2024). https://doi.org/10.1016/j.heliyon.2024.e31634
28. H. Shabbir, E. Csapó & M. Wojnicki. Carbon quantum dots: The role of surface functional groups and proposed mechanisms for metal ion sensing. *Inorganics*, 11(6), 262 (2023). https://doi.org/10.3390/inorganics11060262
29. M. Jorns & D. Pappas. A review of fluorescent carbon dots, their synthesis, physical and chemical characteristics, and applications. *Nanomaterials*, **11**(6), 1448 (2021). https://doi.org/10.3390/nano11061448
30. M. Ullah, U.A. Awan, H. Ali, A. Wahab, S.U. Khan, M. Naeem, M. Ruslin, A.Z. Mustopa & N. Hasan. Carbon dots: New rising stars in the carbon family for diagnosis and biomedical applications. *Journal of Nanotheranostics*, **6**(1), 1 (2025). https://doi.org/10.3390/jnt6010001
31. D. Ozyurt, M.A. Kobaisi, R.K. Hocking & B. Fox. Properties, synthesis, and applications of carbon dots: A review. *Carbon Trends*, **12**, 100276 (2023). https://doi.org/10.1016/j.cartre.2023.100276
32. H. Liu, X. Zhong, Q. Pan, Y. Zhang, W. Deng, G. Zou, H. Hou & X. Ji. A review of carbon dots in synthesis strategy. *Coordination Chemistry Reviews*, **498**, 215468 (2024). https://doi.org/10.1016/j.ccr.2023.215468
33. B.D. Mansuriya & Z. Altintas. Carbon dots: Classification, properties, synthesis, characterization, and applications in health care—An updated review (2018–2021). *Nanomaterials*, **11**(10), 2525 (2021). https://doi.org/10.3390/nano11102525
34. Q. He & L. Zhang. Design of carbon dots as nanozymes to mediate redox biological processes. *Journal of Materials Chemistry B*, **11**, 5071–5082 (2023). https://doi.org/10.1039/d2tb02259a
35. H.H. Jing, F. Bardakci, S. Akgöl, K. Kusat, M. Adnan, M.J. Alam, R. Gupta, S. Sahreen, Y. Chen, S.C.B. Gopinath & S. Sasidharan. Green carbon dots: Synthesis, characterization, properties and

biomedical applications. *Journal of Functional Biomaterials*, **14**(1), 27 (2023). https://doi.org/10.3390/jfb14010027

36. A. Barhoum, A. Meftahi, M.S.K. Sabery, M.E.M. Heravi & F. Alem. A review on carbon dots as innovative materials for advancing biomedical applications: synthesis, opportunities, and challenges. *Journal of Materials Science*, **58**, 13531–13579 (2023). https://doi.org/10.1007/s10853-023-08797-6
37. S. Mourdikoudis, R.M. Pallares & N.T.K. Thanh. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale*, **10**, 12871–12934 (2018). https://doi.org/10.1039/c8nr02278j
38. S. Das, S. Mondal & D. Ghosh. Carbon quantum dots in bioimaging and biomedicines. *Frontiers in Bioengineering and Biotechnology*, **11**, 1333752 (2024). https://doi.org/10.3389/fbioe.2023.1333752
39. P. Lesani, G. Singh, C.M. Viray, Y. Ramaswamy, D.M. Zhu, P. Kingshott, Z. Lu & H. Zreiqat. Two-photon dual-emissive carbon dot-based probe: Deep-tissue imaging and ultrasensitive sensing of intracellular ferric ions. *ACS Applied Materials & Interfaces*, **12**(16), 18395–18406 (2020). https://doi.org/10.1021/acsami.0c05217
40. Y.-Y. Yao, G. Gedda, W.M. Girma, C.-L. Yen, Y.-C. Ling & J.-Y. Chang. Magnetofluorescent carbon dots derived from crab shell for targeted dual-modality bioimaging and drug delivery. *ACS Applied Materials & Interfaces*, **9**(16), 13887–13899 (2017). https://doi.org/10.1021/acsami.7b01599
41. T. Vyas, S. Jaiswal, S. Choudhary, P. Kodgire & A. Joshi. Recombinant organophosphorus acid anhydrolase (OPAA) enzyme–carbon quantum dot (CQDs)–immobilized thin film biosensors for the specific detection of ethyl paraoxon and methyl paraoxon. *Environmental Research*, **243**, 117855 (2024). https://doi.org/10.1016/j.envres.2023.117855
42. X. Bao, Y. Yuan, J. Chen, B. Zhang, D. Li, D. Zhou, *et al. In vivo* theranostics with near-infrared-emitting carbon dots—highly efficient photothermal therapy based on passive targeting after intravenous administration. *Light: Science & Applications*, **7**, 91 (2018). https://doi.org/10.1038/s41377-018-0090-1
43. W.B. Zhao, D.D. Chen, K.K. Liu, Y. Wang, R. Zhou, S.Y. Song, F.K. Li, L.Z. Sui, Q. Lou, L. Hou & C.X. Shan. Near-infrared I/II emission and absorption carbon dots via constructing localized excited/charge transfer state for multiphoton imaging and photothermal therapy. *Chemical Engineering Journal*, **452**(Part 2), 139231 (2023). https://doi.org/10.1016/j.cej.2022.139231
44. K. Negi, N.K. Pathak, U. Tripathy, S.K. Dey & S.K. Sahu. Two-photon NIR-responsive carbon dots incorporated into NMOFs for targeted photodynamic therapy. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **694**, 134163 (2024). https://doi.org/10.1016/j.colsurfa.2024.134163
45. Z.M. Marković, M. Kovačova, P. Humpoliček, M.D. Budimir, J. Vajďák, P. Kubat, *et al.* Antibacterial photodynamic activity of carbon quantum dots/polydimethylsiloxane nanocomposites against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. *Photodiagnosis and Photodynamic Therapy*, **26**, 342–349 (2019). https://doi.org/10.1016/j.pdpdt.2019.04.019
46. G. Çamlik, I. Ozakca, B. Bilakaya, A.T. Ozcelikay, A.J. Velaro, S. Wasnik & I.T. Degim. Development of composite carbon quantum dots–insulin formulation for oral administration. *Journal of Drug Delivery Science and Technology*, **76**, 103833 (2022). https://doi.org/10.1016/j.jddst.2022.103833
47. S. Samimi, M.S. Ardestani & F.A. Dorkoosh. Preparation of carbon quantum dots–quinic acid for drug delivery of gemcitabine to breast cancer cells. *Journal of Drug Delivery Science and Technology*, **61**, 102287 (2021). https://doi.org/10.1016/j.jddst.2020.102287
48. H. Wang, Z. Song, J. Gu, S. Li, Y. Wu & H. Han. Nitrogen-doped carbon quantum dots for preventing biofilm formation and eradicating drug-resistant bacteria infection. *ACS Biomaterials Science & Engineering*, **5**(9), 4739–4749 (2019). https://doi.org/10.1021/acsbiomaterials.9b00583
49. S.D. Hettiarachchi, R.M. Graham, K.J. Mintz, Y. Zhou, S. Vanni, Z. Peng & R.M. Leblanc. Triple conjugated carbon dots as a nano-drug delivery model for glioblastoma brain tumors. *Nanoscale*, **11**(3), 6192–6205 (2019). https://doi.org/10.1039/c8nr08970a
50. P. Tiwari, R.P. Shukla, K. Yadav, N. Singh, D. Marwaha, S. Gautam, A.K. Bakshi, N. Rai, A. Kumar, D. Sharma & P.R. Mishra. Dacarbazine-primed carbon quantum dots coated with breast cancer

cell-derived exosomes for improved breast cancer therapy. *Journal of Controlled Release*, **365**, 43–59 (2023). https://doi.org/10.1016/j.jconrel.2023.11.005

CÓMO CITAR ESTE ARTÍCULO

J. Maldonado-Villamizar. Puntos cuánticos de carbono: Un enfoque nanotecnológico para la encapsulación de fármacos. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 51–67 (2026). https://doi.org/10.15446/rcciquifa.v55n1.120972

Technological research article

Application of high-performance liquid chromatography with diode-array detection (HPLC-DAD) for the determination of sulfadiazine and sulfadoxine in serum

Charli Rose Parra[1], Juan Pablo Betancourt Arango[2]*, Jorge Enrique Gómez Marín[1] & Gonzalo Taborda Ocampo[2]

[1] Molecular Parasitology Research Group (GEPAMOL), Biomedical Research Center, Universidad del Quindío, Armenia, Colombia. Cra 15 Calle 12N, Armenia, Colombia. Tel/Fax: +57 67 359959.
[2] Department of Chemistry, Universidad de Caldas, Calle 65 N. 26-10, A.A. 265, Caldas, Colombia.

* Corresponding author E-mail: chemquantum@outlook.com

Received: March 31, 2025
Corrected: September 14, 2025
Accepted: September 18, 2025

https://doi.org/10.15446/rcciquifa.v55n1.119599

SUMMARY

Introduction. Monitoring serum drug levels in children with congenital toxoplasmosis is essential for determining therapeutic efficacy. This study aimed to standardize the quantification of two sulfonamides, sulfadiazine (SDZ) and sulfadoxine (SDX), using high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD). Key attributes such as linearity, precision, accuracy, selectivity, limit of detection (LOD), and limit of quantification (LOQ) were evaluated, alongside secondary attributes like drug stability and matrix effects. **Methodology**. The quantification of SDZ and SDX was performed using HPLC-DAD, evaluating: linearity: Confirmed a linear correlation between analytical signal and drug concentration, precision: Evaluated using relative standard deviation (%RSD), accuracy: Determined by recovery percentages, selectivity: Ensured no significant interference from the biological matrix, LOD and LOQ: Assessed for method sensitivity. **Results**. The method demonstrated a clear linear relationship between concentration and instrumental response. Precision was within acceptable ranges for bioanalytical studies, with %RSD indicating consistent results. The accuracy was satisfactory with recovery percentages slightly below 90%, which was acceptable considering the complexity of the biological matrix. LOD and LOQ were consistent with previously reported values, confirming high sensitivity. **Conclusions**. The HPLC-DAD method is reliable, robust, and sensitive for monitoring sulfadiazine and sulfadoxine levels in serum. While recovery percentages were slightly below 90%, the method's performance was satisfactory considering the biological matrix. This method is suitable for therapeutic monitoring and can aid in assessing treatment efficacy in congenital toxoplasmosis.

Keywords: RP-HPLC-DAD; sulfadoxine; sulfadiazine; toxoplasmosis.

RESUMEN

Aplicación de la cromatografía líquida de alta resolución con detección por matriz de diodos (HPLC-DAD) para la determinación de sulfadiazina y sulfadoxina en Suero

Introducción. La monitorización de los niveles séricos de fármacos en niños con toxoplasmosis congénita es esencial para determinar la eficacia terapéutica. El objetivo de este estudio fue estandarizar la

cuantificación de dos sulfonamidas, sulfadiazina (SDZ) y sulfadoxina (SDX), mediante cromatografía líquida de alta resolución acoplada a un detector de matriz de diodos (HPLC-DAD). Se evaluaron atributos clave como la linealidad, la precisión, la exactitud, la selectividad, el límite de detección (LOD) y el límite de cuantificación (LOQ), junto con atributos secundarios como la estabilidad del fármaco y los efectos de la matriz. **Metodología.** La cuantificación de SDZ y SDX se realizó mediante HPLC-DAD, evaluando: linealidad: Se confirmó una correlación lineal entre la señal analítica y la concentración del fármaco, precisión: Evaluada mediante la desviación estándar relativa (%RSD), exactitud: Determinada por los porcentajes de recuperación, selectividad: Se garantizó que no hubiera interferencias significativas de la matriz biológica, LOD y LOQ: Se evaluó la sensibilidad del método. **Resultados.** El método demostró una clara relación lineal entre la concentración y la respuesta instrumental. La precisión estuvo dentro de rangos aceptables para estudios bioanalíticos, con %RSD indicando resultados consistentes. La exactitud fue satisfactoria, con porcentajes de recuperación ligeramente inferiores al 90%, lo que resultó aceptable teniendo en cuenta la complejidad de la matriz biológica. El LOD y el LOQ fueron coherentes con los valores comunicados anteriormente, lo que confirma la alta sensibilidad. **Conclusiones.** El método HPLC-DAD es fiable, robusto y sensible para monitorizar los niveles de sulfadiazina y sulfadoxina en suero. Aunque los porcentajes de recuperación fueron ligeramente inferiores al 90%, el rendimiento del método fue satisfactorio teniendo en cuenta la matriz biológica. Este método es adecuado para la monitorización terapéutica y puede ayudar a evaluar la eficacia del tratamiento en la toxoplasmosis congénita.

Palabras clave: RP-HPLC-DAD; sulfadoxina; sulfadiazina; toxoplasmosis.

RESUMO

Aplicação da cromatografia líquida de alta resolução com detecção por matriz de díodos (HPLC-DAD) para a determinação de sulfadiazina e sulfadoxina no soro

Introdução. A monitorização dos níveis séricos dos medicamentos em crianças com toxoplasmose congénita é essencial para determinar a eficácia terapêutica. O objetivo deste estudo foi normalizar a quantificação de duas sulfonamidas, a sulfadiazina (SDZ) e a sulfadoxina (SDX), por cromatografia líquida de alta resolução acoplada a um detetor de díodos (HPLC-DAD). Foram avaliados atributos-chave como a linearidade, a precisão, a exatidão, a seletividade, o limite de deteção (LOD) e o limite de quantificação (LOQ), bem como atributos secundários como a estabilidade do fármaco e os efeitos da matriz. **Metodologia.** A quantificação de SDZ e SDX foi efectuada por HPLC-DAD, avaliando: linearidade: foi confirmada uma correlação linear entre o sinal analítico e a concentração do fármaco, precisão: avaliada pelo desvio-padrão relativo (%RSD), exatidão: determinada pelas taxas de recuperação, seletividade: foi assegurada a ausência de interferências significativas da matriz biológica, LOD e LOQ: foi avaliada a sensibilidade do método. **Resultados.** O método demonstrou uma relação linear clara entre a concentração e a resposta instrumental. A precisão situou-se dentro de intervalos aceitáveis para estudos bioanalíticos, com %RSD a indicar resultados consistentes. A exatidão foi satisfatória, com percentagens de recuperação ligeiramente inferiores a 90%, o que foi aceitável tendo em conta a complexidade da matriz biológica. O LOD e o LOQ foram consistentes com os valores previamente comunicados, confirmando a elevada sensibilidade. **Conclusões.** O método HPLC-DAD é fiável, robusto e sensível para monitorizar os níveis séricos de sulfadiazina e sulfadoxina. Embora as taxas de recuperação tenham sido ligeiramente inferiores a 90%, o desempenho do método foi satisfatório, tendo em conta a matriz biológica. Este método é adequado para a monitorização terapêutica e pode ajudar a avaliar a eficácia do tratamento na toxoplasmose congénita.**Palavras-chave:** RP-HPLC-DAD, sulfadoxina, sulfadiazina, toxoplasmose.

Palavras-chave: RP-HPLC-DAD; sulfadoxine; sulfadiazina; toxoplasmose.

1. INTRODUCTION

Congenital toxoplasmosis is an infection caused by the protozoan *Toxoplasma gondii*, which is transmitted from mother to child during pregnancy [1]. In Colombia, this disease represents a significant public health challenge due to its prevalence and the severe complications it causes in the pediatric population [2]. The estimated prevalence in the country is approximately 0.4 per 1,000 children under the age of five, although other sources report values ranging from 2 to 10 cases per 1,000 newborns. These discrepancies can be attributed to regional variations and methodological differences in data collection [3]. The standard treatment consists of a combination of pyrimethamine with a sulfonamide, such as sulfadiazine (SDZ) or sulfadoxine (SDX) [4]. This treatment must be administered for a full year to maintain therapeutic drug levels, preventing the onset of retinochoroiditis lesions and new neurological complications while avoiding adverse effects such as hematological toxicity at high doses [4, 5].

Accurate quantification of SDZ and SDX in biological fluids is essential for therapeutic drug monitoring (TDM). In *T. gondii* infections, rigorous control of plasma antibiotic levels is necessary to ensure treatment efficacy [6]. However, the complexity of biological matrices, due to the presence of various metabolites and proteins, poses challenges for drug analysis. While methods for sulfonamide quantification in plasma have been reported, validated techniques for their determination in serum are scarce. Serum could be particularly useful for retrospective analyses in cases of therapeutic failure. Additionally, there is evidence that elevated concentrations of these drugs can lead to significant adverse effects [7]. Therefore, a validated methodology is crucial for providing reliable quantitative information on these analytes in biological samples.

Drug analysis in biological fluids presents challenges due to matrix complexity, as interfering metabolites and proteins can affect the quantification of target compounds [8]. Although several techniques for determining sulfonamides in plasma have been reported [9-11], no specific methodologies have been described for serum, which has potential applications in retrospective studies of therapeutic failures. Various analytical techniques have been employed for drug quantification in biological matrices, including gas chromatography (GC), high-performance liquid chromatography (HPLC), and spectrophotometry [8]. Among these, HPLC coupled with a diode array detector (HPLC-DAD) [12-15], stands out for its sensitivity, specificity, and ability to simultaneously analyze multiple compounds.

The validation of analytical methods is essential to ensure the reliability and reproducibility of results [14, 15]. This process involves evaluating parameters such as linearity, precision, accuracy, selectivity, limit of detection, and limit of quantification. In this context, the present study aims to standardize and validate an HPLC-DAD-based analytical method for the simultaneous quantification of SDX and SDZ in serum. Implementing this method will enable more precise monitoring of serum drug levels in pediatric patients with congenital toxoplasmosis, ultimately optimizing treatment and minimizing associated risks.

2. METHODOLOGY

2.1. Standards and Reagents

The solvents used in this study included HPLC-grade methanol (J.T. Baker, CAS: 67-56-1); analytical-grade formic acid (98%) (Merck, CAS: 64-18-6); and analytical-grade perchloric acid

(72%) (Merck, CAS: 7601-90-3) for the extraction process. HPLC-grade water was obtained from a Direct-Q system (Millipore).

2.2. Selection of Drugs

Certified standards of the antibiotics sulfadiazine (SDZ, CAS: 68-35-9) and sulfadoxine (SDX, CAS: 2447-57-6) were used, both purchased from Orbus Pharma Ltda. (Bogotá, D.C.), in solid form and with a purity close to 100%. Additionally, a fixed-dose combination of pyrimethamine-sulfadoxine tablets (500/25 mg, PYR-SDX) was analyzed, obtained from the pharmaceutical company BCN Medical (Bogotá). The initial solutions of each antibiotic were prepared from high-purity standards using HPLC-grade methanol as the primary solvent. The concentration was expressed in μg/mL and calculated according to the following equation:

$$Concentration\left(\frac{\mu g\ of antibiotic}{mL\ of solution}\right) = \frac{m \times \%Purity}{V}$$

Where, m is mass of the analyte (antibiotic) in μg, V is final volumetric capacity (L) and **%** *Purity* is purity percentage of the antibiotic.

Since SDZ and SDX exhibit low solubility in methanol and are practically insoluble in water, individual solutions were prepared at lower concentrations than those reported in the original method (100 mg/mL for SDZ). To enhance solubilization, the solutions were sonicated in a Branson-2510 device at a temperature of 36-37 °C for 5 minutes. The stock solutions prepared were: SDZ 624 μg/mL in methanol and SDX 644 μg/mL in methanol. From these, additional dilutions were prepared: SDZ 516 μg/mL, SDX 530 μg/mL, as well as a mixed solution containing both drugs at a concentration of 130 μg/mL.

2.3. Serum and Plasma Samples

Blood samples were collected from healthy volunteers with no diagnosis of toxoplasmosis and no history of treatment with sulfadiazine, sulfadoxine, or pyrimethamine. A total of 10 mL of venous blood was drawn from the forearm into two types of collection tubes: for serum, a tube without anticoagulant (dry tube) was used, while for plasma, a BD Vacutainer® tube containing lithium heparin as an anticoagulant was employed. The sample processing was performed as follows: Serum samples: Blood in the dry tube was left at room temperature (20-25 °C) for 30 minutes to allow complete coagulation. It was then centrifuged at 1,500 g for 10 minutes, and the supernatant (serum) was carefully recovered. Plasma samples: Blood collected in the lithium-heparin tube was centrifuged directly at 1,500 g for 10 minutes. The plasma (upper layer) was then carefully separated using a Pasteur pipette. Both serum and plasma samples were stored at -20 °C until analysis.

2.4. Chromatographic Conditions

The analyses were performed using an HPLC-DAD system (Shimadzu, Japan) equipped with a Prominence SPD-M20A photodiode array UV-VIS detector (operating at 270 nm), a Shimadzu CTO-10AS VP column oven, a Shimadzu SIL-10AF autosampler, and a Prominence DGU-20A5 degasser. A Waters C18 ODS2 column (250 × 4.6 mm, 5 μm) was used for the separation. Data acquisition and analysis were carried out using LC-Solution software (Shimadzu). A reverse-phase high-performance liquid chromatography (RP-HPLC) method was applied based on a previously described protocol (9), with modifications made to optimize the determination of SDX. Several comparative tests were conducted to assess the influence of the biological matrix (serum and plasma) on antibiotic quantification, as well as to compare cali-

bration curves prepared in serum and mobile phase (methanol). The mobile phase was prepared as follows: Mobile phase A: Methanol, water, and concentrated formic acid in a ratio of 50:950:1 (v/v/v). Mobile phase B: Methanol, water, and concentrated formic acid in a ratio of 500:500:1 (v/v/v). The injection volume was set at 20 μL. The injection port temperature was maintained between 25°C and 30°C, with a column flow rate of 0.5 mL/min and a working pressure range of 0.7–24.7 MPa. HPLC filters were from Millex (13 mm) and Advantec (0.22 μm). Standard solutions and analytes were injected in triplicate into the RP-HPLC-DAD system under the established conditions to determine the characteristic analytical signal and retention time for both individual solutions and the mixture. To evaluate the impact of the biological matrix on the analytical response, solutions were prepared in both serum and plasma, including blank matrices (without antibiotics) and spiked samples, ensuring that the detected signals corresponded exclusively to the analyzed drugs.

2.5. Calibration Curves

2.5.1. Linearity and System Variability Testing

To evaluate the linearity, seven calibration standard levels were prepared with concentrations of 120, 50, 30, 10, 5, 1, and 0.5 μg/mL. These standards were obtained by diluting the sulfadiazine (SDZ, 530 μg/mL) and sulfadoxine (SDX, 1070 μg/mL) stock solutions in methanol. The calibration curve was designed to cover a broad concentration range, from very low values to relatively high levels, within the expected range for therapeutic blood concentrations. To assess system variability (including the pump, detector, and column), the concentration range that satisfies Beer's Law was determined—specifically, the optimal range where the analytical signal is proportional to the concentration of the standards. Each standard of both drugs was injected once, and the resulting signal was averaged. To establish the relationship between the average peak area and the concentration of the standards, the following parameters were calculated using the least squares statistical method: slope of the calibration curve, intercept, correlation coefficient (r), and coefficient of determination (r^2).

2.5.2. Linearity and Variability Curve with Serum and Plasma Matrix

A calibration curve was constructed in a serum matrix, with seven concentration levels ranging from 0.2 μg/mL (the lowest concentration standard) to 50 μg/mL, for both sulfadoxine (SDX) and sulfadiazine (SDZ). These standards were analyzed under the same sample preparation conditions. For the serum calibration curve, two stock solutions were prepared: SDZ 624 μg/mL and SDX 644 μg/mL. From these solutions, two additional dilutions were made: 50 μg/mL and 20 μg/mL, respectively. The standard concentrations were diluted in serum from a volunteer to achieve the different calibration levels. Additionally, a calibration curve was prepared in plasma to assess potential variations in the analytical signal when using a different blood matrix.

2.5.3. Calibration Curve PYR-SDX in Serum

For the pyrimethamine-sulfadoxine (PYR-SDX) combination, a solution was prepared with a final concentration of 250 μg/mL, calculated based on pyrimethamine. The linearity of the analytical response was evaluated by preparing seven calibration levels in the region of highest graphical linearity, with the following concentrations in the serum matrix: 50, 30, 5, 2, 1, 0.5, and 0.2 μg/mL. Each standard was injected in duplicate into the HPLC system. Subsequently, the dependence relationship between the average peak area and the concentration of the

standards was determined, applying the same statistical parameters used for the evaluation of system linearity.

2.6. Statistical Analysis

The results were analyzed using the data processing programs Origin Pro 8 SR0 and GraphPad Prism. The relative standard deviation percentage (% RSD) was calculated using the following equation:

$$\%RSD = \left(\frac{Desviación\ estándar}{Área\ promedio}\right) x\ 100$$

To verify the concentration, range within which the linear model is valid, the following evaluations were conducted: a) Homoscedasticity Analysis: Using a residual plot versus concentration. b) Verification of the Linear Model Validity: Through the correlation coefficient. c) Analysis of Variance (ANOVA) of the regression, evaluating: Proportionality, through a t-Student test for the intercept. Slope and regression, comparing t_{exp} with t_{cal} at a 95% confidence level, where $t_{exp} > t_{cal}$ indicates a significant correlation between the chromatographic peak area and the analyte concentration. Snedecor's F-test to evaluate the equality of variances. Additionally, the limits of detection (LOD) and limits of quantification (LOQ) were calculated to ensure the accuracy of the analytical measurements: Limit of Detection (LOD): Determined by multiplying the average area of the blank signal noise by 4. Limit of Quantification (LOQ): Obtained by multiplying the average area of the blank signal noise by 16. From these values, the following definitions were established: LOD: The minimum detectable amount, which indicates the presence of the analyte, but whose quantitative determination is not valid. LOQ: The minimum quantifiable amount, necessary to perform a reliable prediction of the concentration in the sample.

3. RESULTS AND DISCUSSION

3.1. Qualitative analysis

According to the chromatographic results, it was found that the sulfonamides are easily separated by RP-HPLC-DAD under the established conditions for both mobile phases. However, it was not possible to differentiate the peak between sulfadoxine (SDX) and pyrimethamine (PYR) (Figure 1). For this reason, the measurement of PYR was not analyzed in this study.

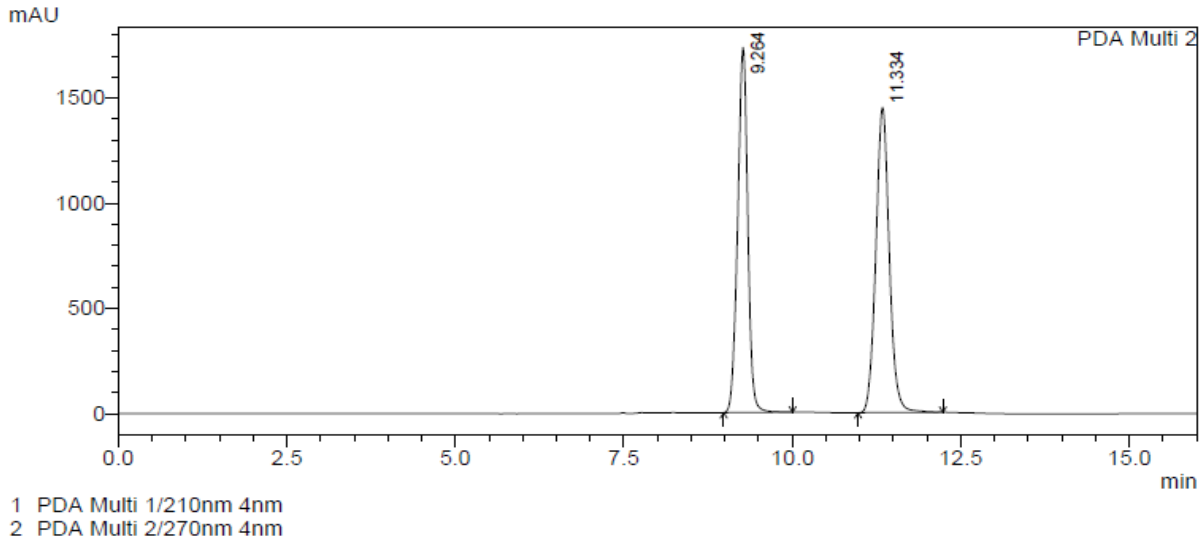


Figure 1. Chromatogram of a volunteer serum sample to which 300 µg/mL SDX, 200 µg/mL SDZ, and 200 µg/mL PYR were added. The peaks corresponding to SDZ and SDX are visible, but PYR is not detected. This likely indicates that the PYR peak elutes at a retention time very close to that of SDX, thus masking it under the current working conditions.

The retention times (RT) of the analytes after mixing were analyzed (**Table 1**). The RT of SDX and SDZ are not close to each other, so there is no overlap or interference between the signals of the two antibiotics. The RT of the fixed combination of PYR-SDX (although it is not possible to differentiate between the PYR and SDX peaks) shows a well-defined analytical signal with a RT similar to that of SDX in the standard solution, confirming the presence of SDX both in the solution and in the commercial tablets of this combination.

Table 1. Comparison of the retention times of the antibiotics in individual solutions and in the mixture.

Standard	Matrix	RT (min) individual	RT (min) in the mixture
Sulfadiazine (SDZ)	Methanol	9.341	9.451
	Serum		9.330
	Plasma		9.418
Sulfadoxine (SDX)	Methanol	11.599	11.658
	Serum		11.528
	Plasma		11.631
Fixed combination Pyrimethamine-Sulfadoxine (PYR-SDX)	Methanol	*11.503	
	Serum	*11.495	
	Plasma	*11.550	

3.2. Variability analysis in methanol matrix

Several calibration curves were prepared in duplicate, and the obtained peak areas were used to calculate the mean, standard deviation, and relative standard deviation percentage (%RSD), as recorded in **Table 2** and **Table 3** for the methanol matrix.

Table 2. Peak areas obtained for sulfadiazine standard in the system linearity assessment using a methanol matrix.

Sulfadiazine (SDZ) - System Linearity						
Level	**[ ] (µg/mL)**	**Area-1**	**Area-2**	**Media**	**SD**	**%RSD**
1	**0.5**	73364	52360	62862	14852.07	19.88
2	**1**	116523	123949	120236	5250.97	3.62
3	**5**	649031	316234	482632,5	235323.02	48.96
4	**10**	1467661	2520778	1994219,5	744666.17	38.84
5	**30**	4429106	4079225	4254165,5	247403.23	6.45
6	**50**	10114695	6872123	8493409	2292844.65	29.48
7	**120**	17909623	17261007	17585315	458640.77	2.78

Table 3. Peak areas obtained for sulfadoxine standard in the system linearity assessment using a methanol matrix.

Sulfadoxine (SDX) - System linearity						
Level	**[ ] (µg/mL)**	**Area-1**	**Area-2**	**Media**	**SD**	**%RSD**
1	**0.5**	86853	62540	74696,5	17191.89	23.02
2	**1**	129977	160046	145011,5	21261.99	14.67
3	**5**	579577	381794	480685,5	139853.70	29.09
4	**10**	1334516	2500208	1917362	824268.72	42.99
5	**30**	3819439	3855412	3837425,5	25436.75	0.66
6	**50**	8956182	6596526	7776354	25436.75	0.32
7	**120**	16772189	16266813	16519501	357354.79	2.16

3.3. Variability analysis in serum matrix

Several calibration curves were prepared in duplicate, and the obtained peak areas were used to calculate the mean, standard deviation, and relative standard deviation percentage (%RSD), as recorded in **Table 3** and **Table 4** for the calibration curve in the serum matrix.

Table 3. Peak areas obtained for the sulfadiazine standard in calibration curves using a serum matrix.

Sulfadiazine (SDZ)						
Level	**[ ] (µg/mL)**	**Area-1**	**Area-2**	**Media**	**SD**	**%RSD**
1	0.2	85930	55656	70793	21406.95	30.24
2	0.5	105626	97568	101597	5697.87	5.61
3	1	247518	248254	247886	520.43	0.21
4	2	464886	461915	463400.5	2100.81	0.45
5	5	1270344	1271269	1270806.5	654.07	0.05
6	30	7572169	7578400	7575284.5	4405.98	0.06
7	50	13182226	13115713	13148969.5	47031.79	0.36

Table 4. Peak areas obtained for the sulfadoxine standard in calibration curves using a serum matrix.

Sulfadoxine (SDX)						
Level	**[ ] (µg/mL)**	**Area-1**	**Area-2**	**Media**	**SD**	**%RSD**
1	0.2	182999	89186	136092.8	66335.81	48.74
2	0.5	131734	140722	136227.9	6355.48	4.67
3	1	236565	237114	236839.5	388.20	0.16
4	2	451158	423493	437325.4	19562.11	4.47
5	5	913446	914157	913801.6	502.75	0.06
6	30	6601460	6615340	6608400.1	9814.64	0.15
7	50	11562666	11528196	11545430.6	24373.97	0.21

3.4. Method linearity

The RP-HPLC-DAD methodology implemented for the detection and quantification of sulfadiazine (SDZ) and sulfadoxine (SDX) proved to be appropriate under the established chromatographic conditions. A strong linear response was observed between the analytical signal in methanol (**Figure 2A** and **Figure 2B**) and the drug concentration in the serum matrix (**Figure 2C** and **Figure 2D**).

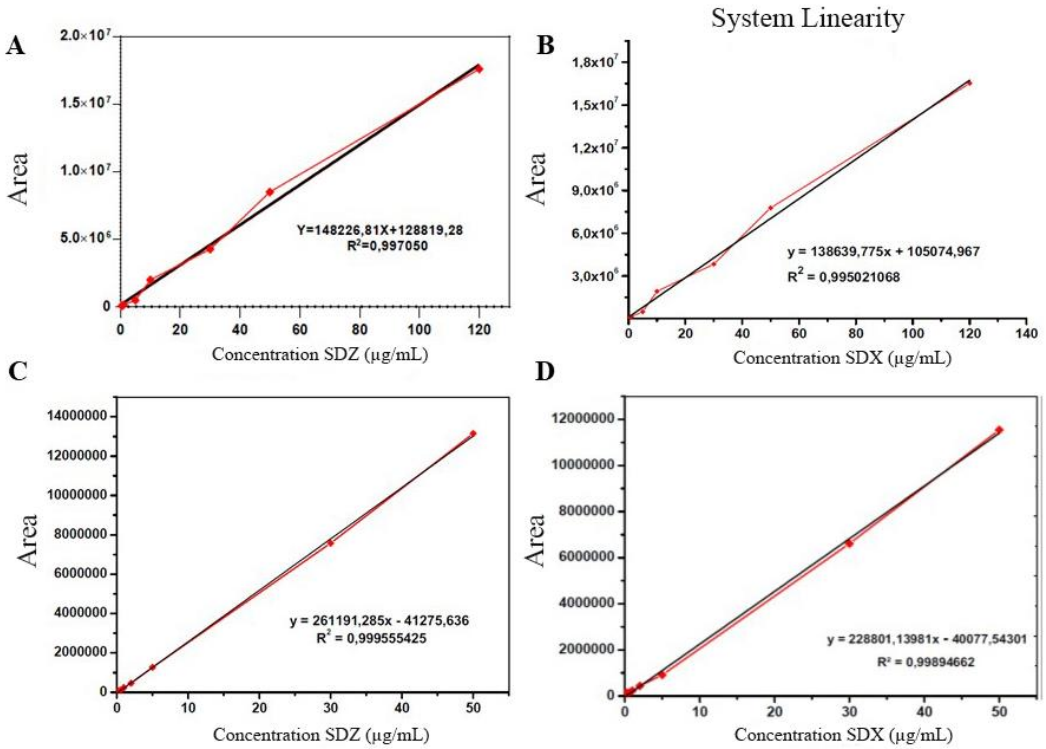


Figure 2. A. Calibration curve for sulfadiazine in methanol matrix. **B.** Calibration curve for sulfadoxine in methanol matrix. **C.** Calibration curve for sulfadiazine in serum matrix. **D.** Calibration curve for sulfadoxine in serum matrix.

For both matrices and both drugs (SDZ and SDX), correlation coefficients exceeded 0.99. The graphical analysis provided a visual evaluation of the lower limits established for the bioanalysis of the standard calibration curves, ensuring the method's linearity in the serum matrix.

Some data points in the system linearity curve (methanol matrix) presented slightly higher values. **Table 5** presents the calculated confidence limits for the correlation coefficients.

Table 5. Correlation coefficients, regression equations, and confidence limits for the studied drugs.

Drug	Correlation coefficient (r)	Confidence limits		Equation of the line $y = a + bx$
		Pending (b)	Ordered at the origin (a)	
Sulfadiazine (serum)	0.997	148226.81±3294.2	128819.28±117877.5	Y=128819.2+148226.8X
Sulfadiazine (methanol)	0.999	261191.29±6332.4	-41275.64±140180.2	Y = -41275.6+261191.2X
Sulfadoxine (serum)	0.997	138639.77±4385.8	105075.3±221947.5	Y = 105075.3+138639.7X
Sulfadoxine (methanol)	0.999	228801.14±8541.2	-40077.5±189076.6	Y = -40077.5+228801.1X

Additionally, **Table 6** summarizes the statistical significance test results performed on each calibration curve.

Table 6. Student's t-test results for the studied drugs and the method's linearity assessment.

Drug	r	r^2	t_{exp}	t_{tab}	H_o	Linear correlation
Sulfadiazine (serum)	0.997050	0.9941080	44.99626	2.57	≠ 0	Significant
Sulfadiazine (methanol)	0.999778	0.9995560	106.02686	2.57	≠ 0	Significant
Sulfadoxine (serum)	0.997507	0.9950211	48.97097	2.57	≠ 0	Significant
Sulfadoxine (methanol)	0.999473	0.9989462	68.859576	2.57	≠ 0	Significant

The statistical Student's t-test was performed for each drug using a 95% confidence level (α = 0.05) and considering seven concentration levels in both the method and system calibration curves. With n = 5 degrees of freedom, the tabulated t-values from the t-distribution table were 2.57 for all calibration curves. Based on the results presented in **Table 6**, a linear correlation was confirmed between the x and y variables for all analyzed calibration curves, both for the system and the method.

As a complementary analysis, homoscedasticity of the residuals was assessed to further verify the linear relationship between the variables in each calibration curve. The adjusted y-values were calculated from the selected regression equation, and residuals were determined as the difference between the adjusted y-value and the analytical signal (peak area). A Residuals vs. Concentration plot was then generated for the methanol calibration curves (**Table S1** and **Table S2**) and serum calibration curves (**Table S3** and **Table S4**). The homoscedasticity analysis confirmed that, for all studied drugs and both the system and method, the distribution of data points in the residual plots was random, without a discernible linear trend. Furthermore, the absence of a pattern in the sign of the residuals (±) supports the validity of the initially established linear model.

3.5. Precision

To estimate precision, an analysis of both instrumental repeatability and overall method reproducibility across the calibration range was conducted. Instrumental repeatability was assessed at three concentration levels by measuring two peak areas for both analytes (**Table 7**).

Table 7. Peak areas obtained at three concentration levels for sulfadiazine and sulfadoxine standards (instrumental repeatability).

Sulfadiazine (SDZ)						
Level	**[ ] μg/mL**	**Area 1**	**Area 2**	**Media**	**SD***	**%RSD****
2	0.5	105626	97568	101597	5697.87	5.61
4	2	464886	461915	463400.5	2100.81	0.45
7	50	13182226	13115713	13148969.5	47031.79	0.36
Sulfadiazine (SDX)						
Level	**[ ] μg/mL**	**Area 1**	**Area 2**	**Media**	**SD***	**%RSD****
2	0.5	131734	140722	136227.9	6355.48	4.67
4	2	451158	423493	437325.4	19562.11	4.47
7	50	11562666	11528196	11545430.6	24373.97	0.21

For SDZ and SDX, a greater dispersion of results was observed at the lowest concentration level. This is likely due to an increase in instrumental noise as the analyte concentration decreases, making it more challenging to fully resolve the chromatographic peaks. To evaluate method repeatability, recovery percentages (%R) were determined using a spiking approach with five concentration levels of standard solutions, analyzed on a single day under identical instrumental conditions in the serum matrix (**Table S5**).

For reproducibility assessment, Table S6 presents peak areas obtained over two different analysis days using real samples treated with Falcidar (PYR-SDX), focusing specifically on sulfadoxine. The samples were analyzed at unique concentration levels for each sample but on different days, covering a range from low to high along the calibration curve. The analyses were conducted 113 days apart, with a corrected injection volume of 20 μL. The acceptance criterion for the relative standard deviation (RSD) depends on the assay's purpose and the complexity of the biological matrix under study. For antibiotic analysis in serum, RSD values up to 15% are generally acceptable. However, for concentrations near the limit of quantification, RSD values of up to 20% may be considered acceptable.

3.6. Selectivity

A wavelength analysis was conducted to determine the optimal conditions for achieving the highest resolution and chromatographic peak distinction. It was found that at lower concentrations, better peak differentiation was obtained at 270 nm (**Figure 3**).

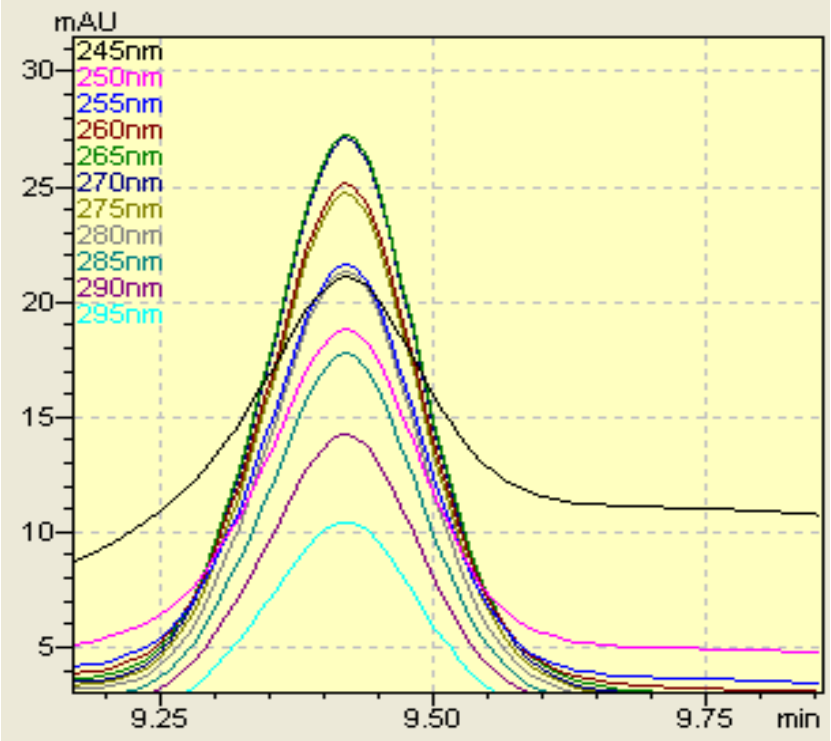


Figure 3. Chromatographic peaks obtained at different wavelengths (245–295 nm) for SDZ in a serum matrix using HPLC-DAD.

3.7. Identity confirmation: Selectivity/Specificity

To ensure that the chromatographic response was solely attributed to the analyzed compounds, all components involved in the sample processing and quantification were tested both together and separately. This included serum and plasma samples (negative controls) without the presence of the drugs (**Figure 4A** and **Figure 4B**). No chromatographic peaks were observed at the retention times of the target analytes, confirming the absence of interference in the method's measurements. In the chromatograms presented, the retention time (t_e) scale was maintained to allow direct comparisons between the observed drug signals.

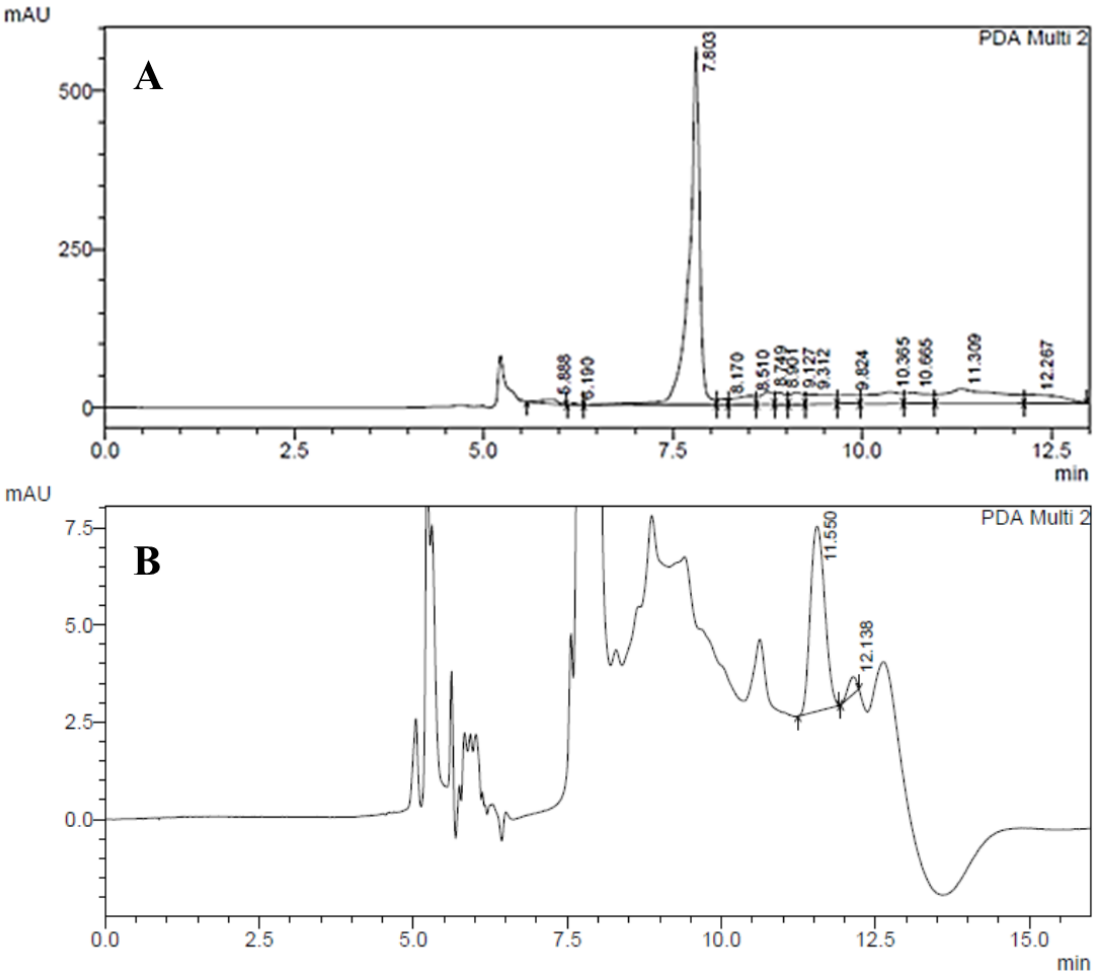


Figure 4. A. Chromatogram of undoped serum matrix. **B.** Chromatogram of undoped plasma matrix.

It is worth noting that during plasma preparation, a metabolite with a distinct peak at approximately 7.8 min may have been removed, leading to the observed chromatogram. However, this peak was not significant due to its signal intensity being comparable to the instrument's baseline noise. In the analyses of undoped matrices, no quantifiable signals were detected except for the peak at 7.8 min, which exhibited high resolution and chromatographic intensity. Nevertheless, this peak did not interfere with the drug signals, which were observed at approximately 9.4 min and 11.6 min for SDZ and SDX, respectively. To further investigate potential matrix effects, SDZ and SDX were spiked into serum (**Figure 5A**) and plasma (**Figure 5B**).

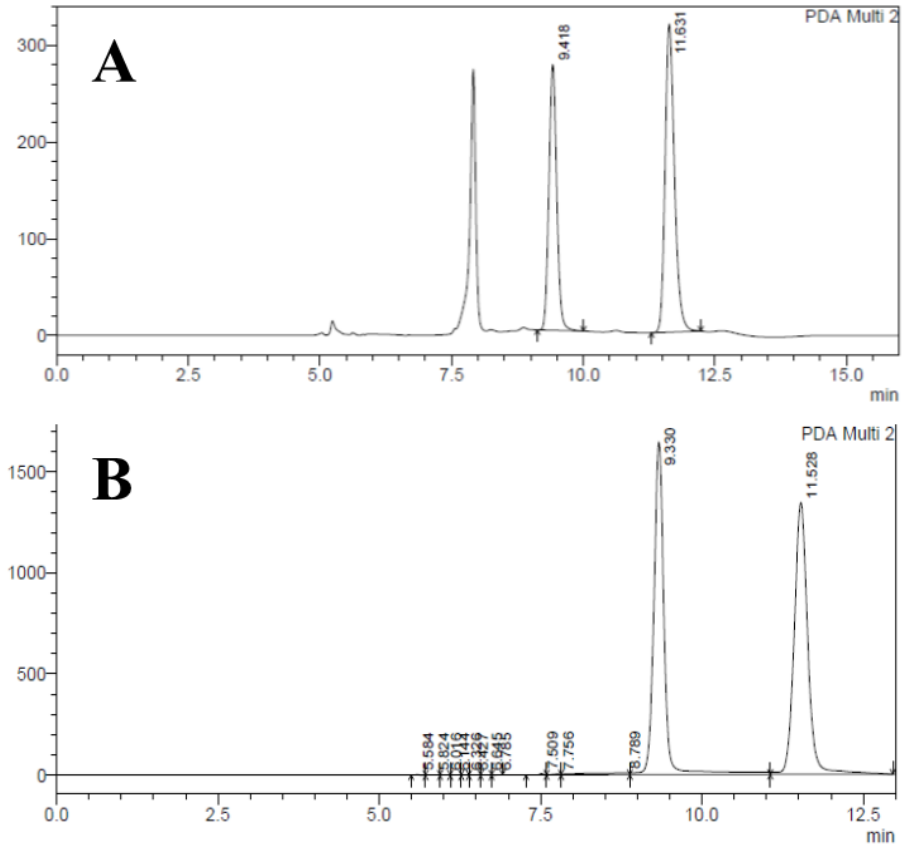


Figure 5. A. SDZ-SDX mixture in serum (130 μg/mL). **B.** SDZ-SDX mixture in plasma (50 μg/mL).

3.8. Accuracy

The statistical determination of accuracy was performed for five concentration levels within the calibration curve range for both antibiotics. A calibration curve was constructed to analyze the proportionality of recovered amounts at each level relative to the others, evaluate the recovery percentage (%R), determine the *t*-experimental value, and compare it to the *t*-tabulated value. A 95% confidence level ($p = 0.05$) was applied, considering five measurements without replicates, and the *F*-experimental value was calculated against the *F*-tabulated value (**Table S7** and **Table S8**).

This statistical procedure serves as an approximation for assessing method accuracy, as no replicates were performed for individual recovery percentage measurements. However, a proportional linear trend was observed in the amount of analyte recovered in each determination. This trend was confirmed by statistical estimators, including the *t*-Student and *F*-Snedecor tests. For both antibiotics, the *t*-Student test showed that a calculated *t* value greater than the tabulated *t* indicates method accuracy at a significance level of $\alpha = 0.05$. This confirms the presence of a statistically significant nonzero slope in the calibration curve.

3.9. Sensitivity

The analytical sensitivity was determined using the signal-to-noise ratio method, employing the instrumental signal provided by the blank sample. In this study, the blank corresponds to serum samples from individuals who have not received the treatment; therefore, no signal should be detected at the specific retention times for SDZ and SDX unless caused by an endogenous compound acting as an interfering signal. This possibility could be explained by the

broad range of the calibration curve. The estimated values for both parameters—Limit of Detection (LD) and Limit of Quantification (LQ)—are summarized in **Table 8**.

Table 8. Limits of detection and quantification for SDZ and SDX.

Drug	Average Area	Average Area	L.D (μg/mL)	L.Q (μg/mL)
Sulfadiazine	0.28409	1.13636	1.35883	4.52945
Sulfadoxine	0.32351	1.29420	2.09227	6.97424

4. DISCUSSION

Based on the obtained data and considering certain experimental limitations, the method was thoroughly evaluated in a biological matrix (blood serum), assessing key quality attributes such as linearity, precision, accuracy, selectivity, and sensitivity. The linearity assessment confirmed a direct correlation between the instrumental analytical signal and the concentration of the studied drugs within the established calibration range and under the applied chromatographic conditions. This clearly indicates that the results are directly proportional to the drug concentrations. Regarding precision, both instrumental and method precision were evaluated, yielding %RSD values within the acceptable ranges for bioanalytical studies. This suggests that the data are closely clustered around the mean value.

Although the recovery percentage was below 90%, considering the nature of the biological matrix, sample preparation and purification steps, dilutions, the actual analyte concentration in the original blood samples, and the analytical signal response, the method's performance is deemed satisfactory. Specifically, the average recovery was 77% for SDZ and 86% for SDX, with a %RSD of approximately 11.2%, which is below the 15% threshold established in the FDA guidelines for bioanalytical method validation in human studies. Based on this information, the analytical method can be considered reliable and sensitive for determining and monitoring SDZ and SDX levels within therapeutic concentrations.

In terms of reproducibility, the %RSD values were higher than those observed in repeatability studies but remained within the acceptance limit. Instrumental repeatability analysis showed %RSD values below 6%, indicating minimal dispersion in the peak areas obtained across different concentration levels in the calibration curves. This suggests a high level of system precision under the specified analytical conditions. The %RSD values for method repeatability were below 15%, although some were higher than those observed for instrumental repeatability. This discrepancy arises because method repeatability accounts for not only the instrument's precision but also all procedural steps involved in sample extraction and purification. The more steps involved, the greater the sample manipulation, leading to increased variability in the results due to potential analyte loss during the method's application. For reproducibility, variations in analysis time could increase %RSD due to potential fluctuations in the instrument's analytical response or signal stability from day to day, leading to data dispersion. However, all results remained below 15% RSD, demonstrating good reproducibility.

Precision is inherently associated with random errors in the determination process, which cause individual results to deviate from the mean value in an uncontrollable manner. Factors such as matrix complexity, analyte concentration, dilution steps, sample preparation, extraction procedures, instrument operating conditions, and analysis time can contribute to variability in the results. Nevertheless, all obtained values fall within the acceptance criteria for antibiotic analysis, indicating minimal variability among results and, therefore, good preci-

sion. Under the specified chromatographic conditions, the method exhibited adequate selectivity, as it allowed for the accurate and specific determination of the studied drugs without significant interference from the biological matrix components. Given the complexity of the serum matrix, interactions between the analytes and reagents used in sample preparation and purification likely played a role in the observed recovery rates. The presence of blood components such as lipids, salts, and hormones can compete with the target analytes for active sites in the stationary phase, reducing available binding sites and leading to analyte loss and lower instrumental responses.

From a clinical perspective, therapeutic drug levels could be considered an acceptance criterion, particularly at concentrations where a positive treatment response is expected. This is supported by Trenque *et al.*, who reported plasma SDX concentrations of 46.1 μg/mL within a broad range, although under different chromatographic conditions [11] (11). Another study reported a median of 42.39 μg/mL for malaria treatment. Given that malaria is also caused by a parasite (*Plasmodium falciparum*), a comparable therapeutic response might be inferred for toxoplasmosis, suggesting that the determined LD and LQ values are appropriate for the study's objectives. In conclusion, the method was successfully standardized and validated for the determination of sulfadiazine and sulfadoxine in serum, demonstrating its suitability for monitoring antibiotic levels in clinical samples.

5. CONCLUSIONS

Based on the obtained data, and considering some experimental limitations, as well as the particular characteristics of the biological matrix (blood serum), the relevant quality attributes of the methodology were thoroughly assessed, including linearity, precision, accuracy, selectivity, and sensitivity. The linearity assessment confirmed a correlation between the instrumental analytical signal and the concentration of the studied drugs within the established calibration range, under the applied chromatographic conditions. This clearly indicates that the results are directly proportional to the concentration of the drugs used.

Regarding precision, both instrumental and method precision were evaluated, with %RSD values within the acceptance range for bioanalytical studies, indicating that the data are closely clustered around the mean value. In the reproducibility study, the %RSD showed values higher than those observed in repeatability but remained below the acceptance limit. Although the recovery percentage was below 90%, considering the biological matrix type, sample preparation and purification processes, dilutions, the actual analyte concentration in the original blood samples, and the analytical signal generated by the analytes under the established chromatographic conditions, it can be concluded that the method developed in this research demonstrates satisfactory performance. The method shows reliability and sensitivity for determining and studying the levels of SDX and SDZ in therapeutic concentrations for treatment.

ACKNOWLEDMENTS

The authors would like to express their gratitude to the Master's program in Chemistry and Biomedicine at the University of Quindío. Additionally, they would like to thank the Chromatography and Related Techniques Research Group (GICTA) at the University of Caldas.

AUTHORS CONTRIBUTIONS

CRP: Development of the experimental part, manuscript draft preparation. **JPBA:** Proofreading, document translation, and validation. **JEGM:** Data evaluation, statistical application, and information confirmation. **GTO:** Project supervisor and manuscript evaluation.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

SUPPLEMENTARY INFORMATION

Table S1. Residual analysis (homoscedasticity) for sulfadoxine (methanol).

X	Y	Y original	Y adjusted
[ ] (µg/mL)	Residue	Area	Area
0.5	59830.68	74697	10962.62
1	12277.09	145012	89320.01
5	27970.25	480686	219915.65
10	-17706.43	1917362	481106.93
30	6125.61	3837426	1264680.79
50	-219178.51	7776354	7794462.91
120	130681.30	16519501	13018288.60

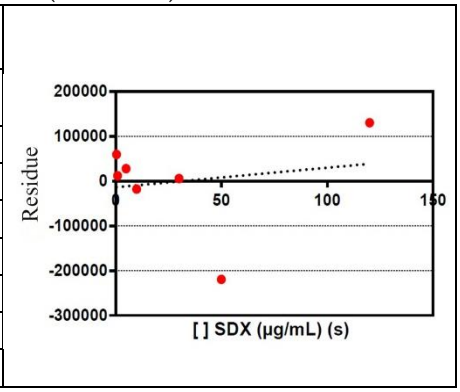


Table S2. Residual analysis (homoscedasticity) for sulfadoxine (serum).

X	Y	Y original	Y adjusted
[ ] (µg/mL)	Residue	Area	Area
0.2	130410.12	136092.8	5682.7
0.5	61904.87	136227.9	74323.0
1	48115.90	236839.5	188723.6
2	19800.66	437325.4	417524.7
5	-190126.6	913801.6	1103928.2
30	-215556.7	6608400.1	6823956.7
50	145451.6	11545431	11399979.4

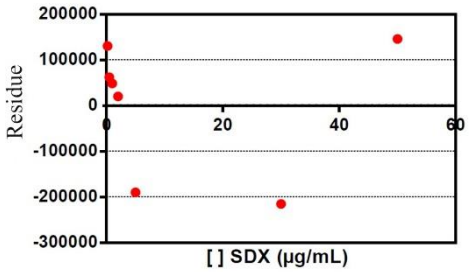


Table S3. Residual analysis (homoscedasticity) for sulfadiazine (methanol).

X	Y	Y original	Y adjusted
[ ] (µg/mL)	Residue	Area	Area
0.2	59830.68	70793.3	10962.62
0.5	12277.09	101597.1	89320.01
1	27970.25	247885.9	219915.65
2	-17706.43	463400.5	481106.93
5	6125.61	1270806.4	1264680.79
30	-219178.51	7575284.4	7794462.91
50	130681.30	13148969.9	13018288.60

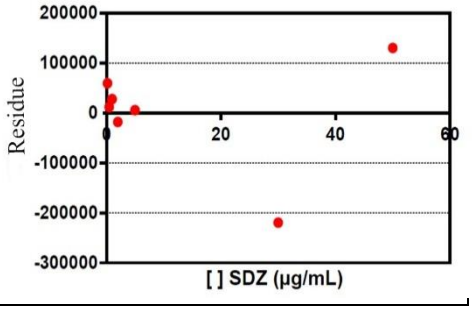


Table S4. Residual analysis (homoscedasticity) for sulfadiazine (serum).

X	Y	Y original	Y adjusted
[ ] (µg/mL)	Residue	Area	Area
0.5	-140070.69	62862	202932.69
1	-156810.10	120236	277046.10
5	-387320.84	482632.5	869953.34
10	383132.11	1994219.5	1611087.39
30	-321458.10	4254165.5	4575623.60
50	953249.18	8493409	7540159.82
120	-330721.56	17585315	17916036.56

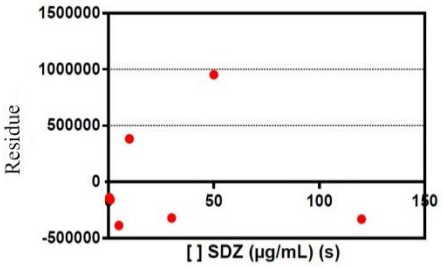


Table S5. Method repeatability after doping in matrix.

Drug	Serum		
	Media	SD*	%RSD**
Sulfadiazine	77.126	8.633309	11.19
Sulfadoxine	86.706	7.303717	8.423

*SD: Standard deviation

** %RSD: percentage relative standard deviation

Table S6. Areas obtained for the drug sulfadoxine, on two different days to assess reproducibility.

[ ] µg/L Calculated	Drug	Day 1	Day 2	%RSD	RT
		Area 1	Area 2		
12.253	M1-SDX	2750704	2763386	0.325	11.4540
18.965	M2-SDX	4263388.4	4299246	0.597	11.4235
30.655	M3-SDX	8195176.8	6973772	11.397	11.4325
33.925	M4-SDX	7460823.2	7721949	2.432	11.4340
39.862	M5-SDX	8902518.8	9080447	1.399	11.4520
42.777	M6-SDX	10667234.8	9747345	6.372	11.4295

Table S7. Recovery percentages obtained for sulfadoxine in the study for accuracy evaluation.

Sulfadoxine (SDX)		
[ ] µg/mL added	**[ ] µg/mL retrieved**	**(%R)**
2	1.940	97
5	3.817	76.36
10	8.620	86.23
20	17.404	87.02
50	44.695	86.92
	$\%\overline{R}$	86.71
	SD	7.303717
	%RSD	8.423
	t exp.	94.64213
	t tab.	3.18245
	F. exp.	8957.1329
	F. tab.	10.13

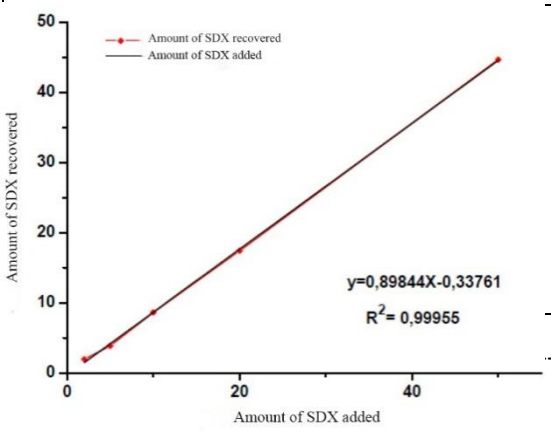
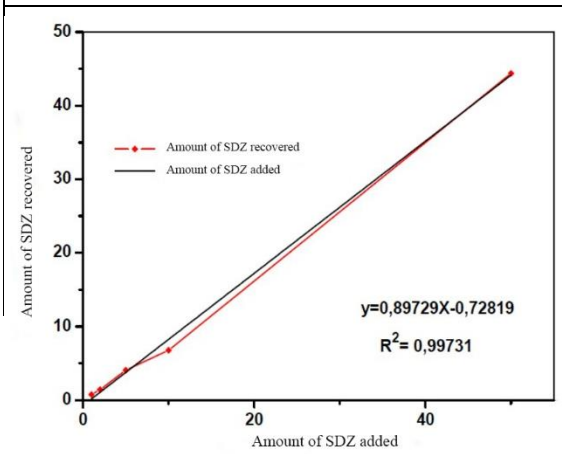


Table S8. Recovery percentages obtained for sulfadiazine in the study for accuracy evaluation.

Sulfadiazine (SDZ)		
[ ] µg/mL added	**[ ] µg/mL retrieved**	**(%R)**
1	0.759	75.9
2	1.411	70.57
5	4.055	81.09
10	6.775	67.75
50	44.375	88.75
	$\%\overline{R}$	77.126
	SD	8.628264
	%RSD	11.19
	t exp.	38.54768684
	t tab.	3.182
	F. exp.	1485.924
	F. tab.	10.13

REFERENCES

1. J. Espinoza-Rojas, E. López-Mora, J. Dabanch-Peña & R. Cruz-Choappa. Recomendaciones para el diagnóstico y tratamiento de la infección por *Toxoplasma gondii*. *Revista Chilena de Infectología*, **39**(2), 132–137 (2022). https://doi.org/10.4067/s0716-10182022000200132
2. J.E. Gómez-Marin, A. de-la-Torre, E. Angel-Muller, J. Rubio, J. Arenas, E. Osorio, *et al.* First Colombian multicentric newborn screening for congenital toxoplasmosis. *PLoS Neglected Tropical Diseases*, **5**(5), e1195 (2011). https://doi.org/10.1371/journal.pntd.0001195
3. G. Pedraza-López, P.A. Otálora-Vargas & L.A. Vargas-Torres. Toxoplasmosis congénita en primera infancia y toxoplasmosis en mujeres en edad reproductiva en Colombia. *Salud y Sociedad UPTC*, **7**(2), 21–35 (2021). https://doi.org/10.19053/uptc.2744953x.15515
4. R. McLeod, K. Boyer, T. Karrison, K. Kasza, C. Swisher, N. Roizen, *et al.* Outcome of treatment for congenital toxoplasmosis, 1981-2004: the national collaborative Chicago-based, congenital toxoplasmosis study. *Clinical Infection Diseases*, **42**(10), 1383–1394 (2006). https://doi.org/10.1086/501360
5. R. McLeod, F. Kieffer, M. Sautter, T. Hosten & H. Pelloux. Why prevent, diagnose and treat congenital toxoplasmosis? *Memórias do Instituto Oswaldo Cruz* (Rio de Janeiro), **104**(2), 320–344 (2009). https://doi.org/10.1590/s0074-02762009000200029
6. J.J. Berzas-Nevado, G. Castañeda-Peñalvo & F.J. Guzmán-Bernardo FJ. Simultaneous determination of sulfaquinoxaline, sulfamethazine and pyrimethamine by liquid chromatography. *Journal of Chromatography A*, **870**(1–2), 169–177 (2000). https://doi.org/10.1016/s0021-9673(99)00880-8
7. D.R. Schmidt, B. Hogh, O. Andersen, S.H. Hansen, K. Dalhoff & E. Petersen. Treatment of infants with congenital toxoplasmosis: tolerability and plasma concentrations of sulfadiazine and pyrimethamine. *European Journal of Pediatrics*, **165**(1), 19–25 (2006). https://doi.org/10.1007/s00431-005-1665-4

8. FDA. *Bioanalytical Method Validation: Guidance for Industry*. Office of Communications, Division of Drug Information Center for Drug Evaluation and Research, Food and Drug Administration. Silver Spring, (MD), 2018; 44 p. URL: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf
9. J.K. Johannessen, I. Christiansen, D.R. Schmidt, E. Petersen & S.H. Hansen. Simultaneous determination of pyrimethamine, sulfadiazine and *N*-acetyl-sulfadiazine in plasma for monitoring infants in treatment of congenital toxoplasmosis. *Journal of Pharmaceutical and Biomedical Analysis*, **36**(5), 1093–1098 (2005). https://doi.org/10.1016/j.jpba.2004.09.001
10. S. Corvaisier, B. Charpiat, C. Mounier, M. Wallon, G. Leboucher, M. Al-Kurdi, J.-F. Chaulet & F. Peyron. Population pharmacokinetics of pyrimethamine and sulfadoxine in children treated for congenital toxoplasmosis. *Antimicrobial Agents and Chemotherapy*, **48**(10), 3794–3800 (2004). https://doi.org/10.1128/aac.48.10.3794-3800.2004
11. T. Trenque, N. Simon, I. Villena, C. Chemla, C. Quereux, B. Leroux, *et al.* Population pharmacokinetics of pyrimethamine and sulfadoxine in children with congenital toxoplasmosis. *British Journal of Clinical Pharmacology*, **57**(6), 735–741 (2004). https://doi.org/10.1111/j.1365-2125.2004.02077.x
12. M.M. Ghanem & S.A. Abu-Lafi. Development and validation of a stability-indicating HPLC method for the simultaneous determination of sulfadiazine sodium and Trimethoprim in injectable solution formulation. *Scientia Pharmaceutica*, **81**(1), 167–182 (2013). https://doi.org/10.3797/scipharm.1210-12
13. J.Q. Sun, D.S. Sun & H. Chen. Simultaneous determination of sulfadiazine and sulfamethoxazole in synthetic samples and pharmaceutical tablets using HPLC-DAD combined with second-order calibration method. *Advanced Materials Research*, **554–556**, 1781–1784 (2012). https://doi.org/10.4028/www.scientific.net/amr.554-556.1781
14. I. García, M.C. Ortiz, L. Sarabia & J.M. Aldama. Validation of an analytical method to determine sulfamides in kidney by HPLC-DAD and PARAFAC2 with first-order derivative chromatograms. *Analytica Chimica Acta*, **587**(2), 222–234 (2007). https://doi.org/10.1016/j.aca.2007.01.054
15. K. Madej, Ł. Paprotny, D. Wianowska, J. Kasprzyk, M. Herman & W. Piekoszewski. A fully validated HPLC–UV method for determination of sulthiame in human serum/plasma samples. *Biomedical Chromatography*, **35**(3), e5002 (2021). https://doi.org/10.1002/bmc.5002

HOW TO CITE THIS ARTICLE

C. Rose-Parra, J.P. Betancourt-Arango, J.E. Gómez-Marín & G. Taborda-Ocampo. Application of high-performance liquid chromatography with diode-array detection (HPLC-DAD) for the determination of sulfadiazine and sulfadoxine in serum. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 68–87 (2026). https://doi.org/10.15446/rcciquifa.v55n1.119599

Clinical research article

Antibiotic pharmacotherapy of pleurisy, pleural empyema, infections of various genesis: ABC and VED analysis

Viktoriia Shapovalova[1a]*, Oleksandr Nevzghoda[2b], Valentyn Shapovalov[1c], Alina Osyntseva[3d], Olena Lavoshnyk[4e] & Valerii Shapovalov[3f]

[1] Private Scientific Institution "Scientific and Research University of Medical and Pharmaceutical Law", Kyiv, Ukraine
[2] Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
[3] Lviv Medical University, Lviv, Ukraine
[4] State Enterprise "Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines", Kharkiv, Ukraine

E-mail addresses:
[a] v.shapovalova@srumpl.org; [b] sashko.nev0703@gmail.com;
[c] office@srumpl.org; [d] osincevaalina6@gmail.com

ORCID IDs:
[a] https://orcid.org/0000-0003-4770-7292; [b] https://orcid.org/0009-0001-2777-7333;
[c] https://orcid.org/0000-0002-9329-0195; [d] https://orcid.org/0000-0003-1655-318X;
[e] https://orcid.org/0009-0000-0023-434X; [f] https://orcid.org/0000-0002-6696-6380

*Corresponding author: Viktoriia Shapovalova

Received: August 14, 2025
Corrected: September 20, 2025
Accepted: September 23, 2025

https://doi.org/10.15446/rcciquifa.v55n1.125038

SUMMARY

Introduction: Antibiotic resistance remains one of the greatest challenges in global health, which necessitates a systemic approach to the rational use of antimicrobial medicines, in particular in patients with infections of various genesis, pleurisy, pleural empyema, tuberculosis and chronic infections. In Latin American countries, in particular in Colombia, the problem of uncontrolled antibiotic use is combined with socio-economic factors that affect access to effective pharmacotherapy, especially in patients with pleurisy, pleural empyema, tuberculosis and chronic infections. **Objective:** To conduct an analysis of antimicrobial medicines registered in Colombia using ABC and VED analysis, to identify priority active substances for further planning of procurement, consumption control strategies, and prescription in the treatment of pleurisy, pleural empyema, tuberculosis and chronic infections. **Methodology:** The study was based on the analysis of 20 antibacterial drugs registered in Colombia, using international databases, price catalogs, and WHO guidelines. An ABC analysis was performed by cost level, ranking by criticality criterion (VED), and the share of each drug in total costs was calculated. Additionally, literature sources on antimicrobial resistance. **Results:** The highest share in costs (category A) was made by Ceftriaxone, Amoxicillin/clavulanic acid, and Azithromycin. Within category B, Ciprofloxacin, Doxycycline, and Cefalexin were identified. Category C drugs had a small share of costs, but a high frequency of use. The relationship between price indicators and prevalence of use indicates the need to optimize procurement policies. **Discussion:** The data obtained emphasize the need to implement antimicrobial stewardship strategies and national monitoring. **Conclusions:** The results of the study have practical

value for health policy, antimicrobial control and the formation of national protocols for the treatment of pleurisy, pleural empyema, tuberculosis and chronic infections. ABC/VED analysis allows to identify critical points in the costs of antibiotics, outlines the prospects for the development of individualized pharmacotherapy in the context of increasing antibiotic resistance.

Keywords: pharmacotherapy; antimicrobial medicines; pleurisy; pleural empyema; tuberculosis; chronic infections; ABC analysis; VED analysis; infections.

RESUMEN

Farmacoterapia antibiótica de la pleuresía, el empiema pleural y las infecciones de diversa etiología: análisis ABC y VED

Introducción: La resistencia a los antibióticos sigue siendo uno de los mayores desafíos para la salud mundial, lo que exige un enfoque sistémico para el uso racional de los medicamentos antimicrobianos, en particular en pacientes con infecciones de diversa etiología, pleuresía, empiema pleural, tuberculosis e infecciones crónicas. En los países latinoamericanos, especialmente en Colombia, el problema del uso incontrolado de antibióticos se combina con factores socioeconómicos que afectan el acceso a una farmacoterapia eficaz, sobre todo en pacientes con pleuresía, empiema pleural, tuberculosis e infecciones crónicas. **Objetivo:** Realizar un análisis de los medicamentos antimicrobianos registrados en Colombia mediante el análisis ABC y VED, para identificar los principios activos prioritarios para la planificación de la adquisición, las estrategias de control del consumo y la prescripción en el tratamiento de la pleuresía, el empiema pleural, la tuberculosis y las infecciones crónicas. **Metodología:** El estudio se basó en el análisis de 20 medicamentos antibacterianos registrados en Colombia, utilizando bases de datos internacionales, catálogos de precios y las guías de la OMS. Se realizó un análisis ABC por nivel de costo, clasificación según el criterio de criticidad (VED) y se calculó la proporción de cada fármaco en el costo total. Adicionalmente, se consultaron fuentes bibliográficas sobre resistencia antimicrobiana. **Resultados:** La mayor proporción en los costos (categoría A) correspondió a ceftriaxona, amoxicilina/ácido clavulánico y azitromicina. Dentro de la categoría B, se identificaron ciprofloxacino, doxiciclina y cefalexina. Los fármacos de categoría C representaron una pequeña proporción de los costos, pero una alta frecuencia de uso. La relación entre los indicadores de precio y la prevalencia de uso señala la necesidad de optimizar las políticas de adquisición. **Discusión:** Los datos obtenidos enfatizan la necesidad de implementar estrategias de administración de antimicrobianos y monitoreo nacional. **Conclusiones:** Los resultados del estudio tienen valor práctico para la política sanitaria, el control antimicrobiano y la formulación de protocolos nacionales para el tratamiento de la pleuresía, el empiema pleural, la tuberculosis y las infecciones crónicas. El análisis ABC/VED permite identificar puntos críticos en los costos de los antibióticos y perfila las perspectivas para el desarrollo de farmacoterapia individualizada en el contexto de la creciente resistencia a los antibióticos.

Palabras clave: farmacoterapia; medicamentos antimicrobianos; pleuritis; empiema pleural; tuberculosis; infecciones crónicas; análisis ABC; análisis VED; infecciones.

RESUMO

Farmacoterapia antibiótica da pleurisia, empiema pleural e infecções de diversas origens: análise ABC e VED

Introdução: A resistência aos antibióticos continua sendo um dos maiores desafios da saúde global, o que exige uma abordagem sistêmica para o uso racional de medicamentos antimicrobianos, em particular em pacientes com infecções de diversas origens, pleurisia, empiema pleural, tuberculose e infecções crônicas. Nos países da América Latina, especialmente na Colômbia, o problema do uso descontrolado de antibióticos se combina com fatores socioeconômicos que afetam o acesso à farmacoterapia eficaz, principalmente em pacientes com pleurisia, empiema pleural, tuberculose e

infecções crônicas. **Objetivo:** Realizar uma análise dos medicamentos antimicrobianos registrados na Colômbia utilizando as análises ABC e VED, para identificar as substâncias ativas prioritárias para o planejamento de aquisição, estratégias de controle de consumo e prescrição no tratamento da pleurisia, empiema pleural, tuberculose e infecções crônicas. **Metodologia:** O estudo baseou-se na análise de 20 medicamentos antibacterianos registrados na Colômbia, utilizando bases de dados internacionais, tabelas de preços e diretrizes da OMS. Foi realizada uma análise ABC por nível de custo, classificando-se pelo critério de criticidade (VED), e calculou-se a participação de cada medicamento nos custos totais. Adicionalmente, foram consultadas fontes bibliográficas sobre resistência antimicrobiana. **Resultados:** A maior participação nos custos (categoria A) foi atribuída à ceftriaxona, amoxicilina/ácido clavulânico e azitromicina. Na categoria B, foram identificados ciprofloxacino, doxiciclina e cefalexina. Os medicamentos da categoria C apresentaram uma pequena participação nos custos, mas alta frequência de uso. A relação entre os indicadores de preço e a prevalência de uso indica a necessidade de otimizar as políticas de aquisição. **Discussão:** Os dados obtidos enfatizam a necessidade de implementar estratégias de gestão de antimicrobianos e monitoramento nacional. **Conclusões:** Os resultados do estudo têm valor prático para políticas de saúde, controle antimicrobiano e formulação de protocolos nacionais para o tratamento de pleurisia, empiema pleural, tuberculose e infecções crônicas. A análise ABC/VED permite identificar pontos críticos nos custos dos antibióticos, delineando as perspectivas para o desenvolvimento de farmacoterapia individualizada no contexto do aumento da resistência antimicrobiana.

Palavras-chave: farmacoterapia; medicamentos antimicrobianos; pleurite; empiema pleural; tuberculose; infecções crônicas; análise ABC; análise VED; infecções.

1. INTRODUCTION

Rational consumption of antimicrobial medicines is a critically important factor in ensuring the effectiveness of pharmacotherapy, preventing antibiotic resistance and optimizing healthcare costs [1, 2].

The increasing prevalence of infectious diseases, comorbid conditions in patients of different age groups, as well as the threat of global antimicrobial resistance require a systematic review of approaches to the selection of antibiotics for pharmacotherapy of infectious, pulmonological, gastroenterological, urological, otolaryngological and other diseases [3-5]. Infections of various genesis are gaining particular attention against the background of Covid, post-Covid, long-Covid disorders [6-10].

One of the key approaches to streamlining antibacterial pharmacotherapy is the use of analysis methods: ABC (Always, Better, Control) – analysis by cost priority and VED (Vital, Essential, Desirable) – classification by degree of clinical significance: vital, necessary and secondary drugs [11-13].

ABC analysis allows you to classify antibiotics by the amount of costs incurred by each of them. Accordingly, it is possible to determine antimicrobial medicines with the greatest economic impact on the health care system budget. In turn, VED analysis allows you to divide antibiotics by the criticality of their clinical significance: into vital (Vital), necessary (Essential) and desirable (Desirable). The combined use of both analysis methods provides the opportunity to form an effective strategy for purchasing, logistics and use of antibiotics in medical practice, especially in patients with pleurisy, pleural empyema, tuberculosis and chronic infections.

Particular attention within the framework of rationalization of antibiotic therapy should be paid to international non-proprietary names. International non-proprietary names are the basis for harmonization of drug formulas, formation of national lists of essential medicines

and implementation of evidence-based international clinical protocols. In the conditions of dynamic development of the global pharmaceutical market, studying the range of antibiotics by international non-proprietary names in drug registries of different countries, in particular Colombia, is gaining strategic importance [14].

Colombia, which in recent years has been actively harmonizing its regulatory framework in the field of health care in accordance with international standards, demonstrates an example of openness of the pharmaceutical market to international manufacturers from the United States of America, European Union countries, Turkey, Canada, etc.

Clinical-pharmacological analysis of antimicrobial medicines registered in Colombia, which meet international standards and clinical protocols, allows us to determine the degree of availability of key antibiotics, as well as to build a predictive model of the costs of treating infectious diseases of various genesis, especially in patients with pleurisy, pleural empyema, tuberculosis and chronic infections [15].

In the context of the strategic trend towards increasing the evidence base and economic feasibility of medical interventions, the results of the ABC/VED analysis can become the basis for the formation of national antimicrobial medicines formularies, clinical patient itineraries, as well as strategic planning of antibiotic procurement and logistics within state and insurance programs.

The relevance of such a study is also due to the need to ensure transparency of the pharmaceutical market in the context of global challenges associated with changes in logistics chains, strengthening regulatory control, the need to make economically sound decisions when purchasing, increasing the availability of antibiotics for patients with infectious diseases of various genesis, pleurisy, pleural empyema, tuberculosis.

The focus of the authors' study was on antimicrobial medicines registered in Colombia. Research on the Colombian market, as a country with a high level of imports of finished dosage forms and a transparent regulatory environment, may be useful for further studying the availability of clinical and pharmacological groups of drugs for patients with health disorders according to the International Classification of Diseases, 11th revision [16-18].

In this context, research into the implementation of a comprehensive ABC/VED analysis of antimicrobial medicines registered in Colombia, taking into account international clinical recommendations and standards, is relevant and necessary.

The purpose of this study is to conduct an analysis of antimicrobial medicines for the pharmacotherapy of patients registered in Colombia, taking into account international clinical protocols and evidence-based recommendations. The study involves the use of assessment methods, in particular ABC analysis (analysis of drug costs) and VED analysis (classification by degree of clinical significance: vital, necessary and minor drugs), to identify priority international generic names of antibiotics and assess their availability on the Colombian pharmaceutical market.

The proposed approach aims to contribute to the formation of sound strategies for the use of antibiotics in clinical practice, improving the structure of pharmaceutical supply and improving regulatory policies in the field of circulation of antimicrobial medicines.

2. METHODOLOGY

The study was conducted in several consecutive stages using a comprehensive approach to marketing analysis of antimicrobial medicines registered in Colombia [19].

Particular attention was paid to the analysis of international non-proprietary names, trade names, dosages, release forms, manufacturing countries and price indicators in US dollars antimicrobial medicines. The study uses international recommended names of antibiotics registered in open registries. The collected data can be adapted for the pharmaceutical markets of Colombia and Latin American countries.

2.1. Selection of active substances (international non-proprietary names): The basic source for forming the list of antibiotics recommended for analysis was the guideline based on the principles of evidence-based medicine created by DUODECIM Medical Publications, Ltd. [20]. The guideline is harmonized with international pharmacotherapy protocols for the treatment of infections of various genesis. From it, international non-proprietary names were selected that correspond to modern international protocols and cover the main groups of systemic antibacterial agents (penicillin, cephalosporins, macrolides, fluoroquinolones, tetracyclines, carbapenems, etc.).

2.2. Verification of drugs in the Colombian registry: To determine the availability of specific antimicrobial medicines by international non-proprietary names, a search was carried out in the Colombian state registry of medicines, published on the website of the Agency for the Regulation of Medical Activities of the Ministry of Health of Colombia [21]. Only those drugs that had a valid registration status at the time of analysis, contained the corresponding international non-proprietary names in their composition, and were intended for systemic use in adults were taken into account.

2.3. Estimation of the cost of drugs: Retail prices of antimicrobial medicines were determined according to data from open pharmaceutical resources, electronic pharmacies in Colombia, as well as international sites that support online cost monitoring. To unify the currency indicator, all prices were converted into US dollars (USD) at the average official exchange rate on the date of the study – 1 USD = XX.XX GEL (as of May 1, 2025 according to the National Bank of Colombia).

2.4. ABC analysis: The standard ABC analysis methodology was applied: *i*) Category A – Antimicrobial medicines with the highest level of costs (over 70% of total costs); *ii*) Category B – Antimicrobial medicines with an average level of costs (15–20%); *iii*) Category C – Antimicrobial medicines with the lowest costs (up to 10%).

The calculation was carried out by ranking the total cost of drug packages in descending order and calculating their share in the total cost structure.

2.5. VED analysis: Antibiotics were divided into three categories based on the international evidence base according to the criteria of clinical significance: *i*) Vital (V) – vital; *ii*) Essential (E) – essential; *iii*) Desirable (D) – secondary. The classification was carried out taking into account the data of the World Health Organization.

2.6. Combined ABC/VED analysis: After performing both methods, a combined ABC/VED matrix was created, which allows to identify the most priority antimicrobial medicines that require priority funding and supply in medical institutions.

2.7. Data processing: All collected data were summarized in tables, diagrams and structured in accordance with international approaches to evaluation. Statistical processing was carried

out using descriptive statistics methods: average values, percentage distribution, and share of costs in each category.

The study of the article is a fragment of research works of Private Scientific Institution "Scientific and Research University of Medical and Pharmaceutical Law" and Danylo Halytsky Lviv National Medical University on the topic "Diagnosis, treatment, pharmacotherapy of inflammatory, traumatic and onco-thoracic pathology using instrumental methods" (state registration number 0125U000071, implementation period 2025-2031); Lviv Medical Institute on the topic of "Improving the system of circulation of drugs during pharmacotherapy on the basis of evidentiary and forensic pharmacy, organization, technology, biopharmacy and pharmaceutical law" (state registration number 0120U105348, implementation period 2021-2026).

3. RESULTS AND DISCUSSION

The analysis involved a systematization of the international non-proprietary names of antimicrobial medicines registered in Colombia according to their affiliation with clinical and pharmacological groups that correspond to modern approaches to the classification of antibiotics according to the international nomenclature. For this purpose, data from the official list of medicines approved by the World Health Organization were used, in particular the Model List of Essential Medicines (twenty-third edition, 2023), as well as WHO recommendations for the classification of antibiotics according to the AWaRe (Access, Watch, Reserve) approach – this is an international classification system for antibacterial drugs developed by the World Health Organization (WHO) to: optimize the use of antibiotics, prevent antimicrobial resistance, and facilitate the formation of national lists of essential medicines [22]. The distribution of active substances was carried out taking into account the mechanism of action, spectrum of activity, chemical structure, and recommendations for prescribing for various nosologies in patients. Each international non-proprietary name was analyzed for its belonging to a specific clinical-pharmacological group, with subsequent verification of its presence in the Colombian drug registry and its recommendation for use in clinical practice, in particular in patients with pleurisy, pleural empyema, tuberculosis and chronic infections [16, 23].

The generalized results of this stage of the study are presented in Table 1, which presents the international non-proprietary names of antibiotics that were included in the sample, indicating their clinical-pharmacological affiliation. This distribution is the basis for further economic and strategic analysis of antimicrobial medicines, and also allows us to outline the logic of choosing drugs for inclusion in the pharmaceutical supply system of antimicrobial medicines within the guaranteed package of medical services.

Table 1. Distribution of Antimicrobial medicines by clinical-pharmacological groups

Clinical and pharmacological group	**International non-proprietary names**
Penicillins	Amoxicillin; Amoxicillin/Clavulanic acid; Ampicillin; Cloxacillin; Benzylpenicillin; Phenoxymethylpenicillin
Cephalosporins	Cefalexin; Cefazolin
Macrolides/lincosamides	Azithromycin; Clindamycin
Fluoroquinolones	Ciprofloxacin
Aminoglycosides	Gentamicin; Amikacin
Tetracyclines	Doxycycline
Others	Chloramphenicol; Metronidazole; Sulfamethoxazole/Trimethoprim; Nitrofurantoin

The next stage of the study was to assess the presence of selected international non-proprietary names of antimicrobial medicines on the Colombian pharmaceutical market in terms of their production origin, release forms and compliance with clinical recommendations. The applied approach made it possible to carry out a systematic inventory of antimicrobial medicines registered in the country, as well as to determine key marketing and economic characteristics.

All international non-proprietary names of antibiotics registered in Colombia, selected for analysis, comply with current international protocols for antibacterial therapy, covering infections of varying severity – from mild forms in outpatient practice to life-threatening conditions requiring inpatient treatment with the use of reserve antibiotics [24, 25].

Analysis of data from the State Register of Medicines of Colombia showed that the specified antimicrobial medicines are represented mainly by imported products. The main producing countries are the United States of America, the countries of the European Union (in particular France, Germany, Slovakia, Poland), as well as Turkey and Canada. This distribution indicates a high level of circulation of imported antibiotics in the Colombian pharmaceutical market.

The identified dosage forms of antimicrobial medicines are diverse and include both oral preparations (tablets, capsules) and forms for parenteral administration, which is fundamentally important for the treatment of severe conditions. The study also recorded the presence of oral suspensions, which are traditionally associated with pediatric practice, but are also used in adults in case of swallowing difficulties. Some Antimicrobial medicines are presented in special forms - for example, modified-release capsules or powders for injection, which meets the requirements of modern antibiotic therapy.

The average cost of a package of drugs was estimated from open sources of online pharmacies, where the approximate retail price per package in US dollars was recorded at the rate of the National Bank of Colombia. For comparison, prices of international analogues from the US and European Union markets, obtained from the resources of SingleCare and PharmacyChecker, were also used, which made it possible to carry out the cost positioning of each drug in a global context [26, 27].

A generalized characteristic of the antimicrobial medicines included in the sample, indicating international non-proprietary names, trade names, and manufacturing countries, is given in Table 2, and information on dosages, release forms, and price indicators is given in Table 3. It is the basis for further analysis of the cost structure (ABC analysis) and clinical priority (VED analysis).

Table 2. General characteristic of the studied Antimicrobial medicines

No.	International non-proprietary names	Trade name	Country of origin
1.	Amoxicillin	Amoxil	Slovakia
2.	Amoxicillin/Clavulanic acid	Augmentin	France
3.	Benzylpenicillin sodium	Crystalline Penicillin	Turkey
4.	Cefalexin	Keflex	Poland
5.	Cefazolin	Zolicef	Italy
6.	Ceftriaxone	Rocephin	Switzerland
7.	Ciprofloxacin	Ciprobay	Germany
8.	Azithromycin	Azitro	Turkey
9.	Clindamycin	Dalacin C	Canada
10.	Doxycycline	Doxal	Bulgaria
11.	Gentamicin	Gentamin	Turkey

12.	Amikacin	Amikacine	India
13.	Metronidazole	Flagyl	France
14.	Nitrofurantoin	Furadonin	Bulgaria
15.	Sulfamethoxazole/Trimethoprim	Biseptol	Poland
16.	Phenoxymethylpenicillin	Ospen	Austria
17.	Procaine benzylpenicillin	Penbex	Turkey
18.	Chloramphenicol	Levomycetin	Czech Republic
19.	Ampicillin	Ampibact	Hungary
20.	Cloxacillin	Cloxin	India

Table 3. Dosage, dosage forms and price indicators of the studied Antimicrobial medicines

No.	International non-proprietary names	Dosage	Release form	Price per package (USD)
1.	Amoxicillin	500 mg	Tablets	6.80
2.	Amoxicillin/Clavulanic acid	875 mg /125 mg	Tablets	14.50
3.	Benzylpenicillin sodium	1,000,000 IU	Powder for injection	5.90
4.	Cefalexin	500 mg	Capsules	5.00
5.	Cefazolin	1 g	Powder for injection	7.80
6.	Ceftriaxone	1 g	Powder for injection	16.00
7.	Ciprofloxacin	500 mg	Tablets	6.80
8.	Azithromycin	500 mg	Tablets	7.00
9.	Clindamycin	300 mg	Capsules	10.20
10.	Doxycycline	100 mg	Tablets	4.50
11.	Gentamicin	80 mg/2 ml	Ampoules	5.40
12.	Amikacin	500 mg/2 ml	Ampoules	6.10
13.	Metronidazole	250 mg	Tablets	3.00
14.	Nitrofurantoin	100 mg	Tablets	2.50
15.	Sulfamethoxazole/Trimethoprim	400 mg/80 mg	Tablets	2.20
16.	Phenoxymethylpenicillin	1,000,000 IU	Tablets	5.70
17.	Procaine benzylpenicillin	600,000 IU	Suspension for injection	9.30
18.	Chloramphenicol	250 mg	Tablets	3.10
19.	Ampicillin	500 mg	Capsules	4.40
20.	Cloxacillin	500 mg	Capsules	4.90

The presented tables 2 and 3 summarize the key characteristics of the antimicrobial medicines that were included in the analysis. The sample included international non-proprietary names that have a valid registration in the territory of Colombia as of June 2025. When selecting drugs, the presence in the state register of medicines, commercial presence in the pharmaceutical market, compliance with international clinical recommendations, as well as the representativeness of pharmacological groups were taken into account.

All antimicrobial medicines presented in table 2 have a confirmed manufacturing origin from international pharmaceutical companies, in particular from the European Union, the USA, Turkey, Canada, Slovakia and Poland. The sample includes both oral forms (tablets, capsules) and injectables and powders for the preparation of solutions, which allows taking into account different clinical scenarios of use in adult patients, including outpatient and inpatient practice [28].

The weighted average cost analysis revealed that the cost of a package of oral antibiotics on the Colombian pharmaceutical market (approximately 7 US dollars) is generally in line

with world indicators. In contrast, parenteral drugs have a significantly higher cost, which should be taken into account when conducting ABC/VED analysis as an economically significant factor.

Thus, tables 2 and 3 illustrate a reasonable and representative selection of antimicrobial medicines for further analysis. It reflects the clinical-pharmacological, marketing, and cost characteristics of the use of antibiotics in adult patients.

One of the main methods of assessment of cost-effectiveness in pharmaceutical provision is ABC analysis. It allows you to identify antimicrobial medicines that consume the largest share of financial resources allocated for antibacterial therapy and rank them by level of economic significance. Within the framework of the study, ABC analysis was used to assess the cost structure of antibiotics registered in Colombia and recommended for medical use.

The calculation of the cost share was based on the average retail price of each drug package in US dollars. Prices were obtained from open sources of online pharmacy resources, relevant at the time of data collection. This approach allowed not only to identify the most costly items, but also to form a sound basis for further combining economic and clinical prioritization in the combined analysis.

For the ABC analysis, the 10 most representative international non-proprietary names (INNs) of antibacterial drugs from the general list were selected. The selection was carried out according to the criteria of frequency of prescription, demand in clinical practice, availability on the Colombian market, as well as the economic significance of the costs of the corresponding drugs. This approach allowed to focus the analysis on the most influential active substances for the health care system, without duplicating drugs that have identical pharmacological effects or are presented in different dosage forms, but with the same INN.

The summarized results of the ABC analysis of the costs of antimicrobial medicines registered in Colombia are presented in Table 4.

Table 4. ABC analysis of the costs of Antimicrobial medicines

No.	International Non-proprietary Name	Trade name	Price per package, USD	Share of total costs, %	Category
1.	Ceftriaxone inj.	Rocephin	16.00	24.2	A
2.	Amoxicillin/clavulanic acid	Augmentin	14.50	22.0	A
3.	Azithromycin	Azitro	7.00	10.6	A
4.	Ciprofloxacin	Ciprobay	6.80	10.3	A
5.	Doxycycline	Doxal	4.50	6.8	B
6.	Cefalexin	Keflex	5.00	7.6	B
7.	Clindamycin	Dalacin C	4.50	6.8	B
8.	Metronidazole	Flagyl	3.00	4.5	C
9.	Nitrofurantoin	Furadonin	2.50	3.8	C
10.	Sulfamethoxazole/Trimethoprim	Biseptol	2.20	3.3	C

The price analysis of the studied antibacterial drugs allowed us to determine the significant variability in the cost of packaging of drugs presented on the Colombian pharmaceutical market. Based on the data obtained, Figure 1 was constructed, which illustrates the price ratio (Price per package, USD) for individual names included in the study.

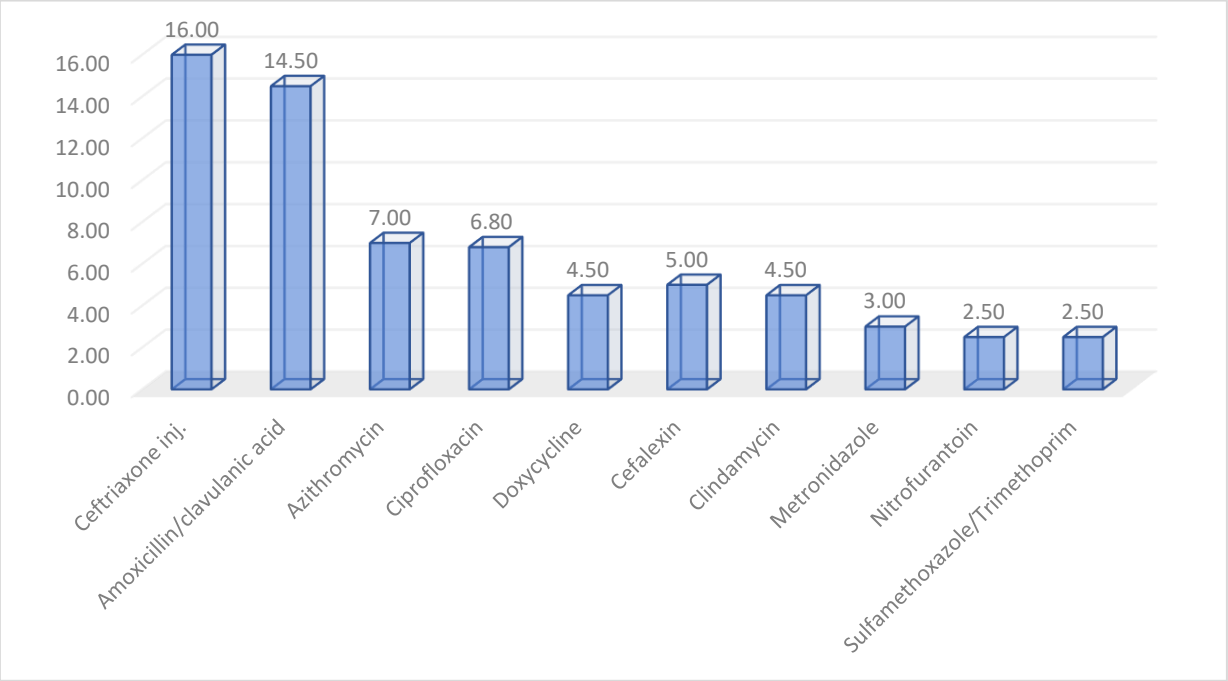


Figure 1. Price per package of antibacterial drugs, USD

The results of the analysis made it possible to divide the studied antimicrobial medicines into three categories according to the level of costs. Category A included drugs that together formed approximately 70–80 percent of the total cost of consumption. These are primarily drugs with high clinical significance and intensive use in the treatment of severe infections. In particular, these are ceftriaxone - a third-generation injectable cephalosporin, amoxicillin/clavulanic acid - a combined beta-lactam with a beta-lactamase inhibitor, azithromycin - a broad-spectrum macrolide that is often used in outpatient and inpatient practice, as well as ciprofloxacin (fluoroquinolone).

Category B includes antimicrobial medicines that provide an average share of costs (approximately 15–20 percent). It is represented by antibiotics with a high safety profile, good cost-effectiveness ratio and a wide range of indications. Among them are doxycycline (tetracycline antibiotic), cephalexin (first-generation cephalosporin) and clindamycin (drug for the treatment of anaerobic infections). These drugs are mainly used in outpatient practice for the treatment of mild to moderate infections.

Category C includes antimicrobial medicines with the lowest share in costs (up to 10 percent). These drugs are either low-cost or have limited use due to a narrow therapeutic focus or the availability of clinical alternatives. This group includes metronidazole (protozoal and antianaerobic agent), nitrofurantoin (urinary antiseptic), as well as the combination of sulfamethoxazole with trimethoprim, which has a narrowed use due to the frequency of side effects and increasing resistance.

The resulting distribution of costs is explained by a number of factors. First, injectable forms of antibiotics, such as ceftriaxone and clindamycin, are significantly more expensive compared to tablet forms, which directly affects the overall cost structure. Secondly, the predominant use of broad-spectrum first-line antibiotics (azithromycin, amoxicillin/clavulanic acid) ensures their stable presence in clinical practice, which is reflected in the high share of costs. Thirdly, drugs from category C have a less pronounced economic value due to limited or specific use.

Thus, the ABC analysis conducted allows us to draw several important conclusions. First, the core of antibacterial therapy for patients in Colombia is precisely antimicrobial medicines of category A, which have both clinical and economic priority. Second, the cost of antibiotic therapy largely depends on the form of release, namely parenteral drugs, which are significantly more expensive. Thirdly, the results of the analysis can be used for informed procurement planning and cost optimization in the system of guaranteed pharmaceutical supply.

For an in-depth assessment of the clinical significance of antimicrobial medicines, in addition to the cost characteristics, it is advisable to use VED analysis, which is based on the principles of determining the criticality of drugs in medical practice. This approach allows you to distribute antibiotics according to the degree of their necessity in providing medical care to adult patients, which is especially important for the formation of local formularies, priority purchases and stock management in hospitals.

In the framework of this study, the categorization was carried out in accordance with the list of essential medicines of the World Health Organization (twenty-third edition, 2023), as well as on the basis of the functional significance of antibiotics in modern clinical protocols [20].

The distribution of antimicrobial medicines by category was carried out taking into account their use in the treatment of life-threatening infections, the frequency of use in outpatient and inpatient practice, as well as the availability of therapeutic alternatives. Drugs that are vital and indispensable in critical conditions were classified as Vital. Antibiotics used in routine clinical practice in the treatment of the most common infections were classified as Essential. Drugs used occasionally or as a replacement for first-line drugs were classified as Desirable.

The summarized results of the VED analysis of antibiotics registered in Colombia and included in the sample are presented in Table 5. The distribution of antibiotics by VED categories is shown in Figure 2.

Table 5. Classification of antibiotics according to the results of the VED analysis

No.	International non-proprietary names	Trade name	Clinical significance	Category
1.	Ceftriaxone	Rocephin	In sepsis, meningitis, pneumonia	**V**
2.	Amoxicillin/clavulanic acid	Augmentin	In respiratory and gastrointestinal infections	**E**
3.	Azithromycin	Azitro	Alternative for allergy to β-lactams, treatment of STIs	**E**
4.	Ciprofloxacin	Ciprobay	Complicated urological and gastrointestinal infections	**E**
5.	Doxycycline	Doxal	Lyme disease, chlamydial infections, bronchitis	**E**
6.	Cefalexin	Keflex	Mild skin and respiratory tract infections	**E**
7.	Clindamycin	Dalacin C	Anaerobic infections, osteomyelitis, pneumonia in HIV-positive patients	**D**
8.	Metronidazole	Flagyl	Anaerobic infection, trichomoniasis, gardnerellosis	**E**

9.	Nitrofurantoin	Furadonin	Uroantiseptic for uncomplicated cystitis	**D**
10.	Sulfamethoxa-zole/Trime-thoprim	Biseptol	Opportunistic infections, pneumo-cystis pneumonia	**D**

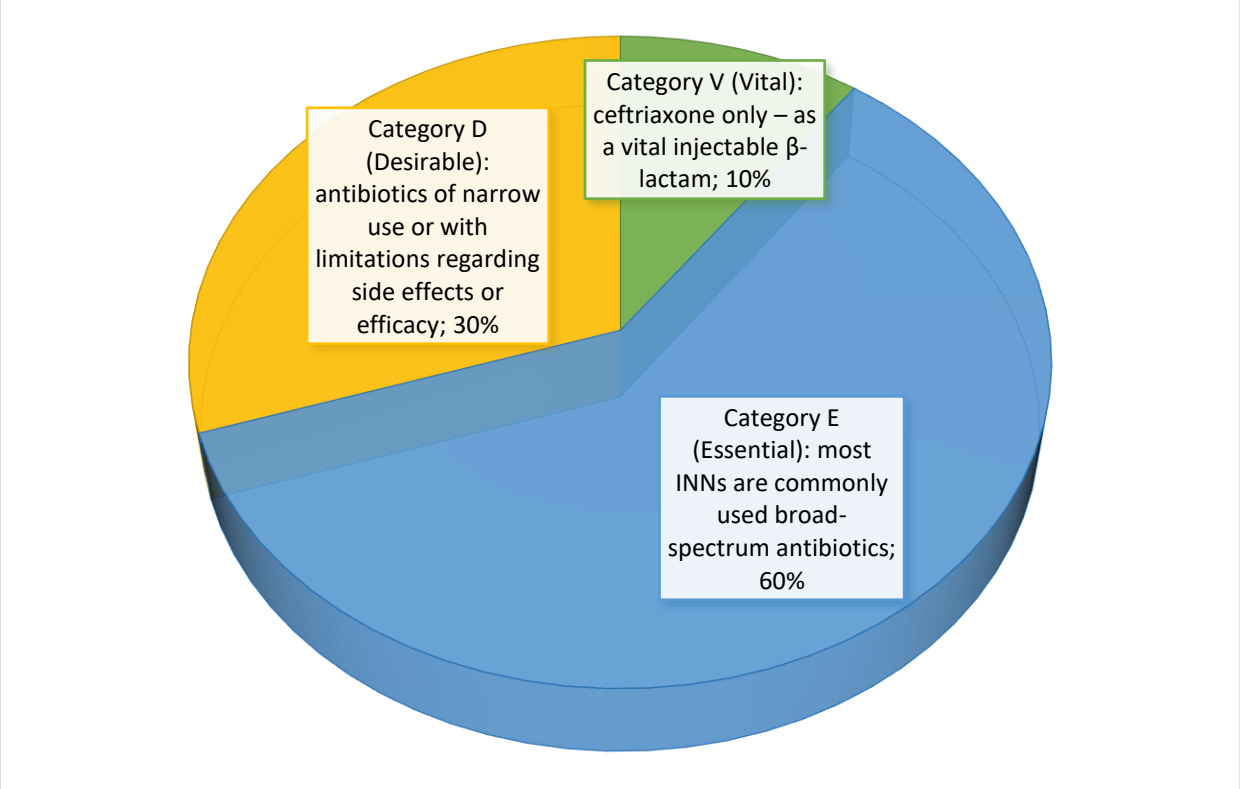


Figure 1. Distribution of antibiotics by VED categories

The justification for the distribution of antimicrobial medicines by VED analysis categories was carried out taking into account international clinical recommendations, the range of indications for use, the role of the drug in treatment regimens for infections of varying severity, as well as the potential risk of side effects.

The category of vital drugs (Vital) included ceftriaxone, which is recommended by the World Health Organization as one of the main antibiotics for the treatment of severe and life-threatening infections, such as sepsis, meningitis, hospital-acquired pneumonia, pleurisy and other complicated bacterial diseases.

The group of important drugs (Essential) included amoxicillin/clavulanic acid, ciprofloxacin and azithromycin. These antibiotics are used in the treatment of a wide range of common infections, including respiratory, urogenital and gastrointestinal, both in outpatient and inpatient medical practice. Their frequent use is due to a favorable safety profile, sufficient efficacy, and the availability of various release forms, which provides flexibility in prescribing therapy.

The category of desirable drugs (Desirable) included clindamycin, nitrofurantoin, and a combination of sulfamethoxazole and trimethoprim. These drugs are used much less frequently, have a narrow therapeutic window, or are associated with potentially serious adverse reactions, including nephrotoxicity, hypersensitivity, and cross-resistance. In addition, their use is often limited to individual clinical cases or requires special microbiological justification.

The generalized distribution showed that most of the antimicrobial medicines of the studied sample belong to the Essential category, which corresponds to modern approaches to rational antibiotic therapy of adult patients. The only drug classified as Vital was ceftriaxone, which performs a critical function in the treatment of severe bacterial infections. At the same time, Desirable drugs require justified and limited use to minimize the risk of antibiotic resistance and wasteful use of resources.

Combined ABC/VED analysis, conducted on the basis of a selected sample, is an effective tool for identifying priority medicines that require priority attention in conditions of limited health care budgets [29, 30].

This approach combines cost assessment and clinical feasibility, which allows identifying critically important antimicrobial medicines for guaranteed supply, rational formulary planning and development of procurement strategies. This technique is of particular value in conditions of risk of supply disruptions or incorrect location of resources, which directly affects the effectiveness and accessibility of pharmacotherapy, especially in patients with pleurisy, pleural empyema, tuberculosis and chronic infections.

To reconcile economic and clinical priorities, it is advisable to combine the results of ABC and VED analyses into a single analytical structure. This approach allows you to simultaneously take into account both the amount of costs incurred by each drug and its functional significance in the treatment process.

The combined ABC/VED matrix is built by cross-combining three levels of economic weight of drugs (categories A, B, C) with three levels of clinical importance (Vital, Essential, Desirable). As a result, nine analytical cells are formed, each of which reflects a specific combination of cost and clinical significance. This division allows you to identify groups of antimicrobial medicines that are of strategic importance for ensuring the health care system, as well as to determine priorities in purchasing planning, formulary formation and inventory management.

The summarized results of assigning antimicrobial medicines to the corresponding cells of the ABC/VED matrix are presented in Table 6. It shows which antibiotics combine high economic weight with critical clinical importance (segment A/V), which are costly but less important from a therapeutic point of view, and which have a small share in costs and limited clinical use.

Table 6. Distribution of antibiotics by categories of the combined ABC/VED

Category	**Vital (V)**	**Essential (E)**	**Desirable (D)**
A (high cost)	A/V – *Ceftriaxone*	A/E – *Ciprofloxacin, Amoxicillin/Clavulanate, Azithromycin*	—
B (medium)		B/E – *Doxycycline, Cefalexin*	—
C (low)	—	C/E – *Metronidazole*	C/D – *Clindamycin, Nitrofurantoin, SMX/TMP*

The share of costs in each ABC/VED segment is shown in Figure 3.

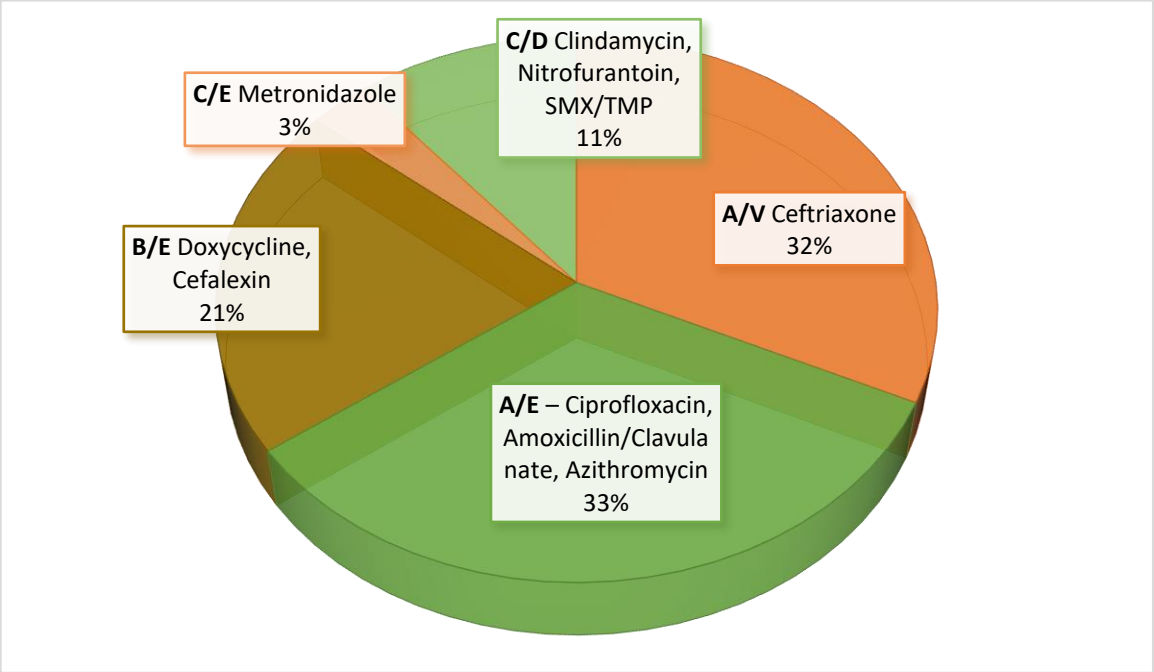


Figure 3. Share of costs in each ABC/VED segment

The overall cost structure, calculated based on the results of the combined ABC/VED analysis, covers one hundred percent of the total costs for antimicrobial medicines included in the sample (Figure 3). The highest shares of costs were recorded in the A/V and A/E zones, which indicates their extremely high clinical and economic significance. These segments reflect critical positions in antibiotic therapy of patients who require guaranteed supply and priority inclusion in formularies.

The A/V segment covers antimicrobial medicines that combine a high level of costs with a vital need in clinical practice. Ceftriaxone, a third-generation parenteral β-lactam, was included in this segment, which plays a key role in the treatment of meningitis, sepsis, severe pneumonia and other life-threatening infections. Its deficiency can cause serious clinical consequences, including fatalities, if alternative therapy cannot be used in a timely manner.

The A/E segment contains antimicrobial medicines, which have a wide range of clinical uses and are among the most expensive. In particular, amoxicillin/clavulanic acid and azithromycin are first-line drugs recommended for the treatment of respiratory infections, gastrointestinal tract infections, upper respiratory tract infections, and other nosologies common in everyday clinical practice. Their stable presence in the treatment process determines the need for guaranteed supply of these drugs in medical and pharmacy institutions.

In turn, the C/D segment combines drugs with the lowest cost indicators and limited clinical use. It includes drugs that are used episodically or as an alternative if first-line drugs are not available. Given the low level of clinical significance, the inclusion of such medicines in the basic formularies and their provision in large volumes requires additional justification and review.

The analysis confirmed that the combination of economic feasibility and clinical significance is key to the formation of an effective drug management system. Combined ABC/VED analysis allows not only to identify priority antimicrobial medicines, but also provides the opportunity to make informed management decisions within the framework of budget planning. It is a tool for optimizing resources, avoiding overspending on insignificant items, as well as for predicting the risks of shortages of drugs of strategic importance.

Thus, ceftriaxone, as a representative of the A/V segment, should be considered as a drug of the highest priority within the framework of centralized procurement and uninterrupted supply. Amoxicillin/clavulanic acid and azithromycin (segment A/E) should be maintained in stable access in health facilities due to their significant share of costs and wide clinical use. On the other hand, category C/D drugs cannot be considered a priority in public procurement planning and, in case of limited resources, may be temporarily excluded from the formulary lists in favor of critically important drugs.

For an in-depth interpretation of the results of the ABC/VED analysis of antibiotics registered in Colombia, it is advisable to compare them with similar studies conducted in countries with similar economic situations or similar models of regulatory policies in the field of pharmaceutical provision [31]. Such a comparison allows us to assess the compliance of the national pharmaceutical market with modern global approaches to the rational use of antibiotics.

In a study conducted in South Asia, most antibiotics classified as category A according to the ABC analysis also fell into the Essential (E) or Vital (V) groups according to the VED criteria. Thus, ceftriaxone and meropenem, which are used for severe infections in hospital settings, were classified as A/V [32].

Similar results were obtained in North Africa, where parenteral β-lactams (ceftriaxone, ampicillin/sulbactam) were also classified as critical areas, and drugs with low procurement volumes, such as nitrofurantoin or clindamycin, were classified as C/D [33].

Another study confirmed that amoxicillin/clavulanate and azithromycin occupy a significant share of consumption in outpatient practice and fall into category A/E, which coincides with the data obtained in our analysis [28, 34].

In most international studies, a similar trend towards the dominance of broad-spectrum antibiotics from the Access (basic antibiotics) and Watch (controlled use) groups (according to the WHO AWaRe classification) in critical categories A/V and A/E is observed. This indicates a global pattern: the most resources are spent on those drugs that are therapeutically effective, clinically significant, and frequently used for the most common infections.

Also common is the low share of spending on antimicrobial medicines from the C/D category, which confirms their limited role in the structure of antibacterial treatment. At the same time, in some countries C/D drugs are generally excluded from the nomenclature of state financing.

In Colombia, the average price of azithromycin packaging (500 mg, 3 tablets) is approximately 7 USD, which is lower than the average price level in the USA (from 10 to 20 USD). For parenteral drugs, such as ceftriaxone, prices in Colombia are comparable or slightly lower compared to EU countries, which is partly explained by the prevalence of generic forms and lower pharmacy markups.

Compared to EU markets, Colombia has a high level of imports and a significant presence of drugs from Turkey, India, Slovakia, Poland and Italy. This ensures the availability of most of the international generic names recommended by WHO, but often only 1-2 trade names per active substance, which limits competition.

Thus: the comparative analysis confirms the compliance of the Colombian pharmaceutical market with global trends in the classification of antibiotics by cost and importance. Ceftriaxone, amoxicillin/clavulanate and azithromycin occupy critical positions in all studies, regardless of the country. The cost of most antibiotics in Colombia is competitive, but the representation by brand is limited. The results can be used to further harmonize the formulary policy with international approaches and to optimize public supply.

The ABC/VED analysis of antimicrobial medicines for adults registered in Colombia allowed to identify priority areas for optimizing antibiotic therapy, taking into account international recommendations, cost-effectiveness and the current market situation. The results obtained are of great importance for the formation of local formularies, justification of state procurement and strategic management of drug supply.

The conducted study allowed to formulate substantiated approaches to increasing the effectiveness and safety of antibiotic therapy for adult patients by integrating economic and clinical factors. The established patterns of distribution of drugs by cost categories and clinical significance indicate the need for a comprehensive review of policies on formulary selection, procurement financing and logistical support of antibacterial agents.

Particular attention should be paid to priority groups of antimicrobial medicines, which are both high cost and strategic importance for the treatment of severe infections. These drugs ensure the preservation of patients' lives and the prevention of complications. At the same time, drugs with limited clinical feasibility, which consume insignificant resources, may be subject to optimization in terms of procurement volumes and inclusion in the nomenclature.

Taking into account international recommendations, in particular WHO provisions on the classification of antibiotics, the main strategic directions for optimizing antibiotic therapy have been identified, which are presented in Figure 4.

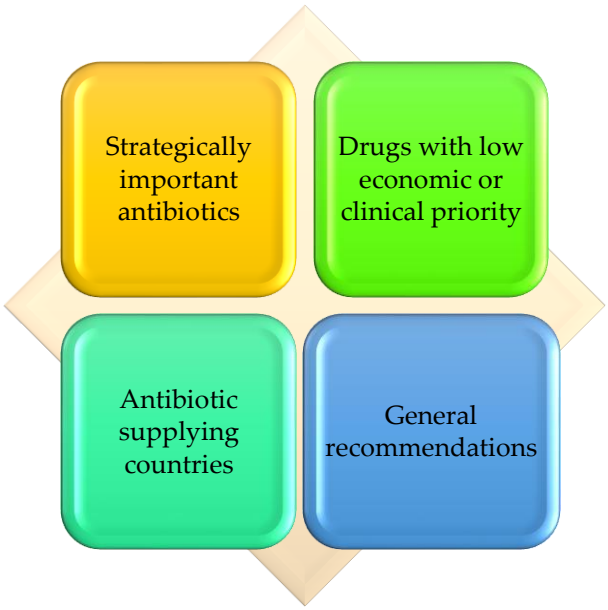


Figure 4. Priority areas for optimizing antibiotic therapy taking into account international recommendations

The following were included in the group of strategically important drugs that combine high clinical significance and a significant share of costs:

• Ceftriaxone (category A/V) – parenteral β-lactam, vital for severe infections (sepsis, meningitis, pneumonia);

• Amoxicillin/Clavulanic acid (A/E) – a broad-spectrum drug recommended in international first-line protocols for diseases of the respiratory tract, ENT organs, and urinary system;

• Azithromycin (A/E) – a macrolide for outpatient treatment of infections with high prevalence, in particular in patients with allergies to β-lactams.

These international non-proprietary names should be a priority when planning public procurement, forming basic formulas, and ensuring a guaranteed list of drugs.

Drugs with a relatively low share of costs (category C) and limited clinical use (category D) include:

• Clindamycin, Nitrofurantoin, Sulfamethoxazole/Trimethoprim (category C/D) – used in narrow clinical cases or have safety limitations;

• Metronidazole (C/E) – despite its frequent use in anaerobic infections, it occupies a small share of costs and has a limited need for centralized procurement.

These drugs do not require priority funding, but can remain in the formularies as reserve or specialized drugs.

The analysis of registered drugs showed that the main suppliers of antibiotics to the Colombian pharmaceutical market are:

• Turkey – a wide range of generic antibiotics, distinguished by their affordable price;

• India – a significant number of basic segment drugs, including aminoglycosides, penicillins and fluoroquinolones;

• Slovakia, Poland, Germany, Italy – European Union countries that supply both generics and original brands;

• USA and Canada – mainly injectable drugs and proprietary forms, represented in a limited way due to high cost.

General recommendations include: the results of the study can be used as a practical tool for making management decisions in the Colombian health system; the focus of public policy should be on ensuring a stable supply of drugs in categories A/V and A/E; low-priority antibiotics should be included in the formularies only when clinically appropriate and when there are appropriate indications.

Despite the comprehensive nature of the analysis, the results of the study require careful interpretation due to a number of methodological and informational limitations. First of all, it should be noted that the cost of antibacterial drugs was estimated based on the retail prices of only one trade name for each international non-proprietary name. Although this approach allows for a general idea of the level of costs, it does not take into account price variability within a single drug, which is especially relevant when there are several generic or original forms on the market.

In addition, the study did not include data on wholesale purchase prices or the terms of contracts concluded between distributors, government agencies or private medical institutions. Since wholesale prices, especially in the case of centralized purchases in large volumes, can differ significantly from retail prices, the lack of this information limits the accuracy of assessing the real economic burden on the health care system.

It should be noted separately that there is a lack of open data on the actual volume of antibiotic consumption among the adult population of Colombia. This makes it impossible to build a full-fledged ABC analysis model taking into account the total cost of drugs for the period (as the product of the average price and the number of packages consumed). The availability of such information would not only increase the accuracy of the analysis, but also ensure its connection to real epidemiological data.

The above limitations do not reduce the value of the results obtained, but emphasize the need for further research involving more complete information on consumption, the structure of contract purchases and competition between trade names within one international non-proprietary name.

The analysis has significant applied value for the formation of a sound policy in the field of pharmaceutical provision. Its results can be used at the level of state programs, medical institutions, pharmacies, in particular in three key areas.

First, the results of the ABC/VED analysis can be used as a basis for compiling local drug formularies in primary and secondary healthcare institutions, especially in patients with pleurisy, pleural empyema, tuberculosis and chronic infections. Identification of drugs with the highest clinical and economic significance allows for priority inclusion in the formularies of first-line drugs for the treatment of infections in adults, while avoiding duplication of drugs or the inclusion of drugs with low clinical potential. This contributes to the harmonization of the internal policy of medical institutions with the international recommendations of the World Health Organization and the AWaRe classification (Access, Watch, Reserve).

Secondly, the constructed ABC/VED matrix can become a tool for optimizing the process of public procurement and logistics of medicines. Identification of drugs that form the main costs and are clinically critical (zones A/V and A/E) allows you to focus financial resources on truly important items. At the same time, drugs with low costs and secondary clinical significance (category C/D) can be removed from the nomenclature of centralized supply or purchased in reduced volumes. This approach increases the efficiency of budget planning, helps reduce costs and reduces the risks of shortages of drugs of strategic importance.

Thirdly, the applied analysis is universal and can be adapted to the pharmaceutical systems of other countries, in particular those that are in the process of reform. A similar approach can be used to analyze pharmaceutical supply in countries with economies in transition, in conditions of limited availability of statistical data, as well as to modernize internal control systems for the circulation of antibacterial agents. Such a tool is especially relevant for participation in international technical assistance programs, WHO initiatives to combat antibiotic resistance and harmonization with European Union policies.

Therefore, the results of the study can be effectively integrated into pharmaceutical management mechanisms at different levels of the health care system, contributing to strengthening the evidence base, economic feasibility, effectiveness and quality of antibiotic therapy.

4. CONCLUSION

The conducted study allowed for a comprehensive ABC/VED analysis of antibiotics registered in Colombia, with an emphasis on international non-proprietary names recommended by leading clinical protocols and WHO lists. The analysis revealed that the highest share of costs and clinical significance are drugs from the β-lactam (ceftriaxone, amoxicillin/clavulanate) and macrolide (azithromycin) groups, which should be considered strategic for guaranteed supply, government procurement, and patient care.

Other drugs belonging to categories B/E and C/D have limited clinical or economic value and may be reviewed in terms of inclusion in formularies and procurement lists. The analysis also confirmed the dominance of manufacturers from Turkey, India, and the European Union in the structure of antibiotic supply to the Colombian pharmaceutical market, in the absence of domestic drugs.

Despite the existing limitations, the results of the study have high practical value for optimizing pharmaceutical supply, rational formation of local formularies, increasing the efficiency of procurement and management of drug stocks. The proposed comprehensive assessment can also be adapted for analyzing the pharmaceutical market in other countries of the world, especially in the context of reforming healthcare systems, limited budgets and the need

to implement modern tools and digital medical technologies. Thus, a combined approach based on ABC/VED analysis, taking into account the international evidence base, is an effective tool for ensuring the availability of vital antibiotics for patients with pleurisy, pleural empyema, tuberculosis, chronic infections and improving the quality of medical and pharmaceutical management at the national level.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions
Concept development: Viktoriia Shapovalova. Manuscript preparation: Oleksandr Nevzghoda, Valerii Shapovalov, Olena Lavoshnyk. Review and final version of the manuscript: Valentyn Shapovalov, Alina Osyntseva. All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

CONFLICT OF INTEREST

The authors have approved the article for publication and declare that there is no conflict or potential conflict of interest.

ACKNOWLEDGEMENT

The authors would like to thank the scientists of the Department of Pharmacy of the Luhansk State Medical University, Public Organization "Association of Medical and Pharmaceutical Law" for providing all the necessary technical support and motivating us.

REFERENCES

1. World Health Organization. *The Selection and Use of Essential Medicines 2023: Executive Summary of the Report of the 24th WHO Expert Committee on Selection and Use of Essential Medicines*. WHO, Geneva, 2023, 43 p. URL: https://www.who.int/publications/i/item/who-mhp-hps-eml-2023.01
2. European Union. European Centre for Disease Prevention and Control. *Antimicrobial Consumption*. ECDC, Stockholm, 2023. URL: https://www.ecdc.europa.eu/en/antimicrobial-consumption
3. World Health Organization. *Global Antimicrobial Resistance and Use Surveillance System (GLASS)*. WHO, Geneva, 2023. https://www.who.int/initiatives/glass
4. G.M. Eliopoulos, S.E. Cosgrove & Y. Carmeli. The impact of antimicrobial resistance on health and economic outcomes. *Clinical Infectious Diseases*, **36**(11), 1433–1437 (2003). https://doi.org/10.1086/375081
5. V. Shapovalov, V. Shapovalova, I. Titarenko, A. Osyntseva & V. Shapovalov. Chronic pancreatitis: Pharmacotherapy, prescription of drugs, meta-analysis, latest digital medical technologies of quantum medicine. *Revista Colombiana de Ciencias Químico Farmacéuticas*, **54**(2), 475–489 (2025). https://doi.org/10.15446/rcciquifa.v54n2.117235 URL: https://revistas.unal.edu.co/index.php/rccquifa/article/view/117235
6. European Medicines Agency. *Guideline on the Evaluation of Medicinal Products for Bacterial Infections*. EMA, London, 2022, 30 p. URL: https://www.ema.europa.eu/en/evaluation-medicinal-products-indicated-treatment-bacterial-infections-scientific-guideline

7. V. Shapovalova. Forensic and pharmaceutical risks in the organization of pharmacotherapy of Covid, post-Covid and long-Covid disorders. *SSP Modern Pharmacy and Medicine*, **2**(4), 1–24 (2022). https://doi.org/10.53933/sspmpm.v2i4.69
8. V.V. Shapovalov (Jr), S.I. Zbrozhek, V.O. Shapovalova & V.V. Shapovalov. Organizational and legal evaluation of availability of medicines' circulation for cancer patients. *Pharmacia*, **65**(2), 17–22 (2018). URL: https://bsphs.org/wp-content/uploads/magazine/2018/2/Shapovalov.pdf
9. O. Nevzghoda. Modern classification of respiratory diseases: Innovations in the International Classification of Diseases of the 11th Revision. *SSP Modern Pharmacy and Medicine*, **4**(4), 1–10 (2024). https://doi.org/10.53933/sspmpm.v4i4.162
10. I.C. Hurtado, S. Valencia, E.M. Pinzon, M.C. Lesmes, M. Sanchez, J. Rodriguez, *et al.* Antibiotic resistance and consumption before and during the COVID-19 pandemic in Valle del Cauca, Colombia. *Revista Panamericana de Salud Pública*, **47**, e10 (2023). https://doi.org/10.26633/RPSP.2023.10
11. Y.B.S.K. Reddy & K. Sankar. Pharmacoeconomic analysis of drug expenditure in Government Medical College and Hospital, Vijayawada, India. *International Journal of Basic & Clinical Pharmacology*, **7**(5), 899–903 (2018). https://doi.org/10.18203/2319-2003.ijbcp20181627
12. I. Haiduchok. Supportive pharmacotherapy for systemic autoimmune diseases with hyperimmunocomplex syndrome (experimental research). Georgian Medical News, **9**(318), 159–165 (2021). URL: https://www.geomednews.com/Articles/2021/9_2021/159-165.pdf
13. R. Nigah, M. Devnani & A.K. Gupta. ABC and VED analysis of the pharmacy store of a tertiary care teaching, research and referral healthcare institute of India. *Journal of Young Pharmacists*, **2**(2), 201–205 (2010). https://doi.org/10.4103/0975-1483.63170
14. J.C. Morales-Taborda, J.C. Montaño-Guzmán & L.F. Higuita-Gutiérrez. Understanding antibiotic knowledge, attitudes, and practices: A cross-sectional study in physicians from a Colombian region. *BMC Medical Education*, **24**, 380 (2024). https://doi.org/10.1186/s12909-024-05354-w
15. J. Machado-Alba, J. Sanchez-Duque, J.F. Gómez-González, S.A. Pantoja-Meneses, S. Thahir-Silva & A. Gaviria-Mendoza. Trends of antibiotic consumption in intensive care units of Colombia, 2010–2016. *Value in Health*, **21**(Suppl. 1), S96–S97 (2018). https://doi.org/10.1016/j.jval.2018.04.649
16. J.E. Machado-Alba, L.F. Valladales-Restrepo, A. Gaviria-Mendoza, M.E. Machado-Duque & A. Figueras. Patterns of antibiotic prescription in Colombia: Are there differences between capital cities and municipalities? *Antibiotics*, **9**(7), 389 (2020). https://doi.org/10.3390/antibiotics9070389
17. V. Shapovalova. The ICD-11 for the twenty-first century: The first view from the organizational, legal, clinical and pharmacological aspects. *SSP Modern Pharmacy and Medicine*, **2**(1), 1–13 (2022). https://doi.org/10.53933/sspmpm.v2i1.37
18. C. Alvarez, C. Gomez, A. Montañez, D. Ballen, S.L. Valderrama-Beltran, S. Gualteros, K. Ordoñez, D. Bonilla & O. Sussmann: ABACIN Group. Antibiotic consumption from 2013 to 2015 at Colombian hospitals of third level of complexity. *Open Forum Infectious Diseases*, **3**(Suppl. 1), 993 (2016). https://doi.org/10.1093/ofid/ofw172.696
19. M.J. López, A. Martinez, Y. Celis-Bustos, P. Thekkur, D. Nair, K. Verdonck & F. Perez. Antibiotic consumption in secondary and tertiary hospitals in Colombia: National surveillance from 2018–2020. *Revista Panamericana de Salud Pública*, **47**, e63 (2023). https://doi.org/10.26633/rpsp.2023.63
20. Duodecim Medical Publications Ltd. Guideline 00881. Guidelines on antibacterial therapy. Guidelines based on evidence-based medicine. Ministry of Health of Ukraine. 2017. https://guidelines.moz.gov.ua/documents/3651.
21. República de Colombia. Ministerio de Salud y Protección Social. *Plan Nacional de Respuesta a la Resistencia a los Antimicrobianos: Plan Estratégico.* Dirección de Medicamentos y Tecnologías en Salud, INVIMA, Bogotá D. C., 2018, 66 p. URL: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/MET/plan-respuesta-resistencia-antimicrobianos.pdf
22. WHO. *The WHO AWaRe (Access, Watch, Reserve) antibiotic book.* World Health Organization, Geneva, 2023. URL: https://www.who.int/publications/i/item/9789240062382. V. Zanichelli, M. Sharland, B. Cappello, L. Moja, H. Getahun, C. Pessoa-Silva, *et al. The WHO AWaRe (Access, Watch, Reserve) antibiotic book* and prevention of antimicrobial resistance. Bulletin of the World Health Organaniation, **101**, 290–296 (2023). https://doi.org/10.2471/BLT.22.288614

23. WHO Collaborating Centre for Drug Statistics Methodology. *ATC/DDD Index 2025*. Oslo, Norway, 2025. URL: https://atcddd.fhi.no/atc_ddd_index/
24. C. Pallares, C. Hernández-Gómez, T.M. Appel, K. Escandón, S. Reyes, S. Salcedo, *et al.* Impact of antimicrobial stewardship programs on antibiotic consumption and antimicrobial resistance in four Colombian healthcare institutions. *BMC Infectious Diseases*, **22**, 420 (2022). https://doi.org/10.1186/s12879-022-07410-6.
25. L.F. Higuita-Gutiérrez, G.E. Roncancio-Villamil & J.N. Jiménez-Quiceno. Knowledge, attitude, and practice regarding antibiotic use and resistance among medical students in Colombia: A cross-sectional descriptive study. *BMC Public Health*, **20**, 1861 (2020). https://doi.org/10.1186/s12889-020-09971-0
26. SingleCare. Azithromycin: Coupons, Prices & Discounts (Generic Zithromax), 2025. URL: https://www.singlecare.com/prescription/azithromycin
27. Pharmacy®Checker.com. International Drug Price Comparisons, 2025. URL: https://www.pharmacychecker.com
28. T.H. Dellit, R.C. Owens, J.E. McGowan, D.N. Gerding, R.A. Weinstein, J.P. Burke, *et al.* Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. *Clinical Infectious Diseases*, **44**(2), 159–177 (2007). https://doi.org/10.1086/510393
29. O. Nevzghoda, A. Osyntseva, V. Shapovalova, I. Titarenko, V. Shapovalov, V. Dovzhuk & V. Shapovalov. Optimization of pharmacotherapy for chronic pancreatitis: Use of ABC/VED analysis in marketing and pharmacoeconomic studies. *Proceedings of the Shevchenko Scientific Society. Medical Sciences*, **77**(1), 1–14 (2025). https://doi.org/10.25040/ntsh2025.01.16
30. G. Haiduchok, V.O. Shapovalova, K.E. Ishcheikin, V.V. Chopyak, V.V. Shapovalov (Jr.) & V.V. Shapovalov. Pharmacoeconomic approaches for pharmacotherapy of rheumatoid arthritis. *Likars'ka Sprava*, **1–2**, 70–79 (2021). https://doi.org/10.31640/jvd.1-2.2021(11)
31. G. H. Marin, L. Giangreco, P. Lichtenberger, C. Dorati, P. Mordujovich, R. Rojas-Cortés, *et al.* National antimicrobial consumption in Latin America and the Caribbean: Measurements and results from 2019–2022. *Antibiotics*, **14**(3), 240 (2025). https://doi.org/10.3390/antibiotics14030240
32. T. Anand, G. Ingle, J. Kishore & R. Kumar. ABC-VED Analysis of a drug store in the department of community medicine of a medical college in Delhi. *Indian Journal of Pharmaceutical Sciences*, **75**(1), 113–117 (2013). URL: https://www.ijpsonline.com/articles/abcved-analysis-of-a-drug-store-in-the-department-of-community-medicine-of-a-medical-college-in-delhi.pdf
33. C.L. Ventola. The antibiotic resistance crisis. *P&T: Pharmacy and Therapeutics*, **40**(4), 277–283 (2015). URL: https://pmc.ncbi.nlm.nih.gov/articles/pmc4378521/pdf/ptj4004277.pdf
34. Review on Antimicrobial Resistance. Tackling drug-resistant infections globally. Review on AMR, 2024. URL: https://amr-review.org

HOW TO CITE THIS ARTICLE

V. Shapovalova, O. Nevzghoda, V. Shapovalov, A. Osyntseva, O. Lavoshnyk & V. Shapovalov. Antibiotic pharmacotherapy of pleurisy, pleural empyema, infections of various genesis: ABC and VED analysis. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 88–108 (2026). https://doi.org/10.15446/rcciquifa.v55n1.125038

Review article

The role of calcium ions and the calcineurin route in fungal virulence: a review

Francisco Patricio de Andrade Júnior*[1,2], Gleice Rayanne da Silva[1], Valgrícia Matias de Sousa [1] & Bagnólia Araújo Costa[1]*

[1] Faculty of Medical Sciences, State University of Piauí, Teresina, Brazil
[2] Postgraduate Program in Natural and Synthetic Bioactive Products, Federal University of Paraíba, João Pessoa, Paraíba, Brazil
*Corresponding author: juniorfarmacia.ufcg@outlook.com

Received: December 28, 2024
Corrected: September 26, 2025
Accepted: September 28, 2025

https://doi.org/10.15446/rcciquifa.v55n1.125068

SUMMARY

Introduction: Calcium ions (Ca^{2+}) play a fundamental role in regulating various cellular processes and are present in all living organisms. In microorganisms such as fungi, the Ca^{2+} signaling pathway supports essential functions for survival, including stress resistance, apoptosis, growth, reproduction, and virulence. **Objective:** This review aims to explore the importance of Ca^{2+} and the calcineurin pathway in the development and maintenance of fungal virulence. **Method:** This is a narrative review based on literature available in the PubMed and ScienceDirect databases, focusing on articles published between 2015 and 2024. **Results:** The findings reveal that the calcineurin pathway is crucial for several processes contributing to fungal virulence. Calcineurin becomes active through calmodulin, a protein that requires calcium ions for its activation. The main processes regulated by calcium and calcineurin include the formation and maintenance of cell walls, hyphae, and biofilms. Moreover, these components are involved in conidia formation and increase thermotolerance in certain pathogenic fungi. It is worth noting that calcineurin inhibitors, such as cyclosporine A and tacrolimus, have shown synergistic effects when combined with antifungal agents like fluconazole and micafungin. **Conclusions:** These findings highlight the potential of Ca^{2+} and the calcineurin pathway as promising pharmacological targets for the development of new antifungal strategies.

Keywords: Calcium; calcineurin; fungi; virulence.

RESUMEN

El papel de los iones de calcio y de la vía de calcineurina en la virulencia fúngica: una revisión

Introducción: Los iones de calcio (Ca^{2+}) desempeñan un papel fundamental en la regulación de diversos procesos celulares y están presentes en todos los organismos vivos. En microorganismos como los hongos, la vía de señalización del Ca^{2+} sostiene funciones esenciales para la supervivencia, incluyendo la resistencia al estrés, la apoptosis, el crecimiento, la reproducción y la virulencia. **Objetivo:** Esta revisión tiene como objetivo explorar la importancia del Ca^{2+} y de la vía de la calcineurina en el desarrollo y mantenimiento de la virulencia fúngica. **Metodología:** Se trata de una revisión narrativa basada en la literatura disponible en las bases de datos PubMed y ScienceDirect, con énfasis en artículos publicados entre 2015 y 2024. **Resultados:** Los resultados revelan que la vía de la calcineurina es crucial para diver-

sos procesos que contribuyen a la virulencia fúngica. La calcineurina se activa a través de la calmodulina, una proteína que requiere iones de calcio para su activación. Los principales procesos regulados por el calcio y la calcineurina incluyen la formación y el mantenimiento de las paredes celulares, las hifas y el biofilm. Además, estos componentes participan en la formación de conidios y aumentan la termotolerancia en ciertos hongos patógenos. Cabe destacar que los inhibidores de la calcineurina, como la ciclosporina A y el tacrolimus, han demostrado efectos sinérgicos cuando se combinan con agentes antifúngicos como el fluconazol y la micafungina. **Conclusiones:** Estos hallazgos destacan el potencial del Ca^{2+} y de la vía de la calcineurina como objetivos farmacológicos prometedores para el desarrollo de nuevas estrategias antifúngicas.

Palabras-clave: Calcio; calcineurina; hongos; virulencia.

RESUMO

O papel dos íons de cálcio e da via de calcineurina na virulência fúngica: uma revisão

Introdução: Os íons de cálcio (Ca^{2+}) desempenham um papel fundamental na regulação de diversos processos celulares e estão presentes em todos os organismos vivos. Em microrganismos como os fungos, a via de sinalização do Ca^{2+} apoia funções essenciais para a sobrevivência, incluindo resistência ao estresse, apoptose, crescimento, reprodução e virulência. **Objetivo:** Esta revisão tem como objetivo explorar a importância do Ca^{2+} e da via de calcineurina no desenvolvimento e manutenção da virulência fúngica. **Método:** Trata-se de uma revisão narrativa realizada com base na literatura disponível nas bases de dados *PubMed* e *ScienceDirect*, com foco em artigos publicados entre 2015 e 2024. **Resultados:** Os resultados revelam que a via de calcineurina é crucial para diversos processos que contribuem para a virulência fúngica. A calcineurina torna-se ativa por meio da calmodulina, uma proteína que necessita de íons de cálcio para sua ativação. Os principais processos regulados pelo cálcio e pela calcineurina incluem a formação e manutenção das paredes celulares, das hifas e do biofilme. Além disso, esses componentes estão envolvidos na formação de conídios e aumentam a termotolerância em certos fungos patogênicos. Vale destacar que os inibidores de calcineurina, como a ciclosporina A e o tacrolimo, demonstraram efeitos sinérgicos quando combinados com agentes antifúngicos como fluconazol e micafungina. **Conclusões**: Esses achados destacam o potencial do Ca^{2+} e da via de calcineurina como alvos farmacológicos promissores para o desenvolvimento de novas estratégias antifúngicas.

Palavras-chave: Cálcio; calcineurina; fungos; virulência.

1. INTRODUCTION

Fungi are considered eukaryotic beings. These microorganisms, called saprophytes, play a fundamental role in the decomposition of organic matter and in the process of photosynthesis for the production of carbon dioxide. They can promote pathogenic activity causing diseases in humans, animals and plants [1, 2].

These microorganisms can present in yeast or filamentous form. Yeasts are classified as single-celled fungi with vegetative growth through budding or fission, developing as a fruitful body through asexual reproduction. These microorganisms are tolerant of low levels of pH, temperature, nutritional conditions (carbohydrates) and oxygen [3]. While filamentous fungi, structurally, have interconnected tubular units called hyphae that adhere to cell surfaces and facilitate penetration through the cell wall, favoring pathogenicity in host cells from the activation of different signaling pathways involved with molecules signaling, such as calcium ions [4].

Calcium ions participate in essential cellular processes in the most diverse types of living beings [5]. Ca^{2+} signaling is conserved evolutionarily from prokaryotes and eukaryotes [6]. In fungi, they can regulate resistance to stress, apoptosis, growth, reproduction and virulence [7-11].

Virulence, is the capacity that the pathogenic microorganism has to invade the host's defenses and create favorable conditions for its colonization, growth and development, causing illness [12, 13]. The morphological change to the parasitic phase requires great transitions in the patterns of gene expression, which promotes the adaptation of the fungus to the host environment with consequent remodeling of the cell wall and metabolism, in order to favor its survival and virulence [14].

The participation of Ca^{2+} in the fungal virulence process occurs at different stages. In the morphological transition, the calcineurin pathway has been shown to be important, with the action of calcium being indispensable [15, 16]. In addition, during the germination of conidia, structures that participate in host recognition and infection, the calcineurin pathway is one of the main routes, confirming the importance of Ca^{2+} [17-19].

In general, in fungi, exist six types of Ca^{2+} transporters have been identified, including Ca^{2+}-ATPase, Ca^{2+}/H^{+} exchange [10], high-affinity calcium uptake system (HACS), low-affinity calcium uptake system (LACS), Transient receptor potential (TRP) and mitochondrial calcium uniporter (MCU) [10, 20, 21].

In the vacuole, the main intracellular calcium reserve in fungi, the transient receptor potential (TRP) channel, *TRPY1* (previously named *YVC1*), was found. Which has been shown to act in the virulence of pathogenic fungi, such as *Candida albicans*, *Aspergillus fumigatus* and *Magnaporthe oryzae* [20, 22-24]. Similarly, the plasma membrane Ca^{2+}-ATPase 1 (Pmc1) regulates cytosolic calcium levels by transporting them into the vacuole. A study in which Pmc1 was subjected to mutation, changing its functionality, found a reduction in virulence, with reduced fungal loads in the lungs and no evidence of brain infection [25].

In this context, the focus of the review involves the description of cellular components, functions and molecular mechanisms of the calcium-calcineurin signaling pathway, highlighting the attributions of this pathway and of calcium ions in fungal virulence.

2. METHODOLOGY

The present research is a narrative literature review [26], in which articles were searched in the databases: PubMed and Science Direct, using the following associated descriptors: "Calcium" AND "Fungal Virulence "," Calcium "AND" Mycelial Growth "," Calcium "AND" Hyphal Growth "," Calcium "and" Thermotolerance "," Calcium "AND" Cell Wall "," Calcium "AND" Biofilm "," Calcineurin "AND" Inhibitors "," Calcineurin "AND" Fungal Virulence "," Calcineurin "AND" Mycelial Growth "," Calcineurin "AND" Hyphal Growth "," Calcineurin "AND" Thermotolerance "," Calcineurin "AND" Cell wall "," Calcineurin " AND "Biofilm".

Articles published between 2015 and 2024, in English, were included, prioritizing the most recent publications that addressed the proposed theme. Works involving bacteria, helminths and protozoa, monographs, dissertations, theses and books, were excluded.

3. LITERATURE REVISION

3.1. General information about calcium in fungi

It has been elucidated that several organelles present in fungi are fundamental for intracellular calcium homeostasis, such as the plasma membrane, vacuoles and endoplasmic reticulum.

The plasma membrane is composed of a lipid bilayer and proteins that form a specialized structure capable of promoting high virulence. In addition, it is responsible for several cellular functions, such as protection barrier, promotion of virulence through the secretion of virulence factors, endocytosis, hyphae production, cell wall synthesis and transport of molecules and nutrients in favor of the concentration gradient [27-30].

On the surface of the plasma membrane, there are calcium channels that will allow the influx of calcium ions and activation of the calcineurin pathway that is involved in several adaptive virulence processes [31].

More specifically in fungi, such as *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, HACS has been identified. This system is formed by the proteins Cch1, Mid1 and Ecm7, where Cch1 is a homologue of the voltage-gated calcium channel α1 subunit in mammals. Cch1 and Mid1, interact with each other, forming the Cch1-Mid1 complex that acts as a channel for calcium in the plasma membrane, while Ecm7 is involved in the influx of Ca^{2+} mediated by HACS [10, 18, 32-34]. This system presents itself as the main alternative for the influx of Ca^{2+} in fungal cells, being activated by low concentrations of extracellular calcium and inactivated when the concentrations of this cation increase [18, 35].

In addition, LACS was identified in pathogenic fungi, which is activated when there is high availability of calcium in the extracellular environment and is composed of a single member, fig1, formed by four transmembrane domains. This system is directly associated with mycelial growth and conidiation [36-38].

Vacuoles, in its turn, are necessary organelles to maintain homeostasis in fungal cells, being involved in several essential biological processes, including endocytosis, maintenance of pH and salt balance, phosphate degradation, transmembrane transport and are characterized by being the main reserve of intracellular Ca^{2+} in fungi. Therefore, changes in this component can influence cellular functionality [39, 40].

In *Saccharomyces cerevisiae*, about 90% of the calcium ions are stored in the vacuole. Oxalate, phosphate and organic acids present in this organelle chelate a high amount of Ca^{2+}, and only a small portion is available for release in response to different stimuli [4].

Among the mechanisms involved in the capture of Ca^{2+} for vacuoles are the fusion of its membrane with secretory vesicles derived from the Golgi apparatus, the action of Ca^{2+}-ATPase mediated by the vacuole membrane Ca2+-ATPase (PMC1) and H^{+}/Ca^{2+}antiporter (VCX1) [41]. VCX1 has been shown to act by recovering basal $[Ca^{2+}]_c$ after a peak of Ca^{2+} in *S. cerevisiae*, while vacuolar Ca^{2+} _ATPase Pmc1 maintains $[Ca^{2+}]_c$ [10].

In *Cryptococcus neoformans*, VCX1 is believed to have the main function of sequestering high concentrations of Ca^{2+} [25]. While a study carried out in Bauveria bassiana demonstrated that the action of Ca2 + _ATPase is important for the total speed of germination and conidiation, resistance to oxidative stress and cell wall, as well as for virulence [9].

In addition to these, TRPY1 or Yvc1, is a Ca^{2+} channel already identified in the vacuolar membrane of some species of fungi, such as *S. cerevisiae* [42]. This channel participates in the control of intracellular Ca^{2+} concentrations, responses to stress and virulence of pathogenic fungi, such as *Candida albicans*, *Aspergillus fumigatus* and *Magnaporthe oryzae* [20, 22].

In fungal cells, the endoplasmic reticulum (ER) is a crucial organelle, performing important functions, such as the metabolism of several proteins and sterols [43]. In addition, it is one of the intracellular compartments that participate in the regulation of intracellular Ca^{2+} [44].

In homeostasis, the concentration of cytosolic calcium in these cells remains at low levels, in contrast to the levels in the ER that are about a thousand times higher. This is mainly due to the action of binding proteins that favor their excess accumulation [43].

However, stimuli can trigger an opening of the calcium channels and a rapid increase in the concentration of calcium in the cytosol, which gives a versatile and universally used signal in fungal cell dynamics [44, 45, 46].

It has been shown that the Ca^{2+}-Calmodulin (CaM) signaling pathway depends on the intracellular calcium of the endoplasmic reticulum, the association of the endoplasmic reticulum and the release of calcium from the lumen of the phagosome, which regulate the recruitment of Rubicon, a negative autophagy regulator, for the sustained production of phosphatidylinositol-3-phosphate (PtdIns3P), assembly of NADPH (nicotinamide and adenine dinucleotide phosphate) oxidase and formation of LAPosome [46, 47].

The continuity of the nuclear membrane with the ER has been described. These contact sites, such as MECA (mitochondria-RE-anchor cortex) and ERMES (ER and mitochondrial meeting structures), connect the ER to the mitochondria [48, 49]. They provide a direct and agile transport of metabolites between organelles [50].

The role of ERMES in Ca^{2+} homeostasis has been seen in previous studies, in which imaging techniques have shown that excess Ca^{2+} in ER may be released into mitochondria, acting in the prevention of possible cytotoxic effects and modulating mitochondrial activity [43]. The action of Ca^{2+} present in the ER is also important for the process of cellular apoptosis, especially produced by stress in this organelle [51].

Thus, different fungal structures present themselves as sources of Ca^{2+} that participate in the calcineurin (CN) activation process. Structurally, CN is a heterodimer containing a catalytic subunit called calcineurin A (CnA) and a regulatory subunit, called calcineurin B (CnB) [31, 37].

Once there is an influx of Ca^{2+} by HACS, LACS or unknown calcium channels or its release by vacuoles and endoplasmic reticulum, four of these ions bind to CaM, forming the $4Ca^{2+}$ - Calmodulin (CaM) complex, which in turn, will be able to stimulate calcineurin (CN), increasing the phosphatase activity of CN, allowing dephosphorylation of the calcineurin-dependent transcription factor (CrzA). This will be translocated to the nucleus allowing the transcription of calcium-responsive genes, by binding to the calcineurin-dependent response element (CDRE) [18, 31, 37]. Another important protein is the root clavata-homolog 1 (Rch1) protein, which acts as a negative regulator of calcium uptake when the intracellular concentrations of this cation are high [38]. In a study carried out in *S. cerevisiae*, it was observed that once CDRE is activated, there will be an induction of Rch1 production in the endoplasmic reticulum (ER), which will be transported through the secretory pathway of the ER / Golgi Complex to the membrane, inhibiting the influx of calcium ions (Figure 1) [38].

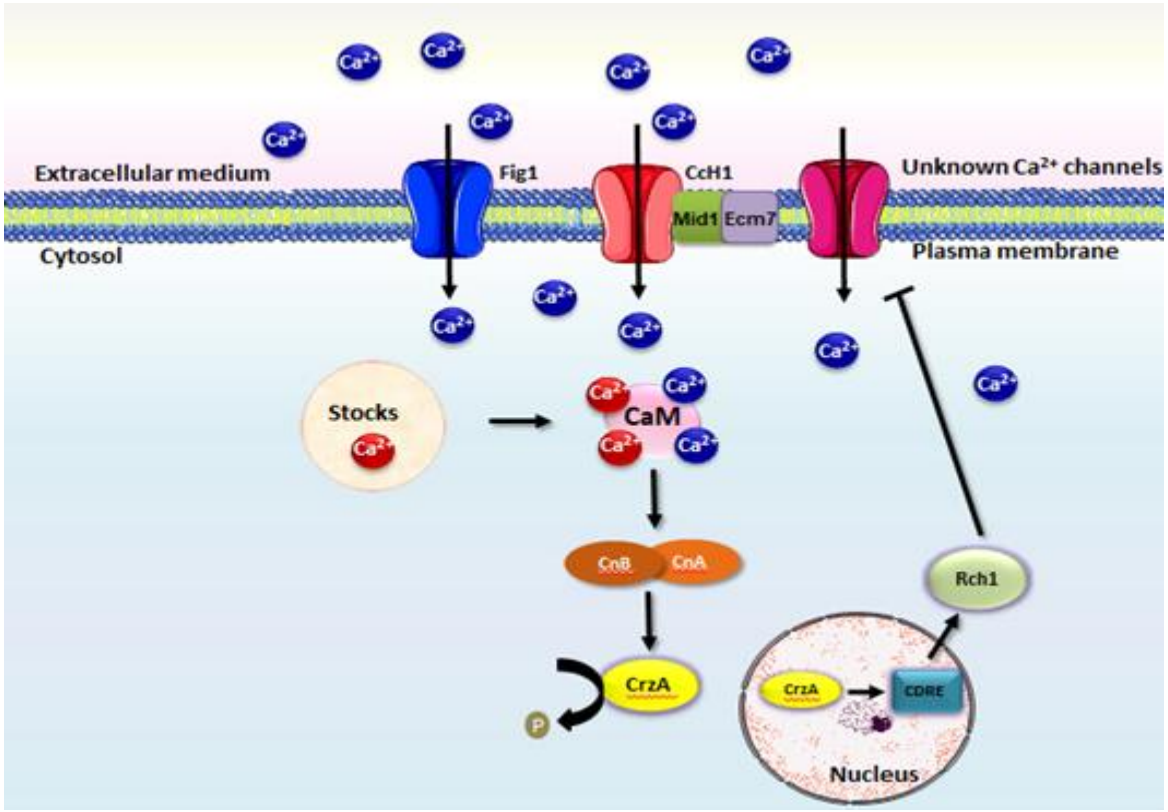


Figure 1. Calcineurin pathway in fungal. Caption: CaM = calmodulin; CDRE = calcineurin-dependent response element; CnA = calcineurin A; CnB = calcineurin B; CrzA = calcineurin-dependent transcription factor; Rch1 = Protein root clavata-homolog 1.

3.2. Cell wall

The fungal cell wall consists of an important structure, being responsible for both protection against stress and the host's immune system. Molecularly, this virulence factor is formed by an internal matrix composed of β-glucans and chitin, which together give the fungal cell rigidity [52].

It has been shown that the deletion of the subunit A of calcineurin has caused a decrease in the ability of strains of *C. albicans* to withstand osmotic stress, indicating that calcium and the calcineurin pathway may influence the plasticity of the cell wall [52].

Studies have indicated that there is participation of the calcineurin pathway in the cell wall integrity pathway (CWI) that is responsible for allowing the formation of specific genes involved in the fungal cell wall biogenesis [53-55].

In *A. fumigatus*, it was evidenced that the deletion of the CnaA gene, responsible for encoding the A subunit of calcineurin, caused a loss of the growth phenotype. This may be associated with the fact that CnaA regulates the phosphorylation of a mitogen activated kinase (MpkA), involved in the CWI pathway. The non-functioning of this kinase results in the absence of expression of genes involved in the synthesis of the cell wall with the emergence of compact colonies and reduced filamentation. In addition, the deletion of CnaA causes in the absence of CrzA that encodes the expression of ChsB (chitin synthase B), responsible for encoding the enzyme chitin synthase involved in the synthesis of chitin, one of the main components of the fungal cell wall [56, 57].

3.3. Conidia

Conidia are specialized structures produced at the end of the asexual life cycle of several filamentous fungi. They have intra and extracellular characteristics that give them the ability to survive in unfavorable conditions such as thermal stress, dehydration, osmotic pressure and variations in pH and temperature. Among its functions, fungi dispersion and environmental persistence stand out [58, 59]. In pathogenic species, they also participate in the host's recognition and infection process, acting as "infectious particles" of fungi [60].

The beginning of the infection process is characterized by the adhesion of the pathogen to the host, being indispensable for growth, persistence and fungal pathogenesis. Studies have shown that conidia preferentially adhere to proteins and carbohydrate fractions, as well as to constituents of the basal lamina. And that, a series of proteins present on the surface of the conidia mediate the adhesion to the cellular components. As examples, we have Lectin A and the extracellular protein of the thaumatin domain AfCalAp (Afu3g09690) [60, 61].

Undoubtedly, conidia formation is of great importance in fungal virulence. Recent studies have reported the influence of calcium ions on a wide range of cellular processes in filamentous fungi, such as production, sporulation and germination of conidia [19, 62].

Magnaporthe oryzae conidia were treated with four Ca^{2+} signaling inhibitors: a Ca^{2+} chelating agent, eghazic acid (EGTA); a calmodulin II kinase (CaMKII) inhibitor, KN-93; a Cch1 calcium channel protein inhibitor, verapamil; and a Ca^{2+}-ATPase inhibitor, CPA. The results showed that all tested inhibitors decreased the germination of conidia. In addition, the elongation of the germ tube was also altered by the inhibitors [19]. Similar results were found by Zhang *et al.* [41] in *Arthrobotrys oligospora*, in which blocking calcium absorption led to a reduction in the rate of vegetative growth, resulting in the absence of conidia.

The involvement of calcineurin in the regulation of the production and germination processes of conidia has been widely described [17]. In *Aspergillus* spp. the CrzA transcription factor acts downstream in order to favor the germination process. The calcineurin signaling pathway is important in morphogenesis/dimorphism and fungal virulence, where its activation is mediated by several other regulatory pathways that depend on stress factors to promote greater transcription [63, 64].

The deletion of the Mid1 and Cch1 genes has been shown to affect HACS and consequently essential processes such as growth and production of conidia, however the importance of HACS for virulence depends directly on the fungal fungal species [10, 41].

The genetic replacement of the CrzA locus in *Aspergillus fumigatus* resulted in a strain with significant problems in the growth of polarized hyphae, in the structure of the cell wall, in asexual development and in the germination of conidia [63].

In the investigation of the heat shock proteins (HSP)-calcineurin pathway, more specifically, in Hsp90 and Hsp70, it was observed that a negative modulation on this pathway, promoting the inhibition of the conidial morphogenesis in *Aspergillus flavus* [11].

3.4. Hyphae

Hyphae and pseudo-hyphae are structures that allow fungi to invade tissues, as well as give these microorganisms resistance to the phagocytosis process. Pseudo-hyphae are morphologically elliptical in shape and long multicellular, with the presence of constriction between the mother cell and the length of the filament, whereas hyphae are multicellular tubular cells, with no constriction between the mother cell and the filament [65-67].

Several factors can contribute to the development of these fungal structures. In the case of pseudo-hyphae, it has been shown that pH 6.0 and temperatures of 35ºC induce growth. Regarding hyphae, growth is benefited from temperatures of 37ºC, N-acetyl glucosamine, hypoxia, hypercapnia and alkaline pH in vitro, in addition to calcium ions [66, 68].

It has been shown that calcium in fungal cells causes the depolymerization of actin, allowing the fusion of secretory vesicles present at the end of the hyphae with consequent secretion of hyphae specific cell wall proteins (CWPs), providing material for the structure of the cell wall, allowing the rapid phases of cell extension. Thus, this process is associated with the rapid growth of these fungal structures. It is believed that the calcium needed to perform this function is derived, mainly from Cch1 and Mid1 and that calcineurin plays a crucial role in this process [69-76].

A study carried out in Aspergillus fumigatus reported that when deleting subunit A or B or both, from the CN, the microorganism showed defects in hyphal growth [71]. Thus, it is evident that mutations or modifications in calcineurin cause inhibition of the extension of hyphae during the invasion process, causing less virulence, lessening the infectious process and consequent decrease in mortality rates [18].

Another component responsible for the control of calcium and hyphal growth is SPF1 which presents itself as a P-type ATPase present in the endoplasmic reticulum of fungi. In C. albicans species, the SPF1 deletion has been shown to affect the growth rate of the microorganism. In addition, it was shown that this deletion resulted in increased chitin synthesis, an important component of the fungal wall. The increase in this polysaccharide is directly associated with stress [75]. Thus, it has been identified that the deletion of SPF1 leads to an increase in the abnormal content of cytoplasmic calcium, with a decreased response to stress, as well as attenuation of virulence [75].

3.5. Biofilm

Biofilms are presented as a set of microbial cells protected by a polymeric extracellular matrix, constituted mainly by polysaccharides that have the ability to strongly adhere to biotic and abiotic surfaces, favoring the development of infections, especially hospital infections [77, 78]. Furthermore, this virulence factor is complex, since it is responsible for considerable resistance to antifungals and the host's defenses [79].

The deletion of genes such as MidA, CchA, CrzA or CnaA in Aspergillus niger, has shown a reduction in the concentration of intracellular calcium, causing an inhibition of biofilm formation [56]. Thus, there was the quantification of several groups of genes ags1, ags2 and ags3 that encode α-1,3-glucan, important for mycelial adhesion; genes related to galactosaminogalactane (GAG) (uge3, uge5, agd3, gtb3), which act in adhesion processes; genes responsible for chitin synthesis (chsB, chsD) and β-1-3-glucan synthesis (Fska). Thus, the authors showed that genes related to GAG and α-1,3-glucan had their expression reduced due to decreased concentrations of intracellular Ca^{2+}, causing a reduction in the biofilm's adhesion capacity, in addition, and chsB and Chsd were regulated negatively, with a decrease in chitin production [56]. Furthermore, it has been elucidated that ueg3 is essential for the biofilm formation process and the reduction of intracellular calcium causes its depletion, causing defects in the constitution of biofilm [80].

One of the most important characteristics for biofilm formation is hydrophobicity, due to the fact that microorganisms must be able to adhere to hydrophobic surfaces, due to the secretion of proteins called hydrophobins. The RodA gene, which expresses the hydrophobin

RodA, showed a reduced level in *A. niger* with deletion of MidA, CchA, CrzA or CnaA [56], due to the reduction of intracellular calcium.

3.6. Thermotolerance

It is believed that when fungi such as *C. neoformans* are exposed to about 37 °C, CN activation occurs, which will cause the migration of Crz1 to the nucleus, however, when excluding Crz1 in mutant microorganisms it was shown that the growth continued for 37ºC, but which was totally inhibited at 39 °C [37].

When studying other targets present in the calcineurin pathway in C. neoformans Park et al. [70] showed that blocking RNA-binding proteins such as Lhp1, responsible for adapting to thermal stress, caused greater sensitivity to temperature changes, suggesting that this pathway contributes to the greater thermotolerance and survival of this pathogen [16, 70, 81-84].

3.7. Calcineurin pathway as a pharmacological target

Calcineurin inhibitors associated with antifungal agents have demonstrated synergistic effects, suggesting that calcineurin may present itself as an interesting target for future antifungal drugs [18].

Calcineurin activity can be inhibited by binding FK506 (tacrolimus) and cyclosporin A (CsA) to the heterodimer [85].

Studies have shown that the use of calcineurin inhibitor CsA and tacrolimus associated with fluconazole, caused a decrease in the Minimum Inhibitory Concentration (MIC) in strains of *C. albicans* [86, 87]. While FK506 showed synergism with fluconazole and micafungin against strains of *C. neoformans* and *M. circinelloides* [15, 18].

In addition to calcineurin itself, other targets are interesting within the pathway, such as heat shock protein 90 (HSP90), which is a molecular chaperone preserved in bacteria and fungi of medical importance, stabilizing calcineurin and contributing to resistance mechanisms [88, 89]. Thus, the inhibition of this protein has demonstrated an improvement in antifungal activity. Currently, several natural products such as ansamycin, geldanamycin and radicicol are able to inhibit it [88].

4. CONCLUSIONS

Further studies are needed to understand the influence of the different elements that compose the calcineurin pathway and those responsible for the regulation of intracellular Ca^{2+} in different fungal genera of medical importance, in order to highlight targets that are increasingly specific against these pathogens and that present themselves as possibilities for new therapeutic approaches in antifungal pharmacotherapy.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

REFERENCES

1. M.A. Ruggiero, D.P. Gordon, T.M. Orrell, N. Bailly, T. Bourgoin, R.C. Brusca, T. Cavalier-Smith, M.D. Guiry & P.M. Kirk. Correction: A higher level classification of all living organisms. *PLoS One*, **10**(4), e0130114 (2015). https://doi.org/10.1371/journal.pone.0130114
2. C.J.A. Silva & D.J.N. Malta. A importância dos fungos na biotecnologia. *Cadernos de Graduação*, **2**(3),

49-66 (2016). URL: https://periodicos.grupotiradentes.com/unitsaude/article/view/3210/2080
3. L. Vuyst, H. Harth, S. Van Kerrebroeck & F. Leroy. Yeast diversity of sourdoughs and associated metabolic properties and functionalities. *International Journal of Food Microbiology*, **239**, 26–34 (2016). https://doi.org/10.1016/j.ijfoodmicro.2016.07.018
4. M. Lange & E. Peiter. Cytosolic free calcium dynamics as related to hyphal and colony growth in the filamentous fungal pathogen *Colletotrichum graminicola*. *Fungal Genetics and Biology*, **91**, 55–65 (2016). https://doi.org/10.1016/j.fgb.2016.04.001
5. E. Carafoli & J. Krebs. Why calcium? How calcium became the best communicator. *The Journal of Biological Chemistry*, **291**(40), 20849–20857 (2016). https://doi.org/10.1074/jbc.R116.735894
6. A, Roy, A. Kumar, D. Baruah & R. Tamuli. Calcium signaling is involved in diverse cellular processes in fungi. *Mycology*, **12**(1), 10–24 (2020). https://doi.org/10.1080/21501203.2020.1785962
7. S. Wang, X. Liu, H. Qian, S. Zhang & L. Lu. Calcineurin and calcium channel CchA coordinate the salt stress response by regulating cytoplasmic Ca^{2+} homeostasis in *Aspergillus nidulans*. *Applied and Environmental Microbiology*, **82**(11), 3420–3430 (2016). https://doi.org/10.1128/AEM.00330-16
8. H. Tian, S. Qu, Y. Wang, Z. Lu, M. Zhang, Y. Gan, P. Zhang & J. Tian. Calcium and oxidative stress mediate perillaldehyde-induced apoptosis in *Candida albicans*. *Applied Microbiology and Biotechnology*. **101**, 3335–3345 (2017). https://doi.org/10.1007/s00253-017-8146-3
9. J. Wang, X.-G. Zhu, S.-H. Ying & M.-G. Feng. Differential roles for six P-type calcium ATPases in sustaining intracellular Ca^{2+} homeostasis, asexual cycle and environmental fitness of *Beauveria bassiana*. *Scientific Reports*. **7**, 1420 (2017). https://doi.org/10.1038/s41598-017-01570-1
10. M. Lange & E. Peiter. Calcium transport proteins in fungi: The phylogenetic diversity of their relevance for growth, virulence, and stress resistance. *Frontiers in Microbiology*, **10**, 3100 (2020). https://doi.org/10.3389/fmicb.2019.03100
11. S.K. Shishodia, S. Tiwari, S. Hoda, P. Vijayaraghavan & J. Shankar. SEM and qRT-PCR revealed quercetin inhibits morphogenesis of *Aspergillus flavus* conidia via modulating calcineurin-Crz1 signalling pathway. *Mycology*, **11**(2), 118–125 (2020). https://doi.org/10.1080/21501203.2020.1711826
12. D. Chinnapun. Virulence factors involved in pathogenicity of dermatophytes. *Walailak Journal of Science and Technology* (*WJST*), **12**(7), 573–580 (2015). URL: https://wjst.wu.ac.th/index.php/wjst/article/view/1473/501
13. R.A. Cordeiro, J.S. Oliveira, D.S. Castelo-Branco, C.E. Teixeira, F.J. Marques, P.V. Bittencourt, *et al.* *Candida tropicalis* isolates obtained from veterinary sources show resistance to azoles and produce virulence factors. *Medical Mycology*, **53**(2), 145–152 (2015). https://doi.org/10.1093/mmy/myu081
14. J.S. Curcio, J.D. Paccez, E. Novaes, M. Brock & C.M.A. Soares. Cell wall synthesis, development of hyphae and metabolic pathways are processes potentially regulated by microRNAs produced between the morphological stages of *Paracoccidioides brasiliensis*. *Frontiers in Microbiology*. **9**, 3057 (2018). https://doi.org/10.3389/fmicb.2018.03057
15. S.C. Lee, A. Li, S. Calo, M. Inoue, N.K. Tonthat, J.M. Bain, *et al.* Calcineurin orchestrates dimorphic transitions, antifungal drug responses and host-pathogen interactions of the pathogenic mucoralean fungus *Mucor circinelloides*. *Molecular Microbiology*, **97**(5), 844–865 (2015). https://doi.org/10.1111/mmi.13071
16. C. Fu, N. Donadio, M.E. Cardenas & J. Heitman. Dissecting the roles of the calcineurin pathway in unisexual reproduction, stress responses, and virulence in *Cryptococcus deneoformans*. *Genetics*, **208**(2), 639–653 (2018). https://doi.org/10.1534/genetics.117.300422
17. A. Shah, S. Kannambath, S. Herbst, A. Rogers, S. Soresi, M. Carby, A. Reed, S. Mostowy, M.C. Fisher, S. Shaunak & D.P. Armstrong-James. Calcineurin orchestrates lateral transfer of *Aspergillus fumigatus* during macrophage cell death. *American Journal of Respiratory and Critical Care Medicine*, **194**(9), 1127–1139 (2016). https://doi.org/10.1164/rccm.201601-0070OC
18. H.S. Park, S.C. Lee, M.E. Cardenas & J. Heitman. Calcium-calmodulin-calcineurin signaling: A globally conserved virulence cascade in eukaryotic microbial pathogens. *Cell Host & Microbe*, **26**(4), 453–462 (2019). https://doi.org/10.1016/j.chom.2019.08.004

19. W. Huang, X. Liu, X. Zhou, X. Wang, X. Liu & H. Liu. Calcium signaling is suppressed in *Magnaporthe oryzae* conidia by *Bacillus cereus* HS24. *Phytopathology*, **110**(2), 309–316 (2020). https://doi.org/10.1094/phyto-08-18-0311-r
20. R. Tisi, M. Rigamonti, S. Groppi & F. Belotti. Calcium homeostasis and signaling in fungi and their relevance for pathogenicity of yeasts and filamentous fungi. *AIMS Molecular Science*, **3**(4), 505-549 (2016). https://doi.org/10.3934/molsci.2016.4.505
21. M. Lange, F. Weihmann, I. Schliebner, R. Horbach, H.B. Deising, S.G. Wirsel & E. Peiter. The transient receptor potential (TRP) channel family in *Colletotrichum graminicola*: A molecular and physiological analysis. *PLoS One*, **11**(6), e0158561 (2016). https://doi.org/10.1371/journal.pone.0158561
22. Y. Gao, W. Li, X. Liu, F. Gao & X. Zhao. Reversing effect and mechanism of soluble resistance-related calcium-binding protein on multidrug resistance in human lung cancer A549/DDP cells. *Molecular Medicine Reports*, **11**(3), 2118–2124 (2015). https://doi.org/10.3892/mmr.2014.2936
23. S. Hamamoto, Y. Mori, I. Yabe & N. Uozumi. *In vitro* and *in vivo* characterization of modulation of the vacuolar cation channel TRPY1 from *Saccharomyces cerevisiae*. *The FEBS Journal*, **285**(6), 1146–1161 (2018). https://doi.org/10.1111/febs.14399
24. P. Thapak, B. Vaidya, H.C. Joshi, J.N. Singh & S.S. Sharma. Therapeutic potential of pharmacological agents targeting TRP channels in CNS disorders. *Pharmacological Research*, **159**, 105026 (2020). https://doi.org/10.1016/j.phrs.2020.105026
25. E.D. Squizani, N.K. Oliveira, J.C.V. Reuwsaat, B.M. Marques, W. Lopes, A.L. Gerber, *et al.* Cryptococcal dissemination to the central nervous system requires the vacuolar calcium transporter Pmc1. *Cellular Microbiology*, **20**, e12803 (2018). https://doi.org/10.1111/cmi.12803
26. F.P. Andrade Júnior, J.M.M. Sousa, H.I.F. Magalhães & E.O. Lima. Sobrevivendo na ciência em tempos de pandemia: como lidar?. *HOLOS*, **37**(4), e11599 (2021). URL: https://www2.ifrn.edu.br/ojs/index.php/HOLOS/article/view/11599/pdf
27. R.A. Hall. Dressed to impress: impact of environmental adaptation on the *Candida albicans* cell wall. *Molecular Microbiology*, **97**(1), 7–17 (2015). https://doi.org/10.1111/mmi.13020
28. A. Rella, A.M. Farnoud & M. Del Poeta. Plasma membrane lipids and their role in fungal virulence. *Progress in Lipid Research*, **61**, 63–72 (2016). https://doi.org/10.1016/j.plipres.2015.11.003
29. Y. Wang, J. Wang, J. Cheng, D. Xu & L. Jiang. Genetic interactions between the Golgi Ca^{2+}/H^{+} exchanger Gdt1 and the plasma membrane calcium channel Cch1/Mid1 in the regulation of calcium homeostasis, stress response and virulence in *Candida albicans*. *FEMS Yeast Research*, **15**(7), fov069 (2015). https://doi.org/10.1093/femsyr/fov069
30. H. Akiyama & Y. Hirabayashi. A novel function for glucocerebrosidase as a regulator of sterylglucoside metabolism. *Biochimica et Biophysica Acta* (*BBA*) - *General Subjects*, **1861**(10), 2507–2514 (2017). https://doi.org/10.1016/j.bbagen.2017.06.003
31. V. Yadav & J. Heitman. Calcineurin: The Achilles' heel of fungal pathogens. *PLoS Pathogens*, **19**(7), e1011445 (2023). https://doi.org/10.1371/journal.ppat.1011445
32. C.V. Giuraniuc, C. Parkin, M.C. Almeida, M. Fricker, P. Shadmani, S. Nye, *et al.* Dynamic calcium-mediated stress response and recovery signatures in the fungal pathogen, *Candida albicans*. *mBio*, **14**(5), e0115723 (2023). https://doi.org/10.1128/mbio.01157-23
33. T. Kato, A. Kubo, T. Nagayama, S. Kume, C. Tanaka, Y. Nakayama, K. Iida & H. Iida. Genetic analysis of the regulation of the voltage-gated calcium channel homolog Cch1 by the γ subunit homolog Ecm7 and cortical ER protein Scs2 in yeast. *PLoS One*, **12**(7), e0181436 (2017). https://doi.org/10.1371/journal.pone.0181436
34. M. Xie, X. Zhou, Y. Xia & Y. Cao. Mid1 affects ion transport, cell wall integrity, and host penetration of the entomopathogenic fungus *Metarhizium acridum*. *Applied Microbiology and Biotechnology*, **103**, 1801–1810 (2019). https://doi.org/10.1007/s00253-018-09589-8
35. H.S. Kim, J.-E. Kim, H. Son, D. Frailey, R. Cirino, Y.W. Lee, R. Duncan, K.J. Czymmek & S. Kang. Roles of three Fusarium graminearum membrane Ca^{2+} channels in the formation of Ca^{2+} signatures, growth, development, pathogenicity and mycotoxin production. *Fungal Genetics and Biology*, **111**, 30–46 (2018). https://doi.org/10.1016/j.fgb.2017.11.005

36. W. Zhang, C. Hu, M. Hussain, J. Chen, M. Xiang & X. Liu. Role of low-affinity calcium system member *Fig1* homologous proteins in conidiation and trap-formation of nematode-trapping fungus *Arthrobotrys oligospora*. *Scientific Reports*, **9**, 4440 (2019). https://doi.org/10.1038/s41598-019-40493-x
37. A. Cacciotti, M. Beccaccioli & M. Reverberi. The CRZ1 transcription factor in plant fungi: regulation mechanism and impact on pathogenesis. *Molecular Biology Reports*, **51**, 647 (2024). https://doi.org/10.1007/s11033-024-09593-4
38. L. Jiang, H. Xu, M. Wei, Y. Gu, H. Yan, L. Pan & C. Wei. Transcriptional expression of *PHR2* is positively controlled by the calcium signaling transcription factor Crz1 through its binding motif in the promoter. *Microbiology Spectrum*, **12**(1), e0168923 (2024). https://doi.org/10.1128/spectrum.01689-23
39. A. Takeuchi, B. Kim & S. Matsuoka. The destiny of Ca^{2+} released by mitochondria. *The Journal of Physiological Sciences*, **65**(1), 11–24 (2015). https://doi.org/10.1007/s12576-014-0326-7
40. Z. Gong & A.J. Karlsson. Translocation of cell-penetrating peptides into *Candida* fungal pathogens. *Protein Science*, **26**(9), 1714–1725 (2017). https://doi.org/10.1002/pro.3203
41. Y. Zhang, H. Jiang & L. Lu. Calcium cation cycling and signaling pathways in fungi. In: D. Hoffmeister & M. Gressler (editors) *Biology of the Fungal Cell*. (The Mycota, vol 8). Springer, Cham, 2019; pp. 111–123. https://doi.org/10.1007/978-3-030-05448-9_7
42. M. Amini, H. Wang, A. Belkacemi, M. Jung, A. Bertl, G. Schlenstedt, V. Flockerzi & A. Beck. Identification of inhibitory Ca^{2+} binding sites in the upper vestibule of the yeast vacuolar TRP channel. *iScience*, **11**, 1–12 (2019). https://doi.org/10.1016/j.isci.2018.11.037
43. D. Kundu & R. Pasrija. The ERMES (Endoplasmic Reticulum and Mitochondria Encounter Structures) mediated functions in fungi. *Mitochondrion*, **52**, 89–99 (2020). https://doi.org/10.1016/j.mito.2020.02.010
44. H. Wang, R. Gao, Y. Zhang & L. Lu. The versatility of the putative transient receptor potential ion channels in regulating the calcium signaling in *Aspergillus nidulans*. *mSphere*, **8**(6), e0054923 (2023). https://doi.org/10.1128/msphere.00549-23
45. X. Zhang, J. Shao, A. Chen, C. Shang, X. Hu, S. Luo, M. Lei, L. Peng & Q. Zeng. Effects of cadmium on calcium homeostasis in the white-rot fungus *Phanerochaete chrysosporium*. *Ecotoxicology and Environmental Safety*, **157**, 95–101 (2018). https://doi.org/10.1016/j.ecoenv.2018.03.071
46. C.V. Giuraniuc, C. Parkin, M.C. Almeida, M. Fricker, P. Shadmani, S. Nye, *et al*. Dynamic calcium-mediated stress response and recovery signatures in the fungal pathogen, *Candida albicans*. *mBio*, **14**(5), e01157-23 (2023). https://doi.org/10.1128/mbio.01157-23
47. I. Kyrmizi, H. Ferreira, A. Carvalho, J.A.L. Figueroa, P. Zarmpas, C. Cunha, *et al.* Calcium sequestration by fungal melanin inhibits calcium-calmodulin signalling to prevent LC3-associated phagocytosis. *Nature Microbiology*, **3**, 791–803 (2018). https://doi.org/10.1038/s41564-018-0167-x
48. A. González-Montoro, K. Auffarth, C. Hönscher, M. Bohnert, T. Becker, B. Warscheid, F. Reggiori, M. van der Laan, F. Fröhlich & C. Ungermann. Vps39 interacts with Tom40 to establish one of two functionally distinct vacuole-mitochondria contact sites. *Developmental Cell*, **45**(5), 621–636.e7 (2018). https://doi.org/10.1016/j.devcel.2018.05.011
49. Y. Tamura, S. Kawano & T. Endo. Organelle contact zones as sites for lipid transfer. *The Journal of Biochemistry*, **165**(2), 115–123 (2019). https://doi.org/10.1093/jb/mvy088
50. S. Cohen, A.M. Valm & J. Lippincott-Schwartz. Interacting organelles. *Current Opinion in Cell Biology*, **53**, 84–91 (2018). https://doi.org/10.1016/j.ceb.2018.06.003
51. A.P. Gonçalves, J. Heller, A. Daskalov, A. Videira & N.L. Glass. Regulated forms of cell death in fungi. *Frontiers in Microbiology*, **8**, 1837 (2017). https://doi.org/10.3389/fmicb.2017.01837
52. N.A.R. Gow & M.D. Lenardon. Architecture of the dynamic fungal cell wall. *Nature Reviews: Microbiology*, **21**(4), 248–259 (2023). https://doi.org/10.1038/s41579-022-00796-9
53. H. Martín & M. Molina. Special issue "The fungal cell wall integrity pathway". *Journal of Fungi* (Basel, Switzerland), **9**(3), 293 (2023). https://doi.org/10.3390/jof9030293
54. I. Geoghegan, G. Steinberg & S. Gurr. The role of the fungal cell wall in the infection of plants. *Trends in microbiology*. **25**(12), 957–967 (2017). https://doi.org/10.1016/j.tim.2017.05.015

55. N.A.R. Gow, J. Latge & C.A. Munro. The fungal cell wall: Structure, biosynthesis, and function. *Microbiology Spectrum*, **5**(3), FUNK-0035-2016 (2017). https://doi.org/10.1128/microbiolspec.funk-0035-2016
56. L. Liu, B. Yu, W. Sun, C. Liang, H. Ying, S. Zhou, H. Niu, Y. Wang, D. Liu & Y. Chen. Calcineurin signaling pathway influences *Aspergillus niger* biofilm formation by affecting hydrophobicity and cell wall integrity. *Biotechnology for Biofuels and Bioproducts*, **13**, 54 (2020). https://doi.org/10.1186/s13068-020-01692-1
57. P. Wang, B. Li, Y.T. Pan, Y.Z. Zhang, D. W. Li & L. Huang. Calcineurin-responsive transcription factor CgCrzA is required for cell wall integrity and infection-related morphogenesis in *Colletotrichum gloeosporioides*. *The Plant Pathology Journal*, **36**(5), 385–397 (2020). https://doi.org/10.5423/ppj.oa.04.2020.0071
58. G.U. Braga, D.E. Rangel, É.K. Fernandes, S.D. Flint & D.W. Roberts. Molecular and physiological effects of environmental UV radiation on fungal conidia. *Current Genetics*, **61**(3), 405–425 (2015). https://doi.org/10.1007/s00294-015-0483-0
59. T.J.H. Baltussen, J. Zoll, P.E. Verweij & W.J.G. Melchers. Molecular mechanisms of conidial germination in *Aspergillus* spp. *Microbiology and Molecular Biology Reviews*, **84**(1), e00049-19 (2019). https://doi.org/10.1128/mmbr.00049-19
60. A. Yoshimi, K. Miyazawa, M. Kawauchi & K. Abe. Cell wall integrity and its industrial applications in filamentous fungi. *Journal of Fungi* (Basel, Switzerland), **8**(5), 435 (2022). https://doi.org/10.3390/jof8050435
61. J.G. Vasselli & B.D. Shaw. Fungal spore attachment to substrata. *Fungal Biology Reviews*, **41**, 2-9 (2022). https://doi.org/10.1016/j.fbr.2022.03.002
62. K. Chittem, M.F.R. Khan & R.S. Goswami. Efficacy of precipitated calcium carbonate in managing fusarium root rot of field pea. *Phytoparasitica*, **44**, 295–303 (2016). https://doi.org/10.1007/s12600-016-0528-z
63. X. Li, C. Zhang & L. Lu. Fungal calcineurin complex as an antifungal target: From past to present to future. *Fungal Biology Reviews*, **43**, 100290 (2023). https://doi.org/10.1016/j.fbr.2022.10.003
64. R. Ancuceanu, M.V. Hovaneț, M. Cojocaru-Toma, A.I. Anghel & M. Dinu. Potential antifungal targets for *Aspergillus* sp. from the calcineurin and heat shock protein pathways. *International Journal of Molecular Sciences*, **23**(20), 12543 (2022). https://doi.org/10.3390/ijms232012543
65. L. Chevalier, M. Pinar, R. Le Borgne, C. Durieu, M. A. Peñalva, A. Boudaoud & N. Minc. Cell wall dynamics stabilize tip growth in a filamentous fungus. *PLoS Biology*, **21**(1), e3001981 (2023). https://doi.org/10.1371/journal.pbio.3001981
66. H. Chen, X. Zhou, B. Ren & L. Cheng. The regulation of hyphae growth in *Candida albicans*. *Virulence*, **11**(1), 337–348 (2020). https://doi.org/10.1080/21505594.2020.1748930
67. R.W. Roberson. Subcellular structure and behaviour in fungal hyphae. *Journal of Microscopy*, **280**(2), 75–85 (2020). https://doi.org/10.1111/jmi.12945
68. T. Gao, L. Shi, T. Zhang, A. Ren, A. Jiang, H. Yu & M. Zhao. Cross talk between calcium and reactive oxygen species regulates hyphal branching and ganoderic acid biosynthesis in *Ganoderma lucidum* under copper stress. *Applied and Environmental Microbiology*, **84**(13), e00438-18 (2018). https://doi.org/10.1128/aem.00438-18
69. N. Takeshita, M. Evangelinos, L. Zhou, T. Serizawa, R.A. Somera-Fajardo, L. Lu, N. Takaya, G.U. Nienhaus & R. Fischer. Pulses of Ca^{2+} coordinate actin assembly and exocytosis for stepwise cell extension. *Proceedings of the National Academy of Sciences of the United States of America*. **114**(22), 5701–5706 (2017). https://doi.org/10.1073/pnas.1700204114
70. H.S. Park, E.W. Chow, C. Fu, E.J. Soderblom, M.A. Moseley, J. Heitman & M.E. Cardenas. Calcineurin targets involved in stress survival and fungal virulence. *PLoS Pathogens*, **12**(9), e1005873 (2016). https://doi.org/10.1371/journal.ppat.1005873
71. P.R. Juvvadi & W.J. Steinbach. Calcineurin orchestrates hyphal growth, septation, drug resistance and pathogenesis of *Aspergillus fumigatus*: Where do we go from here? *Pathogens* (Basel, Switzerland), **4**(4), 883–893 (2015). https://doi.org/10.3390/pathogens4040883

72. Y. Ishitsuka, N. Savage, Y. Li, A. Bergs, N. Grün, D. Kohler, R. Donnelly, G.U. Nienhaus, R. Fischer & N. Takeshita. Superresolution microscopy reveals a dynamic picture of cell polarity maintenance during directional growth. *Science Advances*, **1**(10), e1500947 (2015). https://doi.org/10.1126/sciadv.1500947
73. N. Takeshita. Coordinated process of polarized growth in filamentous fungi. *Bioscience, Biotechnology & Biochemistry*, **80**(9), 1693–1699 (2016). https://doi.org/10.1080/09168451.2016.1179092
74. L. Zhou, M. Evangelinos, V. Wernet, A.F. Eckert, Y. Ishitsuka, R. Fischer, G.U. Nienhaus & N. Takeshita. Superresolution and pulse-chase imaging reveal the role of vesicle transport in polar growth of fungal cells. *Science Advances*, **4**(1), e1701798 (2018). https://doi.org/10.1126/sciadv.1701798
75. Q. Yu, T. Ma, C. Ma, B. Zhang & M. Li. Multifunction of the ER *P*-type calcium pump Spf1 during hyphal development in *Candida albicans*. *Mycopathologia*, **184** ,573–583 (2019). https://doi.org/10.1007/s11046-019-00372-5
76. P. Zheng, T.A. Nguyen, J.Y. Wong, M. Lee, T.A. Nguyen, J.S. Fan, D. Yang & G. Jedd. Spitzenkörper assembly mechanisms reveal conserved features of fungal and metazoan polarity scaffolds. *Nature Communications*, **11**(1), 2830 (2020). https://doi.org/10.1038/s41467-020-16712-9
77. H.-C. Flemming, E.D. van Hullebusch, T.R. Neu, P.H. Nielsen, T. Seviour, P. Stoodley, J. Wingender & S. Wuertz. The biofilm matrix: multitasking in a shared space. *Nature Reviews: Microbiology*, **21**, 70–86 (2023). https://doi.org/10.1038/s41579-022-00791-0
78. C.B. Costa-Orlandi, J.C.O. Sardi, N.S. Pitangui, H.C. Oliveira, L. Scorzoni, M.C. Galeane, *et al.* Fungal biofilms and polymicrobial diseases. *Journal of Fungi* (Basel, Switzerland), **3**(2), 22 (2017). https://doi.org/10.3390/jof3020022
79. J.F. Kernien, B.D. Snarr, D.C. Sheppard & J.E. Nett. The interface between fungal biofilms and innate immunity. *Frontiers in Immunology*, **8**, 1968 (2018). https://doi.org/10.3389/fimmu.2017.01968
80. C.J. Lin, C. Sasse, J. Gerke, O. Valerius, H. Irmer, H. Frauendorf, *et al.* Transcription factor SomA is required for adhesion, development and virulence of the human pathogen *Aspergillus fumigatus*. *PLoS Pathogens*, **11**(11), e1005205 (2015). https://doi.org/10.1371/journal.ppat.1005205
81. V.E. Glazier, J.N. Kaur, N.T. Brown, A.A. Rivera & J.C. Panepinto. Puf4 regulates both splicing and decay of *HXL1* mRNA encoding the unfolded protein response transcription factor in *Cryptococcus neoformans*. *Eukaryotic Cell*, **14**(4), 385–395 (2015). https://doi.org/10.1128/ec.00273-14
82. S. Roque, M. Cerciat, I. Gaugué, L. Mora, A.G. Floch, M. Zamaroczy, V. Heurgué-Hamard & S. Kervestin. Interaction between the poly(A)-binding protein Pab1 and the eukaryotic release factor eRF3 regulates translation termination but not mRNA decay in *Saccharomyces cerevisiae*. *RNA* (New York, N.Y.), **21**, 124–134 (2015). https://doi.org/10.1261/rna.047282.114
83. F. Martani, F. Marano, S. Bertacchi, D. Porro & P. Branduardi. The *Saccharomyces cerevisiae* poly(A) binding protein Pab1 as a target for eliciting stress tolerant phenotypes. *Scientific Reports*, **5**, 18318 (2015). https://doi.org/10.1038/srep18318
84. Y.-E. Son, C. Fu, W.-H. Jung, S.-H. Oh, J.-H. Kwak, M.E. Cardenas, J. Heitman & H.-S. Park. Pbp1-interacting protein Mkt1 regulates virulence and sexual reproduction in *Cryptococcus neoformans*. *Frontiers in Cellular and Infection Microbiology*, **9**, 355 (2019). https://doi.org/10.3389/fcimb.2019.00355
85. P.R. Juvvadi, D. Fox, B.G. Bobay, M.J. Hoy, S.M.C. Gobeil, R.A. Venters, *et al.* Harnessing calcineurin-FK506-FKBP12 crystal structures from invasive fungal pathogens to develop antifungal agents. *Nature Communications*, **10**(1), 4275 (2019). https://doi.org/10.1038/s41467-019-12199-1
86. W. Jia, H. Zhang, C. Li, G. Li, X. Liu & J. Wei. The calcineurin inhibitor cyclosporine A synergistically enhances the susceptibility of *Candida albicans* biofilms to fluconazole by multiple mechanisms. *BMC Microbiology*, **16**(1), 113 (2016). https://doi.org/10.1186/s12866-016-0728-1
87. T. Wibawa, Nurrokhman, I. Baly, P.R. Daeli, G. Kartasasmita & N. Wijayanti. Cyclosporine A decreases the fluconazole minimum inhibitory concentration of *Candida albicans* clinical isolates but not biofilm formation and cell growth. *Tropical Biomedicine*, **32**(1), 176-182 (2015). URL: https://www.cabidigitallibrary.org/doi/pdf/10.5555/20153104132
88. F.H. Schopf, M.M. Biebl & J. Bichner. The HSP90 chaperone machinery. *Nature Reviews: Molecular Cell Biology*, **18**(6), 345-360 (2017). https://doi.org/10.1038/nrm.2017.39

89. K. Liao & L. Sun. Roles of the Hsp90-calcineurin pathway in the antifungal activity of honokiol. *Journal of Microbiology and Biotechnology*, **28**(7), 1086–1093 (2018). https://doi.org/10.4014/jmb.1801.01024

HOW TO CITE THIS ARTICLE

F.P. de Andrade Júnior, G. Rayanne da Silva, V.M. de Sousa & B. Araújo-Costa. The role of calcium ions and the calcineurin route in fungal virulence: a review. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 109–123 (2026). https://doi.org/10.15446/rcciquifa.v55n1.125068

Review article

Saccharomyces boulardii as an agent of systemic infection: an integrative review

Andry Louhanny Mendes Barbosa*, Júlia Beatriz Pereira de Souza & Egberto Santos Carmo

Federal University of Campina Grande – Cuité, PB, Brazil
*Correspondence author: andry.louhanny@estudante.ufcg.edu.br

Received: November 5, 2024
Corrected: September 27, 2025
Accepted: September 28, 2025

https://doi.org/10.15446/rcciquifa.v55n1.125070

SUMMARY

Objective: To analyze possible cases of infection by *Saccharomyces boulardii* worldwide from 2018 to 2023. **Methodology:** An integrative review was conducted using the Virtual Health Library, PubMed, and ScienceDirect databases, with the descriptors "probiotics", "*Saccharomyces boulardii*", and "fungemia," resulting in 11 articles. **Results:** A total of 69 cases of fungemia were identified in patients aged 3.5 to 93 years, with a mean age of 62 years and a male predominance. The main risk factors included antibiotic use, central venous catheterization, cancer, enteral nutrition, intensive care unit admission, and intubation. The most used antifungal was fluconazole. The fatality rate was 36.2%. **Conclusion:** Although probiotics containing *Saccharomyces boulardii* are generally used for their beneficial effects, it was found that, under certain circumstances, they may cause adverse events in some individuals. Therefore, the risk-benefit ratio should be carefully evaluated in each case.

Keywords: Invasive fungal infections; probiotic; fungemia.

RESUMO

***Saccharomyces boulardii* como agente de infecção sistêmica: uma revisão integrativa**

Objetivo: Analisar possíveis casos de infecção por *Saccharomyces boulardii* no mundo, no período de 2018 a 2023. **Metodologia:** Realizou-se uma revisão integrativa, nas bases de dados Biblioteca Virtual em Saúde, PubMed e ScienceDirect, usando os descritores "probiotics", "*Saccharomyces boulardii*" e "fungemia", incluindo ao final 11 artigos. **Resultados:** Observaram-se 69 casos de fungemia em pacientes com idades de 3 anos e meio a 93 anos, com média de 62 anos, e prevalência para o sexo masculino. Os principais fatores de risco foram: uso de antibióticos, uso de cateter venoso central, câncer, em nutrição enteral, estar em unidade de terapia intensiva e intubados. O antifúngico mais utilizado foi o fluconazol. A taxa de letalidade foi 36,2%. **Conclusão:** Embora os probióticos contendo *Saccharomyces boulardii* sejam utilizados por seus efeitos benéficos, averiguou-se que, sob determinadas circunstâncias, podem ocasionar eventos adversos a alguns indivíduos, devendo o risco-benefício ser avaliado em cada caso.

Palavras-chave: Infecções fúngicas invasivas; probiótico; fungemia.

RESUMEN

***Saccharomyces boulardii* como agente de infección sistémica: una revisión integradora**

Objetivo: Analizar los posibles casos de infección por *Saccharomyces boulardii* a nivel mundial, entre 2018 y 2023. **Metodología:** Se realizó una revisión integrativa en las bases de datos Biblioteca Virtual en Salud, PubMed y ScienceDirect, utilizando los descriptores «probióticos», «*Saccharomyces boulardii*» y «fungemia», incluyendo finalmente 11 artículos. **Resultados:** Se observaron 69 casos de fungemia en pacientes de entre 3,5 y 93 años, con una edad media de 62 años y una mayor prevalencia en hombres. Los principales factores de riesgo fueron: uso de antibióticos, catéter venoso central, cáncer, nutrición enteral, ingreso en la unidad de cuidados intensivos e intubación. El antifúngico más utilizado fue el fluconazol. La tasa de letalidad fue del 36,2 %. **Conclusión:** Si bien los probióticos que contienen *Saccharomyces boulardii* se utilizan por sus efectos beneficiosos, se ha descubierto que, en determinadas circunstancias, pueden causar efectos adversos en algunas personas, y la relación riesgo-beneficio debe evaluarse caso por caso.

Palabras clave: Infecciones fúngicas invasivas; probiótico; fungemia.

1. INTRODUCTION

The normal human intestinal microbiota comprises a diverse group of microorganisms that inhabit the gastrointestinal tract, playing a crucial role in maintaining gastrointestinal health and providing systemic benefits to the body through various important functions, including immune modulation. This microbiota is established at birth and evolves throughout an individual's life, with its composition influenced by several factors, including medical conditions [1-3].

Maintaining balance in the composition of the intestinal microbiota, in terms of quantity and quality, is of utmost importance to avoid situations such as dysbiosis and systemic repercussions. This microbiota comprises a diverse array of microorganisms, including bacteria, archaea, protozoa, viruses, and fungi. Since they make up this ecosystem, some of these microbes are ingested in the form of probiotics for health benefits [1-3]. The term "probiotic" refers to products that contain live microorganisms that, when administered appropriately, can help restore the intestinal microbiota. The consumption of probiotics dates to ancient times, primarily through fermented foods [4, 5].

However, these products are not regulated and tested with the same rigor as allopathic medications, resulting in inconsistencies in their mechanisms of action and safety profiles. In terms of composition, these products are manufactured using various bacterial strains, with *Lactobacillus* being the most utilized. The most prevalent fungal strain found in these products is the yeast *Saccharomyces boulardii* [3-7].

Saccharomyces boulardii is a unicellular eukaryotic microorganism, commonly known as "baker's yeast" or "brewer's yeast," due to its historical use in sugar fermentation for food and beverage production. This fungus can colonize various body sites, including the skin, vaginal mucosa, respiratory tract, and digestive tract [8-10].

In the pharmaceutical industry, it is used as an ingredient to produce probiotics, which are ingested to prevent and treat various diarrheal disorders. They are widely used by elderly patients, children, and especially in Intensive Care Units (ICUs) [9-12].

Microorganisms that are part of the human microbiota, such as yeasts of the genus *Candida*, under certain circumstances like immunosuppression, whether due to chemotherapy, organ transplants, or HIV infection, can develop pathogenic potential, causing various infectious

conditions, ranging from vulvovaginal and oral candidiasis to systemic candidiasis. In this regard, this research aimed to analyze possible cases of infection by *Saccharomyces boulardii* worldwide from 2018 to 2023 [13].

2. METHODOLOGY

This study is an integrative literature review designed to compile and analyze data from publications spanning 2018 to 2023, to address the following guiding questions: What are the documented occurrences of systemic infection by *Saccharomyces boulardii*? What risk factors are associated with *Saccharomyces boulardii* infection? What is the lethality rate among affected individuals?

The literature search was conducted from November to December 2023 across the scientific databases PubMed, ScienceDirect, and the Virtual Health Library (BVS). Articles published in English, Spanish, and Portuguese were selected using the keywords "probiotics," "*Saccharomyces boulardii*," and "fungemia." The Boolean operator "AND" was applied in the following combination: Probiotics AND *Saccharomyces boulardii* AND Fungemia.

The selection of articles for this study was conducted in three stages. First, titles were reviewed to identify relevant studies. In the second stage, abstracts were read, and studies not addressing the proposed research questions were excluded. Finally, a thorough reading of the selected articles was conducted for data extraction. Studies without free full-text access or those cataloged repeatedly across databases were excluded from the final sample.

3. RESULTS AND DISCUSSION

After searching for works in the BVS, PubMed, and Science Direct databases, a thorough analysis of the literature found was conducted, resulting in 93 verified works. Initially, 14 studies were excluded due to duplication, resulting in 79 records for analysis.

Subsequently, of the 79 records, 61 studies were excluded after reviewing titles and abstracts, as well as removing publications that required payment for full-text access. This left 18 studies eligible for further assessment; however, 7 of these did not address the study's guiding question and were therefore excluded. As a result, 11 studies were included in this review. Figure 1 details the number of publications analyzed, and the databases used.

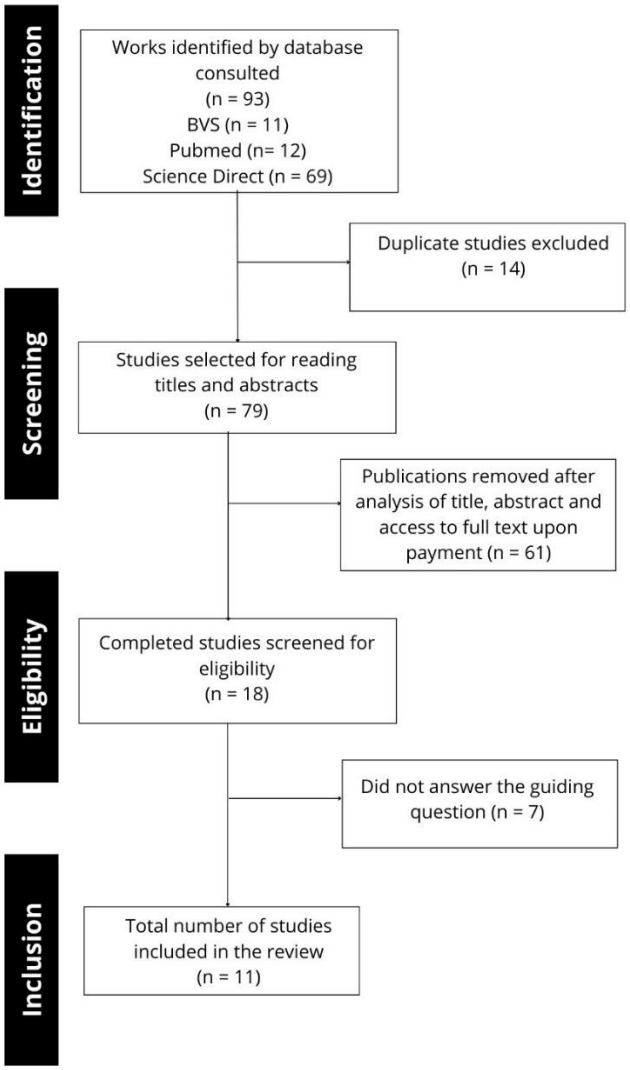


Figure 1. Flowchart illustrating the identification, screening, eligibility, and inclusion process for studies on cases of fungemia caused by *Saccharomyces cerevisiae* var. *boulardii* worldwide from 2018 to 2023.

Analysis of the articles selected in this integrative review enabled the collection of data to synthesize and deepen the understanding of the subject. This facilitated the acquisition of information regarding the clinical profile of individuals affected by fungemia caused by *Saccharomyces cerevisiae* var. *boulardii* worldwide from 2018 to 2023, as summarized in Table 1. The table compiles information from the selected studies, including publication year, title, study country, patient demographics (sex and age), associated risk factors, clinical presentation, diagnostic tests, and mortality. All 11 included studies were published in English. Most studies originated from India (three studies), followed by Turkey and the United States, with two studies each. Most publications were from 2019.

Table 1. Cases of fungemia caused by *Saccharomyces cerevisiae* var. boulardii worldwide from 2018 to 2023.

	Authors, year of publication / article title / study location	**Age – Sex**	**Possible observed risk factors**	**Clinical presentation**	**Death**
1	Kara *et al.*, 2018 / *Saccharomyces cerevisiae* fungemia after probiotic treatment in an intensive care unit patient/ Turkey	88 years – M 38 years – F	• Both used antibiotics • Both patients went to the Intensive Care Unit (ICU) • Intubation: 1 of 2 • Mechanical ventilation: 1 of 2.	• Both used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii* and had positive blood cultures for the fungus.	1(F)
2	Chakravarty, Parashar, Acharyya, 2019 / *Saccharomyces cerevisiae* sepsis following probiotic therapy in an infant/ India	35 years – M	• Use of antibiotics • Admission to the ICU.	• Hospitalized with complaints of watery diarrhea • Malnourished and dehydrated • Used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii*, 250 mg twice a day for 10 days • Erythematous macular rashes on the trunk and limbs • Lymphadenopathy • White blood cell count of 3.4×10^3 /µL with 2.52×10^3 /µL of neutrophils, hemoglobin level of 11.4 g/dL • Enlarged liver without splenomegaly • Lymphadenopathy • Slightly elevated C-reactive protein (CRP) • Blood culture positive for the fungus • Combined T and B cell deficiency	Not reported

Authors, year of publication / article title / study location		Age – Sex	Possible observed risk factors	Clinical presentation	Death
3	Gupta, Singh, Taneja, 2019 / *Saccharomyces*: A Friend or Foe in ICU (A Case Report with Solution)/ India	77 years – F	• Use of antibiotics • Admission to the ICU • Use of a central venous catheter • Diabetes • Intubation.	• Uncontrolled diabetes • Hypertension • Chronic obstructive airway disease • Diagnosis of bilateral pneumonia • Acute kidney injury • Used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii* • Blood culture positive for the fungus	The patient passed away 24 days after admission
4	Chakravarty, Parashar, Acharyya, 2019 / *Saccharomyces cerevisiae* sepsis following probiotic therapy in an infant/ India	88 years – M 38 years – F	• Use of antibiotics • Admission to the ICU.	• Hospitalized with complaints of watery diarrhea • Malnourished and dehydrated • Used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii*, 250 mg twice a day for 10 days • Erythematous macular rashes on the trunk and limbs • Lymphadenopathy • White blood cell count of 3.4×10^3 /μL with 2.52×10^3 /μL of neutrophils, hemoglobin level of 11.4 g/dL; • Enlarged liver without splenomegaly • Lymphadenopathy • Slightly elevated C-reactive protein (CRP) • Blood culture positive for the fungus • Combined T and B cell deficiency	Not reported

Clinical presentation	Death	Authors, year of publication / article title / study location		Age – Sex	Possible observed risk factors	Clinical presentation	Death
Underlying conditions/organs/system compromised: • Digestive tract: 27 of 46 • Neurological: 11 of 46 • Cardiovascular: 8 of 46 • Solid tumor with metastasis: 6 of 46 • Diabetes mellitus (any type): 6 of 46 • Pulmonary: 5 of 46 • Liver: 4 of 46 • Rheumatism: 4 of 46 • Chronic kidney failure: 3 of 46 • 10 did not report the use of the probiotic containing *Saccharomyces cerevisiae* var. *boulardii*, but all had a positive blood culture for the fungus	17	5	Landaburu *et al*., 2020 / Fungemia following *Saccharomyces cerevisiae* var. *boulardii* probiotic treatment in an elderly patient/ Argentina	82 years – F	• Use of antibiotics • Diabetes.	• Alzheimer, hypertension, and diabetes • Hospitalized for the placement of a feeding tube via gastrostomy • Used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii*, 200 mg per day for 6 months • White blood cell count of 13.8 × 10^3 /μL and 12 × 10^3 /μL of neutrophils, platelet count 309 × 10^3 /μL, hemoglobin 11 g/dL • Blood glucose level of 134 mg/dL • Creatinine level of 0.44 mg/dL • Alkaline phosphatase (ALP) 119 U/L • Aspartate aminotransferase (AST) 44 U/L; alanine aminotransferase (ALT) 22 U/L • Blood culture positive for the fungus	None

Possible observed risk factors	Clinical presentation	Death	Authors, year of publication / article title / study location		Age – Sex	Possible observed risk factors
• Use of antibiotics: 3 of 10 • Admission to the Intensive Care Unit: 3 of 10 • Use of central venous catheter: 7 of 10 • Cancer: 3 of 10 • Parenteral nutrition: 3 of 10 • HIV: 1 of 10.	Underlying conditions/organs/compromised systems: • Digestive tract: 2 of 10 • Neurological: 2 of 10 • Cardiovascular: 2 of 10 • Pulmonary: 2 of 10 • All had used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii* and had positive blood cultures for the fungus	5 (3 were male)	6	Rannikko *et al*., 2021 / Fungemia and other fungal infections associated with use of *Saccharomyces boulardii* probiotic supplements/ Finland	30 years – F 39 years – 2(M) 40 years – M 42 years – 2(M) 45 years – M 52 years – 2(M) 55 years – M 56 years – M 58 years – F 59 years – M 60 years – 2(M) 63 years – M 66 years – M 67 years – 2(M)/2(F) 68 years – 2(M) 70 years – F 72 years – 3(F)/1(M) 75 years - F 76 years – F 77 years – 1(F)/1(M) 78 years – F 80 years – 1(F)/1(M) 83 years – 1(F)/1(M) 84 years – M 85 years – M 86 years – F 88 years – 1(F)/1(M) 91 years – 1(M)/1(F) 93 years – M	• Use of antibiotics: 33 of 46 • Use of central venous catheter: 9 of 46 • Cancer: 10 of 46 • HIV: 1 of 46.
• Use of antibiotics • Intubation.	• Closed fracture of the left humerus • After completing the antibiotics and resolution of *C. difficile* colitis, the patient presented with a fever of 41°C, along with altered mental status and nystagmus upon examination. She continued to experience persistent fevers for 24 hours • She had been using the probiotic containing *Saccharomyces cerevisiae* var. *boulardii* before hospitalization • Blood cultures were positive for the fungus	None				

Authors, year of publication / article title / study location		**Age – Sex**	Possible observed risk factors	**Clinical presentation**	**Death**	**Authors, year of publication / article title / study location**		**Age – Sex**
9	Gün *et al*., 2022 / *Saccharomyces cerevisiae* fungemia due to an unexpected source in the pediatric intensive care unit/ Turkey	6 years - M	• Use of antibiotics • Admission to the ICU • Intubation • Use of a central venous catheter • Cancer Use of probiotic by the patient next to him/her.	• Admitted to the ICU on the 14th day post-operation due to respiratory discomfort • Diagnosed with inoperable atypical rhabdoid/teratoid tumor • The patient next to him used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii* • White blood cell count of 5.24 × 10^3 /μL, total neutrophils of 3.63 × 10^3 /μL, hemoglobin level of 9.6 g/dL, hematocrit of 28.4%, and platelet count of 652 × 10^3 /μL • Urea of 7 mg/dL; creatinine of 0.03 mg/dL • Sodium of 138 mmol/L, potassium 5 mmol/L, chloride 104 mmol/L, calcium of 8.1 mg/dL • C-reactive protein (CRP) of 11.3 mg/dL • Arterial blood gas was normal (pH: 7.39, HCO3: 21.8 mmol/L, pCO2: 37 mmHg) • Lactate of 1.6 mmol/L • Catheter tip culture positive for *Saccharomyces cerevisiae* var. *boulardii*	None	7	Poncelet *et al*., 2021 / *Saccharomyces cerevisiae* fungemia: Risk factors, outcome and links with *S. boulardii* - containing probiotic administration/ Belgium	21 years – M 34 years – M 40 years – F 53 years – M 55 years – M 59 years – F 80 years – M 82 years – 2(M) 88 years – F
						8	Shanmukhappa *et al*., 2022 / Unusual presentation of *Saccharomyces* fungemia after probiotics use in a critically ill patient with *Clostridium difficile* infection: a case report/ United States	51 years - F

	Authors, year of publication / article title / study location	Age – Sex	Possible risk factors observed	Clinical presentation	Death
10	Spiliopoulou *et al.*, 2023 / Fungemia due to rare non-*Candida* yeasts between 2018 and 2021 in a Greek tertiary care university hospital/ Greece	2 years – M 48 years – M 71 years – F	• Use of antibiotics; • Admission to the ICU.	• Patient aged 2 years - Diarrhea • Patient aged 48 years - Fabry disease, tetraplegia, gastrostomy, suprapubic catheter, prolonged stay in a rehabilitation facility • Patient aged 71 years - Respiratory failure, intubated, chronic heart failure, hypertension, dyslipidemia, obesity, and long-term hospitalization • Patient aged 2 years - Used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii*; the others did not report using the probiotic containing the fungus • Hemocultures positive for the fungus	1 (F)
11	Tirlangi *et al.*, 2023 / *Saccharomyces* fungemia in two critically ill patients with acute pancreatitis/ India	20 years – M 27 years - F	• Use of Antibiotic: 2 of 2 • Mechanical ventilation: 1 of 2 • Parenteral nutrition: 1 of 2	• Pacient aged 20 years - Diagnosed with moderately severe acute pancreatitis with infected necrosis • Pacient aged 27 years - Severe acute pancreatitis and hospital-acquired pneumonia • Both used the probiotic containing *Saccharomyces cerevisiae* var. *boulardii* • Blood cultures positive for the fungus	Two patients

M: male; **F:** female. **Source:** Own authorship, 2024

During the study period, 69 cases of patients with *Saccharomyces cerevisiae* var. *boulardii* identified in blood or catheter tip cultures were reported, as shown in Table 1. According to the distribution of the reported cases, the patients ranged in age from 3.5 to 93 years, with an average age of 62 for both sexes. Regarding gender, 43(62%) of the patients were male. In the study by Rannikko *et al*. (2021) [12], which included a group of 46 individuals, comprising adults and the elderly of both sexes aged between 30 and 93 years, this higher frequency was also observed, with the majority being male (63%) as the most affected by fungemia. Likewise, Poncelet *et al*. (2021) [11] reported a higher proportion of cases in men, with an average age of 58 years, comparable to the present findings. Further studies are needed to evaluate a potential relationship between fungemia, gender, and age.

Regarding age, the group with the highest incidence of *Saccharomyces* fungemia cases was those over 60 years, representing 59.4% of affected individuals. Several studies indicate that the microbiota of this specific population differs from that of adults, as over time, there is a decrease in bacteria classified as beneficial and an increase in potentially pathogenic enterobacteria [14]. Moreover, excessive antibiotic use, whether combined with or independent of the aging process, can impact the homeostasis of the gut microbiota. As a result, functional foods, especially probiotics, are increasingly used by the elderly population [14]. According to Rannikko *et al*. (2021) [12], in hospitals in the United States, the probiotic containing *Saccharomyces cerevisiae* var. *boulardii* is commonly used, particularly among elderly patients.

The advantage of using a probiotic containing *Saccharomyces cerevisiae* var. *boulardii* is that it functions as an antidiarrheal biotherapeutic agent with proven benefits in various pathological conditions, particularly acute or chronic diarrhea. Unlike antibiotics, it does not alter intestinal morphology or microbiota and shows comparable efficacy to medications used for treating diarrhea. It inhibits the growth of enteric pathogens, can be used concurrently with antibiotics, and helps restore the natural balance of intestinal microbiota [15].

There is a report of a 74-year-old male patient who was admitted to the emergency room with symptoms of abdominal pain, chills, nausea, and vomiting. On the first day of hospitalization, the patient began antibiotic treatment, but no improvement was observed, making intubation and the insertion of a central venous catheter necessary. Blood cultures from days 1 and 2 showed *Saccharomyces* growth, although the patient had not used the probiotic containing *Saccharomyces boulardii* in the hospital. However, the patient reported prolonged use of the probiotic for 7 years before admission [9]. Regarding the use of the probiotic containing this yeast, it was observed that 81.2% of the patients who developed fungemia had used the probiotic. Of these, 29 reported using it at home, in nursing homes, outpatient clinics, or in the Intensive Care Unit (ICU). Among them, 25 individuals were using probiotics in the hospital when their blood cultures tested positive for *Saccharomyces*. Additionally, 48% did not specify where they had used the probiotic, and in one case, the patient had not used it at all.

In the last cited case, a 6-year-old male child was admitted to the ICU on the fourteenth day post-surgery due to respiratory discomfort, with an atypical rhabdoid/teratoid tumor. He was receiving antibiotics, intubated, and had a Central Venous Catheter (CVC). Although he did not use *Saccharomyces cerevisiae var. boulardii*, the culture from the catheter tip tested positive for the fungus on the twenty-fifth day of hospitalization in the ICU [5]. It was noted that this child had a spiky fever the day after a neighboring patient used the probiotic. This raised the possibility that transmission may have occurred through the hands of the ICU staff colonized with the strain.

In the case of the 6-year-old male child, hospitalized on the fourteenth postoperative day in the ICU, as reported by Gün *et al*. (2022) [5], several accounts describe cases of *Saccharomyces*

fungemia in hospitalized individuals who shared a room with patients using the yeast-containing probiotic. Gonsalez *et al*. (2023) [16] reports a potential risk associated with handling sachets and capsules containing the probiotic *Saccharomyces boulardii* in environments where patients are present, regardless of probiotic use. Studies have shown that viable strains can be detected up to one meter from the handling site and remain on surfaces for up to two hours. Thus, it is recommended that, for safety, probiotics be handled in a location distant from patients.

Reports indicate that this yeast can be found in the hands of individuals handling it without gloves, even after thorough handwashing. This presents a potential pathway for infection, which can occur through two primary mechanisms: contamination of the central venous catheter via the hands of healthcare professionals who handled the yeast or through airborne dispersion of strains following the handling of capsules or sachets. Additionally, infection may arise from the intestinal translocation of the ingested microorganism across the enteral or oral mucosa, with evidence suggesting that the risk of intestinal translocation is higher in elderly patients [5, 11, 16].

Some studies outline risk factors for *Saccharomyces* fungemia, noting patients in Intensive Care Units (ICUs) with central venous catheters, on mechanical ventilation, intubated, receiving total parenteral nutrition, using gastrostomy tubes, immunosuppressed due to medication or disease, with uncontrolled diabetes, cancer, prolonged hospital stays, undergoing broad-spectrum antibiotic treatment, or using the probiotic themselves or near other patients in the same unit who are also using it. Reports of *Saccharomyces* fungemia in previously healthy patients remain rare [10, 16-18].

Regarding the use of antibiotics, it was observed that 67% of patients were on these medications, 28% used Central Venous Catheters (CVC), 20% had some cancer, 17% were on enteral nutrition, 12% reported being in the ICU, 11% were intubated, and 6% were receiving total parenteral nutrition. Each of these factors, prolonged hospitalization, mechanical ventilation, HIV, and diabetes, accounted for 3%, while the use of probiotics by neighboring patients was reported in 1.5% of cases.

Saccharomyces should be considered a potential cause of nosocomial infection, as they share similar risk factors with other Healthcare-Associated Infections. These include antimicrobials, failure to adhere to basic control measures, inadequate hand hygiene, immunocompromised hospitalized patients, age over 60, prolonged hospitalization, and invasive medical devices such as central venous catheters, intubation, and mechanical ventilation [16-19].

Probiotics containing *Saccharomyces cerevisiae* var. *boulardii*, whether for treatment or prophylaxis, should be evaluated as a potential risk factor for nosocomial bloodstream infections in individuals with predispositions [16, 20, 21].

Additionally, some patients had prolonged use of the probiotic, including a 3.5-month-old infant who used it for 10 days, an 82-year-old patient who used it for 6 months, and a 74-year-old patient who used it for 7 years. According to a consultation with the electronic formulary of the Brazilian National Health Surveillance Agency (ANVISA), a duration of two to three days is sufficient for treatment. If symptoms persist after 5 days, reevaluation of the diagnosis and modification of therapy are recommended [8, 9, 17, 22].

The total number of deaths is illustrated in Figure 2, which indicates that 36.2% of patients affected by fungemia due to *Saccharomyces cerevisiae* var. *boulardii* died. Among these, 52% were men, with an average age of 72 years. The age group with the highest number of deaths was 60 years and older. When examining the number of deaths by sex and age group, among patients aged 19 to 59 years, 4 out of 7 death cases were female. In contrast, among patients aged 60 years and above, 10 out of 18 death cases were male.

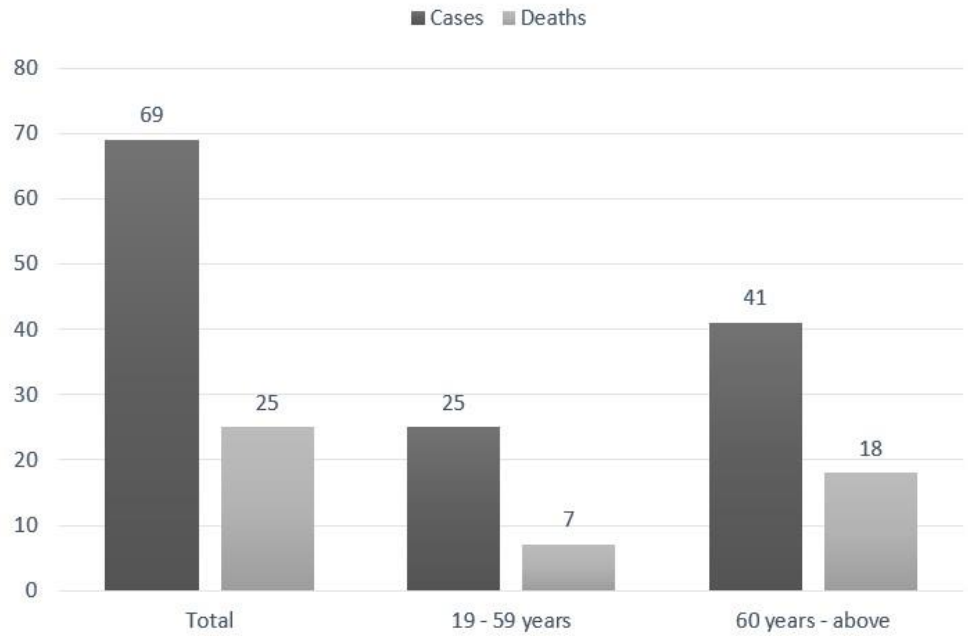


Figure 2. Number of deaths due to fungemia caused by *Saccharomyces cerevisiae* var. *boulardii* worldwide, categorized by age group, from 2018 to 2023.

As individuals' age and life expectancy increases, the immune system undergoes changes that increase susceptibility to various diseases, particularly after age 60. This process of immunosenescence has clinical implications, including increased susceptibility to infections, which contributes to higher morbidity and mortality rates in this population [23].

The occurrence and risk of infectious diseases are significant factors contributing to hospitalization and mortality, particularly in conjunction with advanced age. Furthermore, hospital-acquired infections represent a major cause of morbidity and mortality in the elderly population. A study involving a sample of 322 hospitalized individuals aged 60 years and older reported a mortality rate of 22.9% among patients with hospital-acquired infections [24]. According to the results presented in Figure 3, the distribution of fungemia cases by country varied significantly, with differing numbers of reports over various periods. Initially, Argentina and Spain had the lowest reported cases, with each country documenting only 1 case.

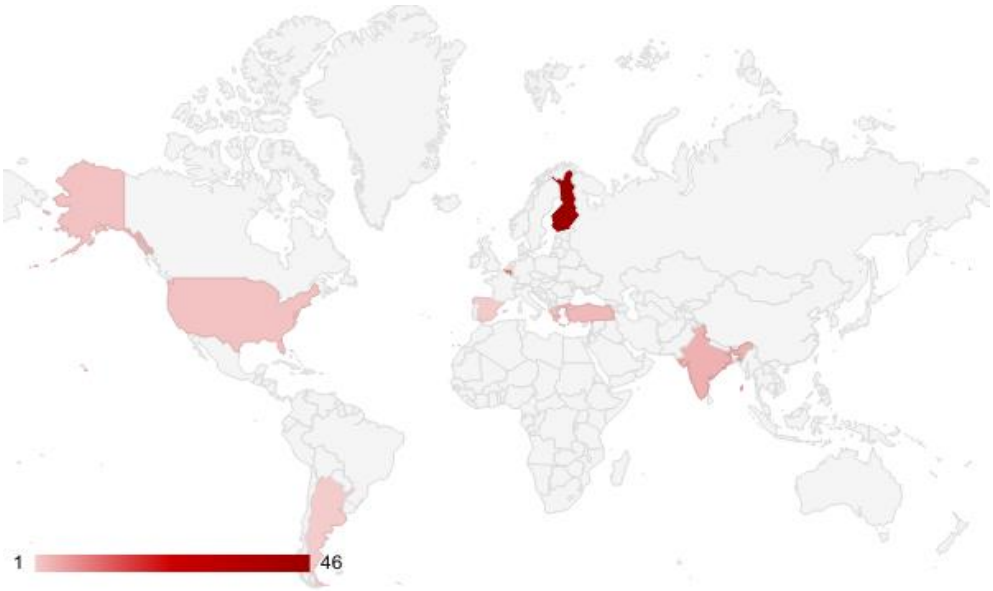


Figure 3. Geographic distribution of fungemia cases by *Saccharomyces cerevisiae* var. *boulardii* worldwide.

This is followed by the United States and Greece, which reported two cases each; Turkey with three cases; India with four reported cases; Belgium with ten occurrences between January 2005 and June 2017; and lastly, with the highest number of affected individuals, Finland with 46 cases of fungemia between January 2009 and December 2018.

The geographic distribution of cases has been explored in only a few studies that consider the number of countries worldwide. It is important to note that many additional cases may be occurring globally across various nations; however, due to a lack of diagnosis or misdiagnosis, this infection is likely underreported. Conversely, when cases are eventually identified, they may not reach the attention of researchers, who could subsequently disseminate such information through scientific publications on the topic.

As shown in Figure 4, the most used antifungal for the treatment of fungemia caused by *Saccharomyces cerevisiae var. boulardii* was fluconazole, with a total of 9 patients treated solely with this antifungal. It was followed by amphotericin B, which was used in 3 cases.

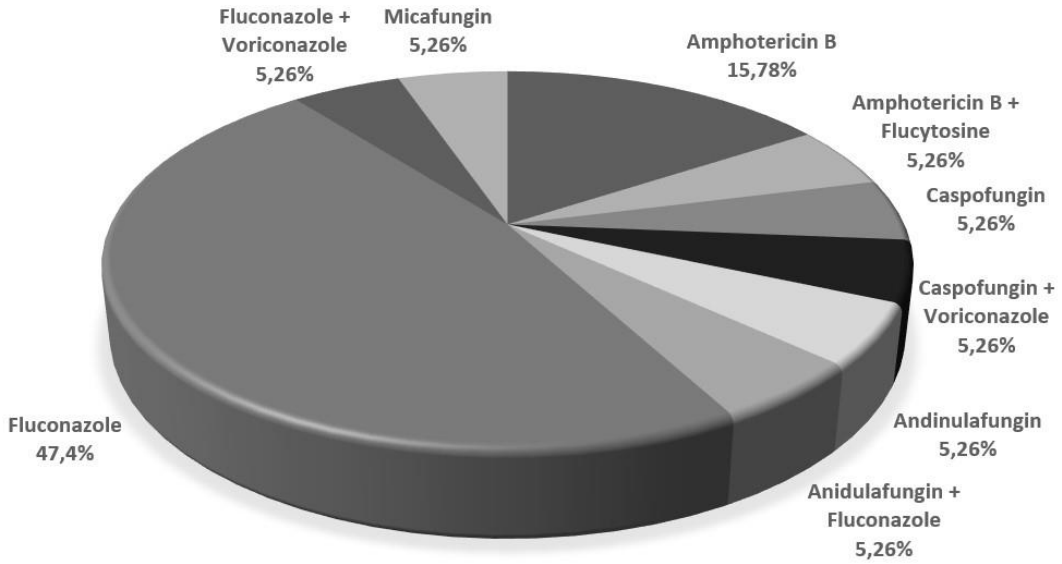


Figure 4. Antifungal agents utilized in the treatment of systemic infections caused by *Saccharomyces cerevisiae* var. *boulardii*.

It is important to note that anidulafungin was utilized in two treatments, one of which was administered concurrently with amphotericin B. Other antifungal combinations included amphotericin B combined with flucytosine. Voriconazole was also used in two treatments, both of which involved concurrent administration of another antifungal; in one instance, voriconazole was used alongside fluconazole, while in another, the patient received voriconazole in combination with caspofungin. Additionally, the treatment type for 46 patients was not specified, and in four cases, individuals did not receive any antifungal therapy, three of whom died, including two males aged 80 and 82 years.

Fluconazole, the most widely used drug, belongs to the azole class and inhibits ergosterol biosynthesis. It is indicated for the treatment of infections such as vaginal candidiasis, and balanitis, as well as for the prophylaxis of recurrent vaginal candidiasis and dermatophytosis like *Tinea pedis*, *Tinea corporis*, *Tinea cruris*, and *Tinea unguium*. Additionally, this antifungal has good pharmacokinetics, being well absorbed in the gastrointestinal tract without interference from gastric pH, and it has good penetration in all studied body fluids, such as cerebrospinal fluid, achieving a serum concentration of up to 70% [25, 26]. It is recommended that for the treatment of bloodstream infections associated with the use of probiotics, the central venous catheter should be removed, and either amphotericin B at a dose of 1 mg/kg/day or fluconazole at 10 mg/kg/day should be used, although there have been reports of strains resistant to both antifungals. Furthermore, studies have shown successful treatment cases of *Saccharomyces cerevisiae var. boulardii* with caspofungin [5].

Additionally, caution is advised when administering probiotics to patients with central venous catheters to prevent potential colonization. Measures such as hand hygiene, use of gloves, and avoiding probiotic handling near patients are recommended. Cases of fungemia have been reported in patients with central venous catheters, including those not receiving *Saccharomyces boulardii*, presenting with fever, and positive cultures for *Saccharomyces* [27].

4. CONCLUSION

The microorganisms that make up the human microbiota can exhibit pathogenic potential under certain conditions. Based on the findings of this research, 69 cases of patients affected by fungemia due to *Saccharomyces cerevisiae* var. *boulardii* were identified from 2018 to 2023.

Furthermore, it was possible to assess the clinical, laboratory, and sociodemographic profile of the affected patients, who had an average age of 62 years, predominantly male. The most common underlying conditions were related to the cardiovascular system, and the only laboratory findings that indicated similar abnormalities in the patients, aside from the positive cultures, were glucose levels, C-reactive protein, and low hemoglobin.

Regarding the observed risk factors, the five most prevalent were antibiotic use, central venous catheterization, cancer, enteral nutrition, and hospitalization in an Intensive Care Unit (ICU). The mortality rate was 36.2%, with the majority being male, particularly those over 60 years of age. Considering the risk factors for fungemia due to *Saccharomyces cerevisiae* var. *boulardii*, it is recommended to adopt certain precautions to prevent the emergence of complications. These include exercising caution when using probiotics containing *Saccharomyces boulardii* in elderly patients, those who are immunocompromised due to disease or medications, diabetics, cancer patients, individuals on antibiotics, those in intensive care, using central venous catheters, intubated, on mechanical ventilation, hospitalized for extended periods, re-

ceiving parenteral nutrition, and those with gastrostomy tubes. Furthermore, capsules and sachets containing the probiotic *Saccharomyces boulardii* should be handled in a separate environment, away from patient rooms, with gloves being changed after each manipulation.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. E.M.M. Quigley. Prebiotics and probiotics in digestive health. *Clinical Gastroenterology and Hepatology*, **17**(2), 333–344 (2019). https://doi.org/10.1016/j.cgh.2018.09.028
2. Y. Bourebaba, K. Marycz, M. Mularczyk & L. Bourebaba. Postbiotics as potential new therapeutic agents for metabolic disorders management. *Biomedicine & Pharmacotherapy*, **153**, 113138 **(**2022). https://doi.org/10.1016/j.biopha.2022.113138
3. F. Cosme, A. Inês & A. Vilela. Consumer's acceptability and health consciousness of probiotic and prebiotic of non-dairy products. *Food Research International*, **151**, 110842 (2022). https://doi.org/10.1016/j.foodres.2021.110842
4. C. de Simone. The unregulated probiotic market. *Clinical Gastroenterology and Hepatology*, **17**(5), 809–817 (2019). https://doi.org/10.1016/j.cgh.2018.01.018
5. E. Gün, H. Özdemir, D.B. Celik, E. Botan & T. Kendirli. *Saccharomyces cerevisiae* fungemia due to an unexpected source in the pediatric intensive care unit. *The Turkish Journal of Pediatrics*, **64**(1), 138–141 (2022). https://doi.org/10.24953/turkjped.2020.1668
6. D. Kothari, S. Patel & S.-K. Kim. Probiotic supplements might not be universally effective and safe: A review. *Biomedicine & Pharmacotherapy*, **111**, 537–547 (2019). https://doi.org/10.1016/j.biopha.2018.12.104
7. A. Sadeghi, M. Ebrahimi, S. Shahryari, M.S. Kharazmi & S.M. Jafari. Food applications of probiotic yeasts; focusing on their techno-functional, postbiotic and protective capabilities. *Trends in Food Science & Technology*, **128**, 278–295 (2022). https://doi.org/10.1016/j.tifs.2022.08.018
8. S. Chakravarty, A. Parashar & S. Acharyya. *Saccharomyces cerevisiae* sepsis following probiotic therapy in an infant. *Indian Pediatrics*, **56**(11), 971–972 (2019). https://doi.org/10.1007/s13312-019-1655-7
9. M. Fadhel, S. Patel, E. Liu, M. Levitt & A. Asif. *Saccharomyces cerevisiae* fungemia in a critically ill patient with acute cholangitis and long-term probiotic use. *Medical Mycology Case Reports*, **23**, 23–25 (2019). https://doi.org/10.1016/j.mmcr.2018.11.003
10. P. Gupta, Y. P. Singh & A. Taneja. *Saccharomyces*: a friend or foe in ICU (a case report with solution). *Indian Journal of Critical Care Medicine*, **23**(9), 430–431 (2019). URL: https://pmc.ncbi.nlm.nih.gov/articles/PMC6775723/pdf/ijccm-23-430.pdf
11. A. Poncelet, L. Ruelle, D. Konopnicki, V. M. Deyi & N. Dauby. *Saccharomyces cerevisiae* fungemia: Risk factors, outcome and links with *S. boulardii*-containing probiotic administration. *Infectious Diseases Now*, **51**(3), 293–295 (2021). https://doi.org/10.1016/j.idnow.2020.12.003
12. J. Rannikko, V. Holmberg, M. Karppelin, P. Arvola, R. Huttunen, E. Mattila, *et al.* Fungemia and other fungal infections associated with use of *Saccharomyces boulardii* probiotic supplements. *Emerging Infectious Diseases*, **27**(8), 2103–2109 (2021). https://doi.org/10.3201/eid2708.210018
13. I.U. Macias-Paz, S. Pérez-Hernández, A. Tavera-Tapia, J.P. Luna-Arias, J.E. Guerra-Cárdenas & E. Reyna-Beltrán. *Candida albicans* the main opportunistic pathogenic fungus in humans. *Revista Argentina de Microbiologia*, **55**(2), 189–198 (2023). https://doi.org/10.1016/j.ram.2022.08.003
14. M.C.F. Figueiredo, D.S. Araújo, J.M.F. Nascimento, F.V.P. Moura, T.R. Silva, F.D.D. Barros, S.R.A. Medeiros, V.A. Oliveira, A.C.P. Sousa & J.A. Pereira-Freire. Efeitos dos probióticos sobre a microbiota intestinal e metabolismo de idosos. *Research, Society and Development*, **9**(4), e133942969 (2020). https://doi.org/10.33448/rsd-v9i4.2969

15. T.R. Lopes & M.A.O. Pinto. Aplicação terapêutica de *Saccharomyces boulardii* em diarreias: uma revisão. *HU Revista* (Juiz de For), **36**(2), 107–122 (2010). URL: https://periodicos.ufjf.br/index.php/hurevista/article/view/875/372
16. C.R. Gonsalez, G.P. Da Silva, R.B. Ralio, N.T. Santos, E.J. Boccardo, L.A. Medeiros & M. Mendonça. Relato de seis casos de fungemia relacionadas à assistência à saúde por *Saccharomyces cerevisiae* (SC) em hospital de São Paulo -SP. *Brazilian Journal of Health Review*, **6**(1), 104–111 (2023). https://doi.org/10.34119/bjhrv6n1-010
17. M.F. Landaburu, G.A.L. Daneri, S. Relloso, L.J. Zarlenga, M.A. Vinante & M.T. Mujica. Fungemia following *Saccharomyces cerevisiae* var. *boulardii* probiotic treatment in an elderly patient. *Revista Argentina de Microbiologia*, **52**(1), 27–30 (2020). https://doi.org/10.1016/j.ram.2019.04.002
18. R.H. Okuhara, E.B.S. Ayub, A. Kataguiri, M.E. Cardoso, L.P. Silva, F.M. Veronese, M. P. Martins, O.H.M. Leite & D.E. Uip. Infecção de corrente sanguínea por *Saccharomyces cerevisiae*: relato de caso. *The Brazilian Journal of Infectious Diseases*, **25**(Suppl. 1), 101456 (2021). https://doi.org/10.1016/j.bjid.2020.101456
19. P.C.A. Martins & A.K.M.G. Vaz. Infecções prevalentes na unidade de terapia intensiva de um hospital universitário. *Enfermagem Brasil*, **19**(3), 238–245 (2020). https://doi.org/10.33233/eb.v19i3.3948
20. W.B. Almeida, N.C.B. Machado, A.P. Rodrigues, I.A. Alves, R.T. Fontana, R.F.F. Monteiro & N.V. Soares. Infecção hospitalar: controle e disseminação nas mãos dos profissionais de saúde de uma Unidade de Terapia Intensiva. *Revista Eletrônica Acervo Saúde*, **11**(2), e130 (2018). https://doi.org/10.25248/reas.e130.2019
21. L.S. Fidelis & E.F.P. Leme. Perfil das infecções de corrente sanguínea em pacientes hospitalizados por covid-19. *The Brazilian Journal of Infectious Diseases*, **26**(Suppl. 1), 101811 (2022). https://doi.org/10.1016/j.bjid.2021.101811
22. Brazil. ANVISA: Agência Nacional de Vigilância Sanitária. Bulário eletrônico Floratil, 2022. URL: https://consultas.anvisa.gov.br/#/bulario/q/?nomeProduto=floratil. Accessed: April 8, 2024.
23. A.S. Leal, B.L. Paoliello, F.B. Da Silva, G.M. Müller, I.C. Carvalho, J.W.S. Neto, *et al.* Os diversos aspectos da imunessenescência: uma revisão sistemática/The various aspects of immunosenescence: a systematic review. *Brazilian Journal of Development*, **8**(3), 15553–15584, (2022). https://doi.org/10.34117/bjdv8n3-006
24. P.J.F.B. Villas & T. Ruiz. Ocorrência de infecção hospitalar em idosos internados em hospital universitário. *Revista de Saúde Pública*, **38**(3), 372–378 (2004). Doi: https://doi.org/10.1590/S0034-89102004000300006
25. Fluconazol: cápsulas. Responsável técnico Ronan Juliano Pires Faleiro. Goiás: Geolab Indústria Farmacêutica S/A, 2018. Bula de remédio. URL: https://geolab.com.br/wp-content/uploads/2021/05/fluconazol-PF.pdf. Accessed: February 12, 2024.
26. T.L. Ferreira. *Avaliação in vitro da atividade antifúngica da mangiferina frente a cepas de Candida spp. resistentes ao fluconazol e seu possível mecanismo de ação*. Tese de mestrado, Universidade Federal do Ceará, Fortaleza, 2023, p. 12. URL: https://repositorio.ufc.br/handle/riufc/73642
27. Floratil: pó oral. Responsável técnico Dra. Marcia Weiss I. Campos. Rio de Janeiro: Farmoquímica S/A, 2021. Bula de remédio. URL: https://www.floratil.com.br/assets/pdf/Bula%20Floratil%20200.pdf. Accessed: April 6, 2024.

HOW TO CITE THIS ARTICLE

A.L. Mendes-Barbosa, J.B. Pereira de Souza & E. Santos-Carmo. *Saccharomyces boulardii* as an agent of systemic infection: an integrative review. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 124–140 (2026). https://doi.org/10.15446/rcciquifa.v55n1.125070

Review article

Use of finasteride and minoxidil for the treatment of androgenetic alopecia: a review

Bruno Francelino da Silva[1], Rafael Gomes Firmino[2], Sarah Rebeca Dantas Ferreira[3] & Francisco Patricio de Andrade Júnior[4]*

[1] School of Higher Education of Agreste Paraibano, Guarabira, Paraíba, Brazil.
[2] State University of Paraíba, Campina Grande, Paraíba, Brazil.
[3] Federal University of Paraíba, João Pessoa, Paraíba, Brazil.
[4] State University of Piauí, 2335 Olavo Bilac Street, Centro (South), Teresina, Piauí, 64001-280, Brazil. E-mail: juniorfarmacia.ufcg@outlook.com

Received: June 25, 2024
Corrected: November 8, 2025
Accepted: November 11, 2025

https://doi.org/10.15446/rcciquifa.v55n1.125079

SUMMARY

Objective: This study aimed to conduct a literature review to investigate the efficacy of Minoxidil and finasteride in the treatment of AGA. **Methods**: It is a narrative review, where articles published between 2013 and 2023 were searched in the LILACS, SciELO, and MEDLINE databases, prioritizing research published in English. **Results**: Pharmacological treatment for AGA mainly involves the use of two approved drugs: Minoxidil and finasteride, available in topical and oral forms. Topically applied Minoxidil, used in concentrations of 2% to 5%, has demonstrated efficacy in promoting hair growth by prolonging the follicle growth phase, with higher concentrations being more effective, however, its use may cause cutaneous adverse effects such as irritation and hypertrichosis. Oral Minoxidil, in concentrations of 0.25-5.0 mg, has been considered as a treatment option, allowing for dose adjustments and better patient adherence compared to the topical formulation, but its cardiovascular effects are concerning. Topical finasteride, 0.25-1.0%, is effective but may be associated with hypertrichosis and cardiovascular effects. Its oral form, 1mg/day, is also effective, but its use is restricted to men due to potential sexual and reproductive problems. Studies suggest an association between Minoxidil and finasteride, whether topical or oral, showing superior efficacy to monotherapy. Final considerations: In summary, pharmacological treatment for AGA presents effective options but also potential adverse effects. Combined therapy may be a promising strategy to improve clinical outcomes and patient adherence while reducing side effects, indicating a multifaceted therapeutic approach to androgenetic alopecia.

Keywords: Alopecia; hair treatment; pharmacological treatment.

RESUMO

Uso de finasterida e minoxidil para o tratamento da alopecia androgenética: uma revisão

Objetivo: Este estudo teve como objetivo realizar uma revisão da literatura para investigar a eficácia do minoxidil e da finasterida no tratamento da alopecia androgenética (AGA). **Métodos:** Trata-se de uma revisão narrativa, na qual foram pesquisados artigos publicados entre 2013 e 2023 nas bases de dados LILACS, SciELO e MEDLINE, priorizando-se pesquisas publicadas em inglês. **Resultados:** O trata-

mento farmacológico da AGA envolve principalmente o uso de dois medicamentos aprovados: minoxidil e finasterida, disponíveis em formas tópica e oral. O minoxidil tópico, utilizado em concentrações de 2% a 5%, demonstrou eficácia na promoção do crescimento capilar, prolongando a fase de crescimento do folículo, sendo as concentrações mais elevadas mais eficazes. No entanto, seu uso pode causar efeitos adversos cutâneos, como irritação e hipertricose. O minoxidil oral, em concentrações de 0,25 a 5,0 mg, tem sido considerado uma opção de tratamento, permitindo ajustes de dose e melhor adesão do paciente em comparação com a formulação tópica, mas seus efeitos cardiovasculares são preocupantes. A finasterida tópica, a 0,25-1,0%, é eficaz, mas pode estar associada à hipertricose e efeitos cardiovasculares. Sua forma oral, 1 mg/dia, também é eficaz, mas seu uso é restrito a homens devido a potenciais problemas sexuais e reprodutivos. Estudos sugerem uma associação entre minoxidil e finasterida, tanto tópica quanto oral, demonstrando eficácia superior à monoterapia. Considerações finais: Em resumo, o tratamento farmacológico para a alopecia androgenética apresenta opções eficazes, mas também potenciais efeitos adversos. A terapia combinada pode ser uma estratégia promissora para melhorar os resultados clínicos e a adesão do paciente, reduzindo os efeitos colaterais, o que indica uma abordagem terapêutica multifacetada para a alopecia androgenética.

Palavras-chave: Alopecia; tratamento capilar; tratamento farmacológico.

RESUMEN

Uso de finasterida y minoxidil para el tratamiento de la alopecia androgenética: una revisión

Objetivo: El presente estudio tuvo como objetivo realizar una revisión de la literatura para investigar la eficacia del Minoxidil y la finasterida en el tratamiento de la AGA. **Métodos**: Se trata de una revisión narrativa en la que se buscaron artículos publicados entre los años 2013 y 2023 en las bases de datos LILACS, SciELO y MEDLINE, priorizando investigaciones en inglés. **Resultados:** El tratamiento farmacológico para la AGA principalmente implica el uso de dos fármacos aprobados: Minoxidil y finasterida, disponibles en formas tópica y oral. El Minoxidil aplicado tópicamente en concentraciones de 2,0% a 5,0% ha demostrado eficacia en la promoción del crecimiento capilar al prolongar la fase de crecimiento de los folículos. Sin embargo, su mayor concentración es la más efectiva, aunque su uso puede causar efectos adversos cutáneos como irritación e hipertricosis. El Minoxidil oral, en concentraciones de 0,25-5,0 mg, se considera una opción de tratamiento que permite ajustes de dosis y mejor adherencia del paciente en comparación con la formulación tópica, aunque sus efectos cardiovasculares son preocupantes. Por otro lado, la finasterida tópica en concentraciones de 0,25-1,0% es efectiva, pero puede asociarse con hipertricosis y efectos cardiovasculares. La finasterida oral, a 1 mg/día, también es efectiva pero su uso está restringido a hombres debido a posibles problemas sexuales y reproductivos. Estudios sugieren que la combinación de Minoxidil y finasterida, ya sea tópica u oral, muestra una eficacia superior a la monoterapia. **Consideraciones finales:** En resumen, el tratamiento farmacológico para la AGA ofrece opciones efectivas, pero también con potenciales efectos adversos. La terapia combinada puede ser una estrategia prometedora para mejorar los resultados clínicos y la adherencia del paciente, al tiempo que se reducen los efectos secundarios, indicando un enfoque terapéutico multifacético para la alopecia androgenética.

Palabras clave: Alopecia; tratamiento capilar; tratamiento farmacológico.

1. INTRODUCTION

Alopecia androgenetica (AGA) is the leading cause of hair loss in individuals with a genetic predisposition. This occurs when genetically sensitive hair follicles are stimulated by dihydrotestosterone (DHT). The presence of DHT leads to a decrease in follicle size, resulting in reduced hair density [1].

This condition is characterized by progressive thinning of terminal hairs after puberty. With a prevalence reaching at least 80% in men and 50% in women by the age of 70, AGA tends to increase in incidence with advancing age. Additionally, it is most commonly observed in individuals of Caucasian descent, followed by Asians and African Americans, with lower incidence among Native Americans and Eskimos [2].

The treatment of AGA is challenging and often requires a multifaceted approach to achieve satisfactory results. Among the available therapeutic options, finasteride and minoxidil emerge as two of the most studied and effective interventions for slowing the progression of hair loss and stimulating hair growth [3].

Finasteride is a selective inhibitor of the enzyme 5-alpha-reductase, responsible for converting testosterone into DHT, thus reducing DHT levels in the body. On the other hand, minoxidil is a vasodilator that works by increasing blood flow to hair follicles and prolonging the hair growth phase [3, 4].

Despite both drugs being widely used against AGA, there is evident scarcity of information among healthcare professionals regarding the efficacy of oral and topical monotherapies, as well as the combination of drugs.

2. METHODOLOGY

2.1. Study Design

The present study is a narrative literature review. According to Andrade Júnior *et al.* [5], this type of research deals with broad questions and typically involves qualitative data. For article searching, the following terms were used: 1) Alopecia; 2) Androgenetic alopecia; 3) Pharmacological treatment; 4) Alopecia; 5) Androgenetic alopecia; 6) Pharmacological treatment. The aforementioned keywords were used in different combinations, utilizing the Boolean operator "AND" [5].

2.2. Inclusion and Exclusion Criteria

Studies addressing drugs used alone or in combination for the pharmacological treatment of androgenetic alopecia were included. Furthermore, articles published in Portuguese and English within the last ten years (2013-2023) were prioritized. Course completion papers, monographs, dissertations, theses, and editorials were excluded from the research.

2.3. Sources of Information

Articles were retrieved from the following databases: LILACS (Latin American and Caribbean Health Sciences) and SciELO (Scientific Electronic Library Online) and MedLine.

2.4. Article Selection and Filtering Process

The initial search in the LILACS, SciELO, and MedLine databases using the established keywords resulted in 48 articles. The filtering process was conducted in three phases. The first phase consisted of an initial screening by title and abstract, in which the articles were reviewed for immediate relevance to Minoxidil or Finasteride in the context of Androgenetic Alopecia (AGA) treatment, excluding those focused solely on hair transplantation, hormonal disorders other than AGA, or animal models. This phase eliminated 18 articles.

In the second phase, a full-text assessment was conducted for the remaining 30 articles, which were read in full. The exclusion criteria applied at this stage included the absence of primary efficacy data, focus on combined therapies without detailing the individual drugs, and non-scientific literature (such as opinion pieces or news articles). This phase excluded 8 articles.

Finally, in the final inclusion phase, a total of 22 articles was included for comprehensive review and data synthesis in the results section, ensuring the inclusion of recent publications (2013–2023) to maintain up-to-date relevance.

This detailed process justifies the selected evidence, allowing the reader to understand the intentional (non-systematic) nature of the inclusion criteria, which is characteristic of a narrative review.

3. RESULTS AND DISCUSSION

In Table 1, it is possible to observe the main information regarding the use of finasteride and minoxidil for the treatment of androgenetic alopecia.

Table 1. Main information on the use of finasteride and minoxidil for the treatment of androgenetic alopecia.

Drug / Type	Key Findings	Mechanism of Action and Concentration/Dose	Common Adverse Effects	Source
Topical Minoxidil	Only topical medication approved by the FDA. Promotes rejuvenation and prolongs the anagen phase (growth). Efficacy proven at 6 months, 1 year, and 5 years vs. placebo.	Mechanism: Potassium channel opener, promoting vasodilation and angiogenesis (improving blood flow). Concentrations: 5% (preferred for men, more effective) and 2% (for women).	Irritation, itching, flaking, contact dermatitis, hypertrichosis (excessive hair growth), reversible hair loss [4, 9-12].	[6-8]
Oral Minoxidil	More convenient, aesthetic, and lower-cost option than topical, resulting in better patient adherence.	Typical Dose: 2.5 mg/day (for men) or 0.25–1.25 mg/day (for women). Can be adjusted up to 5 mg/day [16].	Hypertrichosis (dose-dependent, more common at 5 mg/day). Cardiovascular effects of concern: hypotension, tachycardia, peripheral edema, ECG changes [15, 17].	[13, 15-17]
Oral Finasteride	FDA-approved (1 mg/day) for male AGA. Reduces follicle miniaturization responsible for progressive hair loss.	Mechanism: Selective inhibition of the 5-alpha-reductase Type II enzyme, blocking the conversion of testosterone to DHT (60% to 70% reduction of DHT) [18]. Dose: 1 mg/day (chronic use) [13, 18].	Gynecomastia, muscle atrophy, reduced libido and potency, sexual dysfunction, depression, congenital defects (risk in women). Sexual side effects: affects 1% to 40% [17, 19, 21, 22].	[7, 13, 17-19, 21, 22]
Topical Finasteride	Alternative to avoid systemic effects of the oral route. Efficacy proven in clinical trials, with less pronounced reduction in serum DHT levels.	Concentrations: 0.25% spray or 1% gel (topical application 1-2x/day) [3, 6].	Itching, burning, irritation, contact dermatitis, scalp erythema [3].	[3, 6, 15]
Combined Therapy	Shows significantly greater clinical and instrumental efficacy than monotherapies (individually) [23, 25].	Combination of Topical Minoxidil (5% or 2%) with Topical Finasteride (0.25%) or Systemic Finasteride (1 mg) [1, 23, 25].	Tolerability and safety profile comparable to monotherapies, but with potential for better adherence due to faster efficacy [23].	[1, 23, 25]

Source: own authorship, 2024.

3.1. Topical Therapy

For patients experiencing early hair loss or mild to moderate intensity and prefer to avoid oral medications due to potential systemic side effects, topical therapies may be considered as an effective option, either as initial treatment or as a complement to androgenetic alopecia (AGA) treatment [6].

3.1.1. Minoxidil

Several treatment options exist to control alopecia, though there are significant reports of adverse reactions and lack of efficacy in many cases. Both natural and synthetic medications are utilized, however only two medications (topical minoxidil and oral finasteride) have been FDA-approved to promote hair growth. Due to the diversity of alopecia types, medications are not universally prescribed but rather selected based on the specific type of condition [7].

Minoxidil is the only topical medication FDA-approved for alopecia treatment. Originally developed as an oral treatment for hypertension, Minoxidil was serendipitously discovered as a hair growth promoter during studies to treat high blood pressure [7].

Minoxidil prescriptions are typically available in concentrations of 5% (for male use) and 2% (for female use). This improvement in blood circulation is believed to significantly contribute to hair rejuvenation. Minoxidil is also capable of extending the active growth phase of hair follicles, known as the anagen phase [7]. Additionally, the use of topical minoxidil at a concentration of 5% has been shown to be more effective than the 2% version in promoting changes in terminal hair after 48 weeks of treatment [8].

Topical minoxidil usage presents numerous adverse effects: irritation, itching, flaking, contact dermatitis, reversible hair loss, burning sensation, hypertrichosis, dry scalp, rashes, and redness [4, 9-12]. Adverse cutaneous effects are more common in formulations with higher minoxidil content, possibly due to increased propylene glycol content [6].

Minoxidil should be applied once or twice daily for maximum efficacy. Considering the need for regular treatment application, patient adherence is a critical aspect to consider when recommending minoxidil use. When used appropriately, patients may observe hair growth within a period of 4 to 8 months, reaching stabilization around 12 to 18 months of continuous use. However, if treatment is discontinued, progressive hair loss is expected to occur within 12 to 24 weeks following cessation of medication use [6].

The 5% solution is preferred for male use. Patients should undergo evaluations after six months of treatment. Studies have demonstrated that both the 2% and 5% formulations showed a significant difference in hair growth compared to placebo at six months, one year, and five years after the start of treatment. In men, the 5% solution was significantly more effective than the 2%, while in women, both concentrations, 2% and 5%, showed promising improvements in hair growth area (APF). Although concentrations above 5% may increase efficacy, they also tend to increase the incidence of local irritation [6].

The exact mechanism by which minoxidil stimulates hair growth is still not fully understood, but it is known to act as a potassium channel opener, promoting hyperpolarization of cell membranes. This process results in vasodilation and angiogenesis, thereby improving blood flow and nutrient delivery to hair follicles [7].

Approximately 60% of male patients may not respond to topical minoxidil therapy due to reduced initial levels of sulfotransferase, an enzyme necessary to activate minoxidil into its active metabolite, requiring sometimes resorting to other therapeutic options [13].

3.1.2. Finasteride

Finasteride, a synthetic compound known as 4-azasteroid, is another FDA-approved medication for alopecia treatment. Like minoxidil, finasteride was serendipitously discovered as a hair growth promoter. Before being used in alopecia treatment, finasteride was commonly prescribed to treat prostate enlargement in men. This medication is prescribed only for male patients experiencing hair loss, as it acts by inhibiting the enzyme 5-alpha-reductase, responsible for converting testosterone to dihydrotestosterone (DHT). By blocking this conversion, finasteride helps to reduce the effects of DHT on hair follicles, thus promoting hair growth. It is important to note that this drug does not treat the underlying genetic cause of androgenetic alopecia, and therefore, hair loss may return after treatment discontinuation. Additionally, this medication is contraindicated for women, especially during pregnancy, due to its potential teratogenic effects [7,14]. Regarding the topical use of this drug, it occurs through a 0.25% spray or 1% topical finasteride gel, applied twice daily to the scalp [3].

In recent years, there has been an increase in the use of topical finasteride as a viable alternative to systemic therapies. The efficacy of topical finasteride 0.25% daily has been proven in clinical trials, demonstrating a less pronounced reduction in dihydrotestosterone serum levels compared to oral administration [15]. Additionally, topical finasteride can also be administered once daily but must be used chronically. Furthermore, there are no available data on patient adherence [6]. The most commonly observed adverse effects are itching, burning, irritation, contact dermatitis, and scalp erythema [3].

3.2. Oral Treatment

3.2.1. Minoxidil

Recently, the use of low doses of oral minoxidil has received more attention as a treatment option for androgenetic alopecia (AGA). Oral minoxidil is available in 2.5 mg tablets, which can be divided in half or quarters, allowing dose adjustment according to patient and healthcare provider preferences [3]. However, despite the recommended initial dose to treat male hair loss being 2.5 mg per day, it is often increased to 5 mg per day [16].

Furthermore, recent research indicates that reduced doses of oral minoxidil 2.5-5 mg/day for men and 0.25-1.25 mg per day for women with androgenetic alopecia) may offer safety and efficacy, but should be administered cautiously in patients with a predisposition to cardiovascular events [17].

The advantages of oral minoxidil over topical minoxidil involve its convenience, aesthetic aspect, cost, and treatment adherence. Oral administration of minoxidil is more convenient than its daily topical application. Additionally, from an aesthetic standpoint, oral minoxidil is more effective, as it does not cause changes in the color of gray hair and leaves no product residues as occurs with topical minoxidil. Moreover, due to its convenience, there are reports of good patient adherence to oral minoxidil therapy [13].

The most commonly reported adverse effect of oral minoxidil is hypertrichosis. This occurrence is more frequent among patients receiving higher doses of minoxidil (5 mg per day), while those receiving doses of 2.5 mg per day tend to have a lower incidence of hypertrichosis. Additionally, cardiovascular effects such as hypotension, tachycardia, pericardial effusion, lower extremity edema, and electrocardiogram (ECG) changes (tachycardia, premature ventricular contractions, changes in the T wave) are concerning complications that make oral minoxidil less favorable compared to topical formulation. Overall, the side effects of oral minoxidil are generally dose-dependent and reversible with medication discontinuation [15].

Other unfavorable conditions reported in the literature include: reduced libido, reduced ejaculatory volume, decreased total sperm count, erectile dysfunction and ejaculatory dysfunction, excessive menstruation, growth of hair in distinct areas of the scalp, dizziness, and testicular pain [4, 9-12].

3.2.2. Finasteride

In 1997, finasteride was approved at a dosage of 1 mg/day for the treatment of male AGA. This drug acts as a selective inhibitor of type II 5α-reductase enzyme, also FDA-approved for the treatment of AGA in men. In the scalp, testosterone is converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase. The pathogenesis of AGA suggests that DHT-mediated hair follicle miniaturization is responsible for the condition, resulting in follicles' inability to penetrate fully into the epidermis and, consequently, progressive hair loss, especially in men. This theory is supported by evidence indicating that men with deficiency of type II 5α-reductase enzyme show no signs of AGA [13], it is important to note that 60% to 70% of DHT is reduced during the use of this active ingredient [18].

Oral finasteride is used at a dose of 1 mg/day, allowing the progressive reduction of AGA. The maximum effect of this treatment has been observed after one year of treatment. Additionally, maintenance therapy is essential to promote continued growth [13]. In this context, although finasteride reduces hair loss, promotes hair growth, and reduces DHT levels, it presents a risk of teratogenicity in premenopausal women, making its use cautious in women of reproductive age [17].

Long-term studies have investigated the association between finasteride and prostate cancer. Prostate cancer prevention research has revealed that finasteride may be associated with an increased risk of developing high-grade prostate cancer [19]. Medical treatment should be continued indefinitely, as the benefit will not be maintained after therapy discontinuation. It may take up to a year of treatment before any clinical response is noticeable [20].

The main adverse effects are: gynecomastia, muscle atrophy, reduced libido and potency, sexual dysfunction, depression, and congenital defects, with sexual problems affecting between 1% to 40%, depending on the duration of use and dose [21,22].

3.3. Combination of Minoxidil and Finasteride

The combination of topical minoxidil and oral finasteride becomes interesting, as for patients treated with topical minoxidil, the main reason for discontinuing treatment was the perceived lack of improvement. Therefore, adopting a combined therapy, using topical minoxidil and topical finasteride, may potentially increase adherence to androgenetic alopecia treatment, due to its faster efficacy compared to using either of these treatments alone. Thus, the combination of 5% minoxidil lotion and 0.25% finasteride spray formulation in patients with AGA showed significantly greater clinical and instrumental efficacy compared to monotherapies, with comparable tolerability and safety profile [23].

In a study by Rossi et al. [24], it is evidenced that topical finasteride at 0.5% combined with a 2% minoxidil solution was effective against AGA, however, topical use of finasteride alone is not effective in this case. In other contexts, a combined therapy of 2% minoxidil and systemic 1 mg finasteride is recommended, as it has shown superiority in results compared to monotherapies [25]. Additionally, it is interesting to note that the combination of topical minoxidil and oral finasteride presents good results. However, it is observed that after hair stabilization, oral finasteride can be replaced by topical use as maintenance therapy, contributing to the reduction of possible adverse effects [1].

3.4. Critical Analysis, Limitations, and Controversies in the Literature

A critical analysis reveals that a major limitation observed in many reviewed studies is the lack of standardization in long-term follow-up protocols. Most robust trials report data up to 12 or 24 months, making sustained efficacy and long-term adverse effects less clearly defined, an especially relevant limitation for a chronic condition such as AGA, which requires indefinite treatment.

A significant controversy revolves around Post-Finasteride Syndrome (PFS). Although studies often cite a low incidence of persistent sexual side effects [12], a subset of anecdotal reports and smaller studies [15] suggests severe and persistent emotional, cognitive, and physical impairments. This highlights the need for greater clinical sensitivity and further research using prospective cohorts to fully characterize the prevalence and mechanisms of these long-term symptoms.

Furthermore, the evidence base for women's treatment is notably weaker. While topical Minoxidil is the standard, the use of Finasteride in premenopausal women remains controversial due to teratogenic risk and a less conclusive efficacy profile compared to men [19]. Future research should prioritize high-quality, long-term studies focusing specifically on the female population with AGA. These limitations should be considered when assessing the risk-benefit profile for individual patients, particularly regarding shared decision-making about Finasteride use.

4. FINAL CONSIDERATIONS

The pharmacological treatment of androgenetic alopecia (AGA) has traditionally been based on the use of two licensed and approved medications: minoxidil and finasteride, available in topical and oral forms. Topical therapy, especially with minoxidil, is an effective option for patients with mild to moderate hair loss who wish to avoid potential systemic side effects associated with oral medications. However, it is important to consider potential adverse effects such as scalp irritation and hypertrichosis when using topical minoxidil, especially in formulations with higher concentrations.

Finasteride, whether in oral or topical form, is another widely used option in the treatment of AGA, particularly in men. However, oral finasteride use is associated with potential risks such as sexual side effects and an increased risk of high-grade prostate cancer, highlighting the importance of a careful risk-benefit assessment before prescribing.

The combination of topical minoxidil and oral finasteride may be an interesting strategy to enhance treatment efficacy, especially in patients who do not adequately respond to monotherapy. However, close monitoring of adverse effects and treatment response is crucial, adjusting therapy as needed to optimize outcomes. Additionally, future studies are needed to evaluate the long-term safety and efficacy of this combined approach, as well as to identify potential biomarkers to predict treatment response in different subsets of AGA patients.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. B.S. Chandrashekar, T. Nandhini, R. Sriram & S. Navale. Topical minoxidil fortified with finasteride: An account of maintenance of hair density after replacing oral finasteride. *Indian Dermatology Online Journal*, **6**(1), 17–20 (2015). https://doi.org/10.4103/2229-5178.148925
2. C.H. Ho, T. Sood & P.M. Zito. Androgenetic alopecia. In: *StatPearls* [Internet]. StatPearls Publishing, Treasure Island (FL), 2023. URL: https://pubmed.ncbi.nlm.nih.gov/28613674/
3. S. Devjani, O. Ezemma, K.J. Kelley, E. Stratton & M. Senna. Androgenetic alopecia: therapy update. *Drugs*, **83**(8), 701–715 (2023). https://doi.org/10.1007/s40265-023-01880-x
4. Y. Kelly, A. Blanco & A. Tosti. Androgenetic alopecia: an update of treatment options. *Drugs*, **76**(14), 1349–1364 (2016). https://doi.org/10.1007/s40265-016-0629-5
5. F.P. Andrade Júnior, J.M.M. Sousa, H.I.F. Magalhães & E.O. Lima. Sobrevivendo na ciência em tempos de pandemia: como lidar? *HOLOS*, **37**(4), e11599 (2021). URL: https://www2.ifrn.edu.br/ojs/index.php/HOLOS/article/view/11599/pdf
6. M.S. Nestor, G. Ablon, A. Gade, H. Han & D.L. Fischer. Treatment options for androgenetic alopecia: Efficacy, side effects, compliance, financial considerations, and ethics. *Journal of Cosmetic Dermatology*, **20**(12), 3759–3781 (2021). https://doi.org/10.1111/jocd.14537
7. H. Rambwawasvika, P. Dzomba & L. Gwatidzo. Alopecia types, current and future treatment. *Journal of Dermatology & Cosmetology*, **5**(4), 93–99 (2021). URL: https://medcraveonline.com/JDC/JDC-05-00190.pdf
8. A.K. Gupta, M. Venkataraman & M. Talukder. Relative efficacy of minoxidil and the 5-α reductase inhibitors in androgenetic alopecia treatment of male patients: a network meta-analysis. *JAMA Dermatology*, **158**(3), 266–274 (2022). https://doi.org/10.1001/jamadermatol.2021.5743
9. H. Kaur, S. Kumar, G. Kaur, M. Kaur & M.S. Rathore. Alopecia-factors contributing, diagnosis and treatment. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, **3**(4), 1191–1199 (2013). URL: https://www.ijpcbs.com/articles/alopeciafactors-contributing-diagnosis-and-treatment.pdf
10. I.G. Motofei, D.L. Rowland, S.R. Georgescu, M. Tampa, D. Baconi, E. Stefanescu, B.C. Baleanu, C. Balalau, V. Constantin & S. Paunica. Finasteride adverse effects in subjects with androgenic alopecia: A possible therapeutic approach according to the lateralization process of the brain. *Journal of Dermatology Treatment*, 27(6), 495–497 (2016). https://doi.org/10.3109/09546634.2016.1161155
11. M.Y. Dhariwala & P. Ravikumar. An overview of herbal alternatives in androgenetic alopecia. *Journal of Cosmetic Dermatology*, **18**(4), 966–975 (2019). https://doi.org/10.1111/jocd.12930
12. S. Sheikh, A. Ahmad, S.M. Ali, M.U. Ahmad, M. Paithankar, D. Saptarshi, *et al.* A new topical formulation of minoxidil and finasteride improves hair growth in men with androgenetic alopecia. *Journal of Clinical & Experimental Dermatology Research*, **6**(1), 1000253 (2015). URL: https://www.longdom.org/open-access/a-new-topical-formulation-of-minoxidil-and-finasteride-improves-hair-growth-in-men-with-androgenetic-alopecia-2155-9554.1000253.pdf
13. M. Kaiser, R. Abdin, S.I. Gaumond, N.T. Issa & J.J. Jimenez. Treatment of androgenetic alopecia: current guidance and unmet needs. *Clinical, Cosmetic and Investigational Dermatology*, **16**, 1387–1406 (2023). https://doi.org/10.2147/ccid.s385861
14. Y. Zhang, J. Xu, J. Jing, X. Wu & Z. Lv. Serum levels of androgen-associated hormones are correlated with curative effect in androgenic alopecia in young men. *Medical Science Monitor*, **24**, 7770–7777 (2018). https://doi.org/10.12659/msm.913116
15. M. Vastarella, M. Cantelli, A. Patrì, M.C. Annunziata, P. Nappa & G. Fabbrocini. Efficacy and safety of oral minoxidil in female androgenetic alopecia. *Dermatologic Therapy*, **33**(6), e14234 (2020). https://doi.org/10.1111/dth.14234
16. D. Saceda-Corralo, M. Domínguez-Santas, S. Vañó-Galván & R. Grimalt. What's new in therapy for male androgenetic alopecia? *Archives of Dermatological Research*, **24**(1), 15–24 (2023). https://doi.org/10.1007/s40257-022-00730-y
17. S. Ntshingila, O. Oputu, A.T. Arowolo & N.P. Khumalo. Androgenetic alopecia: An update. *JAAD International*, **13**, 150–158 (2023). https://doi.org/10.1016/j.jdin.2023.07.005

18. P. Sánchez, C. Serrano-Falcón, J.M. Torres, S. Serrano & E. Ortega. 5α-Reductase isozymes and aromatase mRNA levels in plucked hair from young women with female pattern hair loss. *Archives of Dermatological Research*, **310**, 77–83 (2018). https://doi.org/10.1007/s00403-017-1798-0
19. L. Wang, Y. Lei, Y. Gao, D. Cui, Q. Tang, R. Li, D. Wang, Y. Chen, B. Zhang & H. Wang. Association of finasteride with prostate cancer: a systematic review and meta-analysis. *Medicine*, **99**(15), e19486 (2020). https://doi.org/10.1097/md.0000000000019486
20. L. Asfour, W. Cranwell & R. Sinclair. Male androgenetic alopecia. In: K.R. Feingold, S.F. Ahmed, B. Anawalt, *et al.* (editors) *Endotext* [Internet]. MDText.com, Inc., South Dartmouth (MA), 2023. URL: https://www.ncbi.nlm.nih.gov/books/NBK278957/
21. C.A. Ganzer, A.R. Jacobs & F. Iqbal. Persistent sexual, emotional, and cognitive impairment post-finasteride: a survey of men reporting symptoms. *American Journal of Men's Health*, **9**(3), 222–228 (2015). https://doi.org/10.1177/1557988314538445
22. J.M. Hirshburg, P.A. Kelsey, C.A. Therrien, A.C. Gavino & J.S. Reichenberg. Adverse effects and safety of 5-alpha reductase inhibitors (finasteride, dutasteride): a systematic review. *Journal of Clinical and Aesthetic Dermatology*, **9**(7), 56–62 (2016). URL: https://pmc.ncbi.nlm.nih.gov/articles/PMC5023004/
23. A. Rossi, F. Magri, A. D'Arino, F. Pigliacelli, M. Muscianese, P. Leoncini, G. Caro, A. Federico, M.C. Fortuna & M. Carlesimo. Efficacy of topical finasteride 0.5% vs 17alpha-estradiol 0.05% in the treatment of postmenopausal female pattern hair loss: a retrospective, single-blind study of 119 patients. *Dermatology Practical & Conceptual*, **10**(2), e2020039 (2020). https://doi.org/10.5826/dpc.1002a39
24. A. Rossi & G. Caro. Efficacy of the association of topical minoxidil and topical finasteride compared to their use in monotherapy in men with androgenetic alopecia: A prospective, randomized, controlled, assessor blinded, 3-arm, pilot trial. *Journal of Cosmetic Dermatology*, **23**(2), 502–509 (2024). https://doi.org/10.1111/jocd.15953
25. Y. Zhou, C. Chen, Q. Qu, C. Zhang, J. Wang, Z. Fan, Y. Miao & Z. Hu. The effectiveness of combination therapies for androgenetic alopecia: a systematic review and meta-analysis. *Dermatologic Therapy*, **33**(4), e13741 (2020). https://doi.org/10.1111/dth.13741

HOW TO CITE THIS ARTICLE

B.F. da Silva, R. Gomes-Firmino, S.R. Dantas-Ferreira & F.P. de Andrade Júnior. Use of finasteride and minoxidil for the treatment of androgenetic alopecia: a review. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 141–150 (2026). https://doi.org/10.15446/rcciquifa.v55n1.125079

Scientific research article

Cancer drug therapy by Gemcitabine derivatives with PEG linker for gynecological disease, beside speech therapy in implementation of biomedical caring of post-operative

Majid Monajjemi[1]*, Fatemeh Mollaamin[2], Azam Ghadami[3], Reyhaneh Souri[3], Zahra Aghakouchaki[3], Melika Aghaei[4], Zahra Solati[4] & Yasamin Shahverdy[4]

[1] Department of Biology, Faculty of Science, Kastamonu University, Kastamonu, Turkey.
[2] Department of Biomedical Engineering, Faculty of Engineering and Architecture, Kastamonu University, Kastamonu, Turkey.
[3] Department of Polymer Engineering, CT.C., Islamic Azad University, Tehran, Iran.
[4] Department of Chemical Engineering, CT.C., Islamic Azad University, Tehran, Iran.

Corresponding author E-mail: maj.monajjemi@iauctb.ac.ir; m_monajjemi@yahoo.com

Received: March 19, 2025
Corrected: November 11, 2025
Accepted: November 13, 2025

https://doi.org/10.15446/rcciquifa.v55n1.125080

SUMMARY

Background: Although Gemcitabine is applied for chemotherapy as an important drug for breast, head and neck, lung, bladder, as well as acute lymphocytic leukemia, non-Hodgkin's lymphoma, osteosarcoma, and choroid carcinoma, we found derivatives combination of Gemcitabine enable to increase the effectiveness for cancer treatment significantly and these derivatives drugs can be used for decreasing desmoids tumor in the treatment of aggressive fibromatosis. **Aim:** Therefore, our goal was based on analyzing of a series of gemcitabine derivatives containing imine structure (G1-G5), which can be synthesized through reaction of gemcitabine with several aromatic aldehydes for this research and any further activities such as drug design or drug delivery. **Materials and methods:** Furthermore, the structures of the imine derivatives were proved via CHNS/O elemental analysis, FTIR and NMR spectroscopy, which all measurements confirmed the imine derivatives of α-glucosidase inhibition. **Results:** Compound (G5) exhibited higher therapeutic indices, representing possible promising roles. We used gemcitabine derivatives containing Schiff base structure (G5) for patient that could be lead a new design in this study of novel α-glucosidase inhibitor. **Conclusions**: surgery disrupts the physiological balance of the human body and causes great stress and changes hormonal, metabolic, immune and nervous functions, using this drug (G5) during recovery after surgery (ERAS) is a multitasking care process that preserves the normal physiological state and reduces surgical complications of surgery.

Keywords: Gemcitabine derivatives; ERAS guidelines; rehabilitation after surgery; gynecological cancer; post- operative; biomedical caring; oncology; genome map

RESUMEN

Terapia oncológica con derivados de gemcitabina con enlace PEG para enfermedades ginecológicas, además de terapia del habla en la implementación de cuidados biomédicos postoperatorios.

Antecedentes: Si bien la gemcitabina se utiliza en quimioterapia como fármaco importante para el cáncer de mama, cabeza y cuello, pulmón, vejiga, leucemia linfoblástica aguda, linfoma no Hodgkin, osteosarcoma y carcinoma coroideo, hemos descubierto que la combinación de derivados de gemcitabina permite aumentar significativamente la eficacia del tratamiento oncológico. Estos derivados también pueden utilizarse para reducir el tumor desmoide en el tratamiento de la fibromatosis agresiva. **Objetivo:** Por lo tanto, nuestro objetivo se basó en el análisis de una serie de derivados de gemcitabina con estructura imina (G1-G5), sintetizados mediante la reacción de gemcitabina con diversos aldehídos aromáticos, para esta investigación y otras actividades como el diseño o la administración de fármacos. **Materiales y métodos:** Las estructuras de los derivados de imina se confirmaron mediante análisis elemental CHNS/O, espectroscopía FTIR y RMN, mediciones que corroboraron la inhibición de la α-glucosidasa por parte de estos derivados. **Resultados:** El compuesto (G5) mostró índices terapéuticos superiores, lo que sugiere posibles aplicaciones prometedoras. En este estudio, utilizamos derivados de gemcitabina con estructura de base de Schiff (G5) para pacientes que podrían ser candidatos para un nuevo diseño de inhibidor de la α-glucosidasa. **Conclusiones:** La cirugía altera el equilibrio fisiológico del cuerpo humano, provoca un gran estrés y cambios en las funciones hormonales, metabólicas, inmunitarias y nerviosas. El uso de este fármaco (G5) durante la recuperación posquirúrgica (ERAS) constituye un proceso de cuidados integrales que preserva el estado fisiológico normal y reduce las complicaciones quirúrgicas.

Palabras clave: Derivados de gemcitabina; protocolos ERAS; rehabilitación posquirúrgica; cáncer ginecológico; posoperatorio; cuidados biomédicos; oncología; mapeo genómico.

RESUMO

Terapia medicamentosa contra o câncer com derivados de gencitabina com ligante PEG para doenças ginecológicas, além de terapia fonoaudiológica na implementação do cuidado biomédico pós-operatório

Contexto: Embora a gencitabina seja utilizada na quimioterapia como um importante medicamento para câncer de mama, cabeça e pescoço, pulmão, bexiga, bem como leucemia linfoblástica aguda, linfoma não Hodgkin, osteossarcoma e carcinoma de coroide, descobrimos que a combinação de derivados de gencitabina permite aumentar significativamente a eficácia do tratamento do câncer e que esses derivados podem ser usados para reduzir o tumor desmoide no tratamento da fibromatose agressiva. **Objetivo:** Portanto, nosso objetivo foi analisar uma série de derivados de gencitabina contendo estrutura imina (G1-G5), que podem ser sintetizados por meio da reação da gencitabina com diversos aldeídos aromáticos, para esta pesquisa e quaisquer atividades futuras, como o planejamento ou a administração de fármacos. **Materiais e métodos:** Além disso, as estruturas dos derivados de imina foram comprovadas por meio de análise elementar CHNS/O, espectroscopia FTIR e RMN, cujas medições confirmaram a inibição da α-glicosidase pelos derivados de imina. **Resultados:** O composto (G5) apresentou índices terapêuticos mais elevados, representando possíveis aplicações promissoras. Utilizamos derivados de gencitabina contendo estrutura de base de Schiff (G5) em pacientes, o que pode levar a um novo desenvolvimento de inibidores da α-glicosidase neste estudo. **Conclusões:** A cirurgia perturba o equilíbrio fisiológico do corpo humano e causa grande estresse, além de alterar as funções hormonais, metabólicas, imunológicas e nervosas. O uso deste fármaco (G5) durante a recuperação pós-cirúrgica (ERAS) é um processo de cuidado multifuncional que preserva o estado fisiológico normal e reduz as complicações cirúrgicas.

Palavras-chave: Derivados de gencitabina; diretrizes ERAS; reabilitação pós-cirúrgica; câncer ginecológico; pós-operatório; cuidados biomédicos; oncologia; mapeamento genômico

1. INTRODUCTION

1.1. Gemcitabine & Methotrexate for chemotherapy drugs

Gemcitabine as a chemotherapy medication generally applied breast, ovarian cancer, lung cancer, and pancreatic cancer, so this drug is mostly has been used for gynecological cancer through intravenous infusion [1]. The side effects of this drug related to the bone marrow problem, liver and kidney disease, nausea, fever, rash, shortness of breath, mouth sores, diarrhea, neuropathy, and hair loss [2]. Although this drug was synthesized in 1983, was not confirmed up to 1995 [1, 2]. This drug enables to block the creation of new DNA that causes in cell death [3]. Gemcitabine operated as a first-line treatment for pancreatic cancer and also metastatic cancer such as advanced bladder through combination with cisplatin (or with Methotrexate). Moreover, enable for treatment in combination with carboplatin for ovarian cancer and in combination with paclitaxel for metastatic breast cancer instead of surgically removed [3], finally it is dangerous for women who taking gemcitabine should not become pregnant. Gemcitabine inside DNA permits a native nucleoside base to be added in the strains and mask the chain as a "faulty" base, and eludes the cell's normal repair system (base-excision repair), consequently creates an irreparable error that causes to inhibition of further DNA synthesis towards cell death [1-6] (Figure 1).

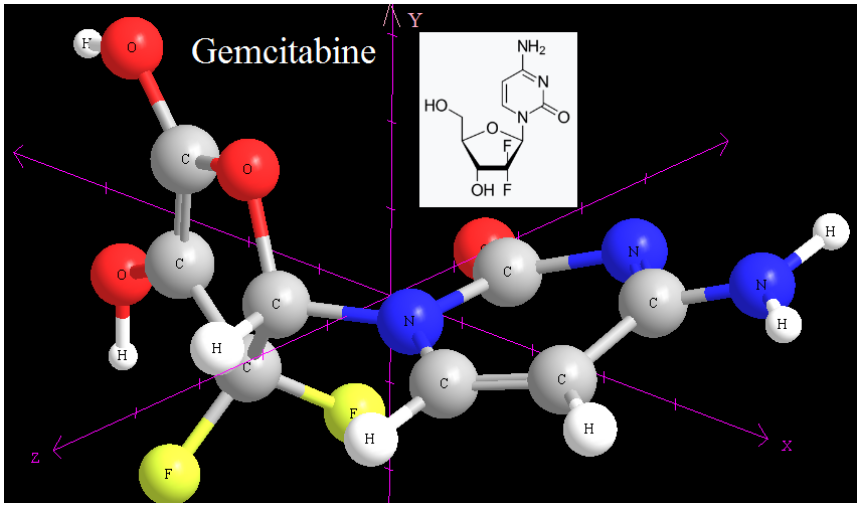


Figure 1. Gemcitabine optimized by B3LYP/6-31g* level of theory.

1.2. Biological effective of a bipill, with gemcitabine (GEM) and methotrexate (MTX)

Recent works reported the biological evaluation of a bipill, via appending two different anticancer agents, viz., gemcitabine (GEM) and methotrexate (MTX), to the distal ends of poly (ethylene glycol) (PEG) spacer. These type covalent binding between GEM and MTX through PEG linker not only transformed the solubility profiles of constituent drug molecules, but also considerably improved their stability in the presence of plasma. In vitro cytotoxicity studies confirmed that GEM-PEG-MTX treats higher cytotoxicity in gynecological cancers such as adenocarcinoma MCF-7 cell lines, when compared to free drug congeners, i.e., free GEM and free MTX. Consequently, dual drug conjugation is an effective means to synergize the therapeutic indices of potential drug candidates while alleviating drug-associated side effects [7] (Figure 2).

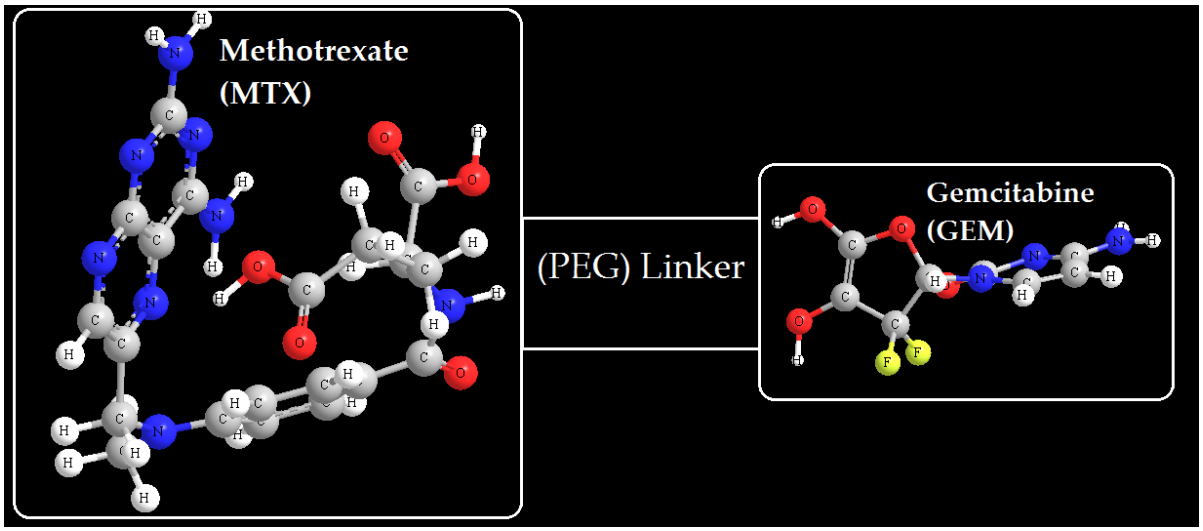


Figure 2. Biological structure of a bipill, with gemcitabine (GEM) and methotrexate (MTX).

2. BACKGROUND OF CHEMICAL SYNTHESIS AND MECHANISM

2.1. Gemcitabine synthesis

The initial synthesis of gemcitabine was done in the Lilly research laboratories, and was illustrated by Hertel *et al.* in 1988 (Figure 3). A Linear Synthesis of Gemcitabine was also accomplished by Kylie Brown *et al.* in 2015 [8]. The synthesis begins by D-glyceraldehyde that could be provided from D-mannitol in two reactions. Using ethyl bromic-di-fluoro-acetate, under standard temperature and pressure by yielded a 3:1 mixture. Gemcitabine is hydrophilic therefore inserted inside the cells through material transporters such as SLC29A1, SLC28A1, and SLC28A3. During inserting slowly linked to phosphate of DNA to produce gemcitabine monophosphate (dFdCMP and this step is a major process for definition of reaction velocity, which is catalyzed by the enzyme deoxycytidine kinase (DCK). After attaching of two other phosphates the three phosphates gemcitabine complex is produced that structurally active for reaction and known as (dFdCTP). Due to the thrice phosphorylated, this complex can masquerade as deoxycytidine triphosphate in DNA structures as new DNA strands during synthesized of the cell replicates [9].

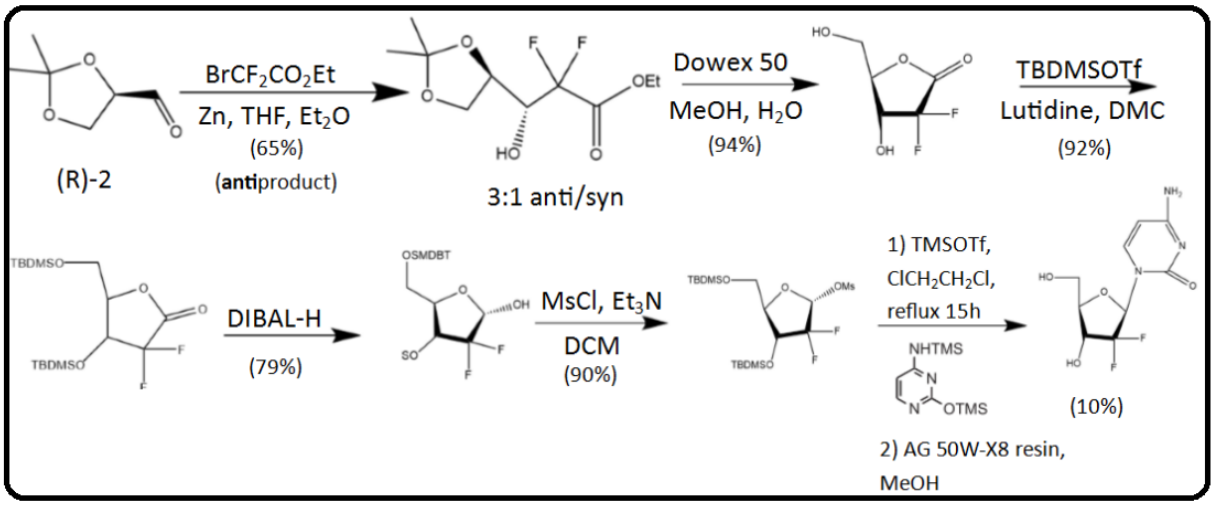


Figure 3. First synthesis of Gemcitabine process by Hertel *et al.* at Lilly laboratories in 1988.

The compound **8** has been employed as starting material in the synthesis of gemcitabine and homo-gemcitabine, also with a Reformatsky reaction as the key step (Figure 3). However, no diastereomeric ratio was given. Presumably, the re-crystals step after the actinide hydrolysis/ sequence allowed separation of the diastereomers, leading to diastereomerically pure **10**. Protection and lactone reduction then gave the difluorolactol **11**. In addition to ethyl- bromic-difluoro-acetate as fluorinated building block, 2-deoxy-2,2-difluororibose has also been synthesized using Reformatsky-type reactions with other reagents (Figure 3). Treatment of bromic-di-fluoro-acetylene **12** with zinc, followed by reaction with D-glyceraldehyde actinide (R)-2, led to the addition product **13** in 50% yield, as a 3/1 anti/syn ratio **14**. Diastereomeric separation using flash chromatography was possible, and anti-**13** was subjected to partial alkyne hydrogenation, ozonolysis /Me2S treatment, with final protection of the obtained product **14** as the triacetate **15** (Figure 4) [10, 11].

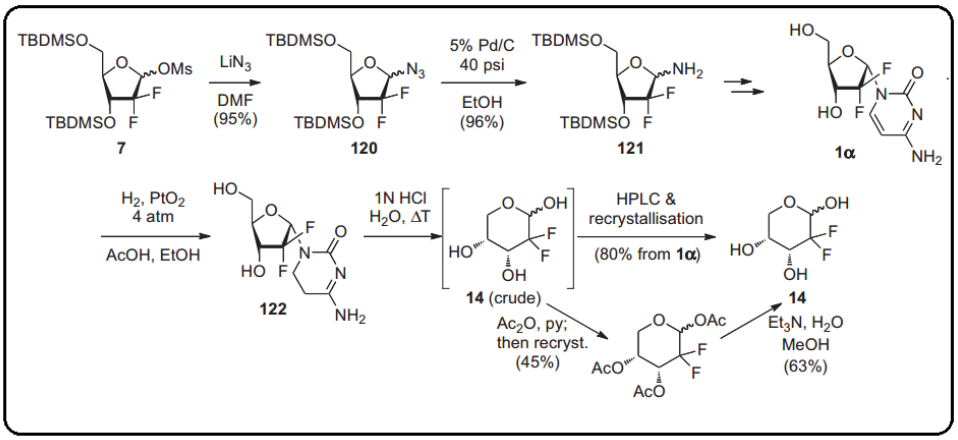


Figure 4. Reformatsky reaction on L-threose derivative and alternative Reformatsky reagents **12** and **16** in the synthesis of 2-deoxy-2,2-difluororibose.

2.2. Aldol reactions

Various systems exhibit the reaction of glycerol-de-hydro-acetate with di-fluoro-acetate from enolate type compounds (Figure 5). The lithium enolate **23** from ethyl di-fluoro-acetate **18** exhibits hampered via Claisen self-condensation side reaction. Beginning from t-butyl-difluoro-thiol-acetate **19**, reached to the formation of lithium enolate **24**, which it is structured through adding **19** to a slight excess of LDA at 80 °C, and then quickly reacted with the electrophile. It is notable the Claisen product is produced by a partial amount (10%) yet and but avoided from forming the corresponding ketene silyl O,S-acetyl **25** (Figure 5).

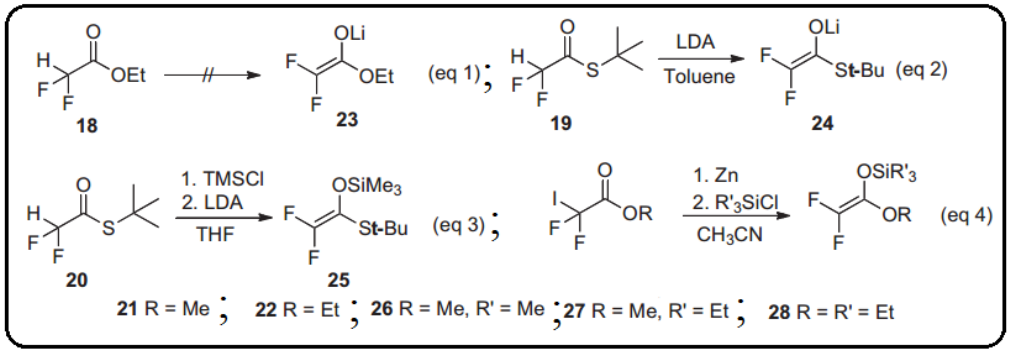


Figure 5. Synthesis of the difluoro-enolate derivatives.

Equation 4 denotes to the formation of ketene silyl acetyls **26–28** via a modification of the Reformatsky conditions, in which a tri-alkyl-chlorosilane was included in the reaction mixture before addition of the electrophile. Similar approaches were used from **22** instead of the **21** to give the silyl ketene acetyl **28**. The resulting product prepared in a lower ratio despite a cyclohexylidene acetyl protected glyceraldehyde [12]. This amount of relationship from rationalize are typically obtained with this protecting group compared to the corresponding actinide. The reactions containing **26–28** (entries are basically Mukaiyama aldol reactions, and these were reached without the addition of a Lewis-acid. *In situ* it was formed to ZnI_2, which was responsible for activating the aldehyde group. Matsumura *et al.* [13] studied on the influence of Lewis acid addition in the presence of BF_3OEt_2, similar anti/syn selectivity was obtained, but in lower yield (Figure 6).

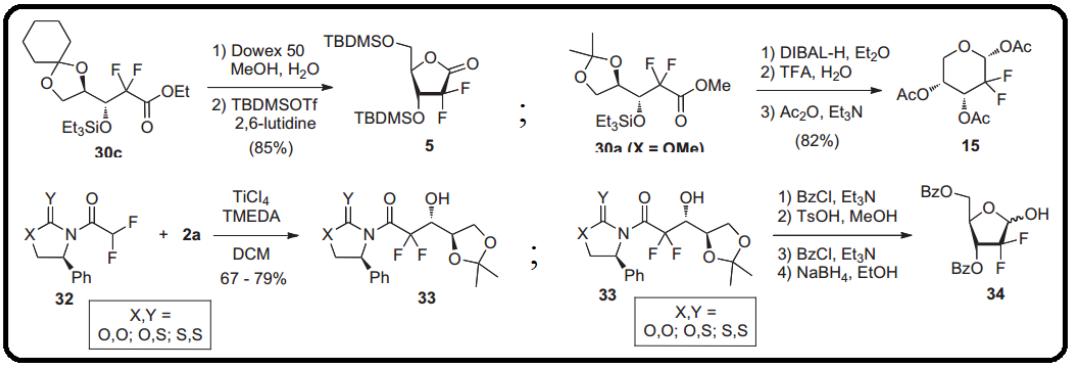


Figure 6. Homo-chiral enolate derivatives and further functional of the aldol products to di-fluoro-ribose derivatives.

3. MATERIAL AND METHODS

3.1. Imine derivatives of Gemcitabine

Configuration and designing of this work have been accomplished based on some of our previous works [14-23]. We also used Silica-Gel (From Germany known as SG-40 Merck) in thin layer chromatography (TLC) with distillated solvents. Infrared spectra were measured by ALPHA II- FT-IR spectrometer from Bruker Company. CHNS/O elemental analysis, determines the percentage of Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S) and Oxygen (O) present in a sample, which this technique is reliable and cost-effective to assess the purity and chemical composition of compounds. These analyzing were measured by EMA 502 Elemental Analyzer CHNS-O. NMR spectrums were measured by Avance NMR Spectrometer (Bruker company) (^{1}H-NMR 500 MHz and ^{13}C-NMR 75 MHz). An amount by 0.02 moles of gemcitabine was mixed by 0.02 moles for each of several benzaldehydes including (G1): 4-methylbenzaldehyde, (G2): 4-bromobenzaldehyde, (G3): 4-chlorobenzaldehyde, (G4): *N,N*-di-methyl-benzaldehyde, and (G5): 4-hydroxybenzaldehyde, inside 40 mL C_2H_5OH. Gradually and slightly glacial acetic acid was added to the mixture and keep out at 90 °C. During monitoring the reaction (by TLC), the product was separated with 80 mL of diethyl ether. After evaporated diethyl ether the product was dried and purified (Figure 7).

3.1.1. "G1": Gemcitabine derived by 4-methylbenzaldehyde

This compound was prepared through mentioned instruction by Formula: $C_{17}H_{17}F_2N_3O_4$; during temperature around 130 °C, with purification of 80%. FTIR results indicated a 3409 cm^{-1} for OH stretching, 3119 cm^{-1} for C-H bound in aromatic position, 2939 cm^{-1} for C-H bound in

aliphatic stretching, 1669 cm^{-1} for C=O stretching, 1579 cm^{-1}, 1541 cm^{-1} for C=N and C=C, respectively. As well as proton NMR in DMSO solvent beside TMS reference, indicated the chemical shifts as follows δ = 8.51 for HC=N imine, 7.69 for HC=N pyrimidine ring, and 7.29-7.19 for Ar-H. In addition, ^{13}C-NMR at (75 MHz) exhibited δ=165.02, 149.99, 139.84, 139.21, 129.61, and 130.01 for those bonds, respectively.

3.1.2. "G2": Gemcitabine derived by 4-bromobenzaldehyde

This compound was prepared through mentioned instruction by formula $C_{16}H_{14}BrF_2N_3O_4$; during temperature around 140 °C with purification of 70%; FTIR results indicated 3429 cm^{-1} for OH hydroxyl group, 3139 cm^{-1} for C-H aromatic group stretching, 2939 cm^{-1}, 2859 cm^{-1} due to C-H groups, 1669 cm^{-1} for C=O carbonyl group, 1591, 1551, 1469 for C=N and C=C groups; As well as proton NMR in DMSO solvent beside TMS reference, indicated the chemical shifts as follows δ = 8.49 for HC=N imine, 7.71 HC=N pyrimidine ring , and 7.38-7.19 cm^{-1} for Ar-H , 6.39 cm^{-1} for HC=N pyrimidine ring. In addition, ^{13}C-NMR at (75 MHZ) exhibited δ =165.01, 149.72, 145.12, 129.75, 129.48, and 130.84 for those bonds, respectively.

3.1.3. "G3": Gemcitabine derived by 4-chlorobenzaldehyde

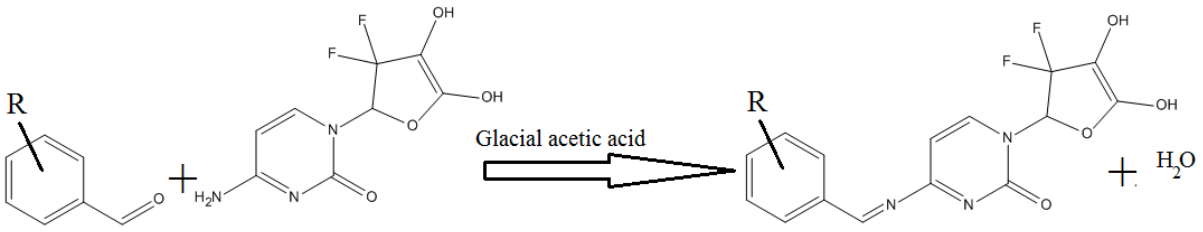
This compound was prepared through mentioned instruction by formula: $C_{16}H_{14}ClF_2N_3O_4$; during temperature around 170 °C with purification of 80%**;** FTIR results indicated 3425 cm^{-1} for OH hydroxyl group, 3119 cm^{-1} for C-H aromatic group stretching, 2945 cm^{-1}, 2832 cm^{-1} due to C-H groups, 1590, 1540, 1490 for C=N and C=C groups. As well as proton NMR in DMSO solvent beside TMS reference, indicated the chemical shifts as follows δ = 8.59 for HC=N imine, 7.69 for HC=N pyrimidine ring, and 7.29-7.19 cm^{-1} for Ar-H, 6.38 cm^{-1} HC=N pyrimidine ring. In addition, ^{13}C-NMR at (75 MHZ) exhibited δ =165.21, 151.72, 143.15, 132.70, 131.33, and 129.64 for those bonds, respectively.

3.1.4. "G4": Gemcitabine derived by N, N- di-methyl-benzaldehyde

This compound was prepared through mentioned instruction by formula:$C_{18}H_{20}F_2N_4O_4$ during temperature around 188 °C with purification of 80%**;** FTIR results indicated 3449 cm^{-1} for OH hydroxyl group, 3120 cm^{-1} for C-H aromatic group stretching, 2959 cm^{-1}, 2850 cm^{-1} due to C-H groups, 1669 for C=O stretching, 1589, 1580 for C=N and C=C groups. As well as proton NMR in DMSO solvent beside TMS reference, indicated the chemical shifts as follows δ = 8.39 for HC=N imine, 7.70 for HC=N pyrimidine ring, and 7.28-7.18 cm^{-1} for Ar-H, 6.39 cm^{-1} HC=N pyrimidine ring. In addition, ^{13}C-NMR at (75 MHz) exhibited δ =167.01, 154.89, 143.99, 139.98, 130.43, and 130.11 for those bonds, respectively.

3.1.5. "G5": Gemcitabine derived by 4-hydroxybenzaldehyde

This compound was prepared through mentioned instruction by formula: $C_{16}H_{15}F_2N_3O_5$; during temperature around 170 °C with purification of 69%**;** FTIR results indicated 3429 cm^{-1} for OH hydroxyl group, 3119 cm^{-1} for C-H aromatic group stretching , 2960 cm^{-1}, 2870 cm^{-1} due to C-H groups, 1580, 1540 for C=N and C=C groups. As well as proton NMR in DMSO solvent beside TMS reference, indicated the chemical shifts as follows δ = 8.38 for HC=N imine, 8.01 for HC=N pyrimidine ring, and 7.30-7.19 cm^{-1} for Ar-H, 5.80 cm^{-1} HC=N pyrimidine ring. In addition, ^{13}C-NMR at (75 MHZ) exhibited δ =165.31, 154.99, 152.98, 143.89, 130.99, and 130.01 for those bonds, respectively.



R=4-N,N-dimethyl ;4-Br ;4-Cl; 4-OH; 4-methyl

Figure 7. Schiff base derivatives of Gemcitabine.

Imine derivatives of compounds of gemcitabine have been prepared by reaction gemcitabine and several aromatic benzaldehydes. The chemical structures of all the end products were identified by some spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR, FTIR, and elemental analysis. The ^{1}H-NMR spectrum of compounds exhibited lack of the amines' proton at δ=6.8 ppm in gemcitabine (Figure 8), but instead of that exhibited a new signal at 8.51, 8.49, 8.59, 8.39, and 8.01 for G1 to G5, ppm respectively for the protons of imine group N=CH

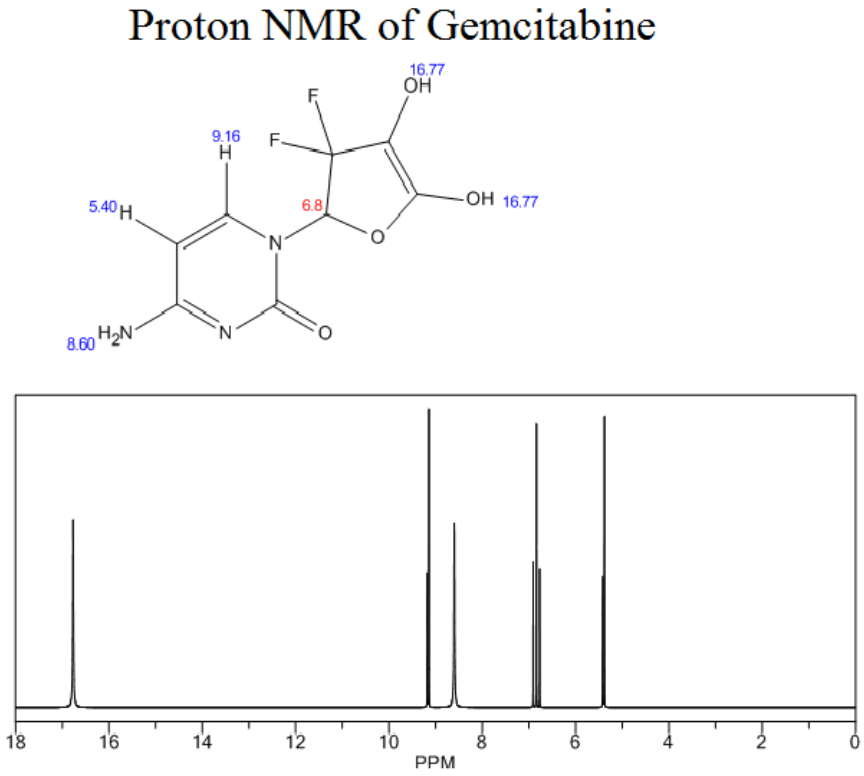


Figure 8. Proton NMR of pure Gemcitabine.

The ^{13}C-NMR spectrum of imine groups exhibited several new signals of the carbon imine group N=CH at 165.02 ppm, 165.01 ppm, 165.21 ppm, 167.01 ppm, and 165.31 ppm for G1 to G5, respectively these signals can be compared with ^{13}CNMR spectrum of pure Gemcitabine (Figure 9). On the other hand, the FT-IR spectra exhibited the stretching vibration of the imine group in complexes for G1-G5 and also two absorption bands of the asymmetric and symmetric stretching vibration of NH_2 group from gemcitabine disappeared after reaction of gemcitabine with several benzaldehydes (G1-G5) (Figure 9).

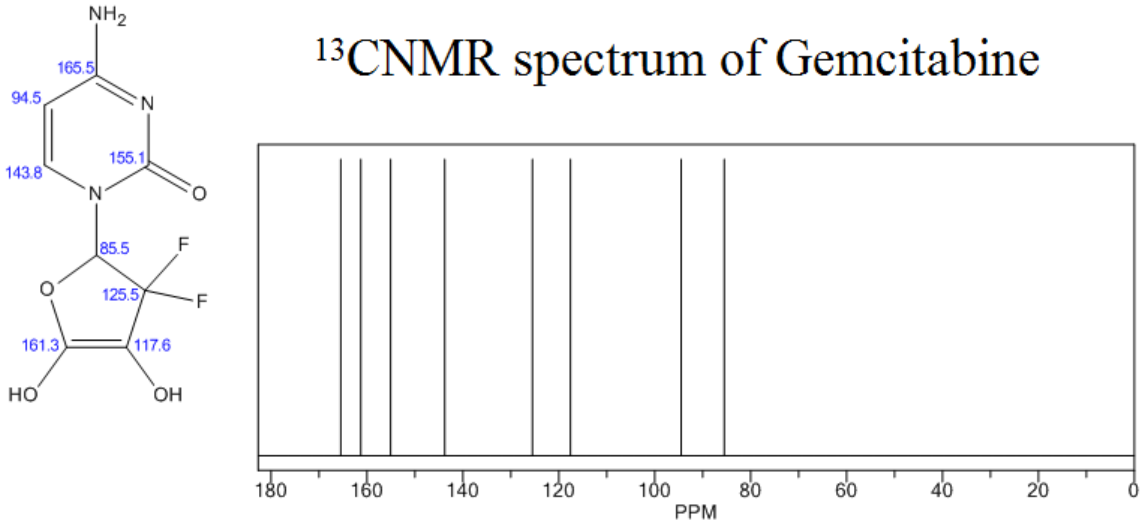


Figure 9. ^{13}C-NMR spectrum of pure Gemcitabine.

3.2. Poly (ethylene glycol) (PEG) spacer and linker for conjugate drugs

Poly (ethylene glycol) (PEG) is the most important polymer in delivering anticancer drugs clinically. PEGylation which means the covalent attachment of PEG to segment of peptides proteins drugs, is known to enhance the aqueous solubility of hydrophobic such drugs. This system increase circulation time and decreases nonspecific uptake, and achieve specific tumor target-ability through the enhanced permeability and retention effect. Currently various PEG-treatments has been developed, and several have received market approval. A vast amount of clinical experience has been gained which has helped to design PEG prodrug conjugates with improved therapeutic efficacy and reduced systemic toxicity. However, more efforts in designing PEG-based prodrug conjugates are anticipated. According to these properties we suggest a combination of Methotrexate with PEG spacer to link to the gemcitabine derivatives that can be used for any further testing for gynecological disease are shown in (Figure 10). Due to each of these structural properties, it can be predicted and then tested for various oncology cancers. These synthetic advances in PEG prodrug conjugation methodologies with varied bioactive components of clinical relevance could be synthesized for any approving by FDA-towards intended clinical applications, and formulations under clinical trials.

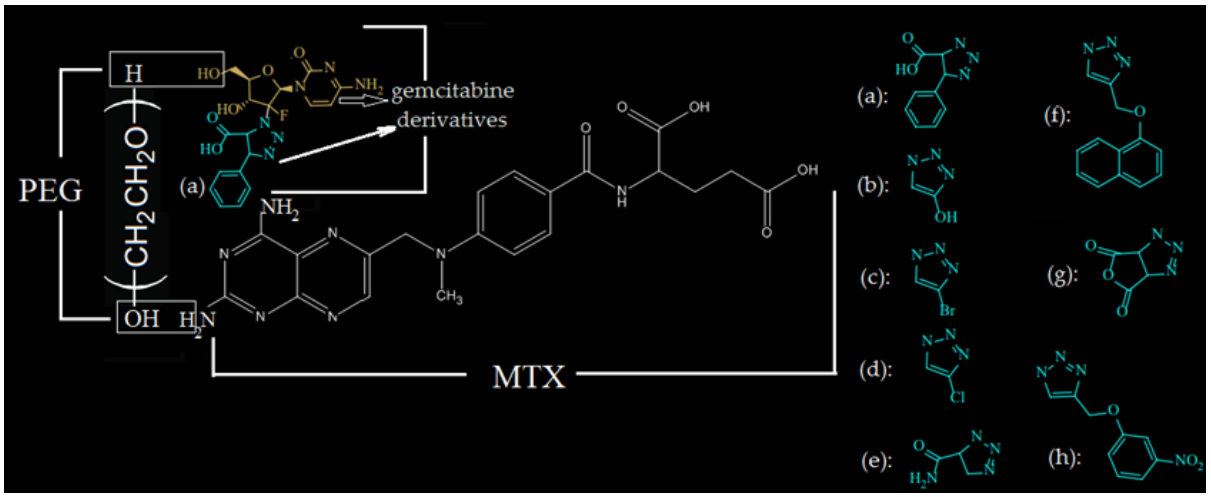


Figure 10. Methotrexate (MTX) with PEG spacer to link to the gemcitabine (GEM) derivatives.

3.3. Ligand and protein preparation

Chemical structures were drawn by ChemDraw (version 19.0.1.8, PerkinElmer) and then open into Chem3D Ultra (version 19.0.1.8, PerkinElmer). First optimization was accomplished using MM2 and MMFF9 force felids and modified in accurate form of three-dimensional geometries for precise structural analysis, and saved in Zmt format. Finally, through Gaussian 2016 ab-initio optimization was done in b3LYP/6-31g* level of theory. Two important gynecological disease proteins one for each cancer cell line (2Q79, 5J6R) was selected for molecular docking simulations. The docking efficiency was confirmed by re-docking the sample ligands of 4ZXT and 7LXL, according to the resulting RMSD at 1.79 Å and 1.20 Å, respectively.

4. RESULTS AND DISCUSSION

4.1. Proteins based on relevance to gynecological cancers

The goal for choosing of the proteins were based on relevance to gynecological disease of several cancers using the Protein Data Bank (PDB), and the mechanism of each system were considered according to critical oncogenic signaling pathways. 2Q79 is related to the crystal structure of single chain E2C from HPV16 [24, 25]. The E2 DNA-binding is related to the cervical cancer-associated strain human papillomavirus type 16 (HPV-16) in gynecological disease. The papillomavirus E2 proteins regulate transcription from all viral promoters, which belong to a group of viral proteins in dimeric β-barrels form that apply surface α-helices during DNA intercalation. Although all E2 proteins are identical in palindromic DNA sequence, proteins of various viral strains differ in their potential to discriminate among their specific DNA-binding sites. The protein data x-ray indicates that though the folding of HPV-16 E2 DNA-binding domain resembles replant to the viral strain papillomavirus type 1, the accurate configuration for the recognition helices is completely different, due to the charge distribution on the DNA-binding area and less electropositive surface for E2. Therefore, HPV-16 E2 is thus less able to utilize charge neutralization of the phosphate groups on DNA to induce bending which causes reducing affinity between HPV-16 E2 and flexible DNA target sequences, in other hands enhanced affinity towards A-tract-containing, pre-bent sequences (Figure 11-a).

5J6R is related to the crystal structure of human papillomavirus type 59 L1 (Figure 11-b). This virus belongs to the Papillomaviridae category that consists of 199 different genotypes containing different tropism and pathogenesis. Generally, they categorized as low and high risk dangerous based on their ability to induce cancer. Carcinogenic HPV types include HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV52, HPV56, HPV51, HPV58,

and HPV59. Two other types which known as HPV68 and HPV66, although often exhibits medium-risk, have a dangerous capacity to induce cancer. Although most of these viruses affected to various cancers, oncogenic types have been linked to cervix, anal, vulva, vagina, penile. Worldwide, HPV infection still causes up to 4.5% (630 000 cases) of all new cancer cases worldwide (8.6% in females; 0.9% in males), representing 29.5% of all infection-related cancers.

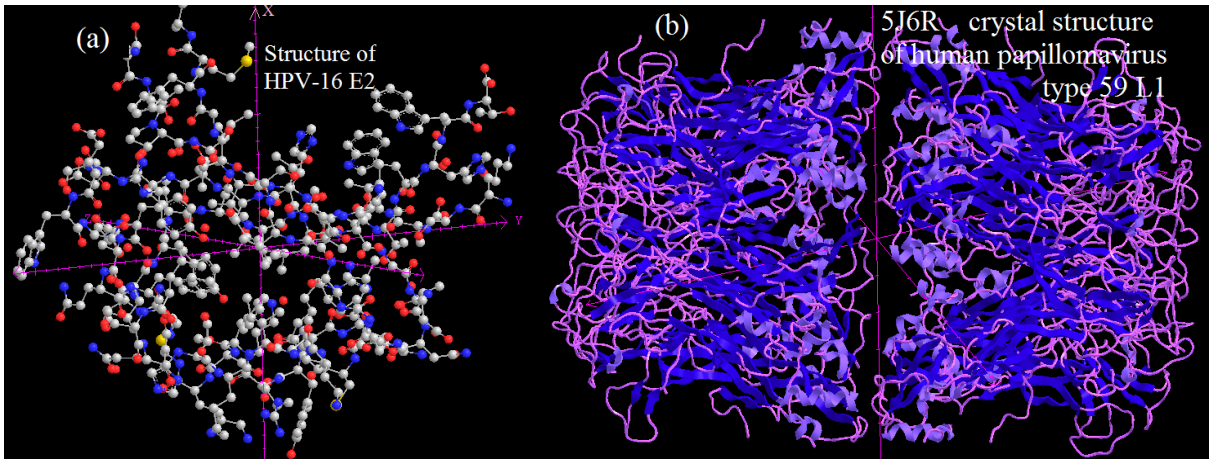


Figure 11. (a) Optimized stricture of HPV-16 E2 with charm software; (b) human papillomavirus type 59 L1 from protein data bank.

Prophylactic HPV vaccines have been progressively introduced worldwide since 2006. Large phase III randomized clinical trials and post-licensure studies and trials have proven high efficacy, effectiveness, and safety records in the prevention of vaccine-type HPV infection and cancer precursor lesions (cervical intraepithelial neoplasia. Although currently, three HPV vaccines are commercially available that include of virus-like particles (VLPs) of HPVs 16/18, the four valent and nine valent vaccines also prepared by VLPs from HPVs 6/11. The bivalent type has exhibited a suitable index against whole of gynecological cancers. This cross-protection has been recently corroborated in observational studies from Scotland. World public health reported, which these vaccines offer comparable immunogenicity, efficacy, and effectiveness for the prevention of cervical cancer, mainly caused by HPV16 and HPV18. In accordance with the genotype attribution to different cancer sites, the existing vaccines could prevent up to 90% of HPV-associated cancers and related pre-neoplastic lesions. Here by we studied 7 central signals with frequent tumor genome alterations, with keyword oncology discovered in these pathways in previous GOA work, and focused on various pathways that are potential drivers of cancer. The routs analyzed are: cell cycle, gynecologic signaling, Myc 4) oxidative stress response / NRF2, PI-3-Kinase signaling, receptor-tyrosine kinase (RTK) / RAS / MAP-Kinase signaling, TGFβ signaling, P53, and β-catenin / WNT were signaling. Alterations in DNA repair pathways, epigenetic modifiers, splicing, and other cellular mechanisms have not been implicated in cancer because these mechanisms mainly underlie genomic instability compared with specific proliferative potential. We began by reviewing the complete set of tumor-disease pathway mappings from the GOA compendium between 2010 and 2023, each of which included pathway genes genetically altered in tumor types. These route diagrams are publicly available as predefined network templates on the www (Figure 12). Considering the association of pathway members across multiple GOA studies, we generated a consolidated list of candidate member genes for each of the 7 pathways. It was then organized based on the updated literature.

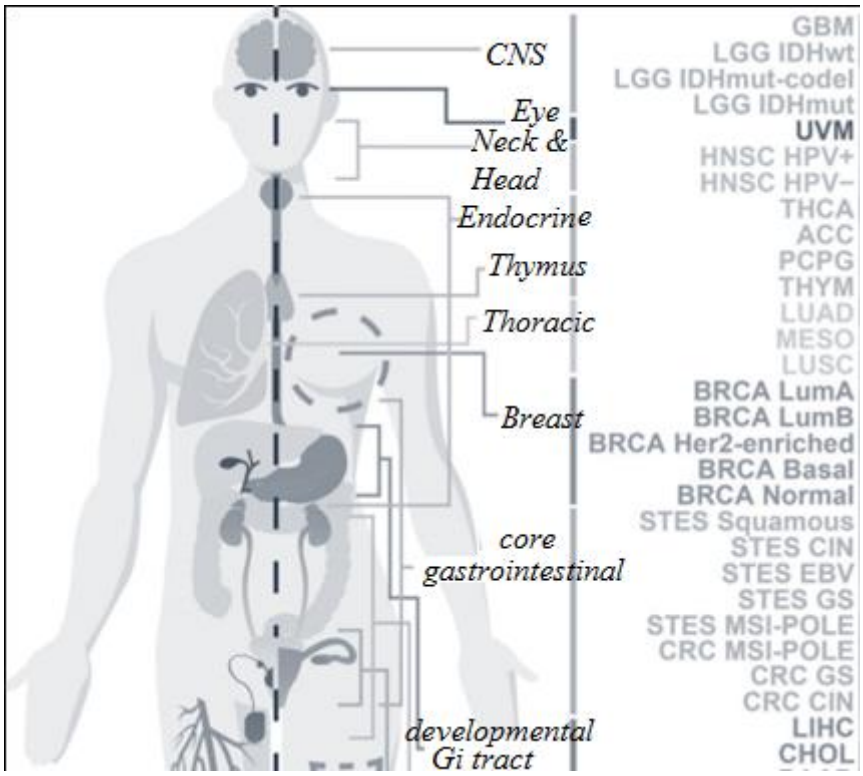


Figure 12. Changed samples in pathway of gynecologic oncology types and tumor subtype.

4.2. Docking simulation

We simulated our model based on our previous works [26-32]. Favorable interactions with the major active site residues were also visualized by binding mode inspection. The computational technique MVD was used for 6 compounds against several different cancer cell lines including 2Q79, 5J6R, and and6BIM from protein data bank. The threshold for docking scores was established by comparing the docking scores of the test compounds with the reference drug gemcitabine and G1 up to G5 compounds. All five complexes exhibited more negative amounts than gemcitabine and g5 was considered to have better binding affinities compared with others (Figure 13). The main target was to determine the stable structure including minimum potential energy, where MVD operates by replacing cavities on the protein active sites, generating poses, and selecting the best pose based on the MolDock score (Figures 13-15).

iGEMDOCK

Protein Ligand Docking/Screening | Docked Poses/Post-Screening Analysis | About

1. Prepare Screening Data

Load | Binding Site | esktop/2Q79.pdb | Docked Poses

Generate | Interaction Profile | Interaction Analysis

Output

C:/Users/sahar/Desktop/iGEMDOCKv2.1/output/ | Set Output Path

Save | Summary table | Text | Excel

Select | All | / | None | Compounds | Display | Sort | Compound | Clean Table

	Compound	Energy	VDW	HBond	Elec
Doc -1	2Q79-G1	-64.41	-42.41	-22.31	-17.43
Doc -2	2Q79-G 2	-62.33	-41.45	-21.73	-17.11
Doc -3	2Q79-G 3	-60.45	-40.55	-20.85	-16.87
Doc -4	2Q79-G 4	-67.39	-46.93	-23.61	-17.93
Doc - 5	2Q79-G 5	-70.66	-49.66	-25.44	-20.47
Doc - 6	2Q79-Gemcitabine	-55.88	-39.98	-19.45	-15.67

Figure 13. Docking simulation data for G1 to G5 including pure Gemcitabine.

Using Molegro Virtual Docker, several possible binding sites were identified in the crystal structure (PDB ID: 2Q79). The MVD workspace was then exported to Discovery Studio, providing a more visually intuitive representation of interactions between protein and ligand, elucidating their type, category, and bond distances. Additionally, all 2Q79-binding compounds formed stable hydrogen bonds and hydrophobic contacts with essential residues binding pocket, including 2Q79, 5J6R, and 6BIM (interaction details in Figure 13). Gemcitabine has a demonstrated inhibitory effect on the reduction in ERK2 phosphorylation, in various types of cancer cells.

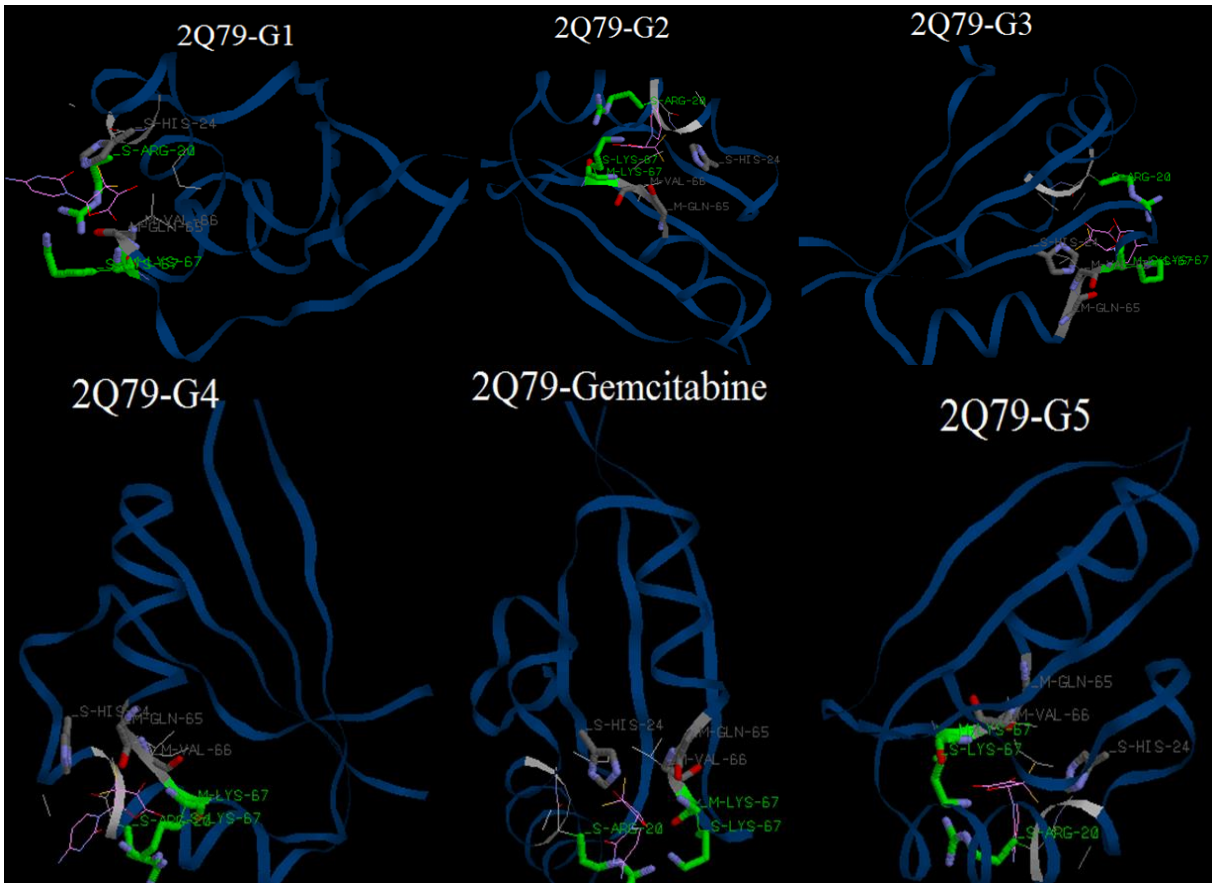


Figure 14. Favorable interactions with the major active site residues were also visualized by binding mode inspection for 6 positions.

Five protein–ligand complexes emerged as leads based on their scores. Investigating, which 2Q79-G5 gave the highest Mol-Dock score energies of −77.66 compared with 2Q79-Gemcitabine which is -55.88. The rest were also over 2Q79-Gemcitabine with sequence as: 2Q79-Gemcitabine <2Q79-G3 <2Q79-G2 <2Q79-G1 <2Q79-G4 <2Q79-G-5. Remarkably, 2Q79-G-5 demonstrated a total of 8 interactions, including hydrogen bonds and carbon–hydrogen bonds with amino acids H-S-Arg20, H-M LYS67, H-S LYS67, V-S-Arg20, V-S-His24, V-M-Gln65, V-M-Val66, and V-S-Lys67 (Figure 15).

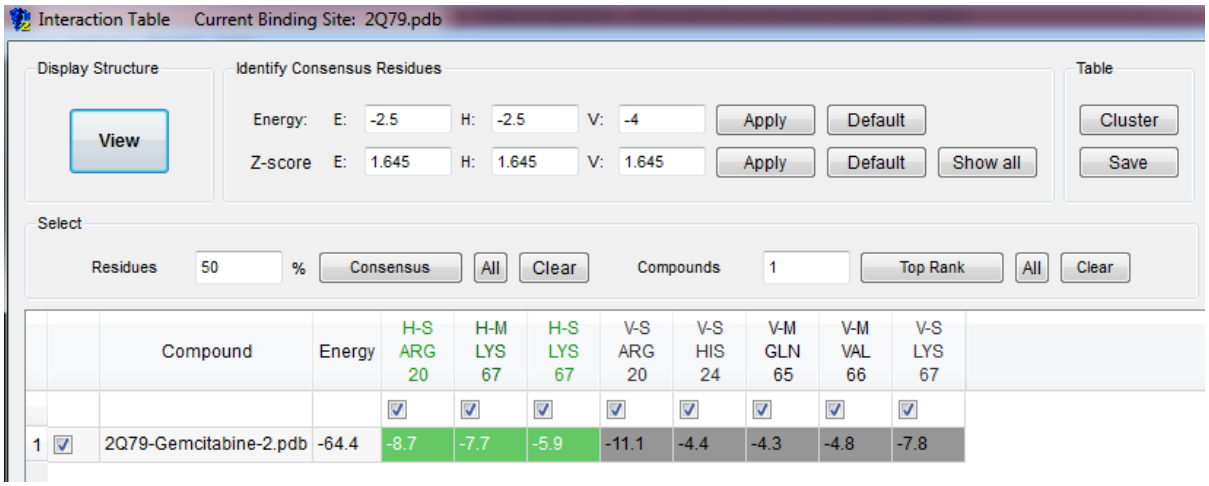


Figure 15. 2Q79-G-5 demonstrated a total of 8 interactions, including hydrogen bonds and carbon–hydrogen bonds with amino acids.

Some of them had shown a total of 6 interactions, including hydrogen bonds, carbon–hydrogen bonds, π–sigma, π–sulfur, π–lone pair, π–π T-shaped, and π–alkyl interactions with amino acids L.

5. CONCLUSION

Oncology genetic counseling has become more common for women with significant risks for certain genetic diseases, especially for women with concerns about potentially fatal conditions such as Tay-Sachs, lifelong health risks from sickle cell anemia, or BRCA gene mutations, which increases the risk of breast cancer. If obstetrics and gynecology patients could receive personalized risk assessments for these conditions without considering physician services, wouldn't they be able to make smarter choices about their health or family planning. For example, insurers and health care providers who increasingly bet their financial resources on patient outcomes may be less willing to treat or cover patients of Gynecologic oncology they perceive to be at higher-than-average risk of developing a disease. And physicians already overwhelmed with patient-generated health data, reports and reminders from other providers, and the daily burden of electronic health record use, population health management, and intense financial pressures may not know what to do with this information.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. K. Brown, A. Weymouth-Wilson & B. Linclau. A linear synthesis of gemcitabine. *Carbohydrate Research*, **406**, 71–75 (2015). https://doi.org/10.1016/j.carres.2015.01.001
2. K.K.Y. Cham, J.H. Baker, K.S. Takhar, J.A. Flexman, M.Q. Wong, D.A. Owen, *et al.* Metronomic gemcitabine suppresses tumour growth, improves perfusion, and reduces hypoxia in human pancreatic ductal adenocarcinoma. *British Journal of Cancer*, **103**, 52–60 (2010). https://doi.org/10.1038/sj.bjc.6605727
3. G. Bocci, R. Danesi, G. Marangoni, A. Fioravanti, U. Boggi, I. Esposito, *et al.* Antiangiogenic versus cytotoxic therapeutic approaches to human pancreas cancer: an experimental study with a vascular

endothelial growth factor receptor-2 tyrosine kinase inhibitor and gemcitabine. *European Journal of Pharmacology*, **498**(1-3), 9–18 (2004). https://doi.org/10.1016/j.ejphar.2004.07.062

4. S.A. Veltkamp, J.H. Beijnen & J.H.M. Schellens. Prolonged versus standard gemcitabine infusion: translation of molecular pharmacology to new treatment strategy. *The Oncologist*, **13**(3), 261–276 (2008). https://doi.org/10.1634/theoncologist.2007-0215
5. L.J. Scott & C.M. Spencer. Miglitol: a review of its therapeutics potential in type 2 diabetes mellitus. *Drugs*, **59**(3), 521–549 (2000). https://doi.org/10.2165/00003495-200059030-00012
6. B. Mayur, S. Sandesh, S. Shruti & S. Sung-Yum. Antioxidant and α-glucosidase inhibitory properties of *Carpesium abrotanoides*. *Journal of Medicinal Plants Research*, **4**(15), 1547–1553 (2010). URL: https://academicjournals.org/article/article 1380703440_ Mayur %20et%20al%20PDF.pdf
7. M. Das, R. Jaine, A.K. Agrawal, K. Thanki & S. Jain. Macromolecular bipill of gemcitabine and methotrexate facilitates tumor-specific dual drug therapy with higher benefit-to-risk ratio. *Bioconjugate Chemistry*, **25**(3), 501–509 (2014). https://doi.org/10.1021/bc400477q
8. K. Brown, A. Weymouth-Wilson & B. Linclau. A linear synthesis of gemcitabine. *Carbohydrate Research*, **406**, 71–75 (2015). https://doi.org/10.1016/j.carres.2015.01.001
9. S. Yang, D. Luo, N. Li, C. Li, S. Tang & Z. Huang. New mechanism of gemcitabine and its phosphates: DNA polymerization disruption via 3′–5′ exonuclease inhibition. *Biochemistry*, **59**(45), 4344–4352 (2020). https://doi.org/10.1021/acs.biochem.0c00543
10. A. El-Laghdach, M.I. Matheu, S. Castillón, C. Bliard, A. Olesker & G. Lukacs. A new and extremely fast synthesis of 2-deoxy-2,2-difluoro-d-arabino-hexose (2-deoxy-2,2-difluoro-d-glucose). *Carbohydrate Research*, **233**, C1–C3 (1992). https://doi.org/10.1016/S0008-6215(00)90944-3
11. A. Rouf, M.A. Aga, B.Kumar, S.Taneja, (R)-2,3-Cyclohexylideneglyceraldehyde, a Chiral Pool Synthon for the Synthesis of 2-Azido-1,3-diols, *Helvetica Chimica Acta*, **98**(6) (2015). DOI:10.1002/hlca.201400344
12. J.A. Weigel. A new method for the synthesis of α,α-difluoro-β-hydroxy esters through the enolization of s-tert-butyl difluoroethanethioate. *The Journal of Organic Chemistry*, **62**(18), 6108–6109 (1997). https://doi.org/10.1021/jo9711596
13. Y. Matsumura, H. Fujii, T. Nakayama, Y. Morizawa & A.J. Yasuda. Titanium-promoted highly stereoselective synthesis of α,α-difluoro-β,γ-dihydroxyester. Simple route to 2-deoxy-2,2-difluororibose. *Journal of Fluorine Chemistry*, **57**(1-3), 203–207 (1992). https://doi.org/10.1016/s0022-1139(00)82832-8
14. M. Monajjemi, M.H. Razavian, F. Mollaamin, F. Naderi & B. Honarparvar. A theoretical thermochemical study of solute-solvent dielectric effects in the displacement of codon-anticodon base pairs. *Russian Journal of Physical Chemistry A,* **82**(13), 2277–2285 (2008). https://doi.org/10.1134/s0036024408130207
15. F. Mollaamin, S. Shahriari & M. Monajjemi. Therapeutic role of medicinal plants against viral diseases focusing on COVID-19: Application of computational chemistry towards drug design. *Revista Colombiana de Ciencias Químico-Farmacéuticas*, **53**(1), 19–43 (2024). https://doi.org/10.15446/rcciquifa.v53n1.112978
16. F. Mollaamin & M. Monajjemi. Drug delivery using doping of boron nitride nanosensor towards releasing chloroquine drug in the cells: A promising method for overcoming viral disease. *Revista Colombiana de Ciencias Químico-Farmacéuticas*, **53**(2), 430–454 (2024). https://doi.org/10.15446/rcciquifa.v53n2.114450
17. M. Monajjemi, H. Aghaie & F. Naderi. Thermodynamic study of interaction of TSPP, CoTsPc, and FeTsPc with calf thymus DNA. *Biochemistry Moscow*, **72**(6), 652–657 (2007). https://doi.org/10.1134/s0006297907060089
18. F. Mollaamin & M. Monajjemi. Molecular modelling framework of metal-organic clusters for conserving surfaces: Langmuir sorption through the TD-DFT/ONIOM approach. *Molecular Simulation*, **49**(4), 365–376 (2023). https://doi.org/10.1080/08927022.2022.2159996
19. F. Mollaamin & M. Monajjemi. Transition metal (X = Mn, Fe, Co, Ni, Cu, Zn)-doped graphene as gas sensor for CO_2 and NO_2 detection: a molecular modeling framework by DFT perspective. *Journal of Molecular Modeling*, **29**(4), 119 (2023). https://doi.org/10.1007/s00894-023-05526-3

20. M. Monajjemi, F. Naderi, F. Mollaamin & M. Khaleghian. Drug design outlook by calculation of second virial coefficient as a nano study. *Journal of the Mexican Chemical Society*, **56**(2), 207–211 (2012). https://doi.org/10.29356/jmcs.v56i2.323
21. M. Monajjem, N. Karachi & F. Mollaamin. The investigation of sequence-dependent interaction of messenger RNA binding to carbon nanotube. *Fullerenes, Nanotubes and Carbon Nanostructures*, **22**(7), 643–662 (2014). https://doi.org/10.1080/1536383x.2012.717557
22. M. Monajjemi. Quantum investigation of non-bonded interaction between the $B_{15}N_{15}$ ring, and BH_2NBH_2 (radical, cation, anion) systems: A nano molecularmotor. *Structural Chemistry*, **23**(2), 551–580 (2012). https://doi.org/10.1007/s11224-011-9895-8
23. M. Monajjemi & J.E. Boggs. A new generation of BnNn rings as a supplement to boron nitride tubes and cages. *Journal of Physical Chemistry A*, **117**(7), 1670–1684 (2013). https://doi.org/10.1021/jp312073q
24. I. Zehbe, E. Wilander, H. Delius & M. Tommasino. Human papillomavirus 16 E6 variants are more prevalent in invasive cervical carcinoma than the prototype. *Cancer Research*, **58**(4), 829–833 (1998). URL: https://pubmed.ncbi.nlm.nih.gov/9485042/
25. L.F.Xi, G.W. Demers, L.A. Koutsky, N.B. Kiviat, J. Kuypers, D.H. Watts, K.K. Holmes & D.A. Galloway. Analysis of human papillomavirus type 16 variants indicates establishment of persistent infection. *The Journal of Infectious Diseases*, **172**(3), 747–755 (1995). https://doi.org/10.1093/infdis/172.3.747
26. F. Mollaamin, J. Najafpour, S. Ghadami, A.R. Ilkhani, M. Akrami & M. Monajjemi. The electromagnetic feature of $B_{15}N_{15}Hx$ (x = 0, 4, 8, 12, 16, and 20) nano, rings: Quantum theory of atoms in molecules/NMR approach. *Journal of Computational and Theoretical Nanoscience*, **11**(5), 1290–1298 (2014). https://doi.org/10.1166/jctn.2014.3495
27. M. Monajjemi & F. Mollaamin. Intermolecular simulation of nanobiological structures in point of potential energy and second virial coefficient. *Journal of Computational and Theoretical Nanoscience*, **9**(12), 2208–2214 (2012). https://doi.org/10.1166/jctn.2012.2640
28. F. Mollaamin & M. Monajjemi. Electric and magnetic evaluation of aluminum–magnesium nanoalloy decorated with germanium through heterocyclic carbenes adsorption: A density functional theory study. *Russian Journal of Physical Chemistry B*, **17**(3), 658–672 (2003). https://doi.org/10.1134/s1990793123030223
29. M. Monajjemi, S. Ketabi, M.H. Zadeh & A. Amiri. Simulation of DNA bases in water: Comparison of the Monte Carlo algorithm with molecular mechanics force fields. *Biochemistry Moscow*, **71**(Suppl. 1), S1–S8 (2006). https://doi.org/10.1134/s0006297906130013
30. F. Mollaamin & M. Monajjemi. Graphene-based resistant sensor decorated with Mn, Co, Cu for nitric oxide detection: Langmuir adsorption & DFT method. *Sensor Review*, **43**(4), 266–279 (2023). https://doi.org/10.1108/SR-03-2023-0040
31. M. Monajjemi, B. Honarparvar, S.M. Nasseri & M. Khaleghian. NQR and NMR study of hydrogen bonding interactions in anhydrous and monohydrated guanine cluster model: A computational study. *Journal of Structural Chemistry*, **50**(1), 67–77 (2009). https://doi.org/10.1007/s10947-009-0009-z
32. B. Khalili-Hadad, F. Mollaamin & M. Monajjemi. Biophysical chemistry of macrocycles for drug delivery: A theoretical study. *Russian Chemical Bulletin*, **60**(2), 238–241 (2011). https://doi.org/10.1007/s11172-011-0039-5

HOW TO CITE THIS ARTICLE

M. Monajjemi, F. Mollaamin, A. Ghadami, R. Souri, Z. Aghakouchaki, M. Aghaei, Z. Solati & Y. Shahverdy. Cancer drug therapy by Gemcitabine derivatives with PEG linker for gynecological disease, beside speech therapy in implementation of biomedical caring of post-operative. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 151–166 (2026). https://doi.org/10.15446/rcciquifa.v55n1.125080

Review article

Historical analysis and social and health impact of fentanyl use in Colombia

Jorge Iván Sierra Duran[1], Marcia Andrea Yucuma-Guzmán[2]*, Diego Ivan Caviedes-Rubio[2], Daniel Ricardo Delgado[2] & Luis Manuel Lozano Ramos[1]

[1] Grupo de Investigación Innovación y Tecnología, Escuela Antidrogas "Mayor Wilson Quintero Martínez" (ESAND), Bogotá, Colombia

[2] Grupo de Investigación de Ingenierías UCC-Neiva, Facultad de Ingeniería, Sede Neiva, Universidad Cooperativa de Colombia, Neiva 410001, Colombia

*Corresponding author E-mail: marcia.yucuma@ucc.edu.co

Received: October 17, 2025
Corrected: November 19, 2025
Accepted: November 26, 2025

https://doi.org/10.15446/rcciquifa.v55n1.125082

SUMMARY

Introduction: Fentanyl, a synthetic opioid up to 100 times more potent than morphine, has ignited a global public health crisis. In Colombia, its increasing diversion from the medical system to illicit markets represents an emerging threat that demands in-depth analysis. **Purpose:** The objective of this research was to conduct a comprehensive analysis of the fentanyl phenomenon in the country, covering its history, current socio-health impact, and the systemic failures that facilitate its spread. **Methodology:** A qualitative methodology was employed, combining narrative documentary analysis with interviews of key informants directly involved in the legal control and institutional response to the opioid. **Results:** The results reveal that Colombia is in an early phase, defined by pharmaceutical diversion rather than clandestine production. A complex trafficking network exists, involving both legal and illegal actors, with hospitals and clinics being the primary point of leakage according to 67% of those interviewed. This diversion causes a severe health impact, including deaths by overdose (a link made by 78% of experts), and disproportionately affects at-risk youth (44%) and marginalized communities (33%). **Conclusions:** The study concludes that this problem is a symptom of deep-seated institutional flaws and social vulnerabilities. It highlights a critical window of opportunity to act, making a comprehensive response that combines public health strategies with the rigorous strengthening of controls imperative to contain the threat and prevent an escalation of the crisis.

Keywords: Fentanyl; illicit trafficking; non-medical use; overdose; pharmaceutical diversion; public health; social vulnerability.

RESUMEN

Análisis histórico e impacto social y sanitario del consumo del fentanilo en Colombia

Introducción: El fentanilo, un opioide sintético hasta 100 veces más potente que la morfina, ha provocado una crisis de salud pública global. En Colombia, su desvío desde el sistema médico hacia mercados ilícitos constituye una amenaza emergente que requiere un análisis profundo. **Objetivo:** Esta investigación tuvo como objetivo analizar integralmente el fenómeno en el país, abarcando su historia, impacto

sociosanitario y las fallas sistémicas que facilitan su propagación. **Metodología:** Se utilizó una metodología cualitativa que combinó análisis documental narrativo y entrevistas a informantes clave directamente involucrados en el control legal y la respuesta institucional al opioide. **Resultados:** Los resultados revelan que Colombia está en una fase inicial, definida por el desvío farmacéutico en lugar de la producción clandestina. Existe una compleja red de comercialización con actores tanto legales como ilegales, siendo los hospitales y clínicas el principal punto de fuga según el 67% de los entrevistados. Este desvío provoca un grave impacto sanitario, incluyendo muertes por sobredosis (vinculado por el 78% de los expertos), y afecta desproporcionadamente a jóvenes en riesgo (44%) y comunidades marginadas (33%). **Conclusiones:** Se concluye que esta problemática es un síntoma de profundas fallas institucionales y vulnerabilidades sociales. El estudio destaca una ventana de oportunidad crítica para actuar, siendo imperativa una respuesta integral que combine estrategias de salud pública y un fortalecimiento riguroso de los controles para contener la amenaza y evitar una escalada de la crisis.

Palabras Clave: Fentanilo; tráfico ilícito; uso no médico; sobredosis; desviación de productos farmacéuticos; salud pública; vulnerabilidad social.

RESUMO

Análise histórica e impacto social e na saúde do uso de fentanil na Colômbia

Introdução: O fentanil, um opioide sintético até 100 vezes mais potente que a morfina, desencadeou uma crise global de saúde pública. Na Colômbia, seu crescente desvio do sistema médico para mercados ilícitos representa uma ameaça emergente que exige uma análise aprofundada. **Objetivo:** O objetivo desta pesquisa foi realizar uma análise abrangente do fenômeno do fentanil no país, abordando sua história, o impacto socio-sanitário atual e as falhas sistêmicas que facilitam sua disseminação. **Metodologia:** Foi empregada uma metodologia qualitativa, combinando análise documental narrativa com entrevistas com informantes-chave diretamente envolvidos no controle legal e na resposta institucional ao opioide. **Resultados:** Os resultados revelam que a Colômbia está em uma fase inicial, definida pelo desvio de medicamentos em vez da produção clandestina. Existe uma complexa rede de tráfico, envolvendo atores legais e ilegais, sendo hospitais e clínicas os principais pontos de desvio, segundo 67% dos entrevistados. Esse desvio causa um grave impacto na saúde, incluindo mortes por overdose (relação apontada por 78% dos especialistas), e afeta desproporcionalmente jovens em situação de risco (44%) e comunidades marginalizadas (33%). **Conclusões:** O estudo conclui que esse problema é sintoma de falhas institucionais profundas e vulnerabilidades sociais. Ele destaca uma janela de oportunidade crítica para agir, tornando imperativa uma resposta abrangente que combine estratégias de saúde pública com o fortalecimento rigoroso dos controles para conter a ameaça e evitar a escalada da crise.

Palavras-chave: Fentanil; tráfico ilícito; uso não médico; overdose; desvio de medicamentos; saúde pública; vulnerabilidade social.

1. INTRODUCTION

Fentanyl, a synthetic opioid initially developed as a powerful analgesic for controlled clinical settings, now represents one of modern pharmacology's greatest paradoxes [1-3]. Its extraordinary potency, 50 to 100 times greater than that of morphine, makes it an indispensable therapeutic tool but also places it at the epicenter of a devastating global public health crisis [4-6]. Fueled by its illicit manufacture and distribution, this substance has caused a large-scale health emergency in North America, and its presence is increasingly notable in Latin America [7, 8]. In Colombia, the growing shift of fentanyl from the medical sphere to illegal markets has raised alarm bells, highlighting a worrisome trend where synthetic opioids, often mixed with other drugs, are beginning to displace traditional substances and exponentially multiply the risk of fatal overdoses [9, 10].

Although the fentanyl crisis has been extensively documented in countries like the United States and Mexico, there is a lack of comprehensive, specific analysis of its impact in Colombia [7]. Most reports focus on seizure statistics or general epidemiological alerts [11, 12]. A thorough investigation is needed to connect the drug's historical journey—from its therapeutic origins to its current status as a threat—with the unique characteristics of the Colombian context [10]. There is a need for a study that examines the interconnectedness of institutional failures, socioeconomic vulnerabilities, and local drug trafficking dynamics that are facilitating its spread and use in the country.

The primary objective of this research is to comprehensively analyze the fentanyl phenomenon in Colombia, detailing its historical evolution, its current impact on public health and public safety, and the systemic failures that have allowed it to take root. The goal is to understand the structural factors that promote its diversion and illicit use in order to provide an integrated diagnosis that can serve as a foundation for designing more effective public policies. This study is crucial given the sustained increase in fentanyl-related cases in Colombia, as warned by the Drug Observatory [10]. The complexity of the phenomenon—spanning from pharmacology to criminology and sociology—demands a multidisciplinary approach that moves beyond fragmented perspectives. Understanding the deep-seated causes of its expansion is critical not only for mitigating immediate harm through public health strategies but also for anticipating and preventing a crisis of the magnitude seen in other regions.

The growing fentanyl crisis in Colombia is not an isolated event or a simple extension of traditional drug trafficking; it is the predictable consequence of a set of interconnected systemic failures. It is postulated that the combination of deficient pharmaceutical regulation, drug control strategies focused on interdiction rather than public health, and profound socioeconomic vulnerabilities (such as lack of access to mental health services) creates an environment conducive to the rapid expansion of this synthetic opioid's use [13].

This article traces the journey of fentanyl from its creation in the Janssen laboratories to its current central role in the global public health crisis. Its pharmacology will be analyzed to understand the root of its potency and lethality. Subsequently, the focus will shift to the Colombian landscape, examining how illicit market dynamics and institutional weaknesses have shaped a high-risk scenario. Finally, current responses will be evaluated, and a paradigm shift toward an integrated approach of public health and harm reduction will be proposed as the only effective path to contain this growing threat.

1.1. Historical development of fentanyl

The story of fentanyl begins in the late 1950s at the Janssen Pharmaceutica laboratory in Beerse, Belgium, under the leadership of its founder, Dr. Paul Janssen [14, 15]. A visionary chemist and pharmacologist, Janssen aimed to synthesize an opioid analgesic that would overcome the limitations of morphine, the gold standard of the era (Figure 1) [1]. The objectives were clear: create a molecule with greater potency, a faster onset of action, and, crucially, an improved safety profile, particularly regarding cardiovascular effects. Morphine was known to induce histamine release, which could cause vasodilation and hypotension—undesirable complications during surgical procedures [4, 5].

In 1959, after systematically synthesizing and evaluating a series of compounds, Janssen's team achieved its goal with the creation of fentanyl [16-18]. The new molecule proved to be 50 to 100 times more potent than morphine, with an almost immediate onset when administered intravenously and a shorter duration of action, allowing for more precise control by anesthesiologists [19, 20]. Furthermore, it did not cause significant histamine release, providing the desired hemodynamic stability. Following rigorous clinical trials, fentanyl was introduced into

medical practice in Europe in 1963 and received approval from the U.S. Food and Drug Administration (FDA) in 1968, marketed under the brand name Sublimaze® [21-23]. Its arrival marked a milestone in modern anesthesiology and it quickly became one of the most widely used opioids in operating rooms worldwide [24, 25].

The success of fentanyl in surgery prompted Janssen Pharmaceutica and other companies to explore new delivery methods to expand its therapeutic utility beyond the operating room [26-28]. This innovation led to the development of various formulations designed to manage different types of severe pain [19, 20]. One of the most significant innovations came in the 1990s with the introduction of the fentanyl transdermal patch, marketed as Duragesic® [29, 30]. This delivery system revolutionized the treatment of chronic pain, especially persistent cancer-related pain [31, 32]. The patch, which adheres to the skin, contains a fentanyl reservoir that is released slowly and steadily, absorbing through the skin into the bloodstream over 48 to 72 hours. This provided constant, sustained pain relief, significantly improving patients' quality of life and freeing them from the need for frequent oral analgesics [3, 29, 33].

Subsequently, to address "breakthrough pain"—acute, transient exacerbations of pain in patients with otherwise controlled chronic pain—fast-acting formulations were developed [34]. Among these were transmucosal oral lozenges, such as Actiq®, popularly known as "fentanyl lollipops," which consisted of a fentanyl matrix on an oral applicator [35]. As it dissolved in the mouth, the drug was rapidly absorbed through the oral mucosa, providing almost immediate relief. This was followed by other formulations like buccal tablets (Fentora®), sublingual tablets (Abstral®), and nasal and sublingual sprays, all designed for the same purpose: rapid drug delivery to control peaks of intense pain [36-38].

However, while fentanyl is a promising tool against pain, it has generated one of the most devastating public health crises in history [39]. The fentanyl crisis did not emerge in a vacuum; it is the most recent and lethal phase of an opioid epidemic that has evolved in three distinct, overlapping waves in the United States—a public health catastrophe largely incubated within the healthcare system and the pharmaceutical industry itself [39].

The first wave began in the 1990s, driven by a paradigm shift in pain management and, critically, by the business practices of the pharmaceutical industry. In 1995, Purdue Pharma launched OxyContin, an extended-release formulation of the opioid oxycodone [40]. The company undertook an unprecedented and highly aggressive marketing campaign targeting both doctors and patients, in which the drug's addiction risk was systematically downplayed. The idea was promoted that due to its slow-release mechanism, the potential for abuse was minimal. Sales representatives assured doctors that less than 1% of users would develop an addiction, despite the company's own internal studies from as early as 1999 indicating the true figure could be as high as 13%. This misinformation, coupled with lobbying to treat pain as the "fifth vital sign," led to the massive over-prescription of opioid analgesics for a wide range of conditions, many of which did not warrant such a powerful treatment. By 2012, at the peak of this wave, 259 million opioid prescriptions were issued in the United States—almost one for every person in the country. This practice created a vast population of patients with iatrogenic dependence, an addiction caused by medical treatment [41].

The second wave began around 2010 as a direct consequence of the first. As the scale of the addiction crisis became apparent, health and regulatory authorities began taking steps to restrict opioid prescribing. Stricter guidelines and monitoring systems were implemented, making it harder for many who had already developed a dependency to obtain prescriptions. Without adequate access to treatment for opioid use disorder, a large number of people were forced to seek alternatives on the illegal market. Heroin, being cheaper and more accessible,

became the primary option, leading to a resurgence in its use and a rise in related overdose deaths [42, 43].

The third and most deadly wave began around 2013 with the influx of illicitly manufactured fentanyl (IMF) into the drug market. Traffickers quickly realized the economic advantages of fentanyl: its synthetic production in a lab was cheaper, faster, and more scalable than cultivating poppies for heroin, and its extreme potency allowed for a much larger number of doses to be trafficked in a smaller physical volume. IMF began appearing as an adulterant in the heroin supply, often without the user's knowledge, triggering an unprecedented and exponential spike in overdose deaths. Between late 2013 and 2014, over 700 fentanyl-related deaths were already recorded in the U.S., a figure that would only escalate dramatically in the following years [44].

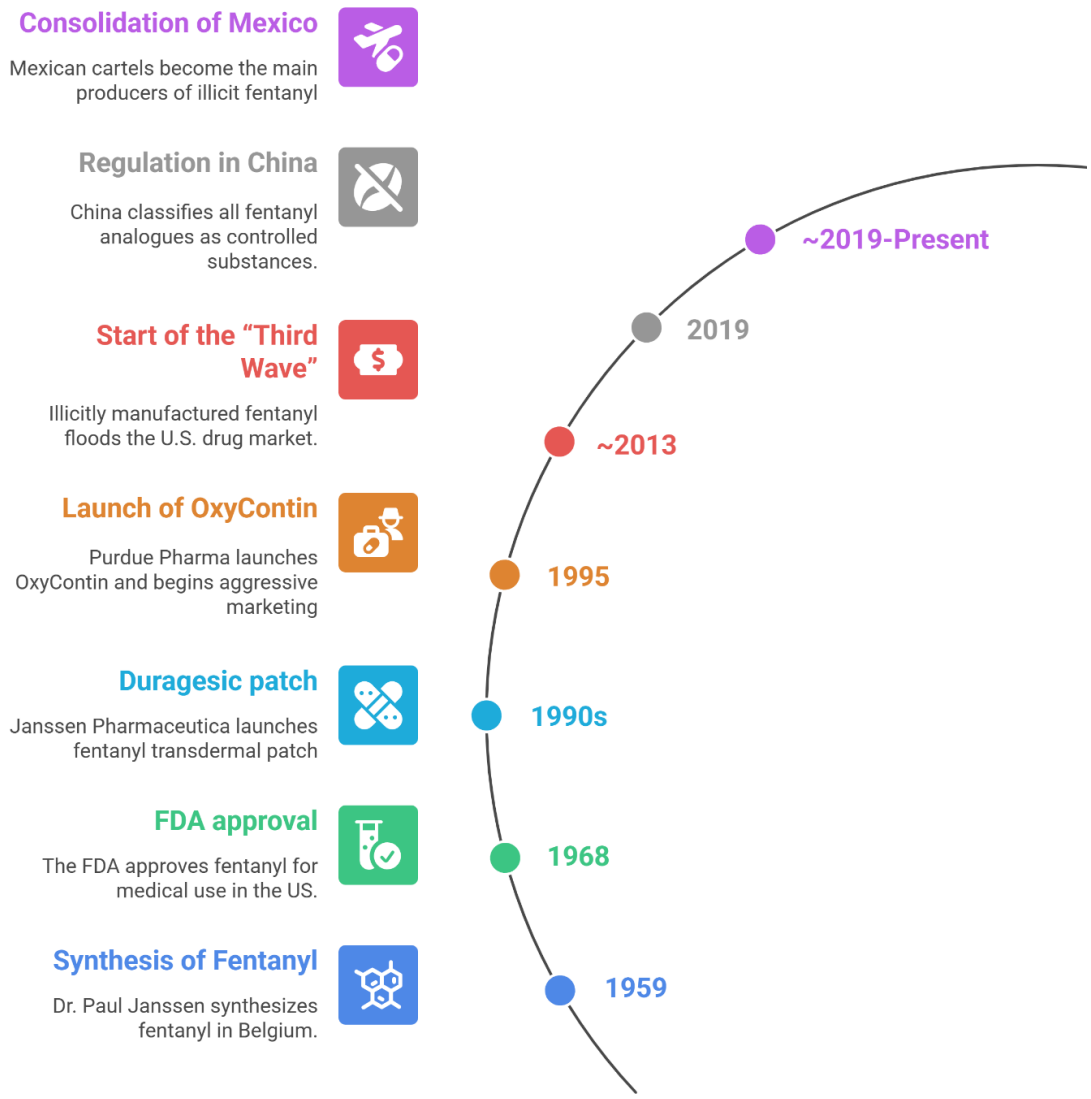


Figure 1. Key milestones in the history of fentanyl.

1.2. Chemistry, synthesis, and licit *vs.* illicit routes

From a chemical perspective, fentanyl (Figure 2), systematically named *N*-(1-(2-phenethyl)-4-piperidinyl-*N*-phenyl-propanamide (Molecular formula: $C_{22}H_{28}N_2O$), belongs to the 4-anilinopiperidine family [1]. Its structure is fundamentally different from natural and semi-syn-

thetic opiates like morphine, codeine, or heroin, which are based on the phenanthrene skeleton. Fentanyl is a fully synthetic compound derived from meperidine, giving it distinct pharmacological properties [45, 46].

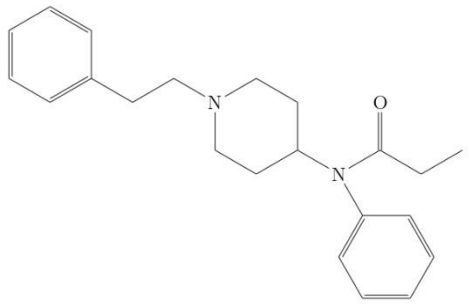


Figure 2. Chemical structure of fentanyl.

The structure of fentanyl has served as a molecular scaffold for developing numerous analogs by selectively modifying parts of the molecule to modulate its potency, onset, and duration of action. In the clinical setting, this has resulted in a family of highly useful opioids [47]:

i). Alfentanil and Sufentanil (Figure 3 and 4): Analogs with an even faster onset and shorter duration, ideal for brief surgical procedures [48, 49].

ii). Remifentanil (Figure 5): A unique analog with an ester linkage that is rapidly hydrolyzed by plasma esterases, giving it an ultra-short half-life (3-5 minutes) and allowing for very precise control of anesthesia without accumulation [50, 51].

iii). Carfentanil (Figure 6): An extremely potent analog, approximately 10,000 times more potent than morphine, whose use is restricted to tranquilizing large animals like elephants. Its appearance on the illicit drug market has caused clusters of mass overdoses due to its incredibly narrow safety margin [52, 53].

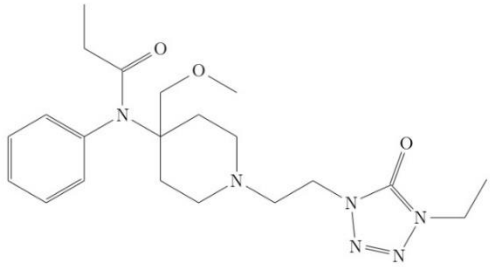


Figure 3. Chemical structure of Alfentanil.

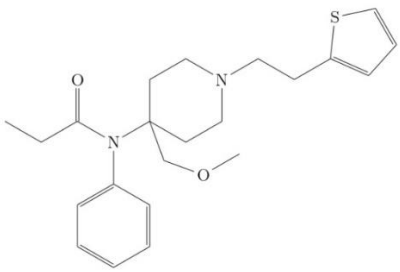


Figure 4. Chemical structure of Sufentanil.

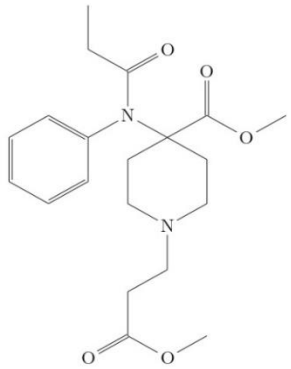


Figure 5. Chemical structure of Remifentanil.

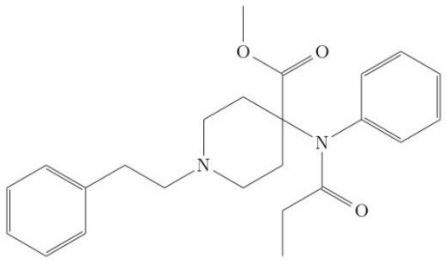


Figure 6. Chemical structure of Carfentanil.

These analogs, along with many others created in clandestine labs and known as "designer drugs," demonstrate the versatility of the fentanyl structure for chemical manipulation [54, 55]. The original synthesis developed by Paul Janssen is an example of elegance in organic chemistry, but its complexity and the reagents it requires make it impractical for large-scale clandestine production. The classic route, described in scientific literature, involves several steps [55, 56]:

i). Condensation of 1-benzyl-4-piperidone with aniline to form a Schiff base.
ii). Reduction of the resulting imine double bond to obtain 1-benzyl-4-anilinopiperidine.
iii). Acylation of this intermediate with propionic anhydride.
iv). Debenzylation of the protecting group on the piperidine nitrogen via catalytic hydrogenation, using a palladium-on-carbon (Pd/C) catalyst. This step produces the key precursor known as norfentanyl.
v). Finally, *N*-alkylation of norfentanyl with a 2-phenethyl halide to yield fentanyl.

Although efficient in a pharmaceutical lab setting, the use of precious metal catalysts like palladium and the need for high-pressure hydrogenation equipment make this route costly and technically demanding, pushing it out of favor with illicit producers.

Unlike pharmaceutical labs, clandestine laboratories have adopted alternative synthesis routes that prioritize simplicity, low cost, and, above all, the use of chemical precursors that, until recently, were not strictly regulated. The two best-known methodologies are the "Siegfried route" and the "Gupta route". The Siegfried route, in particular, has become the most prevalent. This route often begins with a key precursor: N-phenethyl-4-piperidone (NPP) (Figure 7) [57, 58].

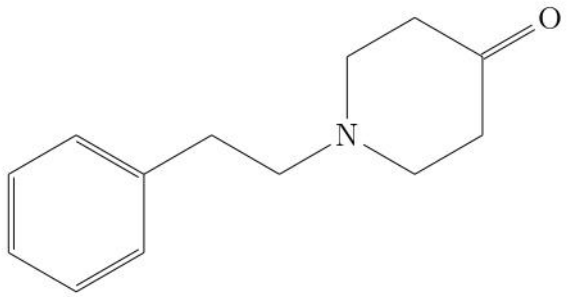


Figure 7. Chemical structure of *N*-phenethyl-4-piperidone.

From NPP, the synthesis can proceed in two main ways [47]:

i). Two-step pathway: First, a condensation of NPP with aniline followed by a reduction (e.g., with sodium borohydride) to form the intermediate 4-anilino-N-phenethylpiperidine (ANPP). Then, the ANPP is acylated with propionyl chloride or propionic anhydride to produce fentanyl.
ii). One-step pathway (reductive amination): NPP reacts directly with aniline in the presence of a reducing agent to form ANPP, which is then acylated as in the previous pathway.

Due to their central role in illicit production, both NPP and ANPP have been subjected to strict international control and are included in Schedule I of the 1988 United Nations Convention [59]. In response, other precursors and intermediates like norfentanyl, 4-anilinopiperidine (4-AP), and 1-boc-4-AP have also been added to control lists in recent years. While controlling precursors is a necessary tool, it has proven to be an inherently reactive strategy with limited effectiveness. Transnational organized crime has demonstrated remarkable chemical agility, consistently outmaneuvering the slow bureaucracy of international regulation in a perpetual

game of "cat and mouse". The process is cyclical and predictable: law enforcement and international bodies identify an essential chemical precursor, like NPP. After a lengthy diplomatic and regulatory process, the substance is finally added to UN control lists, restricting its legal trade. However, chemists working for criminal organizations employ two main strategies to evade these controls. The first is the use of "masked" or "designer" precursors [60, 61].

This technique is based on the organic synthesis concept of "protecting group chemistry". A controlled precursor, like 4-anilinopiperidine (4-AP), is taken, and a "protecting group" is added to a reactive part of the molecule. A common example is the tert-butoxycarbonyl (Boc) group, which is added to the piperidine nitrogen to create 1-boc-4-AP [62]. This new molecule, though chemically very similar, is not (initially) on the list of controlled substances and can be traded with fewer restrictions. For the clandestine producer, removing the "Boc" group is a simple, high-yield reaction step that regenerates the necessary 4-AP precursor to continue the synthesis [62].

The second strategy is to move "upstream" in the synthesis chain. Instead of trying to acquire the controlled precursor (e.g., NPP), clandestine labs acquire basic chemical building blocks—which have widespread industrial use and are virtually impossible to regulate—and synthesize the precursor themselves. This "upstream synthesis" capability gives them extraordinary resilience against precursor control efforts. This adaptability shows that an approach focused solely on regulating a limited number of chemicals is destined for partial and temporary success. The true strength of the criminal system lies in its chemical knowledge and capacity for innovation—a challenge that current policies struggle to counter effectively.

2. METHODOLOGY

2.1. Research design

This research is part of a narrative literature review, a methodological approach that allows for a comprehensive exploration and synthesis of a topic from various perspectives. This approach identifies the topic's historical development, current debates, and emerging areas of research. Then, it connects the topic with information generated by experts. This method was chosen because it is well-suited to integrating knowledge from multiple disciplines, including pharmacology, public health, sociology, and history, to construct a cohesive analysis of the impact of fentanyl in Colombia.

2.1.1. Search strategy and source selection

A systematic and multifaceted search strategy was designed to ensure a comprehensive and relevant review of the literature. The search was conducted between July and September 2025, with no initial publication date restrictions, to include foundational historical documents on the synthesis and development of fentanyl. However, priority was given to works published in the last two decades to analyze its more recent social and health impacts.

The selection of articles was based on an iterative process. Initially, seminal articles and comprehensive reviews were identified. Subsequently, a "snowballing" technique was applied, reviewing the reference lists of key documents to find additional relevant sources.

2.1.2. Databases searched

High-impact global and regional databases were consulted to ensure broad and diverse coverage. The global databases consulted were Scopus, Web of Science (WoS), PubMed/MEDLINE and Google Scholar. The Latin American Databases consulted were: SciELO (Scientific Electronic Library Online), LILACS (Latin American and Caribbean Health Sciences Literature)

and Redalyc (Network of Scientific Journals from Latin America and the Caribbean, Spain, and Portugal).

2.1.3. Search terms

Combinations of search terms were used in both English and Spanish, employing Boolean operators (AND, OR). The keywords included in English were: "Fentanyl", "Fentanyl history", "Fentanyl synthesis", "Opioid crisis", "Synthetic opioids", "Drug policy", "Public health", "Social impact", "Colombia", "Latin America"; and the keywords included in Spanish were: "Fentanilo", "Historia del fentanilo", "Crisis de opioides", "Opioides sintéticos", "Salud pública", "Impacto social", "Política de drogas", and "Colombia".

2.1.4. Inclusion and exclusion criteria

The following criteria were applied for source selection:

2.1.4.1. Inclusion criteria:

i). Articles addressing the history, pharmacology, chemical synthesis, or analogs of fentanyl.
ii). Studies on the social, health, and security impacts of fentanyl and other synthetic opioid use.
iii). Documents on drug policies in Colombia and Latin America.
iv). Official reports from governmental and non-governmental organizations (e.g., Ministry of Justice and Law of Colombia, INCB).
v). Articles in English and Spanish.

2.1.4.1. Exclusion criteria:

i). Articles with a purely clinical focus (e.g., specific anesthesia protocols) that did not discuss the broader context of fentanyl use.
ii). Studies with a strictly veterinary focus.
iii). Editorials, letters to the editor, or conference abstracts without substantial data.

2.1.5. Information synthesis

After selection, relevant information was extracted from each of the 64 documents included in this review. The information was organized and synthesized narratively, grouped into the following thematic categories to structure the article: 1) The history and pharmaceutical development of fentanyl and its analogs; 2) The transition from medical to illicit use and the global public health crisis; and 3) The current landscape of fentanyl in Colombia, including its social and health impacts and institutional responses. This approach allows for connecting historical and scientific evidence with the specific social and political context of Colombia.

Furthermore, a qualitative approach with an exploratory and descriptive scope complemented this study. This design was considered the most suitable for addressing an emerging and complex phenomenon, allowing for a deep dive into the social, health, and institutional dimensions of fentanyl use in the country. The qualitative perspective facilitated the analysis of perceptions, experiences, and narratives of key actors, as well as the interpretation of the regulatory and institutional context surrounding the issue.

2.2. Phases of the research process

The study was structured in four sequential and complementary phases to ensure rigor and systematicity, as described in Table 1.

Table 1. Phases of the research process.

Phases	Description	Objective
Phase I	Semi-structured interviews with key informants.	Obtain primary data on the experiences, practices, and perceptions of professionals with direct involvement in the control and management of fentanyl.
Phase II	Documentary review and analysis (official reports, statistics, scientific literature, and regulatory frameworks).	Contextualize, triangulate, and validate qualitative findings with robust secondary sources.
Phase III	Thematic content analysis of interviews and documents.	Identify recurring patterns, emerging categories, and significant relationships within the data corpus.
Phase IV	Synthesis and integrative interpretation of findings.	Formulate evidence-based conclusions and recommendations for public policy and prevention

2.2.1. Unit of analysis and participant selection

The unit of analysis consisted of key informants, defined as professionals and officials with direct involvement in the legal control, oversight, and institutional response to fentanyl in Colombia. A non-probabilistic purposive sampling method was used, selecting individuals who, due to their role and experience, possessed specialized knowledge and a privileged perspective on the phenomenon.

A total of ten (10) interviews were conducted. This number, though small, is justified by the highly specialized nature of the topic. The objective was not statistical representativeness but rather informational saturation and conceptual depth. Given that the circle of experts with direct responsibility for regulating and controlling fentanyl at the national level is limited, priority was given to the quality and richness of the information this select group could provide. The strategy sought depth over breadth, enabling a dense and detailed dialogue that would not be achievable with a larger but less specialized sample. Exclusion criteria were the absence of a direct professional link to the topic and an unwillingness to participate under the terms of informed consent.

2.3. Data collection techniques and instruments

The main data collection techniques were:

1. Semi-Structured Interview: An open-ended question guide was designed to direct the conversation while allowing the flexibility to explore emerging themes. This instrument was validated by expert review (a qualitative research specialist and a professional from the pharmaceutical sector) to ensure its relevance and clarity.
2. Documentary Analysis: A series of documents were collected and analyzed, including reports from the Colombian Drug Observatory, resolutions from the Ministry of Health and Social Protection, and peer-reviewed scientific literature.

The combination of these techniques (source triangulation) allowed for the cross-referencing and complementing of information, thereby strengthening the validity and reliability of the study's findings.

2.4. Data analysis strategy

The collected information was processed through thematic content analysis. The procedure was conducted manually, beginning with the literal transcription of the interviews. Subse-

quently, an open coding process was applied to identify units of meaning and relevant concepts. These codes were grouped into emerging thematic categories, which allowed for the identification of recurring discourses, institutional tensions, and significant patterns in the data. This interpretive approach facilitated a deep understanding of the participants' narratives.

2.5. Ethical considerations

The research adhered strictly to the ethical principles of research involving human subjects. Written informed consent was obtained from all participants before each interview, ensuring their understanding of the study's objectives and their voluntary participation. The confidentiality and anonymity of the informants were guaranteed by assigning alphanumeric codes to unlink their identities from their testimonies. The project and its instruments were submitted for review to ensure compliance with ethical and methodological standards.

3. RESULTS

The findings of this study, based on interviews with key actors, expose recurring patterns in fentanyl use that reveal profound social and health complexities. These patterns underscore the critical challenges Colombia faces in both public health and security.

The fentanyl phenomenon in Colombia presents a distinctive case study, with a nature that differs significantly from the crises observed in Mexico and the United States. The Colombian situation is in an early stage, characterized not by clandestine production but predominantly by the diversion of pharmaceutical products. This context offers a crucial window of opportunity for implementing preventive intervention and containment strategies.

Field findings from interviews with key informants reveal a sophisticated trafficking network involving both actors from the legal circuit and criminal organizations. The analysis of the identified actors shows the following distribution:

i). 44% are pharmaceutical importers and distributors, indicating a critical vulnerability in the legal supply chain.

ii). 44% is composed of micro-trafficking networks, the National Narcotics Fund, and some clinics, showing the permeability between the health system and illicit markets.

iii). 11% is attributed to illegal groups specializing in the diversion of medicines.

This configuration highlights the structural weaknesses of the Colombian health system in the face of drug trafficking. The fact that 67% of interviewees indicate that fentanyl is primarily diverted from hospitals and clinics confirms that the epicenter of the problem lies in failures of control, monitoring, and possible corruption within the health institutions themselves.

The pharmaceutical diversion thesis is corroborated by the nature of the seizures. Unlike in North America, where clandestinely produced powder and pills predominate, the overwhelming majority of seizures in Colombia are vials of injectable solution. Authorities have confirmed that, to date, no fentanyl synthesis labs have been detected in the country. The volume of these seizures shows an alarming upward trend. While reports in 2018 and 2019 were of quantities smaller than 10 vials, since 2023 significant seizures have been recorded, such as 233 vials in Bogotá, 455 in Cartagena, and 2,000 in Maicao in February 2024 [10].

Regarding mortality, although the figures are low, the trend is also concerning. Between 2013 and 2023, 30 fentanyl-associated deaths were reported, but more than half of them (16 cases) occurred between 2021 and 2023, indicating a clear acceleration of the problem. Further-

more, the mixing with other drugs such as heroin, cocaine, and primarily ketamine was detected in one-third of fatal cases and mentioned by 11% of interviewees as a practice that considerably increases the risk of lethal overdose.

In response to this emerging threat, authorities have begun to mobilize:

i). **Health surveillance:** INVIMA has issued health alerts about fraudulent batches, while the National Institute of Health (INS) maintains surveillance of poisonings through the SIVIGILA system.

ii). **Security strategy:** In 2023, the National Police presented the "Anticipate to Prevent" plan, a comprehensive approach that ranges from prevention and harm reduction to interinstitutional cooperation.

iii). **Legislative reforms:** Bills have been introduced (PL 194 and PL 227 of 2023, [63]) that seek to classify fentanyl trafficking as a standalone crime with significantly higher penalties, in line with its lethality.

The current problem, centered on diversion from the health system, presents a critical opportunity to act. Future success will depend on the state's ability to rigorously strengthen controls over the pharmaceutical supply chain while simultaneously expanding public health strategies, such as risk education and overdose management training. The risk of escalation is imminent; the country's criminal networks could venture into large-scale synthesis or importation if they identify a profitable market. The actions taken today will determine whether Colombia manages to contain this crisis in its current phase or moves toward a public health scenario of the magnitude seen in North America (Figure 8).

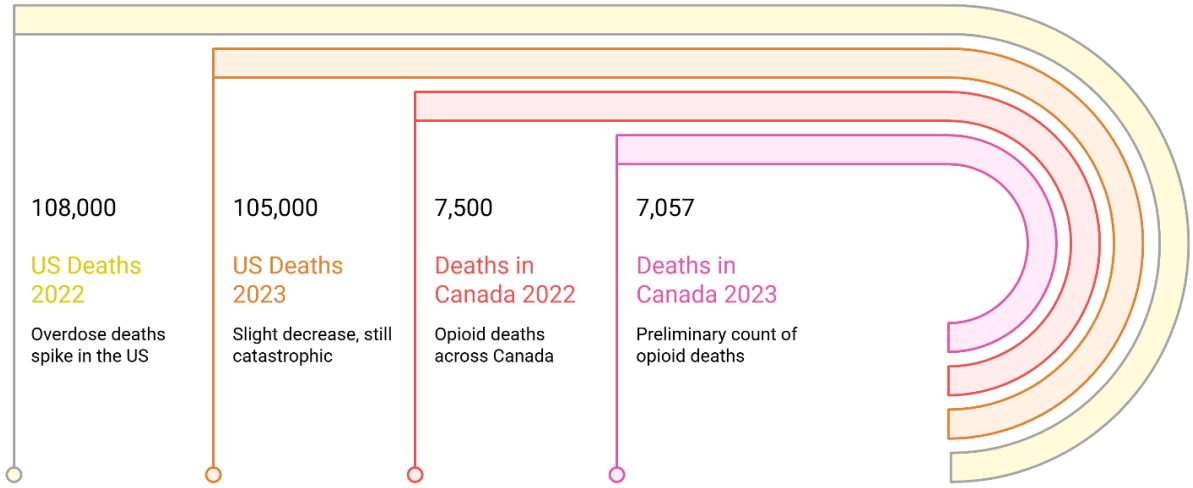


Figure 8. Opioid-related deaths in North America.

3.1. Analysis of the socio-health impact and systemic repercussions of fentanyl use

Fentanyl use generates profound and multifaceted consequences that transcend mortality statistics, permeating the social fabric and overburdening public institutions (Figure 9). The perception of its severity is nearly unanimous: 78% of the experts interviewed state that the primary health impact is the increased prevalence of deaths by overdose. However, this direct effect is merely the epicenter of a crisis with much broader systemic repercussions.

The impact extends to the core of society, where 33% of participants associate opioid use with serious social problems such as family disintegration and rising crime. This disintegration materializes as an increase in children entering the foster care system and a significant loss of economic productivity due to disability and premature death in the working-age population. At the community level, the phenomenon generates collective trauma, fosters stigma,

and erodes social cohesion, which worsens the perception of insecurity and degrades the quality of life, as noted by Vega-Pulido and Estrella (2013) regarding violence associated with drug trafficking [64].

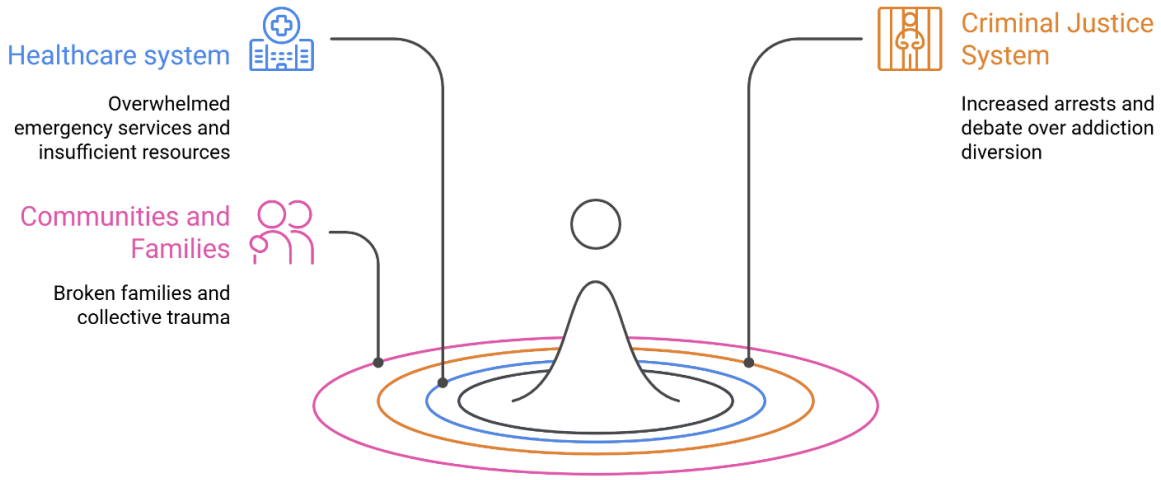


Figure 9. Consequences of the fentanyl crisis.

This crisis places an unsustainable strain on public systems. The healthcare system is overwhelmed, with emergency services and intensive care units saturated by the treatment of acute overdoses and their long-term complications, such as anoxic brain injury. Simultaneously, addiction treatment and mental health centers operate with insufficient resources for a demand that outstrips their capacity. Meanwhile, the criminal justice system faces an increase in drug-related arrests, perpetuating a cycle of criminalizing addiction that sparks debate about the need to shift from a punitive approach to a public health one.

Vulnerability to these impacts is not uniform. The data reveal that the most affected groups are at-risk youth (identified by 44% of interviewees) and marginalized communities (33%). Structural inequality, lack of opportunities, and institutional weakness act as predisposing factors, turning substance use into an escape from precarious living conditions.

Faced with this complex landscape, experts propose a multi-sectoral preventive approach. A majority (56%) consider educational campaigns to be the priority strategy. However, the need for a comprehensive approach is recognized, complemented by strict monitoring in pharmacies (22%) and specialized training in public health (22%). These findings reaffirm the imperative to articulate a state response that integrates public education with the strengthening of institutional controls and international cooperation, in order to mitigate the expansion of use, especially among the most vulnerable populations.

4. DISCUSSION

While a global phenomenon, the fentanyl crisis acquires particularly complex contours in Colombia that demand an analysis beyond traditional criminology. From a psychosocial perspective, the problem is not simply a new substance on the illicit market, but a symptom of deep-seated institutional flaws and pre-existing social vulnerabilities. The findings of this research unveil a perverse dynamic where the infrastructure designed to heal—the healthcare system—becomes a key vector of the crisis. The results are interpreted below through this lens.

The research shows that the fentanyl trafficking network is a hybrid structure, where the porosity between the legal and illegal is the norm, not the exception. The involvement of actors from the pharmaceutical sector and clinics, alongside criminal networks, cannot be seen as a

set of isolated failures. Sociologically, this points to a structural weakness in the state's mechanisms of control and traceability. Phenomena such as corruption and the lack of rigorous oversight, cited by Albores-García and Cruz (2023), are the catalysts that allow a controlled medication to be massively diverted to the black market [65]. This "institutional leakage" is the first link in a chain that ends in a severe public health crisis.

This systemic failure is aggravated by the methods of trafficking. The diversion from hospitals and the subsequent mixing of fentanyl with other drugs like heroin or cocaine is a practice that maximizes both the trafficker's profits and the user's risk of death. This adulteration, documented internationally by authors such as Misailidi *et al.* (2017) [66], creates an extremely high-risk scenario where the consumer has no real knowledge of the potency of the dose they are taking, which exponentially multiplies the probability of a fatal overdose. For health authorities, this represents a major challenge, as they must combat the distribution not just of one substance, but of an unpredictable and lethal cocktail.

The repercussions of this dynamic transcend mortality statistics. While the health impact is devastating—with rising deaths and an overburdened healthcare system—the psychosocial impact is perhaps more profound and lasting. Family disintegration, increased crime, and the erosion of community trust are wounds in the social fabric that take generations to heal. The fentanyl crisis, therefore, should not be framed solely as a health problem, but as an accelerator of social fracture. This reinforces the need for a comprehensive response that, as suggested by Piedrahíta-Bustamante (2014) [67], effectively articulates the public health, criminal justice, and social welfare systems.

Finally, it is crucial to understand who the primary victims of this institutional and social collapse are. Our findings are clear: at-risk youth and marginalized communities bear the heaviest burden of the crisis. From a sociological perspective, their consumption cannot be reduced to an individual choice; it is conditioned by structural determinants such as exclusion, lack of opportunities, and poor access to basic health and education services. In these contexts, fentanyl can emerge as a tragic form of self-medication against the pain of hopelessness. Therefore, any effective prevention strategy must go beyond informational campaigns. It is imperative, as argued by López-Villegas and Sánchez-Sandoval (2024) [68], to implement community intervention and empowerment programs directly in these environments, addressing the root causes of vulnerability and not just its symptoms.

5. CONCLUSIONS

The fentanyl phenomenon in Colombia presents a distinctive nature and is in an incipient phase, defined primarily by the diversion of pharmaceutical products from the legal supply chain rather than by large-scale clandestine production. This central finding reveals that the crisis is not merely a drug trafficking problem but a symptom of deep-seated institutional fissures and pre-existing social vulnerabilities. The porosity between legal and illegal actors, coupled with structural failures in the health system's control and traceability mechanisms, has created an environment where the healthcare infrastructure becomes an unintentional vector of the problem. The consequences of this dynamic are devastating, manifesting as a severe health impact due to the high risk of fatal overdoses, exacerbated by mixing with other substances, and as a profound fracture of the social fabric. This impact disproportionately affects vulnerable populations, such as at-risk youth and marginalized communities, whose consumption is conditioned by structural determinants like exclusion and lack of opportunity. Finally, a paradigm shift in drug policy is imperative, moving toward a comprehensive and multi-sectoral response. This strategy must urgently articulate, *i)* the rigorous strengthening

of controls on the pharmaceutical supply chain to prevent diversion; *ii)* the expansion of public health and harm reduction policies; and *iii)* the implementation of prevention programs focused on the structural causes of vulnerability. Containing the threat in its current phase is crucial to prevent an escalation into a public health crisis of the magnitude observed in other regions.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. K.E. Dunn, H.E. Bird & C.J. Durgin. Fentanyl. In: K.E. Dunn (editor). *The Oxford Handbook of Opioids and Opioid Use Disorder*. Oxford University Press, 2023; pp. 317–353. https://doi.org/10.1093/oxfordhb/9780197618431.013.13
2. P. Armenian, K.T. Vo, J. Barr-Walker & K.L. Lynch. Fentanyl, fentanyl analogs and novel synthetic opioids: A comprehensive review. *Neuropharmacology*, **134**(Part A), 121–132 (2018). https://doi.org/10.1016/j.neuropharm.2017.10.016
3. T.H. Stanley. The fentanyl story. *The Journal of Pain*, **15**(12), 1215–1226 (2014). https://doi.org/10.1016/j.jpain.2014.08.010
4. R.J. Fleischman, D.G. Frazer, M. Daya, J. Jui & C.D. Newgard. Effectiveness and safety of fentanyl compared with morphine for out-of-hospital analgesia. *Prehospital Emergency Care*, **14**(2), 167–175 (2010). https://doi.org/10.3109/10903120903572301
5. E.R. Weldon, R.E. Ariano & R.A. Grierson. Comparison of fentanyl and morphine in the prehospital treatment of ischemic type chest pain. *Prehospital Emergency Care*, **20**(1), 45–51 (2016). https://doi.org/10.3109/10903127.2015.1056893
6. G. Lindbeck, M.I. Shah, S. Braithwaite, J.R. Powell, A.R. Panchal, L.R. Browne, *et al.* Evidence-based guidelines for prehospital pain management: Recommendations. *Prehospital Emergency Care*, 27(2), 144–153 (2023). https://doi.org/10.1080/10903127.2021.2018073
7. M.A. Rech, E. Donahey, J.M. Cappiello-Dziedzic, L. Oh & E. Greenhalgh. New drugs of abuse. *Pharmacotherapy*, **35**(2), 189–197 (2015). https://doi.org/10.1002/phar.1522
8. M.P. Prekupec, P.A. Mansky & M.H. Baumann. Misuse of novel synthetic opioids: A deadly new trend. *Journal of Addiction Medicine*, **11**(4), 256–265 (2017). https://doi.org/10.1097/adm.0000000000000324
9. L.J. Tanz, A. Stewart, R.M. Gladden, J.Y. Ko, L. Owens & J. O'Donnell. Detection of illegally manufactured fentanyls and carfentanil in drug overdose deaths — United States, 2021–2024. *MMWR: Morbidity and Mortality Weekly Report*, **73**(48), 1099–1105 (2024). https://doi.org/10.15585/mmwr.mm7348a2
10. República de Colombia, Ministerio de Justicia y Derecho. *Situación actual de la problemática del fentanilo y opioides sintéticos con fines no médicos en Colombia*. Observatorio de Drogas de Colombia – ODC, Bogotá, 2024; 15 p. URL: https://www.minjusticia.gov.co/programas-co/ODC/Documents/Publicaciones/Fentanilo.pdf
11. L. Beletsky & C.S. Davis. Today's fentanyl crisis: Prohibition's Iron Law, revisited. *International Journal of Drug Policy*, **46**, 156–159 (2017). https://doi.org/10.1016/j.drugpo.2017.05.050
12. D. Ciccarone. The triple wave epidemic: Supply and demand drivers of the US opioid overdose crisis. *International Journal of Drug Policy*, **71**, 183–188 (2019). https://doi.org/10.1016/j.drugpo.2019.01.010
13. W. Cadena-Afanador & C.A. Devia-Garzón. La lucha contra las drogas en América Latina y las iniciativas generadas desde la UNASUR y la OEA. *Revista Científica General José María Córdova*, **11**(12), 39–59 (2013). https://doi.org/10.21830/19006586.186

14. T.H. Stanley, T.D. Egan & H. Van Aken. A tribute to Dr. Paul A. J. Janssen: Entrepreneur extraordinaire, innovative scientist, and significant contributor to anesthesiology. *Anesthesia & Analgesia*, **106**(2), 451–462 (2008). https://doi.org/10.1213/ane.0b013e3181605add
15. T.H. Stanley. The history of opioid use in anesthetic delivery. In: E. Eger II, L. Saidman & R. Westhorpe (editors). *The Wondrous Story of Anesthesia*. Springer, New York (NY), 2014; p.p. 641–659. https://doi.org/10.1007/978-1-4614-8441-7_48
16. J. Patocka, W. Wu, P. Oleksak, R. Jelinkova, E. Nepovimova, L. Spicanova, P. Springerova, S. Alomar, M. Long & K. Kuca. Fentanyl and its derivatives: Pain-killers or man-killers? *Heliyon*, **10**(8), e28795 (2024). https://doi.org/10.1016/j.heliyon.2024.e28795
17. R.S. Vardanyan & V.J. Hruby. Fentanyl-related compounds and derivatives: Current status and future prospects for pharmaceutical applications. *Future Medicinal Chemistry*, **6**(4), 385–412 (2014). https://doi.org/10.4155/fmc.13.215
18. A. Poklis. Fentanyl: A review for clinical and analytical toxicologists. *Journal of Toxicology: Clinical Toxicology*, **33**(5), 439–447 (1995). https://doi.org/10.3109/15563659509013752
19. P.J. Pascoe. Opioid analgesics. *Veterinary Clinics of North America: Small Animal Practice*, **30**(4), 757–772 (2000). https://doi.org/10.1016/s0195-5616(08)70005-6
20. T.H. Stanley. Anesthesia for the 21st Century. *Baylor University Medical Center Proceedings*, **13**(1), 7–10 (2000). https://doi.org/10.1080/08998280.2000.11927635
21. R.D. Mellon. The neurotoxic potential of opioids including fentanyl and fentanyl analogs. *Advances in Neurotoxicology*, **8**, 105–165 (2022). https://doi.org/10.1016/bs.ant.2022.04.006
22. J. Woodcock. A difficult balance — Pain management, drug safety, and the FDA. *The New England Journal of Medicine*, **361**, 2105–2107 (2009). https://doi.org/10.1056/nejmp0908913
23. T.W. Quinn. Anesthetic considerations in the surgical excision of impacted teeth. *Dental Clinics of North America*, **23**(3), 461–470 (1979). https://doi.org/10.1016/s0011-8532(22)01212-5
24. A.J. Walz & F.-L. Hsu. An operationally simple synthesis of fentanyl citrate. *Organic Preparations and Procedures International*, **49**(5), 467–470 (2017). https://doi.org/10.1080/00304948.2017.1374129
25. C.A. Valdez. Gas chromatography-mass spectrometry analysis of synthetic opioids belonging to the fentanyl class: A review. *Critical Reviews in Analytical Chemistry*, **52**(8), 1938–1968 (2022). https://doi.org/10.1080/10408347.2021.1927668
26. P.R. Keefe. *El imperio del dolor: La historia secreta de la dinastía que reinó en la industria farmacéutica*. Reservoir Books, 2022; 688 p.
27. J.A. Cartwright, S. Aniskevich III, C.B. Robards, H. Ramakrishna, S.J. Brull & S.R. Clendenen. Advances in drug safety. *Current Pharmaceutical Design*, **18**(38), 6266–6283 (2012). https://doi.org/10.2174/138161212803832416
28. R.U. Ayres. Anesthesia, surgery, and modern medicine. In: *The History and Future of Technology: Can Technology Save Humanity from Extinction?* Springer, Cham, 2021; pp. 223–250. https://doi.org/10.1007/978-3-030-71393-5_11
29. M.E. Lane. The transdermal delivery of fentanyl. *European Journal of Pharmaceutics and Biopharmaceutics*, **84**(3), 449–455 (2013). https://doi.org/10.1016/j.ejpb.2013.01.018
30. D.T. Anderson & J.J. Muto. Duragesic® transdermal patch: Postmortem tissue distribution of fentanyl in 25 cases. *Journal of Analytical Toxicology*, **24**(7), 627–634 (2000). https://doi.org/10.1093/jat/24.7.627
31. E. Prommer. The role of fentanyl in cancer-related pain. *Journal of Palliative Medicine*, **12**(10), 947–954 (2009). https://doi.org/10.1089/jpm.2009.0051
32. A.P. Adams & D.A. Pybus. Delayed respiratory depression after use of fentanyl during anaesthesia. *British Medical Journal*, **1**(6108), 278–279 (1978). https://doi.org/10.1136/bmj.1.6108.278
33. J.P. Caulkins. Radical technological breakthroughs in drugs and drug markets: The cases of cannabis and fentanyl. *International Journal of Drug Policy*, **94**, 103162 (2021). https://doi.org/10.1016/j.drugpo.2021.103162
34. M.P. Davis. Fentanyl for breakthrough pain: a systematic review. *Expert Review of Neurotherapeutics*, **11**(8), 1197–1216 (2011). https://doi.org/10.1586/ern.11.63

35. R.M. Dsida, M. Wheeler, P.K. Birmingham, T.K. Henthorn, M.J. Avram, C. Enders-Klein, J. Maddalozzo & C.J. Cote. Premedication of pediatric tonsillectomy patients with oral transmucosal fentanyl citrate. *Anesthesia & Analgesia*, 86(1), 66–70 (1998). https://doi.org/10.1213/00000539-199801000-00013
36. P. Watts, A. Smith & M. Perelman. Nasal delivery of fentanyl. *Drug Delivery and Translational Research*, **3**, 75–83 (2013). https://doi.org/10.1007/s13346-012-0078-y
37. E. Zecca, C. Brunelli, F. Centurioni, A. Manzoni, A. Pigni & A. Caraceni. Fentanyl sublingual tablets versus subcutaneous morphine for the management of severe cancer pain episodes in patients receiving opioid treatment: A double-blind, randomized, noninferiority trial. *Journal of Clinical Oncology*, **35**(7), 759–765 (2017). https://doi.org/10.1200/jco.2016.69.9504
38. J. Messina, M. Darwish & P.G. Fine. Fentanyl buccal tablet. *Drugs of Today*, **44**(1), 41–54 (2008). https://doi.org/10.1358/dot.2008.44.1.1178469
39. C. Blanco, T.R.A. Wiley, J.J. Lloyd, M.F. Lopez & N.D. Volkow. America's opioid crisis: the need for an integrated public health approach. *Translational Psychiatry*, **10**, 167 (2020). https://doi.org/10.1038/s41398-020-0847-1
40. A. Van Zee. The promotion and marketing of oxycontin: Commercial triumph, public health tragedy. American Journal of Public Health (AJPH), **99**, 221–227 (2009). https://doi.org/10.2105/ajph.2007.131714
41. C.A. Pérez-Ricart & A. Ibarrola-García. La transición hacia el fentanilo: Cambios y continuidades del mercado de drogas en México (2015-2022). *Revista de Ciencias Sociales*, **36**(53), 15–36 (2023). https://doi.org/10.26489/rvs.v36i53.1
42. T.H. Stanley. The history and development of the fentanyl series. *Journal of Pain and Symptom Management*, **7**(3, Suppl.), S3–S7 (1992). https://doi.org/10.1016/0885-3924(92)90047-l
43. C. Rius, A.E. Serrano-López, R. Lucas-Domínguez, A. Pandiella-Dominique, C. García-Zorita & J.C. Valderrama-Zurián. Fentanyl research: Key to fighting the opioid crisis. *Journal of Clinical Medicine*, **14**(15), 5187 (2025). https://doi.org/10.3390/jcm14155187
44. J. Friedman & C.L. Shover. Charting the fourth wave: Geographic, temporal, race/ethnicity and demographic trends in polysubstance fentanyl overdose deaths in the United States, 2010–2021. *Addiction*, **118**(12), 2477–2485 (2023). https://doi.org/10.1111/add.16318
45. P.F.J. Lipiński & J. Matalińska. Fentanyl structure as a scaffold for opioid/non-opioid multitarget analgesics. *International Journal of Molecular Sciences*, **23**(5), 2766 (2022). https://doi.org/10.3390/ijms23052766
46. P. Armenian, K.T. Vo, J. Barr-Walker & K.L. Lynch. Fentanyl, fentanyl analogs and novel synthetic opioids: A comprehensive review. *Neuropharmacology*, **134**(Part A), 121–132 (2018). https://doi.org/10.1016/j.neuropharm.2017.10.016
47. R.S. Vardanyan & V.J. Hruby. Fentanyl-related compounds and derivatives: Current status and future prospects for pharmaceutical applications. *Future Medicinal Chemistry*, **6**(4), 385–412 (2014). https://doi.org/10.4155/fmc.13.215
48. J.P. Monk, R. Beresford & A. Ward. Sufentanil: A review of its pharmacological properties and therapeutic use. *Drugs*, **36**, 286–313 (1988). https://doi.org/10.2165/00003495-198836030-00003
49. S. Bower & C.J. Hull. Comparative pharmacokinetics of fentanyl and alfentanil. *British Journal of Anaesthesia*, **54**(8), 871–877 (1982). https://doi.org/10.1093/bja/54.8.871
50. S.S. Patel & C.M. Spencer. Remifentanil. *Drugs*, **52**, 417–427 (1996). https://doi.org/10.2165/00003495-199652030-00009
51. M. Kamata & J.D. Tobias. Remifentanil: applications in neonates. *Journal of Anesthesia*, **30**, 449–460 (2016). https://doi.org/10.1007/s00540-015-2134-5
52. J.L.S. Leen & D.N. Juurlink. Correction to: Carfentanil: a narrative review of its pharmacology and public health concerns. *Canadian Journal of Anesthesia/Journal Canadien d'Anesthésie*, **66**, 625 (2019). https://doi.org/10.1007/s12630-019-01310-1
53. J.L.S. Leen & D.N. Juurlink. Carfentanil: a narrative review of its pharmacology and public health concerns. *Canadian Journal of Anesthesia/Journal Canadien d'Anesthésie*, **66**, 414–421 (2019). https://doi.org/10.1007/s12630-019-01294-y

54. R.F. Staack & H.H. Maurer. Metabolism of designer drugs of abuse. *Current Drug Metabolism*, **6**(3), 259–274 (2005). https://doi.org/10.2174/1389200054021825
55. G. Henderson. Designer drugs: Past history and future prospects. *Journal of Forensic Sciences, 1972-2005*, **33**(2), 569–575 (1988). https://doi.org/10.1520/jfs11976j
56. S.M. Burns, C.W. Cunningham & S.L. Mercer. DARK classics in chemical neuroscience: Fentanyl. *ACS Chemical Neuroscience*, **9**(10), 2428–2437 (2018). https://doi.org/10.1021/acschemneuro.8b00174
57. S. Shell, C. Clinton-Frazee & U. Garg. Quantification of fentanyl and norfentanyl in whole blood using liquid chromatography-tandem mass spectrometry. In: U. Garg (editor). *Clinical Applications of Mass Spectrometry in Drug Analysis.* Methods in Molecular Biology, vol. 2737. Humana, New York (NY), 2024; pp. 275–282. https://doi.org/10.1007/978-1-0716-3541-4_25
58. S.R. Vaughan, A.C. Fulton & L.E. DeGreeff. Comparative analysis of vapor profiles of fentalogs and illicit fentanyl. *Analytical and Bioanalytical Chemistry*, **413**, 7055–7062 (2021). https://doi.org/10.1007/s00216-021-03670-4
59. United Nations: International Narcotics Control Board. *Precursors and chemicals frequently used in the illicit manufacture of narcotic drugs and psychotropic substances*. Vienna, 2023; 98 p. URL: https://unis.unvienna.org/unis/uploads/documents/2023-INCB/INCB_precursors_report-English.pdf (accessed October 5, 2025).
60. J. Suzuki & S. El-Haddad. A review: Fentanyl and non-pharmaceutical fentanyls. *Drug and Alcohol Dependence*, **171**, 107–116 (2017). https://doi.org/10.1016/j.drugalcdep.2016.11.033
61. R. Venkatapathy, R. Kauchak, J. Botkins & M.L. Magnuson. Degradation of immediate precursors of fentanyl and fentalogs. *Forensic Chemistry*, **39**, 100573 (2024). https://doi.org/10.1016/j.forc.2024.100573
62. M.M. Vandeputte, A.J. Krotulski, F. Hulpia, S. Van Calenbergh & C.P. Stove. Phenethyl-4-ANPP: A marginally active byproduct suggesting a switch in illicit fentanyl synthesis routes. *Journal of Analytical Toxicology*, **46**(4), 350–357 (2022). https://doi.org/10.1093/jat/bkab032
63. Congreso de la República de Colombia. J.A. Castellanos. Proyecto de Ley 194 de 2023: Por la cual se crea la conducta penal de tráfico, venta o comercialización indebida de fentanilo como delito autónomo y se dictan otras disposiciones. Colombia, 2023; 21 p. URL: https://leyes.senado.gov.co/proyectos/index.php/textos-radicados-senado/p-ley-2023-2024/3088-proyecto-de-ley-194-de-2023 (accessed October 5, 2025).
64. W. Vargas-Pulido & E. Godoy-Estrella. Impacto del gasto de la defensa en el crecimiento económico de Colombia en los últimos veinte años. *Revista Científica General José María Córdova*, **11**(11), 227–257 (2013). URL: http://www.scielo.org.co/pdf/recig/v11n11/v11n11a11.pdf (accessed October 5, 2025).
65. D. Albores-García & S.L. Cruz. Fentanyl and other new psychoactive synthetic opioids. Challenges to prevention and treatment. *Revista de Investigación Clínica*, **75**(3), 93–104 (2023). https://doi.org/10.24875/ric.23000109
66. N. Misailidi, I. Papoutsis, P. Nikolaou, A. Dona, C. Spiliopoulou & S. Athanaselis. Fentanyls continue to replace heroin in the drug arena: the cases of ocfentanil and carfentanil. *Forensic Toxicology*, **36**, 12–32 (2018). https://doi.org/10.1007/s11419-017-0379-4
67. P. Piedrahíta-Bustamante. Las redes del crimen global y su impacto en Colombia. *Analecta Política*, **4**(6), 121–141 (2014). https://revistas.upb.edu.co/index.php/analecta/article/view/2666 (accessed October 5, 2025).
68. A. López-Villegas & Y. Sánchez-Sandoval. Intervenciones psicoeducativas sobre salud mental con adolescentes en contextos escolares: una revisión sistemática. *Revista de Psicología Clínica Con Niños y Adolescentes*, **11**(1), 33–41 (2024). https://doi.org/10.21134/rpcna.2024.11.1.4

HOW TO CITE THIS ARTICLE

J.I. Sierra-Duran, M.A. Yucuma-Guzmán, D.I. Caviedes-Rubio, D.R. Delgado & L.M. Lozano-Ramos. Historical analysis and social and health impact of fentanyl use in Colombia. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 167–184 (2026). https://doi.org/10.15446/rcciquifa.v55n1.125082

Short research article

Antifungal effectiveness of *Citrus sinensis* against *Candida albicans* in the dental field. *In vitro* study

Ivanna del Cisne Samaniego Ramírez[1], Miriam Lima-Illescas[2], Ebingen Villavicencio-Caparó[2], Edisson Pacheco-Quito[2] & Katherine Cuenca-León[1]*

[1] Academic Unit of Health and Wellness, Faculty of Dentistry, Catholic University of Cuenca, Cuenca 010105, Ecuador

[2] Pharmaceutical Innovation and Development in Dentistry Research Group, Faculty of Dentistry, Catholic University of Cuenca, Cuenca 010105, Ecuador

*Correspondence author E-mail: kcuencal@ucacue.edu.ec

Received: January 31, 2025
Corrected: November 20, 2025
Accepted: November 27, 2025

https://doi.org/10.15446/rcciquifa.v55n1.118606

SUMMARY

Introduction: This study focuses on the antifungal effectiveness of *Citrus sinensis* essential oil against *Candida albicans*, a common fungal infection in the oral cavity. **Objective:** To analyze the efficacy of *Citrus sinensis* essential oil against *C. albicans* at different concentrations. **Methodology:** A microbiological analysis using different concentrations of *Citrus sinensis* essential oil and the evaluation of its effect by measuring inhibition halos were included. Non- parametric statistical methods, such as the Kruskal-Wallis test, were applied to analyze the relationship between the concentration of the oil and its antifungal efficacy. **Results:** It was shown that, although *Citrus sinensis* oil did not present statistically significant differences in its antifungal activity at the different concentrations, an increasing trend in efficacy was observed as the concentration increased. This suggests that its use at higher concentrations could be beneficial in complementary treatments. **Conclusion:** *Citrus sinensis* essential oil could be considered as a complementary option in the treatment of fungal infections, but more research is needed to understand its synergy with other oils and its application in dentistry.

Keywords*: Candida albicans*; oral candidiasis; *Citrus sinensis*; microbial sensitivity.

RESUMEN

Eficacia antifúngica de *Citrus sinensis* contra *Candida albicans* en la cavidad bucal. Estudio *in vitro*

Introducción: Este estudio se centra en la eficacia antifúngica del aceite esencial de *Citrus sinensis* contra *Candida albicans*, una infección fúngica común en la cavidad bucal. **Objetivo:** Analizar la eficacia del aceite esencial de *Citrus sinensis* contra *C. albicans* en diferentes concentraciones. **Metodología:** Se realizó un análisis microbiológico con diferentes concentraciones de aceite esencial de *Citrus sinensis* y se evaluó su efecto mediante la medición de halos de inhibición. Se aplicaron métodos estadísticos no paramétricos, como la prueba de Kruskal-Wallis, para analizar la relación entre la concentración del aceite y su eficacia antifúngica. **Resultados:** Se demostró que, si bien el aceite de *Citrus sinensis* no presentó diferencias estadísticamente significativas en su actividad antifúngica a las diferentes concentraciones, se observó una tendencia creciente en la eficacia a medida que aumentaba la concentración. Esto sugiere

que su uso a concentraciones más altas podría ser beneficioso en tratamientos complementarios. **Conclusión:** El aceite esencial de *Citrus sinensis* podría considerarse una opción complementaria en el tratamiento de infecciones fúngicas, pero se necesita más investigación para comprender su sinergia con otros aceites y su aplicación en odontología.

Palabras clave: *Candida albicans*; candidiasis oral; *Citrus sinensis*; sensibilidad microbiana.

RESUMO

Eficácia antifúngica do óleo essencial de *Citrus sinensis* contra *Candida albicans* no ambiente odontológico. Estudo *in vitro*

Introdução: Este estudo concentra-se na eficácia antifúngica do óleo essencial de *Citrus sinensis* contra *Candida albicans*, uma infecção fúngica comum na cavidade oral. **Objetivo:** Analisar a eficácia do óleo essencial de *Citrus sinensis* contra *C. albicans* em diferentes concentrações. **Metodologia:** Foi realizada uma análise microbiológica utilizando diferentes concentrações do óleo essencial de *Citrus sinensis* e a avaliação do seu efeito pela medição dos halos de inibição. Métodos estatísticos não paramétricos, como o teste de Kruskal-Wallis, foram aplicados para analisar a relação entre a concentração do óleo e sua eficácia antifúngica. **Resultados:** Demonstrou-se que, embora o óleo de *Citrus sinensis* não tenha apresentado diferenças estatisticamente significativas em sua atividade antifúngica nas diferentes concentrações, observou-se uma tendência crescente de eficácia com o aumento da concentração. Isso sugere que seu uso em concentrações mais elevadas pode ser benéfico em tratamentos complementares. **Conclusão:** O óleo essencial de *Citrus sinensis* pode ser considerado uma opção complementar no tratamento de infecções fúngicas, mas são necessárias mais pesquisas para compreender sua sinergia com outros óleos e sua aplicação na odontologia.

Palavras-chave: *Candida albicans*; candidíase oral; *Citrus sinensis*; sensibilidade microbiana.

1. INTRODUCTION

Oral diseases, according to the World Health Organization, dental caries, periodontal disease and candidiasis are part of a public health problem [1]. The etiologic agent of oral candidiasis, *C. albicans*, an opportunistic dimorphic yeast, is part of the normal oral microbiota in 30-60% of healthy adults [2, 3]. However, in circumstances such as immunosuppression or the use of dental prostheses may provide its pathogenic transformation, causing recurrent fungal infections [4–6]. Oral candidiasis, which presents as white plaques on the oral mucosa, is clinically diagnosed and treated with topical or systemic antifungals in severe cases [6, 7].

C. albicans has the ability to form biofilms, which increases resistance to conventional treatments, particularly with polyenes and azoles, due to mechanisms such as efflux pumps, gene regulation and enzyme mutations [1, 8, 9]. This phenomenon highlights the need to explore new therapeutic alternatives to combat antifungal resistance and avoid the toxicity generated by these conventional therapies [10, 11].

Phytotherapy, based on natural compounds derived from plants, has gained attention for its antimicrobial potential [12]. Essential oils present antifungal, anti-inflammatory and antioxidant properties acting through mechanisms such as changes in cell membranes and formation of reactive oxygen species [13–16]. These mechanisms act as cell membrane variation and the reproduction of oxygen in reactive species. In this research we will focus on the essential oil of *Citrus sinensis*, where previous in vitro studies have reported its efficacy against various Candida species [16–18]. In view of the above, the present investigation was aimed at evaluating the antifungal effectiveness of *Citrus sinensis* essential oil against *C. albicans* at different concentrations, applying it in dentistry.

2. METHODOLOGY

This is a longitudinal laboratory study, carried out in the laboratories of the Center for Research, Innovation and Technology Transfer of the Catholic University of Cuenca (CIITT), under the approval of CEISH-UCACUE code 108-2024, with exempt category, this project responds to one of the objectives of a research project approved in the call for Sustainable Development Goals (ODS-UCAUCE) whose coding corresponds to PICODS21-21.

2.1. Inclusion criteria:

Candida albicans ATCC 60193 (resistant strain), pure and certified oils (*Citrus sinensis*).

2.2. Exclusion criteria:

Oils with other compounds, sensitive *C. albicans ATCC* strain, clinical isolation of *C. albicans* of human origin and contaminated samples.

2.3. Microbiological analysis

2.3.1. Activation and seeding of Candida albicans: The strain was activated and cultured MuellerHinton (MH) agar medium, prepared according to the manufacturer's technical specifications, ensuring the quality and reproducibility of the culture medium. Before inoculation, dilution of the strain was performed by adjusting it to a density of 0.5 on the McFarland scale, which is approximately equivalent to 1.5×10^8 colony forming units per milliliter (CFU/mL), ensuring a standard concentration of microorganisms for microbiological tests.

Inoculation was carried out using the mass seeding technique inside a laminar flow cabinet (BIOAIR-TopSafe), which allowed uniform distribution of the microorganism over the surface of the culture medium, minimizing contamination and ensuring consistent results. Subsequently, the inoculated plates were incubated in a laboratory oven (Memmert model UF110) at a controlled temperature of 25 °C, for a period of 24 to 48 hours, sufficient time to observe the growth and inhibitory effects of the substances under study.

2.4. Preparation of concentrations and controls:

Solutions of the oil to be evaluated were prepared in concentrations of 25%, 50%, 75% and 100%, using fluconazole (positive control) and distilled water (negative control). All concentrations were worked in triplicate to guarantee the reproduction and precision of the results. The technique used for the inoculation of *Candida albicans* was by mass seeding in the triplicate boxes. Blank sensitivity discs were placed on the medium and the different concentrations of the oil and the controls were deposited on the corresponding discs.

2.5. Observation, measurement and analysis of results:

After the incubation time, the formation of inhibition halos was evaluated in each of the boxes. The measurements of the halos were recorded and analyzed to determine the antifungal activity of the different concentrations of the oil, comparing the results with the positive controls. This procedure allowed an analysis and control of the antifungal activity, ensuring optimal conditions for reproducibility and reliability of the results (Figure 1).

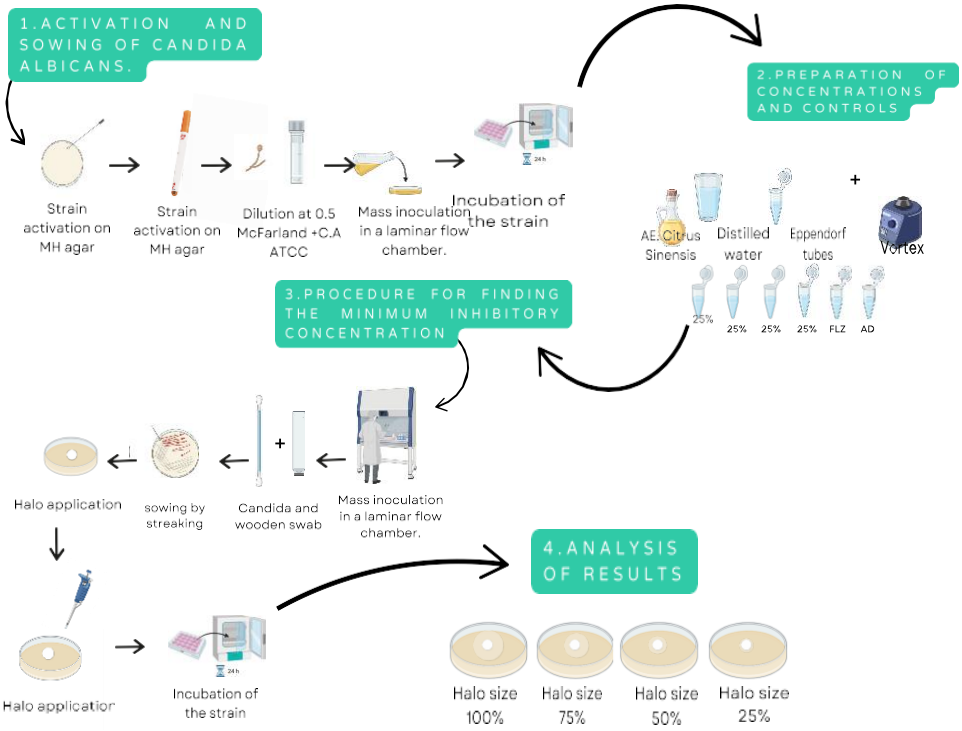


Figure 1. Flow chart of the microbiological analysis and antifungal effectiveness of the essential oil *Citrus Sinensis*. Authors' elaboration.

3. RESULTS

A statistical analysis was performed using non-parametric methods to evaluate the relationship between the variables, concentration and halo. For this purpose, the Kruskal-Wallis test was implemented and the data were analyzed by means of a box plot. All the analysis was performed using SPSS version 26 software, guaranteeing accuracy and reliability in the statistical calculations. The data did not have normality, so nonparametric analysis of the independent variable was performed. The Null Hypothesis states that the halo distribution is the same among the different *Citrus sinensis* concentration categories. To evaluate this hypothesis, a Kruskal-Wallis test for independent samples was carried out. The significance value (Sig.) obtained was 0.069. Based on this result, it was decided to retain the null hypothesis. The data did not have normality, so the nonparametric analysis of the independent variable was performed. The significance value of 0.069 shows that there is not enough statistical evidence to reject the null hypothesis at the 0.05 significance level. Under the conditions of the study, *Citrus sinensis* essential oil showed no significant difference in its antifungal activity against *Candida albicans* among the different concentrations evaluated.

Table 1. Inhibition halo measurements according to *Citrus sinensis* concentrations and controls.

Plant species	Concentrations (%)	Measuring ranges (mm)
Citrus sinensis	25	5
	25	5
	25	6
	50	9
	50	9
	50	6
	75	13
	75	11
	75	6
	100	13
	100	13
	100	9
Positive control (Flz)		19
Negative control (Distilled water)		0

The measurements and concentrations presented show that for the 5 mm value the concentrations are 25% on two occasions; for the 6 mm value, there are concentrations of 25% on one occasion and 50% on two occasions; then, for the 9 mm measurement, there are concentrations of 50% on two occasions and 100% on one occasion. The 11mm measurement has a concentration of 75%, while for the 13 mm value, there are concentrations of 75% on one occasion and 100% on two occasions. In the 19 mm measurement, a fluconazole value is mentioned (positive control) and finally, the 0 mm measurement represents distilled water (negative control), indicating no concentration.

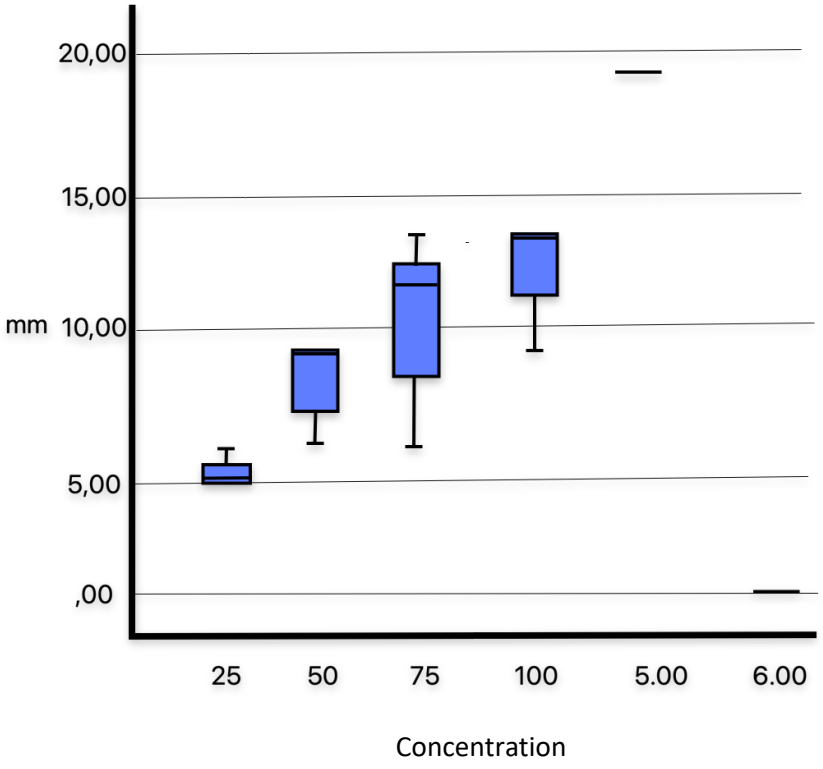


Figure 2. This graph shows the results with respect to the inhibition halos found.

Concentrations of 25%, 50%, 75% and 100% inhibition halo were evaluated by the KirbyBauer method to analyze the antifungal effect. Controls were established, where the positive control (5) showed a high performance, indicating a significant effect against *Candida albicans,* and the negative control (6) showed a null effect, showing a lack of antifungal activity. As for the results by concentration, at 25% there was a minor inhibition halo, indicating limited antifungal activity. At 50%, a modest increase in activity, while at 75% a significant increase was achieved compared to the lower concentrations. Finally, at 100%, the highest antifungal activity was reflected, approaching the results of the positive control. The results show a clear increasing trend in antifungal activity with increasing concentration of *Citrus sinensis* essential oil, suggesting a cumulative effect. This analysis was carried out using the latest generation SPSS software, which allowed a more accurate interpretation of the data.

3.1. Analysis of results

At the 25% concentration, the median is 5 mm, indicating low antifungal activity. The dispersion of the data is very narrow, with a small interquartile range (IQR), suggesting that the measurements are homogeneous and show little variability. Furthermore, no outliers are identified, reinforcing the consistency of the results. When increasing to 50%, the median reaches approximately 7 mm, suggesting an improvement in antifungal activity, and although the scatter increases, showing longer whiskers on the graph, no visible outliers are observed, maintaining the relative consistency of the data [19]. At the 75% concentration, the median is noticeably higher, close to 10 mm, reflecting evident antifungal activity. However, the variability is greater than at the 50% concentration, with a wide IQR and longer whiskers, suggesting considerable scatter in the data; here an outlier is detected near 20 mm, indicating the need for further analysis to understand its origin. Finally, at the 100% concentration, the median resembles that of 75%, albeit with a slightly lower range, evidencing a high level of antifungal activity. The variability is lower compared to the previous concentration, with a smaller IQR and shorter whiskers, indicating more concentrated data in its distribution, and no outliers are observed, suggesting greater stability in the comparative results [20].

4. DISCUSSION

This research focuses on the antifungal activity of *Citrus sinensis ATCC* essential oil, with the objective of analyzing the efficacy of the essential oil against *C. albicans* at different concentrations.

The results obtained for *Citrus sinensis* essential oil indicate that, although no statistically significant differences were found in its antifungal activity at different concentrations, an increasing trend in efficacy was observed as the concentration increased. This suggests that, although *Citrus sinensis* essential oil may not be the most potent, its use at higher concentrations could be beneficial, especially in a context of complementary treatments. However, it was noted that at concentrations of 50% and 75% the effect was minimal.

In comparison with the study of Ortiz-Timbi, the essential oil of *Cinnamomum verum* showed remarkable antifungal activity, with a minimum inhibitory concentration (MIC) of 39 ppm, indicating a high sensitivity of *Candida albicans* [21]. Similarly, in the study by Velasteguí-Pazos, *Melaleuca alternifolia* oil proved to be effective at concentrations of 75% and 100%, presenting significant inhibition halos [22]. These findings position cinnamon oil and tea tree

oil as more potent options in the fight against fungal infections. According to research conducted by Guazanda-Orrala, the essential oil of *Piper imperiale* also showed considerable potential, with a MIC of 6894.286 μg/mL, suggesting that it may be a viable alternative to traditional antifungal treatments [23]. The ability of this oil to inhibit the growth of *Candida spp*. highlights the importance of exploring essential oils as therapeutic options.

On the other hand, citing the work of Vasquez-Gavidia, the essential oil of *Matricaria chamomilla* (chamomile) was shown to have a significant antifungal effect, especially at higher concentrations, suggesting its potential in the treatment of fungal infections [24]. Likewise, according to Valverde-Quinaluisa, oregano oil has been recognized for its antimicrobial properties, making it an interesting candidate for inclusion in antifungal treatments [25].

According to Lalangui-Pazmiño and E.W. Palacios-Paredes alike to Echeverría-Erazo, *Schinus molle* and *Eucalyptus globulus* oils, known for their antimicrobial properties, also deserves attention, although its specific efficacy against *Candida spp.* requires further research [26, 27]. Moreover, analysis reported by Alberca-Torres and Dilas-Castillo regarding ginger oil (*Zingiber officinale*), its antifungal potential has been documented, suggesting that it could be useful in the formulation of combination treatments [28].

The research by Paucar-Rodríguez *et al.* indicates that the essential oil of *Minthostachys mollis* showed remarkable inhibitory activity against several pathogens, including *Candida albicans*, suggesting its potential in the treatment of fungal infections [29]. Finally, Churata-Oroya *et al.* indicated that *Citrus paradisi* (grapefruit) oil presented the most effective antifungal activity against clinical strains of *Candida albicans* at a concentration of 25% [30].

Analysis of *Citrus sinensis* essential oil showed moderate antifungal activity that improved with concentration, with inhibition halos of 5 mm at 25% and 10 mm at 75%. At 100%, the activity remained high, indicating a positive cumulative effect. In comparison, *Cinnamomum verum* stands out with an MIC of 39 ppm, demonstrating high sensitivity against *Candida albicans*, consolidating itself as one of the most effective oils. Similarly, *Melaleuca alternifolia* showed significant efficacy at concentrations of 75% and 100%, positioning itself next to cinnamon oil in effectiveness. On the other hand, *Piper imperiale* has a MIC of 6894.286 μg/mL, suggesting lower effectiveness compared to the previous ones, although, its potential as an alternative is recognized, so *Citrus sinensis* oil offers moderate antifungal activity, while *Cinnamomum verum* oil and *Melaleuca alternifolia* oil are more potent options for the treatment of fungal infections.

5. CONCLUSION

Research on the antifungal effectiveness of *Citrus sinensis* essential oil against *Candida albicans ATCC* 60193 has revealed important findings. Although *Citrus sinensis* oil shows moderate antifungal activity compared to other essential oils and no significant differences in efficacy were detected among the concentrations studied, its safety profile and availability make it an interesting candidate for use in combination with more effective oils.

It is essential to continue exploring natural alternatives such as essential oils, not only to diversify therapeutic options, but also to address the increasing resistance to conventional antifungals. Future research could focus on the synergy between *Citrus sinensis* essential oil and other oils, as well as its application in complementary treatments for fungal infections, especially in patients with compromised immune systems. Although the current results do not support the oil as a conclusive solution against candidiasis, they open the door to further research that could expand our knowledge and offer new hope in the treatment of these infections.

ACKNOWLEDGMENTS

To the Coordination of the Center for Research, Innovation and Technology Transfer of the Catholic University of Cuenca (CIITT), Biotechnology Laboratory.

FINANCING

The publication expenses were financed by the Catholic University of Cuenca, Ecuador, and are anchored to the project entitled: "Design and preparation of a mouth rinse with natural active ingredients to neutralize and eliminate oral fungi in patients with partial and/or total prosthesis", approved under code: PICODS21-21.

CONFLICT OF INTEREST

It is explicitly stated that there is no conflict of interest in conducting this research.

REFERENCES

1. V. Anuța, M.-T. Talianu, C.-E. Dinu-Pîrvu, M.V. Ghica, R.M. Prisada, M.G. Albu-Kaya & L. Popa. Molecular mapping of antifungal mechanisms accessing biomaterials and new agents to target oral candidiasis. *International Journal of Molecular Sciences*, **23**(15), 8212 (2022). https://doi.org/10.3390/ijms23147520
2. A.P. Cortés-Hidalgo, O.H. Roa-Dueñas, Y.R. Méndez-Fandiño & C.A. Álvarez-Moreno. Opciones terapéuticas frente a especies de *Candida* resistentes a equinocandinas. *Universitas Medica*, **59**(2), 1–15 (2018). https://doi.org/10.11144/javeriana.umed59-2.cand
3. M. Taylor, M. Brizuela, A. Raja, M. Brizuela, S. Coleman & M.V. Pellegrini. *Oral Candidiasis*. Continuing Education Activity. StatPearls [Internet], Treasure Island (FL), 2025. URL: https://www.statpearls.com/dentist/ce/activity/113501
4. E. Serra, L.A. Hidalgo-Bastida, J. Verran, D. Williams & S. Malic. Antifungal activity of commercial essential oils and biocides against *Candida albicans*. *Pathogens*, **7**(1), 15 (2018). https://doi.org/10.3390/pathogens7010015
5. D.C. Gheorghe, A.G. Niculescu, A.C. Bîrcă & A.M. Grumezescu. Biomaterials for the prevention of oral candidiasis development. *Pharmaceutics*, **13**(6), 803 (2021). https://doi.org/10.3390/pharmaceutics13060803
6. T. Vila, A.S. Sultan, D. Montelongo-Jauregui & M.A. Jabra-Rizk. Oral candidiasis: A disease of opportunity. *Journal of Fungi*, **6**(1), 15 (2020). https://doi.org/10.3390/jof6010015
7. N.R. Arya & N.B. Rafiq. *Candidiasis*. Continuing Education Activity. StatPearls [Internet], Treasure Island (FL), 2025. URL: https://www.ncbi.nlm.nih.gov/books/NBK560624/
8. J.B. Taheri, S. Azimi, N. Rafieian & H.A. Zanjani. Herbs in dentistry. *International Dental Journal*, **61**, 287–296 (2011). https://doi.org/10.1111/j.1875-595X.2011.00064.x
9. R.-A. Milutinovici, D. Chioran, R. Buzatu, I. Macasoi, S. Razvan, R. Chioibas, *et al.* Vegetal compounds as therapeutic agents in dentistry. *Plants*, **10**(10), 2148 (2021). https://doi.org/10.3390/plants10102148
10. C. Lumbreras, M. Lizasoain & J.M. Aguado. Antifúngicos de uso sistémico. *Enfermedades Infecciosas y Microbiología Clínica*, **21**(7), 366–380 (2002). URL: https://www.elsevier.es/es-revista-enfermedades-infecciosas-microbiologiaclinica-28-articulo-antifungicos-uso-sistemico-13050528
11. G.L.S. Ferreira, A.L.A.d.L. Pérez, Í.M. Rocha, M.A. Pinheiro, R.D. De Castro, H.L. Carlo, E.d.O. Lima & L.R. Castellano. Natural products in oral candidiasis treatment: A systematic review. *Evidence-Based Complementary and Alternative Medicine*, **2015**, 147804 (2015).

https://doi.org/10.1155/2015/147804

12. K. Cuenca-León, E.M. Pacheco-Quito, Y. Granda-Granda, E. Vélez-León & A. Zarzuelo-Castañeda. Phytotherapy: A solution to decrease antifungal resistance in dentistry. *Biomolecules*, **12**(3), 386 (2022). https://doi.org/10.3390/biom12030386 https://doi.org/10.3390/biom12060789
13. V.T. Alexa, A. Galuscan, C.M. Soica, A. Cozma, D. Coricovac, F. Borcan, I. Potescu, A. Mioc, C. Szuhanek & C.A. Dehelean. Cytotoxic and antiproliferative profile of natural preparations containing bergamot, orange and clove oils. *Molecules*, **27**(3), 990 (2022). https://doi.org/10.3390/molecules27030848 https://doi.org/10.3390/molecules27030990
14. C. Cadena-Viteri, M. Lima-Illescas, E.M. Pacheco-Quito, M.C. Balseca-Ibarra, F. Sacoto-Figueroa & K. Cuenca-León. Inhibitory effect of *Schinus molle* L. essential oil against periodontal pathogens. *Revista Colombiana de Ciencias Químico Farmacéuticas*, **53**(2), 414–429 (2024). https://doi.org/10.15446/rcciquifa.v53n2.114449
15. N.J. Ruiz-Pérez, M. González-Ávila, J. Sánchez-Navarrete, J.D. Toscano-Garibay, M.A. Moreno-Eutimio, T. Sandoval-Hernández & M. Arriaga-Alba. Antimycotic and genotoxic evaluation of *Citrus sinensis* and *Citrus latifolia* essential oils. *Scientific Reports*, **6**, 25371 (2016). https://doi.org/10.1038/srep25371
16. O. Atolania, N. Adamub, O.S. Oguntoyea, M.F. Zubairb, O.A. Fabiyic, R.A. Oyegoked, O.S. Adeyemie, E.T. Areha, D.E. Tarighaa, L. Kambizif & G.A. Olatunji. Chemical characterization, antioxidant, cytotoxicity, Anti-*Toxoplasma gondii* and antimicrobial potentials of the *Citrus sinensis* seed oil for sustainable cosmeceutical production. *Heliyon*, **6**(2), e03399 (2020). https://doi.org/10.1016/j.heliyon.2020.e03399
17. M. Patel. Oral cavity and *Candida albicans*: Colonization to infection. *Pathogens*, **11**(3), 335 (2022). https://doi.org/10.3390/pathogens11030335
18. M. Perić, B. Miličić, J. Kuzmanović-Pfićer, R. Živković & V. Arsić-Arsenijević. Review of denture stomatitis: Predisposing factors, etiology and global distribution. *Journal of Fungi*, **10**(5), 328 (2024). https://doi.org/10.3390/jof10050328
19. E. Villavicencio-Caparó, M. Alvear-Córdova, K. Cuenca-León, M. Calderón-Curipoma, D. Palacios-Vivar & A. Alvarado-Cordero. Diseños de estudios clínicos en odontología. *Revista OACTIVA UC Cuenca*, **1**(2), 81–84 (2016). URL: https://oactiva.ucacue.edu.ec/index.php/oactiva/article/view/163/284
20. E. Villavicencio-Caparó, K. Cuenca-León, E. Vélez-León, J. Sayago-Heredia & A. Cabrera-Duffau. Pasos para la planificación de una investigación clínica. *Odontología Activa UCACUE*, **1**(1), 72–75 (2016). URL: https://oactiva.ucacue.edu.ec/index.php/oactiva/article/view/186/321
21. M.A. Ortiz-Timbi. *Actividad antifúngica "in vitro" del aceite esencial y extracto alcohólico del Cinnamomum verum "canela" sobre Candida albicans cepa ATCC 10231*. B. Sc. Tesis. Universidad Nacional de Chimborazo, Riobamba, Ecuador, 2017; 80 p. URL: http://dspace.unach.edu.ec/bitstream/51000/4380/1/UNACH-EC-FCS-ODOT-2017-0034.pdf
22. A.N. Velasteguí-Pazos. *Eficacia antifúngica e inhibitoria in vitro de nistatina versus aceite esencial de Melaleuca alternifolia sobre Candida albicans*. B. Sc. Tesis. Universidad Nacional de Chimborazo, Riobamba, Ecuador, 2022; 60 p. http://dspace.unach.edu.ec/handle/51000/9666
23. L.E. Guaranda-Orrala. *Evaluación de la eficacia antimicótica del aceite esencial Piper imperiale frente a Candida spp. provenientes de muestras clínicas: Utilizando dos técnicas de sensibilidad fúngica validadas en un hospital público de la provincia de Santa Elena*. B. Sc. Thesis. Universidad Politécnica Saleciana, sede Guayaquil, Ecuador, 2024; 101 p. URL: https://dspace.ups.edu.ec/bitstream/123456789/27702/1/UPS-GT005166.pdf
24. C.R. Vásquez-Gavidia. *Efecto antimicótico in vitro de diferentes concentraciones del aceite esencial de la flor de Matricaria chamomilla (Manzanilla) en cultivo de Candida albicans cepa ATCC 10231*. B. Sc. Thesis. Universidad Católica Los Ángeles de Chimbote, Trujillo, Perú, 2018; 65 p. URL: https://repositorio.uladech.edu.pe/handle/20.500.13032/5259
25. P.Y. Valverde-Quinaluisa. *Efectividad antimicótica del aceite esencial de orégano de las provincias de Chimborazo y Santa Elena al 100% de concentración sobre Candida albicans*. B. Sc. Thesis. Universidad Central del Ecuador, Quito, Ecuador, 2015; 103 p. URL: https://www.dspace.uce.edu.ec/entities/publication/30085a3a-4845-4c5d-91f7-ac4d07d8358d

26. G.G. Lalangui-Pazmiño & E.W. Palacios-Paredes. Efecto inhibitorio del aceite esencial de *Schinus molle* a diferentes tiempos y concentraciones, sobre *Candida albicans*. *RECIMUNDO: Revista Científica Mundo de la Investigación y el Conocimiento*, **5**(2), 398–406 (2021). https://doi.org/10.26820/recimundo/5.(2).abril.2021.398-406
27. A.E. Echeverría-Erazo. *Actividad anti fúngica "in vitro" de aceite esencial y extracto alcohólico de eucalipto "Eucalyptus globulus" sobre Candida albicans cepa ATCC 10231*. B. Sc. Thesis. Universidad Nacional de Chimborazo, Riobamba, Ecuador, 2017; 68 p. URL: http://dspace.unach.edu.ec/bitstream/51000/4454/1/UNACH-EC-FCS-ODT-2017-0034.pdf
28. E. Alberca-Torres & R. Dilas-Castillo. Evaluación del potencial antimicótico del aceite esencial de *Zingiber officinale* frente a cepas de *Candida albicans*. *Estudio in vitro*. *Epistemia Revista Científica*, **8**(2), 1–12 (2024). https://doi.org/10.26495/erc.2785
29. E. Paucar-Rodríguez, N. Peltroche-Adrianzen & C.F. Cayo-Rojas. Actividad antibacteriana y antifúngica del aceite esencial de *Minthostachys mollis* frente a microorganismos de la cavidad oral. *Revista Cubana de Investigaciones Biomédicas*, **40**(5), e1450 (2021). URL: https://revibiomedica.sld.cu/index.php/ibi/article/view/1450/972
30. D.E. Churata-Oroya, D. Ramos-Perfecto, H. Moromi-Nakata, E. Martínez-Cadillo, A. Castro-Luna & R. Garcia-de-la-Guarda. Efecto antifúngico del *Citrus paradisi* "toronja" sobre cepas de *Candida albicans* aisladas de pacientes con estomatitis subprotésica. *Revista Estomatológica Herediana*, **26**(2) 78–84 (2016). https://revistas.upch.edu.pe/index.php/REH/article/view/2869/2737

HOW TO CITE THIS ARTICLE

I.d.C. Samaniego-Ramírez, M. Lima-Illescas, E. Villavicencio-Caparó, E. Pacheco-Quito & K. Cuenca-León. Antifungal effectiveness of *Citrus sinensis* against *Candida albicans* in the dental field. *In vitro* study. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 185–194 (2026). https://doi.org/10.15446/rcciquifa.v55n1.118606

Artículo de revisión

¿Son realmente seguros los rellenos dérmicos?

Laura Estela Castrillón Rivera*, Alejandro Palma Ramos, Jorge Ismael Castañeda Sánchez & Violeta Espinosa Antúnez

Laboratorio de Inmunología. Departamento de Sistemas Biológicos. Universidad Autónoma Metropolitana Unidad Xochimilco, México

*Autora de correspondencia: lcrivera@correo.xoc.uam.mx

Recibido: 30 de junio de 2025
Corregido: 9 de diciembre de 2025
Aceptado: 16 de diciembre de 2025

https://doi.org/10.15446/rcciquifa.v55n1.125089

RESUMEN

Introducción: La inyección de rellenos dérmicos son procedimientos comunes en la práctica dermatológica cosmética porque son utilizados principalmente como tratamiento antienvejecimiento para corregir depresiones, arrugas, surcos, cicatrices, ángulos y flacidez entre otros. Dado que algunos rellenos dérmicos se absorben naturalmente con el tiempo, es posible que los pacientes deban repetir el procedimiento después de un período de tiempo para mantener el efecto deseado. Ya que los métodos de aplicación son mínimamente invasivos, pero como cualquier procedimiento médico, existen riesgos involucrados con su aplicación, naturaleza del material y respuesta individual del paciente, en consecuencia el uso de este tipo de rellenos tienen el potencial de producir una respuesta inflamatoria reconocida como reacción a cuerpo extraño, o presentar respuestas inmunológicas conocidas como granulomas inflamatorios que pueden cursar con complicaciones tardías en rangos de leve a severo o aún ser riesgosos para la vida. **Desarrollo del tema:** La presente revisión bibliográfica aborda los siguientes aspectos relacionados con: a) Clasificación de los tipos de rellenos dérmicos, b) Respuesta tisular, c) Complicaciones tempranas y tardías, d) Respuesta inmune, e) Prevención de complicaciones, f) Tratamiento y g) Perspectivas. **Conclusión:** La disposición de tipos y número de rellenos dérmicos continúa en aumento, por tanto, se debe entender sus riesgos y como tratar la aparición de sus complicaciones. Sin embargo, aunque se asume que los materiales utilizados son principalmente biocompatibles, existe cada vez más gran cantidad de reportes de las principales complicaciones y su tratamiento.

***Palabras clave*:** Rellenos dérmicos; reacción a cuerpo extraño; nódulos; biopelículas.

SUMMARY

¿Are dermal fillers really safe?

Introduction: Dermal filler injections are common procedures in cosmetic dermatology practice because they are used to correct depressions, wrinkles, furrows, scars, angles and sagging, among others. Since some dermal fillers are naturally absorbed over time to maintain the desired effect. Since the application methods are minimally invasive, but like any medical procedure there are risks involved with the application, nature of the material and individual patient response, consequently the use of this type of fillers has the potential to produce an inflammatory response recognized as a foreign body reaction, or present immunological responses known as inflammatory granulomas that can present with late com-

plications ranging from mild to severe or even be life-threatening. **Topic development**: This bibliographic review addresses the following aspects related to: a) Classification of dermal fillers types, b) Tissue response, c) Early and late complications, d) Immune response, e) Prevention of complications, f) Treatment and g) Perspectives. **Conclusion**: The availability of types and number of dermal fillers continues to increase; therefore, it is important to understand their risks and how to treat the appearance of their complications. However, although it is assumed that the materials used are mainly biocompatible, there are an increasing number of reports of the main complications and treatments.

Keywords: Dermal fillers; foreign body reaction; nodules; biofilms

RESUMO

Os Preenchimentos Dérmicos São Realmente Seguros?

Introdução: As injeções de preenchimento dérmico são procedimentos comuns na dermatologia estética, sendo utilizadas principalmente como tratamento antienvelhecimento para corrigir depressões, rugas, sulcos, cicatrizes, ângulos e flacidez, entre outros problemas. Como alguns preenchimentos dérmicos são absorvidos naturalmente com o tempo, os pacientes podem precisar repetir o procedimento após um período para manter o efeito desejado. Embora os métodos de aplicação sejam minimamente invasivos, como em qualquer procedimento médico, existem riscos envolvidos na aplicação, na natureza do material e na resposta individual do paciente. Consequentemente, o uso desses tipos de preenchimentos tem o potencial de produzir uma resposta inflamatória conhecida como reação a corpo estranho, ou apresentar respostas imunológicas conhecidas como granulomas inflamatórios, que podem levar a complicações tardias que variam de leves a graves, ou mesmo serem fatais. **Desenvolvimento do Tema:** Esta revisão de literatura aborda os seguintes aspectos relacionados a: a) Classificação dos tipos de preenchedores dérmicos, b) Resposta tecidual, c) Complicações precoces e tardias, d) Resposta imune, e) Prevenção de complicações, f) Tratamento e g) Perspectivas. **Conclusão:** A disponibilidade e o número de tipos de preenchedores dérmicos continuam a aumentar; portanto, é essencial compreender seus riscos e como manejar o desenvolvimento de complicações. Contudo, embora os materiais utilizados sejam geralmente considerados biocompatíveis, há um número crescente de relatos sobre as principais complicações e seus respectivos tratamentos.

Palavras-chave: Preenchimentos dérmicos; reação a corpo estranho; nódulos; biofilmes.

1. INTRODUCCIÓN

La inyección de rellenos dérmicos son procedimientos comunes en la práctica dermatológica cosmética, también conocidos como implantes inyectables, rellenos de tejidos blandos, rellenos de labios y faciales o rellenos de arrugas, se utilizan principalmente como tratamiento antienvejecimiento donde la piel se vuelve más delgada y pierde textura y elasticidad en consecuencia aparecen arrugas, atrofia de la grasa subcutánea disminución de colágeno y elastina, se manifiesta por ingravedad muscular por lo que aparecen pliegues y flacidez de la piel. Estos productos son utilizados para corregir depresiones, arrugas, surcos, cicatrices, ángulos y flacidez para ayudar a crear una apariencia más suave y/o más completa en la cara, incluidos los pliegues nasolabiales, las mejillas, el mentón, los labios y la parte de atrás de las manos [1]. Los resultados exitosos dependerán de la estructura del tejido más profundo y del volumen y tipo de relleno utilizado. El tiempo que dura el efecto depende del material de relleno y de la zona donde se inyecta. En el uso de los rellenos dérmicos son considerados como dispositivos médicos tipo II por la FDA y se consideran relativamente seguros.

Dado que algunos rellenos dérmicos se absorben naturalmente con el tiempo, es posible que los pacientes deban repetir el procedimiento después de un período de tiempo para mantener el efecto deseado ya que los métodos de aplicación son mínimamente invasivos, pero como cualquier procedimiento médico, existen riesgos involucrados con su uso [2]. Sin embargo, aunque los rellenos dérmicos tienen un perfil de seguridad excelente para el rejuvenecimiento, son mínimamente invasivos, éstos pueden cursar con complicaciones tardías en rangos de leve a severo o aún ser riesgosos para la vida [3]. Por otra parte, cualquier relleno dérmico tiene el potencial de producir una respuesta inflamatoria reconocida como reacción a cuerpo extraño y el cuerpo intentará eliminarlo causando por efecto del trauma inflamación aguda caracterizada por hinchazón o eritema. La intensidad y tipo de reacciones inflamatorias o inmunológicas dependen de las características del relleno, además, a mayor permanencia del relleno, debido a sus características de biodegradabilidad, existe la posibilidad de mayor riesgo de complicaciones [4].

Aquellos pacientes que desarrollan reacción inmunológica subaguda pueden presentar una reacción inflamatoria de larga duración y causar nódulos, granuloma a cuerpo extraño o formación de biopelículas entre otros.

2. CLASIFICACIÓN DE MATERIALES DE RELLENO

Los materiales de relleno se pueden clasificar según la profundidad de la implantación dérmica (dérmicos, subdérmicos y periósticos), la duración (temporales, semipermanentes y permanentes), su naturaleza (naturales, semisintéticos y sintéticos), su composición y su comportamiento-estímulo (rellenos bioestimulantes) [5-7]. La mayoría de los rellenos dérmicos más utilizados en la dermatología cosmética tienen un efecto temporal, ya que contienen materiales que el cuerpo absorbe con el tiempo. Por su tiempo de permanencia, se clasifican en biodegradables de corta duración o no biodegradables de larga duración. Los rellenos no degradables provocan reacciones a cuerpo extraño estimulando el depósito de colágeno alrededor de las microesferas como hidrogel de poliacrilamida hidrófila (Aquamid®) y polimetacrilato (Artecoll®) causan complicaciones y difícil tratamiento. Las principales marcas comerciales de estos rellenos se presentan en la Tabla 1.

Tabla 1. Nombres comerciales de rellenos dérmicos

Tipo de implante	Nombre comercial del producto
Injerto de grasa	Implante autólogo
Colágeno	Zyderm®, Zyplast®, Alloderm®, CosmoDerm®, CosmoPlast®, Facin®, Cymetra®
Colágeno más polimetilmetacrilato	Artecoll®, MetaCrill®, Artefill®, Arteplast®
Ácido hialurónico	Hylaform®, Restylane®, Captique®, Perlane®, Juvederm®, Prevell®, Puragen®, Elevess®, Surgyderm®, Rofilan Hylan Gel®, Visagel®, Hyla-system®, Teosyal®, Matridur®, Puragen®, Glytone®, Isogel®
Hidroxiapatita	Hidroxiapatita (HA)®, Hidroxiapatita Coralina (CH)®, Radiesse®, Beauty Fill®
Silicona	Implantech®, Allied Biomedical®, Advanced Bio-Technologies®, Inamed® Aesthetics®
Politetrafluoroetileno expandido (ePTFE)	Gore-Tex®, SoftForm®
Polietileno	Medpor®
Polimetilmetacrilato (PMMA)	Artecoll®, Artesense®, Artefill®
Poliacrilamida hidrófila (PAAH)	Hidrogel®, HPG®, Argiform®, Bioformacryl®, DermaLive®, Aquamid®
Ácido poli-L-láctico (PLLA)	Sculptra®, New-Fill®
Polialquilimida	Bio-alcamid®

2.1. Materiales absorbibles (temporales)

2.1.1. Ácido hialurónico (AH): Es un tipo de azúcar (polisacárido glucosaminoglicano) con alta biocompatibilidad. Está presente en tejidos corporales, como la piel y el cartílago. En forma de gel, se combina con el agua y forma un gel viscoso y transparente cuya turgencia es muy parecida al tejido circundante y una vez que se hincha, causa un efecto de alisado y relleno.

El ácido hialurónico o sus derivados son el tipo de relleno dérmico más utilizado (85%) se considera el estándar de oro, puede provenir de bacterias o de animales. En algunos casos esta sustancia utilizada en los rellenos dérmicos se puede modificar químicamente formando una red que se compone de fracciones entrecruzadas y no entrecruzadas para prolongar su duración en el organismo porque que se ha demostrado que la fracción no entrecruzada puede ser susceptible a la acción de la enzima hialuronidasa por lo que puede ser eliminada rápidamente [8, 9]. Los efectos de este material duran aproximadamente de 6 a 12 meses.

La cantidad inyectada, depende del tamaño y la profundidad del defecto [10], debido a que este relleno es capaz de estimular la síntesis de colágeno como resultado de la expansión mecánica de los tejidos y la subsecuente activación de fibroblastos por lo que es muy utilizado para la rehabilitación facial [11].

2.1.2. Hidroxiapatita de calcio (CaHA): Es un gel utilizado para tejidos blandos, es elaborado de manera artificial a partir de minerales óseos. La hidroxiapatita de calcio es un mineral que se encuentra comúnmente en los dientes y huesos humanos. Tiene propiedades visco-elásticas muy marcadas formado de esferas de hidroxiapatita suspendidas en un gel acuoso de glicerina y carboximetilcelulosa. Actúa como bioestimulante por la formación de tejido fibrohistiocítico nuevo con fibras de colágeno sin formar granulomas ni ser osteogénico. Los efectos de este material duran aproximadamente 18 meses. Una desventaja para su uso es que se requiere de anestesia para su aplicación, empleando la infiltración de anestésico local o el bloqueo regional.

2.1.3. Ácido poli-L-láctico (PLLA): El PLLA es un polímero sintético que se utiliza como micropartículas biodegradables y reabsorbibles. Cada molécula de PLLA tiene un peso molecular de 140,000 daltons con forma cristalina irregular con 2-50 micras de diámetro. Se metaboliza de la misma forma de lactato a piruato y su uso no afecta los niveles séricos de lactato y se reabsorbe a través de mecanismos no enzimáticos mediante hidrólisis. Este material tiene amplios usos en suturas absorbibles y tornillos óseos. El PLLA es un material de relleno de larga duración que se administra mediante una serie de inyecciones de manera estricta en el tejido adiposo subyacente a la dermis y es indicado para el tratamiento de hipertrofia de mejillas y surcos nasogenianos. Los efectos del PLLA generalmente se hacen más evidentes con el tiempo (a lo largo de varias semanas) y pueden durar hasta dos años.

2.1.4. Colágeno bovino-humano. Es el principal componente de la dermis humana y es responsable de la firmeza y el soporte de la piel. Sin embargo, el riesgo de presentar alergia al colágeno bovino es de 1,3 a 6,2% por lo que debe realizarse la prueba de alergia antes de aplicar el producto en el caso del colágeno humano no requiere de prueba de alergia y su duración es de 4 a 7 meses.

2.2. Materiales absorbibles (semi-permanentes)

2.2.1. Perlas de polimetilmetacrilato (microesferas de PMMA): El PMMA es un polímero sintético, biocompatible y no biodegradable. Es un material bifásico compuesto por microesferas lisas de PMMA suspendidas en una solución de colágeno bovino. Este material se utiliza en otros dispositivos médicos, como cemento óseo y lentes intraoculares.

2.2.2. Hidroximetilmetacrilato – fragmentos de etilmetacrilato con ácido hialurónico (AH): Son materiales de relleno bifásico es un hidrogel acrílico de etilmetacrilato (EMA) e hidroximetilmetacrilato (HEMA) con ácido hialurónico. Dermalive® se utiliza para los lentes intraoculares en cataratas.

2.3. Materiales absorbibles (permanentes)

2.3.1. Hidrogel de poliacrilamida (PAAH). Son polímeros sintéticos de hidrogeles de poliacrilamida al 2,5% en agua. Se han utilizado en pacientes con lipo-atrofia facial asociada el tratamiento con antirretrovirales o malformaciones faciales adquiridas o congénitas.

2.3.2. Gel de polialquilamida. Es un gel translúcido compuesto de un polímero de polialquilamida al 4% en agua. Utilizado para corregir irregularidades en lipo-escultura, cicatrices deprimidas, atrofia subcutánea post-traumática y malformaciones óseas. Se considera una prótesis ya que después de su inyección, el tejido circundante reacciona produciendo una cápsula fibrosa y delgada de colágeno que cubre por completo el tejido implantado.

2.3.3. Silicón líquido (polidimetilsilicona). Este material nunca llega a desaparecer, produce efectos adversos años después de su aparición, los granulomas permanentes hicieron que la FDA suspendiera su uso en el ámbito de la cosmética.

2.3.4. Tejido adiposo autólogo. Se inyecta tejido adiposo en la dermis y se produce la formación de colágeno, es el material ideal porque no produce alergias, es biocompatible y produce resultados duraderos.

Entre los mismos productos se pueden presentar diferentes comportamientos debido a las variaciones en su proceso de producción, diferencias en el tamaño molecular o su manufactura como partículas. Se pueden presentar proteínas residuales en cierto tipo de rellenos como resultado de su elaboración, las cuales pueden inducir respuestas biológicas indeseables. Sin embargo, a pesar de las afirmaciones de los productores y diferentes autores de que los rellenos no son inmunogénicos o que las complicaciones son raras, las reacciones adversas siguen ocurriendo [12].

3. APLICACIONES DE LOS RELLENOS DÉRMICOS

El propósito primario para el uso del relleno se debe a la corrección de depresiones (arrugas) o al aumento del volumen de la piel. Entre los principales criterios para la elección de un tipo de relleno son: El sitio y técnica de inyección, condición del tejido blando, propiedades del relleno estimulo post inyección y grado de reacción tisular dentro del cuerpo.

Los rellenos más firmes se prefieren para mantener la forma en capas profundas, sin embargo, aumentan el riego de complicaciones, empeoran la circulación sanguínea y la reacción tisular debido al aumento de presión a tejidos circundantes.

3.1. Limitaciones del uso de rellenos dérmicos

Entre las principales limitaciones para el uso de rellenos dérmicos se encuentran las reacciones de toxicidad, respuestas tisulares, complicaciones clínicas observadas como reacciones tempranas y/o tardías y las respuestas inflamatorias como consecuencia de la respuesta inmune.

3.1.1. Respuesta tisular

El análisis de la imagen histológica de la inducida por los diferentes materiales de relleno permite comprender mejor su interacción e integración tisular, así como las posibles reacciones indeseables [13, 14]. La formación de cápsulas de colágeno y el reemplazo gradual del material

de relleno con tejido autólogo en rellenos del ácido hialurónico demuestra ser un proceso dinámico de integración y degradación. Las implicaciones clínicas de estos cambios histológicos son significativas e influencian los resultados a largo plazo y la satisfacción del paciente [8].

En el año 2020 se ha reportado la apariencia histológica de granulomas inducidos por diversos rellenos describiendo que para el implante de silicón se observan vacuolas vacías rodeadas de células inflamatorias [15]. En el nódulo de rellenos de partículas acrílicas, aparecen numerosos espacios pseudoquísticos con cuerpos translúcidos poligonales de diferente tamaño y forma y no son refringentes bajo la luz polarizada. Los nódulos formados por ácido poliláctico presentan células gigantes multinucleadas que rodean partículas translúcidas fusiformes u ovales refringentes bajo la luz polarizada a diferencia de los nódulos inducidos por ácido hialurónico que forma escamas basófilas no refringentes sin células inflamatorias (o escasas) que los rodeen. Con estos resultados el autor señala las diferencias del tipo e intensidad de reacción que dependen del tipo de relleno como consecuencia de la respuesta al trauma y permanencia del material.

3.1.2. Complicaciones clínicas

Las manifestaciones clínicas se pueden clasificar como tempranas que aparecen en horas o días y pueden ser reversibles o tardías que pueden aparecer por semanas o años (ver figura 1). Entre los principales efectos comunes se encuentran el enrojecimiento y erupción y como efectos raros se pueden presentar sobrerreacción, nódulos palpables, granulomas, infección, heridas abiertas, reacciones alérgicas, necrosis, migración, daño del flujo sanguíneo, cicatrización hipertrófica y telangiectasias cuando aparecen biopelículas [16-23].

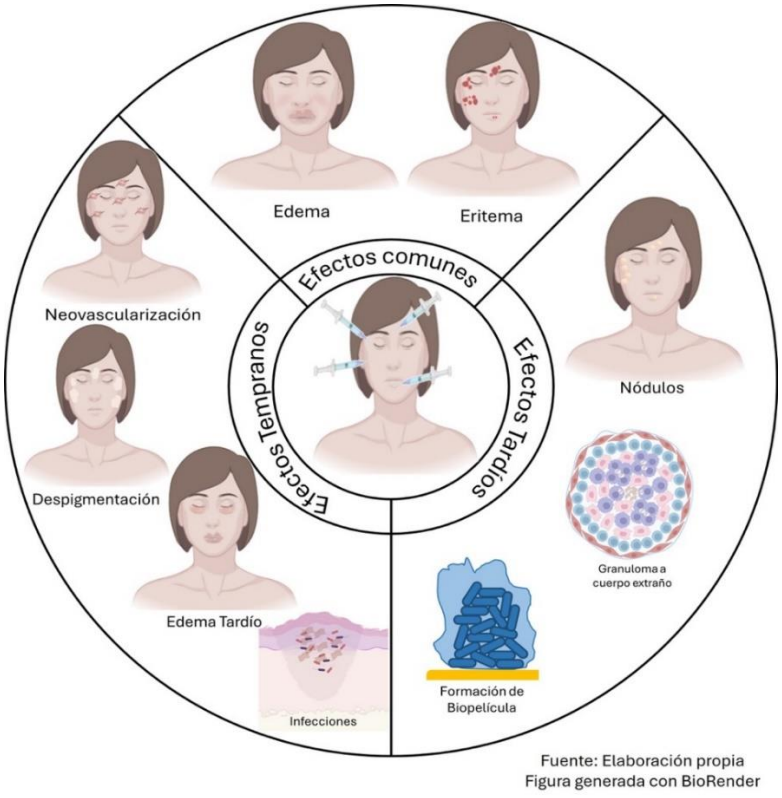


Figura 1. Principales complicaciones del uso de rellenos dérmicos. Cualquier relleno puede presentar efectos comunes relacionados con su aplicación. Como efectos tempranos dependiendo del tipo de relleno pueden (o no) aparecer reacciones relacionadas con el material utilizado y/o complicaciones por la técnica de aplicación. Los efectos tardíos dependen principalmente por la susceptibilidad del paciente y por posibles contaminantes presentes en el relleno.

Entre las clasificaciones clínicas asociadas con el tratamiento por rellenos dérmicos se encuentran:

3.1.2.1. Edema: Es normal que ocurra con cualquier tipo de relleno que debe desaparecer en una semana.

3.1.2.2. Angioedema: Mediada por inmunoglobulina E (hipersensibilidad tipo I) causado por la desgranulación de mastocitos o células cebadas por liberación de proteasas, heparina, histamina, citocinas, prostaglandinas, leucotrienos y factor activador de plaquetas causando edema, eritema, dolor y comezón. Aparecen en horas post aplicación, con reacción severa por varias semanas. Puede haber obstrucción de vías aéreas.

3.1.2.3. Edema malar: Puede ser consecuencia de la aplicación de volumen excesivo o de un producto menos elástico. En el compartimento de grasa orbicular superficial pueden comprimir a los vasos linfáticos y comprometer su drenaje.

3.1.2.4. Edema tardío: Asociado con reacciones de hipersensibilidad mediado por células T que ocurren un día post aplicación de rellenos o después de semanas y pueden permanecer por meses. La respuesta de hipersensibilidad tardía no responde a los antihistamínicos.

3.1.2.5. Eritema: El enrojecimiento es inmediato a la aplicación, si es persistente por días es posible que se trate de una reacción de hipersensibilidad.

3.1.2.6. Neovascularización: Aparecen pequeños vasos sanguíneos por días o semanas después del procedimiento y disminuyen por 3 a 12 meses. Se utilizan tratamientos por láser para las telangiectasias su selección depende del tamaño del vaso sanguíneo comprometido.

3.1.2.7. Despigmentación: Cuando el ácido hialurónico no es aplicado correctamente en la dermis superficial o epidermis aparece una tonalidad azul (efecto Tyndall) que corresponde a la dispersión de la luz por partículas en suspensión.

3.1.2.8. Parestesia: Es un daño de los nervios que pueden ser traspasados o parcialmente lacerados por la inyección. El daño puede ser transitorio o permanente, si es reversible el regreso de las sensaciones puede aparecer después de varios meses.

3.1.2.9. Compromiso vascular: El riesgo más preocupante asociado con el uso de los rellenos dérmicos es la inyección involuntaria en un vaso sanguíneo (arterias y venas), lo que provoca el bloqueo de los vasos sanguíneos y como consecuencia hay disminución de sangre a los tejidos. Es la principal complicación inmediata como resultado de la inyección en arterias causando embolismo que impide el flujo sanguíneo anterógrada y retrógrada resultado en necrosis. Son pocos los reportes de pérdida de visión y hemiplejía después de la inyección de grasa facial como resultado de embolismo ocular y cerebral. Los pacientes presentan dolor intenso, palidez, hinchazón de vénulas, llenado lento de capilares y de coloración azul-rojiza oscura, reacciones tardías como áreas necróticas, pequeñas ampollas blancas y desprendimiento de tejidos por obstrucciones embólicas arteriales [24].

3.1.2.10. Pérdida de visión: Ocurre cuando hay oclusión arterial y flujo retrógrado del material en el sistema arterial. La presión de la aguja promueve el flujo retrógrado por una cantidad elevada de relleno aplicado donde hay menor resistencia a través de los vasos más proximales que puede regresar al flujo anterógrado en vasos distantes del sitio de inyección como la arteria oftálmica que posee muchas ramas que se proyectan en áreas fuera de la ocular sobre la nariz y frente. Entre 1906 y 2029 se han reportado 190 casos de ceguera secundaria por tratamientos estéticos por procedimientos estéticos por inyecciones de grasa autóloga. El trata-

miento requiere de consulta oftálmica de emergencia, masaje ocular, gota para reducir la presión intraocular, oxígeno hiperbárico, diuréticos, corticosteroides sistémicos y tópicos, anticoagulación y descompresión con aguja de la cámara anterior.

3.1.2.11. Reacciones alérgicas: Los pacientes deben someterse a pruebas de alergias antes de usar rellenos hechos con ciertos materiales, especialmente materiales derivados de animales (por ejemplo, de vaca (bovino). Es el resultado de las proteínas residuales con posibilidad de generar respuestas de hipersensibilidad tipo I y tipo IV, muchas son leves y se resuelven espontáneamente (horas o días) se deben utilizar antihistamínicos cuando la respuesta sea del tipo I y esteroides orales cuando se trate de respuestas de hipersensibilidad tipo IV. Se desconocen los riesgos asociados con los usos no aprobados de los rellenos dérmicos o con el uso de los productos no aprobados [25].

3.1.2.12. Infecciones: Cualquier procedimiento que rompe la superficie de la piel como una inyección en la dermis, se asocia con un riesgo de infecciones de bacterias, hongos o virus. Cuando se presentan nódulos inflamatorios con eritema, edema, suaves y sensibilidad se puede sospechar de la aparición de una infección.

La flora normal de la piel y las bacterias residentes en las glándulas apocrinas y sebáceas que pueden jugar un papel en el desarrollo de abscesos secundarios a los rellenos de tejidos blandos y las inyecciones múltiples en el mismo sitio pueden ser estimuladas por traumas repetidos [26].

Entre las bacterias más frecuentes se encuentran *Staphylococcus epidermidis, Staphylococcus aureus, Streptomyces pyogenes, Cutibacterium acnes* y micobacterias atípicas, en un granuloma infeccioso se caracteriza como granuloma supurativo o caseoso e infiltrado neutrofílico que puede resultar en acantosis, hiperqueratosis e hiperqueratosis folicular [27, 28].

Entre las complicaciones tempranas que son reversibles, aparecen en el sitio de aplicación y se presentan como efectos comunes y leves entre los que se encuentran la presencia de brotes herpéticos por la infección viral por *Herpex simplex* por reactivación de contacto previo cuando los pacientes han referido tener antecedentes de herpes labial por reactivación, se debe considerar el uso de valaciclovir el día anterior y 3 días posteriores a la administración del relleno como medida profiláctica. En pacientes con lesiones activas del virus *Herpes simplex* la aplicación de relleno debe retrasarse hasta la resolución completa.

Cuando se realiza un drenaje e incisión de un absceso, el material debe llevarse a cultivo para el aislamiento e identificación del microorganismo responsable, así como su sensibilidad a antimicrobianos para determinar la terapia adecuada o bien, abscesos o celulitis cuando hay infección por *Mycobacterium.*

3.1.3. Efectos tardíos

Al igual que con cualquier procedimiento médico, existen riesgos relacionados con el uso de rellenos dérmicos. Es importante comprender sus límites y probables riesgos. Los rellenos pueden presentar variedad de riesgos a pesar de que se asuman como inertes y no inmunogénicos, por tanto, es importante entender sus propiedades y riesgos asociados para su manejo. En general los rellenos a tejidos blandos son bien tolerados y las reacciones adversas son raras. La mayoría de los efectos secundarios asociados con los rellenos dérmicos ocurren poco después de la inyección y muchos se resuelven en unos pocos días o semanas, pero también pueden manifestarse como respuestas tardías que pueden aparecer por semanas, meses o años [29, 30].

Aunque las complicaciones tardías son eventos raros aún su origen no es completamente conocido. La FDA reconoce otros riesgos raros que han sido también informados como son el choque anafiláctico, migración del material de relleno, fuga o ruptura del implante, lesiones asociadas con el suministro de sangre generando necrosis tisular o muerte. Entre los factores

etiopatológicos tardíos más importantes se encuentran la los nódulos, granulomas a cuerpo extraño y biopelículas (ver Figura 2).

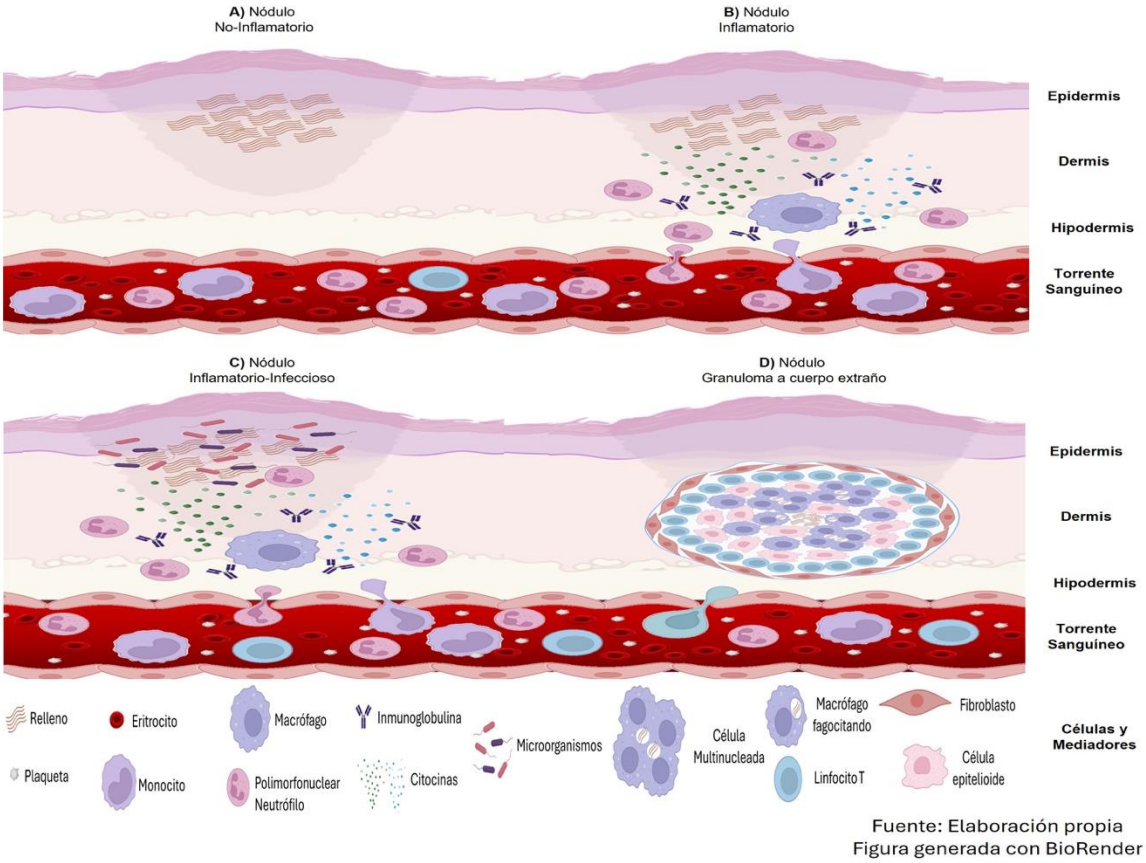


Figura 2. Respuestas biológicas asociadas con el uso de rellenos dérmicos. La generación de nódulos es consecuencia del tipo de relleno utilizado y de la participación de la respuesta inmune del paciente. Los nódulos no-inflamatorios (A) corresponden a la presencia del material inyectado, la aparición de nódulos inflamatorios (B) dependen de la respuesta inmune asociada a una respuesta a cuerpo extraño iniciada por el material inyectado. Los nódulos inflamatorios infecciosos (C) corresponden a una respuesta a cuerpo extraño y a la presencia de microrganismos endógenos (microbiota natural) o exógenos como resultado de la técnica de aplicación o material contaminado. La formación de granulomas (D) son respuestas inmunitarias tardías.

3.1.3.1. Nódulos

En la práctica diaria muchos términos se usan de manera intercambiable para describir un nódulo como masa, bulto, induración, absceso o granuloma y aunque todos tienen diferente significado no son fácil de ser distinguidos. Por esta razón, muchos estudios no pueden ser comparables ya que la definición entre nódulo y granuloma no es clara y se utilizan de manera equivalente.

Un nódulo es un bulto visible o palpable bajo la piel como resultado del exceso de relleno, aplicación del plano incorrecto o como resultado de una reacción temprana a cuerpo extraño o infección, en cambio un granuloma es el resultado del mecanismo de protección para lograr aislar el cuerpo extraño previniendo la migración para rechazar el implante. La diferenciación entre ellos depende del análisis histológico ya que es una herramienta esencial para permitir el diagnóstico adecuado para orientar la terapéutica ya que un granuloma verdadero debe confirmarse por la presencia de células gigantes multinucleadas alrededor del producto basófilo.

De acuerdo a las respuestas biológicas que inducen los nódulos ser pueden clasificar como inflamatorios y no inflamatorios también pueden ser clasificados de acuerdo a su tiempo de aparición post inyección como tempranos (horas o días) o subagudos o tardíos (meses/años) [15]. a) Nódulos no-inflamatorios: Se generan cuando la inyección de material es excesiva o como errores en la técnica de aplicación en áreas no apropiadas que llevan a la agregación dinámica del relleno. Se presentan en pocas semanas post aplicación, se vuelve como un nódulo duro y confinado localmente, son fríos al tacto y su tamaño se conserva hasta que el material se absorba o se remueva. b) Nódulos inflamatorios: Pueden aparecer tempranamente desde los 15 días post aplicación del relleno. No son nódulos simétricos y son el resultado de infecciones generalmente se asocian con la formación de biopelículas que deben tratar con antibióticos y drenaje. c) Nódulos infecciosos: La simetría es importante porque permite la diferenciación de los nódulos infecciosos, sus bordes no están definidos ya que reflejan una respuesta sistémica porque es el resultado de una respuesta infecciosa localizada. Estos nódulos son eritematosos, edematosos, tensos y en algunos casos aparecen púrpuras debido al congestionamiento de capilares, también se observa un crecimiento o persistir o bien resolverse espontáneamente después de su aparición. Los nódulos infecciosos de aparición tardía se asocian con la formación de biopelículas y al ser resistentes al tratamiento con antibióticos, pueden ser reactivados por trauma o por procedimientos de rellenos dérmicos adicionales.

3.1.3.2. Granulomas a cuerpo extraño

Se considera la reacción adversa más frecuente al granuloma a cuerpo extraño [29, 31, 32], el cual es diferente a un nódulo porque su tamaño aumenta con el tiempo y se desarrolla simultáneamente en múltiples sitios donde se aplica la inyección Una reacción a cuerpo extraño por granuloma ocurre después de la inyección de rellenos dérmicos, mostrando características histológicas dependiendo del tipo de relleno [13, 14]. Se caracteriza como una reacción inflamatoria crónica por la presencia de macrófagos fusionados para formar células multinucleadas que aparecen meses o años post aplicación del relleno. Estos granulomas aparecen como pápulas rojas, nódulos o placas (con o sin induración) y cultivo negativo, estas lesiones se pueden volver firmes debido a la fibrosis y pueden ocurrir con cualquier tipo de relleno [33, 34] (ver Figura 1).

Es muy importante conocer si la reacción a cuerpo extraño se resuelve con el tiempo, persiste o crece causando la formación de granulomas tardíos en donde ocurre inflamación aguda resultando en celulitis o infección tardía Su origen es poco comprendido y raramente se describe, por tanto, este fenómeno es subestimado.

Esta reacción aparece como consecuencia por la incapacidad del sistema inmune en donde los fagocitos son incapaces de degradar el material o posiblemente por la presencia de impurezas en el material utilizado. Los macrófagos al ser activados, secretan una gran variedad de citocinas y los productos inflamatorios resultantes atraen más macrófagos que se vuelven más grandes (histiocitos epiteloides) o se fusionan y forman células gigantes multinucleadas que son las células características de un granuloma. En la mayoría de los pacientes, un granuloma a cuerpo extraño a menudo es desencadenado por una infección bacteriana sistémica.

Los granulomas a cuerpo extraño se pueden clasificar como [35]: a) Granuloma quísitico que se forma con el uso de geles biológicos como el ácido hialurónico y colágeno. Su apariencia es roja, indurados o fluctuantes (absceso estéril), son pequeños y superficiales, aparecen durante el primer año y desaparecen espontáneamente al año siguiente. b) Granuloma edematoso/eritematoso: referido como lipogranuloma asociado con fluidos artificiales como aceite de silicón y poliacrilamidas asociado con los rellenos particulados estimulatorios como el PMMA. Aparecen repentinamente años después de la inyección con inflamación extensa y

rodeados e infiltrados por células mononucleares e inflamatorias. c) Granuloma esclerósico: referido como sarcoide o xantelásmico. Está asociado con rellenos particulados estimulatorios como PMMA, CaHA y PLLA, son implantes de larga duración, no tienen una tasa alta de granulomas a cuerpo extraño si se comparan con los rellenos de ácido hialurónico, sin embargo, su apariencia es más pronunciada y persisten más tiempo si se dejan sin tratamiento.

3.1.3.3. Formación de biopelículas

Cuando un material se inyecta en la piel o tejido subcutáneo se recubre con bacterias y se forma una biopelícula que secreta una matriz adhesiva y protectora que permite la adherencia irreversible a estructuras vivas o inertes y puede surgir una infección resistente al tratamiento por antibióticos [36, 37].

Las bacterias inoculadas durante la inyección pueden ser parte del microbioma de la piel [26] y pueden formar biopelículas que crean una matriz que inhibe la hialuronidasa natural, esta organización bacteriana induce una mínima infección con poca respuesta del huésped pasando como asintomática por meses o años y aunque la formación de biopelículas no sea muy reconocida, es un riesgo para complicaciones tardías relacionada por contaminación o bacterias residentes [38-40].

Las bacterias en una biopelícula pueden permanecer latentes y mucho tiempo y reactivarse en un entorno favorable cuando la infección granulomatosa, nódulos, abscesos o enfermedades recurrentes. Los nódulos suelen ser negativos y el cultivo y tratamiento suele ser complicado debido a la capacidad protectora del exopolisacárido característico de estas biopelículas.

Las biopelículas son difíciles de estudiar y tienen desafíos técnicos que retrasan su aislamiento e identificación microbiana por los métodos convencionales por esta razón las técnicas de cultivo estándar fallan para identificar a los microorganismos responsables y se requieren estudios adicionales para su identificación tales como técnicas de PCR, pirosecuenciación, hibridación fuorescente *in situ* y sonicación ultrasónica entre otros que llevan tiempo, mano de obra especializada y costos [41-43].

Existen un único reporte en donde no se consideran a las biopelículas como responsables de la formación de granulomas asociadas con los rellenos dérmicos y la respuesta biológica se explica como una reacción a cuerpo extraño que puede desaparecer con el tiempo y al no contar con cultivos positivos de la muestra de pacientes, se recomienda que el absceso removido sea enviado a bacteriología para la identificación por ultrasonicación y microscopía electrónica lo que dificulta contar con estas estrategias metodológicas para confirmar la organización microbiana como biopelículas [44].

Por otra parte, existen varias publicaciones en donde se considera la formación de biopelículas como una de las principales complicaciones con el uso de rellenos para tejidos blandos [45, 46]. Para demostrar la participación de la formación de biopelículas asociadas con el uso de rellenos dérmicos de ha desarrollado un modelo murino evaluando a diferentes tipos de rellenos degradables (ácido hialurónico), semi-degradables (hidroxiapatita) y permanentes (PAA) para probar si un número bajo de bacterias podrían iniciar la infección o bien si los geles podrían mantener esta infección y evaluar además su posible tratamiento con antibióticos. Con este modelo se logró demostrar la presencia de biopelículas con *Pseudomonas aeruginosa* y con el uso de antibióticos se logró reducir la densidad microbiana pero no su eliminación, sin embargo, un pretratamiento con antibióticos logró evitar su formación [47].

Es importante el reconocimiento de biopelículas como resultado de la infección en los rellenos dérmicos para comprender el origen de la falla en la terapia antimicrobiana con antibióticos y se recomienda para identificar la presencia de biopelículas el uso de sonicación que

desaloja a las bacterias adheridas al implante y ser recuperadas directamente del fluido sonicado [28, 48].

4. RESPUESTA INMUNE ASOCIADA CON EL USO DE RELLENOS DÉRMICOS

La inflamación alrededor del implante es normal y eventualmente lleva a su reabsorción en el caso de materiales biodegradables, inmunológicamente inertes que estimulan la neogénesis de colágeno. Los rellenos permanentes ideales, deben causar muy poca reacción tisular adversa que permita prolongar la persistencia del producto y evitar una reacción crónica o granulomas recurrentes que sean una molestia para el paciente [12].

Cuando una bacteria se introduce en la dermis, la falta de circulación dentro del relleno, es posible que los leucocitos no ataquen a la bacteria y escapen del reconocimiento del sistema inmune [49]; sin embargo, si los granulocitos que son la primera línea de defensa innata llegan al tejido blando, si estas células no logran fagocitar las partículas de los rellenos, son los monocitos/macrófagos que se vuelven la población dominante, al ser activados liberan citocinas y son estas respuestas las que determinan la longevidad de los rellenos. La falla en la fagocitosis efectiva lleva a la formación de granuloma con agregados de macrófagos que cambian su morfología y favorecen el infiltrado de linfocitos T productores de citocinas que llevan a la activación de macrófagos amplificando así la respuesta inflamatoria. El granuloma se va rodeando de un borde fibrótico y en el caso de las infecciones el centro se vuelve necrótico (ver Figura 2).

El tamaño de partícula de los rellenos determina el tipo de respuesta inmune ya que partículas mayores de 5 μm inducen células gigantes a cuerpo extraño mientras aquellas de 15-20 μm son transportadas al nódulo linfático para inducir respuestas inmunes adaptativas [50].

Se ha demostrado que el tamaño de los fragmentos del ácido hialurónico (HA) puede presentar efectos contrarios ya que existen reportes por estimulación *in vitro* de células inmunes en donde las fracciones de bajo peso molecular no logran estimular la respuesta inflamatoria a menos que exista la presencia de bacterias, considerando este hecho un factor de riesgo para la inducción inmunitaria y generando un papel moderador del ácido hialurónico [51]; en contraste con este reporte, se ha demostrado la capacidad del HA como activador de la respuesta inmune, por la liberación de fragmentos de bajo peso molecular resultante de la degradación del polímero que son proinflamatorios iniciando la estimulación de receptores Toll (TLR2 y TLR4 asociados con la inmunidad innata) [52] madurando células dendríticas y estimulando citocinas proinflamatorias como IL-1β, IL-6, IL-12, IL-8, TNFα, metaloproteinasas y TGFβ. En cambio, los fragmentos de alto peso molecular del HA inhiben la producción de mediadores proinflamatorios, reduce la expresión de receptores tipo Toll y regula la angiogénesis por lo que estos fragmentos tienen actividad antiinflamatoria [53]. Por lo tanto, el porcentaje de entrecruzamiento de este polímero genera cambios en su conformación natural, así como en su capacidad inmunogénica [54].

Otro efecto inmunitario importante que se ha reportado es el síndrome autoinmune/inflamatorio inducido por adyuvantes o ASIA (por sus siglas en inglés) provocado por el uso de HA cuyo mecanismo induce la desregulación de los linfocitos T y B, la incapacidad de reconocer antígenos propios, inflamación y daño a los tejidos propios [55].

4.1. Prevención de complicaciones

La aparición de complicaciones es consecuencia de factores relacionados con el paciente por la naturaleza del producto o por el procedimiento de aplicación. Por esta razón se debe conocer

la historia clínica del paciente, condiciones de la piel, alergias, enfermedades sistémicas, medicación actual y procedimientos estéticos previos [23, 56-58].

La aplicación de rellenos está contraindicada en enfermedades autoinmunes, se debe conocer el volumen y técnica de aplicación idónea para su aplicación y selección adecuada del tipo de producto. Se pueden evitar los efectos adversos por el uso de la visualización ultrasónica de los vasos sanguíneos previa aplicación del relleno, uso de anestésico local, inyección de pequeños volúmenes y aspiración previa a la inyección.

Para el caso de los rellenos de ácido hialurónico se han publicado guías y algoritmos útiles con información relacionada con la profundidad de inyección, tipo de aguja, técnica de aplicación, volumen recomendado para evitar complicaciones y mejorar la satisfacción del paciente [4, 59-61], así como del manejo de sus complicaciones cuando éstas se presentan.

4.2. Tratamiento

Entre las principales complicaciones relacionadas con la aplicación de rellenos se encuentran: a) Infecciones principalmente por microflora normal o por biopelículas, b) Granulomas debido a la respuesta a cuerpo extraño, c) Nódulos inflamatorios generados por la activación del sistema inmune sin formación de granuloma como abscesos estériles in presencia de microorganismos y d) Mala aplicación del relleno (efecto Tyndall). Por tanto, se debe conocer el origen de estas respuestas biológicas para una adecuada selección de tratamiento [15].

Se debe diferenciar entre los nódulos inflamatorios de los no-inflamatorios ya que los primeros aparecen en tiempos muy cortos después de la aplicación del relleno y generalmente se relacionan como consecuencia de la técnica de aplicación, en cambio, los inflamatorios se observan días, meses o años post aplicación y varían de acuerdo a su etiología [62].

Es difícil distinguir entre los nódulos inflamatorios que resultan de un proceso infeccioso, versus los que son mediados por la respuesta inmune, en ambos casos, se debe realizar el estudio microbiológico y en base a estos, dirigir la terapia adecuada, para ello se han diseñado algoritmos de tratamiento que pueden ser consultados para la mejor toma de decisión.

Es importante mencionar que el papel de la contaminación bacteriana nunca puede ser ignorada, las biopsias raramente son obtenidas y examinadas y la comprensión del origen del problema se basa solamente en la respuesta al tratamiento. Además, tomar en cuenta que un cultivo negativo no necesariamente corresponde a la ausencia microbiana sino a la presencia de biopelículas que no pueden ser detectadas por las técnicas convencionales del aislamiento bacteriano por lo que se requiere de metodología especializada [63, 64].

Se sugiere que cuando se sospecha de biopelículas con cultivo negativo y la presentación sugiere granuloma a cuerpo extraño y se utilizan antibióticos, éstos retrasan el tratamiento efectivo del granuloma con esteroides intralesionales por meses, en contraste, si se confirma la presencia bacteriana **No se deben usar esteroides** porque deteriora la condición clínica del paciente [65].

Dada la importancia y riesgo de progresión de las lesiones de granulomas a cuerpo extraño, se han sugerido como principales tratamientos [6, 33].

4.2.1. Inyecciones intralesionales: Es el tratamiento primario con corticosteroides que interfieren con las actividades de fibroblastos, macrófagos, células gigantes y síntesis de colágeno. Se utilizan altas dosis de tiamcinolona mezclada con lidocaína para prevenir recurrencia. El uso de bleomicina en cicatrices keloides o hipertróficas ha sido exitoso, así como el antimitótico 5 fluorouracilo. Otros fármacos usados en casos aislados son alopurinol, colchicina o isotretinoína.

4.2.2. Terapia sistémica: Altas dosis de esteroides como prednisolona, minociclina combinada con esteroides orales o intralesionales para evitar la recurrencia de granulomas, también se ha reportado el uso de alopurinol, colchicina y ciclosporina.

4.2.3. Cirugía: No es la primera terapia de elección debido a que un granuloma es invasivo sin bordes confinados que rodean al tejido recomendado solo en casos de absceso estéril.

El uso de ultrasonido de alta frecuencia para aumentar la precisión de la inyección y detectar y manejar complicaciones potenciales, además, se ha asociado el tratamiento con hialuronidasa para el tratamiento de abscesos generados por contaminación o para reacciones adversas [66, 67].

Para el tratamiento de oclusión ocular como resultado de la inyección accidental en arterias por excesiva presión o por volúmenes altos, la administración rápida o profunda se puede causar isquemia, dolor, hipoxia y muerte celular causando necrosis. La compresión y la oclusión son indistinguibles y su tratamiento inmediato consiste en el cese inmediato de la inyección, masaje ocular y cuando el producto sea ácido hialurónico se debe utilizar 400 – 600 unidades de hialuronidasa sin diluir en el área afectada, otras opciones son el uso de nitropaste, compresas calientes, aspirina, sildenafil y oxígeno hiperbárico [68, 69]. Si ocurre oclusión arterial por ácido hialurónico, el procedimiento es urgente y depende de la inyección de hialuronidasa retrobulbar o peribulbar y debe realizarse por especialistas oftalmológicos [70].

Se han publicado resultados de grupos de consenso globales, algoritmos y guías que permiten establecer recomendaciones de prevención y tratamiento de rellenos dérmicos utilizados para tejidos blandos principalmente de ácido hialurónico con información relacionada con la profundidad de inyección, tipo de aguja, técnica de aplicación y volumen recomendado para evitar complicaciones y mejorar la satisfacción del paciente, además se han evaluado y clasificado las principales reacciones, las estrategias para mitigar las secuelas indeseables, cuando éstas se presentan [4, 22, 59, 60, 71-76].

5. PERSPECTIVAS

El diseño de nuevos productos utilizados en la dermatología cosmética es un campo en amplio desarrollo, como sustituto de los procedimientos cosméticos quirúrgicos lo que ha dado lugar a una gran cantidad de investigaciones.

La tendencia va hacia el desarrollo de productos de última generación con uso de materiales naturales y sostenibles con propiedades de reticulación mejoradas que logren resultados más duraderos y se centren en perfeccionar los efectos estimulantes sobre la producción de colágeno. Entre estos productos se encuentran los rellenos híbridos, que no solo rellenan, sino que también estimulan la producción de colágeno y mejoran la calidad de la piel con el tiempo. Un ejemplo de esto es la policaprolactona, un relleno que actúa en dos fases: primero da volumen inmediato, y luego activa fibroblastos para generar colágeno nuevo.

La búsqueda del relleno dérmico ideal debe cumplir con los siguientes requisitos: Resultados más naturales, menor riesgo de efectos adversos, mayor duración, mejora progresiva de la piel y personalización de acuerdo a cada paciente. Por esta razón, se han diseñado nanomateriales como vesículas de hidrogel de ácido hialurónico (HA) o su combinación con hidroxiapatita, complejos de HA con polinucleótidos que favorecen la estimulación de colágeno por los fibroblastos entre otros.

Los laboratorios ya están trabajando en fórmulas que combinen incluso más funciones: relleno, bioestimulación, antioxidación y liberación controlada de principios activos. Se habla

también de rellenos con tecnología inteligente, que puedan adaptarse al movimiento muscular y a las expresiones faciales con aún más precisión.

Sin embargo, estos preparados han probado su seguridad en modelos animales (estudios preclínicos) que deben asegurar la ausencia de toxicidad, seguridad y ausencia de inmunogenicidad mediante estudios clínicos para ser autorizados por las agencias regulatorias y posteriormente comercializados para su uso humano.

6. CONCLUSIÓN

La disposición de tipos y número de rellenos dérmicos continúa en aumento, por tanto, se debe entender cómo se previenen y tratan la aparición de sus complicaciones. Sin embargo, aunque se asume que los materiales utilizados son biocompatibles, existe cada vez más gran cantidad de reportes de las principales complicaciones y su tratamiento.

Es muy importante reconocer que el conocimiento y registro de las reacciones adversas asociadas con los materiales utilizados como rellenos cosméticos a la fecha está limitado, principalmente por dos razones, la primera es que estos eventos no siempre son reportados a las agencias regulatorias que son críticos para conocer la seguridad real a los pacientes [77] y la segunda es que muchas de las revisiones sistemáticas (meta-análisis) tienen criterios de selección muy estrictos de los artículos publicados ya que eliminan mucha información que puede ser importante.

Aunque los eventos adversos son raros, se debe considerar que se pueden presentar complicaciones serias principalmente en el diagnóstico de los nódulos como inflamatorios, no inflamatorios o infecciosos para su manejo y prevención adecuados.

Por otra parte, la formación de biopelículas como evento tardío pueden representar una causa poco reconocida y su diagnóstico es difícil de establecer, de ahí que no se puede descartar su incidencia y también ser considerarlas como responsables de reacciones que pueden evolucionar y comprometer la salud dérmica de los pacientes.

CONFLICTO DE INTERÉS

Los autores declaran que no tienen ningún conflicto de interés, financiero o de otro tipo, que pudieran influir en los resultados de esta revisión.

REFERENCIAS

1. A. Goldman & U. Wollina. Facial rejuvenation for middle-aged women: a combined approach with minimally invasive procedures. *Clinical Interventions in Aging*, **5**, 293–299 (2010). URL: https://doi.org/10.2147/cia.s13215
2. FDA U.S. Food & Drug Administration. Rellenos dérmicos (rellenos de tejidos blandos) https://www.fda.gov/medical-devices/aesthetic-cosmetic-devices/dermal-fillers-soft-tissue-fillers
3. Z.-F. Liao, L.-Y. Cong, F.-W. Li, S.-W. Wang, F.-C. Liu, H.-B. Wang & S.-K. Luo. The research trend of soft tissue filler injection from 2000 to 2022: A bibliometric and visualized analysis. *Plastic & Reconstructive Surgery-Global Open*, **12**(2), e5579 (2024). Doi: https://doi.org/10.1097/gox.0000000000005579
4. M.H. Graivier, L.M. Bass, Z.P. Lorenc, R. Fitzgerald, D.J. Goldberg & G. Lemperle. Differentiating nonpermanent injectable fillers: prevention and treatment of filler complications. *Aesthetic Surgery Journal*, **38**(Suppl_1), S29–S40 (2018). Doi: https://doi.org/10.1093/asj/sjy032

5. I. Cheng-Flores & J.L. Moreno-González. Materiales de relleno en dermatología. *Dermatología Cosmética, Médica y Quirúrgica*, 9(4), 275–283 (2011). URL: https://www.medigraphic.com/pdfs/cosmetica/dcm-2011/dcm114g.pdf
6. I. Sánchez-Carpintero, D. Candelas & R. Ruiz-Rodríguez. Materiales de relleno, tipos, indicaciones y complicaciones. *Actas Dermo-Sifiliográficas*, **101**(5), 381–393 (2010). Doi: https://doi.org/10.1016/j.ad.2010.01.004
7. D.H. Jones. Semipermanent and permanent injectable fillers. *Dermatologic Clinics*, **27**(4), 433–444 (2009). Ddoi: https://doi.org/10.1016/j.det.2009.08.003
8. G.-W. Hong, J. Wan, K. Chang, Y. Park & K.-H. Yi. Decomposition and changes in *in vivo* post-HA filler injection: A review. *Journal of Cosmetic Dermatology*, **24**(1), e16652 (2025). Doi: https://doi.org/10.1111/jocd.16652
9. A. Tezel & G.H. Fredrickson. The science of hyaluronic acid dermal fillers. *Journal of Cosmetic and Laser Therapy*, **16**(1), 45 (2014). Doi: https://doi.org/10.1080/14764170701774901
10. M. Gold. The science and art of hyaluronic acid dermal filler use in esthetic applications. *Journal of Cosmetic Dermatology*, **8**(4), 301–307 (2009). Doi: https://doi.org/10.1111/j.1473-2165.2009.00464.x
11. U. Wollina, H. Kocic & A. Goldman. Hyaluronic acid in facial rehabilitation—A narrative review. *Cosmetics*, **10**(2), 61 (2023). Doi: https://doi.org/10.3390/cosmetics10020061
12. J. Alijotas-Reig M.T. Fernández-Figueras & L. Puig. Inflammatory, immune-mediated adverse reactions related to soft tissue dermal fillers. *Seminars in Arthritis and Rheumatism*, **43**(2), 241–258 (2013). Doi: https://doi.org/10.1016/j.semarthrit.2013.02.001
13. C. Faus-Alcañiz & C. Martínez-Ciarpaglini. Revisión de la respuesta tisular a los materiales de relleno. *Medicina Estética*, **37**(4), 46–54 (2013). Doi: https://doi.org/10.48158/medicinaestetica.037.01
14. E. Haneke. Adverse effects of fillers and their histopathology. *Facial Plastic Surgery*, **30**(6), 599–614 (2014). Doi: https://doi.org/10.1055/s-0034-1396755
15. A. Modarressi, C. Nizet & T. Lombardi. Granulomas and nongranulomatous nodules after filler injection: Different complications require different treatments. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, **73**(11), 2010–2015 (2020). Doi: https://doi.org/10.1016/j.bjps.2020.08.012
16. M.H. Abduljabbar & M.A. Basendwh. Complications of hyaluronic acid fillers and their managements. *Journal of Dermatology & Dermatologic Surgery*, **20**(2), 100–106 (2016). Doi: https://doi.org/10.1016/j.jdds.2016.01.001
17. K. Singh & S. Nooreyezdan. Nonvascular complications of injectable fillers-Prevention and management. *Indian Journal of Plastic Surgery*, **53**(3), 335–343 (2020). Doi: https://doi.org/10.1055/s-0040-1721872
18. K. Rentfro, D. Clarey, E.J. Glenn, R. Sulewski & A. Wysong. Soft tissue dermal filler-associated necrosis and impending necrosis: A systematic review of the literature. *Dermatologic Surgery*, **48**(10), 1051–1056 (2022). Doi: https://doi.org/10.1097/dss.0000000000003574
19. R.A. Machado, L.Q. Rocha de Oliveira, H. Martelli-Júnior, Fábio Ramoa-Pires, J. Badin-Carvas, V.E. Rogerio, V. de Azevedo-Rabelo & R.D. Coletta. Adverse reactions to the injection of face and neck aesthetic filling materials: a systematic review. *Medicina Oral, Patología Oral y Cirugía Bucal*, **28**(3), e278–e284 (2020). Doi: https://doi.org/10.4317/medoral.25713
20. C. DeLorenzi. Complications of injectable fillers, Part 2: Vascular complications. *Aesthetic Surgery Journal*, **34**(4), 584–600 (2014). Doi: https://doi.org/10.1177/1090820x14525035
21. L. Requena, C. Requena, L. Christensen, U.S. Zimmermann, H. Kutzner & L. Cerroni. Adverse reactions to injectable soft tissue fillers. *Journal of the American Academy of Dermatology*, **64**(6), 1178 (2011). Doi: https://doi.org/10.1016/j.jaad.2010.02.064
22. D. Funt & T. Pavicic. Dermal fillers in aesthetics: an overview of adverse events and treatment approaches. *Clinical, Cosmetic and Investigational Dermatology*, **2013**(6), 295–316 (2013). Doi: https://doi.org/10.2147/ccid.s50546
23. F. Bachmann, R. Erdmann, V. Hartmann, L. Wiest & B. Rzany. The spectrum of adverse reactions after treatment with injectable fillers in the glabellar region: results from the Injectable Filler Safety Study. *Dermatologic Surgery*, **35**(Suppl 2), 1629–1634 (2009). Doi: https://doi.org/10.1111/j.1524-4725.2009.01341.x

24. C. DeLorenzi. Complications of injectable fillers, part I. *Aesthetic Surgery Journal*, **33**(4), 561–575 (2013). Doi: https://doi.org/10.1177/1090820x13484492
25. S.H. Dayan, J.P. Arkins & R. Brindise. Soft tissue fillers and biofilms. *Facial Plastic Surgery*, **27**(1), 23–28 (2011). Doi: https://doi.org/10.1055/s-0030-1270415
26. M. Brandwein, D. Steinberg & S. Meshner. Microbial biofilms and the human skin microbiome. *NPJ Biofilms and Microbiomes*, **2**, 3 (2016). Doi: https://doi.org/10.1038/s41522-016-0004-z
27. A.V. Gannesen, V. Borrel, L. Lefeuvre, A.I. Netrusov, V.K. Plakunov & M.G.J. Feuilloley. Effect of two cosmetic compounds on the growth, biofilm formation activity, and surface properties of acneic strains of *Cutibacterium acnes* and *Staphylococcus aureus*. *Microbiology Open*, **8**(3), e00659 (2019). Doi: https://doi.org/10.1002/mbo3.659
28. T. Coenye, K.J. Spittaels & Y. Achermann. The role of biofilm formation in the pathogenesis and antimicrobial susceptibility of *Cutibacteriumacnes*. *Biofilm*, **4**, 100063 (2021). Doi: https://doi.org/10.1016/j.bioflm.2021.100063
29. L.N. Trinh, K.C. McGuigan & A. Gupta. Delayed granulomas as a complication secondary to lip augmentation with dermal fillers: A systematic review. *The Surgery Journal*, **8**(1), e69–e79 (2022). Doi: https://doi.org/10.1055/s-0042-1743524
30. C. Convery, E. Davies, G. Murray & L. Walker. Delayed-onset nodules (DONs) and considering their treatment following use of hyaluronic acid (HA) fillers. *The Journal of Clinical and Aesthetic Dermatology*, **14**(7), E59–E67 (2021). URL: https://pmc.ncbi.nlm.nih.gov/articles/PMC8570356/
31. M.A. Izquierdo & L. Requena. Granulomas por cuerpos extraños. *Actas Dermo-Sifiliográficas*, **90**(11), 543–557 (1999). URL: https://www.actasdermo.org/es-granulomas-por-cuerpos-extranos--articulo-13003554
32. J. Aróstegui-Aguilar, A. Diago, R. Carrillo-Gijón, M. Fernández-Figueras, J. Fraga, A. García-Herrera, *et al.* Granulomas en dermatopatología: principales entidades. Parte II. *Actas Dermo-Sifiliográficas*, **112**(8), 705–724 (2021). Doi: https://doi.org/10.1016/j.ad.2021.04.001
33. J.M. Lee & Y.J. Kim. Foreign body granulomas after the use of dermal fillers: pathophysiology, clinical appearance, histologic features, and treatment. *Archives of Plastic Surgery*, **42**(2), 232–239 (2015). Doi: https://doi.org/10.5999/aps.2015.42.2.232
34. S.-K. Lee, S.M. Kim, S.H. Cho, J.D. Lee & H.S. Kim HS. Adverse reactions to injectable soft tissue fillers: memorable cases and their clinico-pathological overview. *Journal of Cosmetic and Laser Therapy*, **17**(2), 102–108 (2015). Doi: https://doi.org/10.3109/14764172.2014.968584
35. G. Lemperle, N. Gauthier-Hazan, M. Wolters, M. Eisemann-Klein, U. Zimmermann U & D.M. Duffy. Foreign body granulomas after all injectable dermal fillers: Part 1. Possible causes. *Plastic and Reconstructive Surgery*, **123**(6), 1842–1863 (2009). Doi: https://doi.org/10.1097/prs.0b013e31818236d7
36. S. Sharma, J. Mohler, S.D. Mahajan, S.A. Schwartz, L. Bruggemann & R. Aalinkeel. Microbial biofilm: A review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*, **11**(6), 1614 (2023). Doi: https://doi.org/10.3390/microorganisms11061614
37. A.B. Sadashivaiah & V. Mysore. Biofilms: their role in dermal fillers. *Journal of Cutaneous and Aesthetic Surgery*, **3**(1), 20–22 (2010). Doi: https://doi.org/10.4103/0974-2077.63257
38. Y.L. Zhang, Z.S. Sun, W.J. Hong, Y. Chen, Y.F. Zhou & S.K. Luo. Biofilm formation is a risk factor for late and delayed complications of filler injection. *Frontiers in Microbiology*, **14**, 1297948 (2024). Doi: https://doi.org/10.3389/fmicb.2023.1297948
39. M.T. Imen. Delayed infectious reactions of dermal filler injections in the face: Causes and management. *Journal of Stomatology, Oral and Maxillofacial Surgery*, **124**(2), 101334 (2023). Doi: https://doi.org/10.1016/j.jormas.2022.11.010
40. M. Saththianathan, K. Johani, A. Taylor, H. Hu, K. Vickery, P. Callan & A.K. Deva. The role of bacterial biofilm in adverse soft-tissue filler reactions: A combined laboratory and clinical study. *Plastic Reconstructive Surgery*, **139**(3), 613–621 (2017). Doi: https://doi.org/10.1097/prs.0000000000003067
41. A. Hassan, J. Usman, F. Kaleem, M. Omair, A. Khalid & M. Iqbal. Evaluation of different detection methods of biofilm formation in the clinical isolates. *The Brazilian Journal of Infectious Diseases*, **15**(4), 305–311 (2011). Doi : https://doi.org/10.1016/S1413-8670(11)70197-0

42. Y. Xu, Y. Dhaouadi, P. Stoodley & D. Ren. Sensing the unreachable: challenges and opportunities in biofilm detection. *Current Opinion in Biotechnology*, **64**, 79–84 (2020). Doi: https://doi.org/10.1016/j.copbio.2019.10.009
43. K. Conrad, R. Alipasha, S. Thiru & T. Kandasamy. Abscess formation as a complication of injectable fillers. *Modern Plastic Surgery*, **5,** 14–18 (2015). Doi: https://doi.org/10.4236/mps.2015.52003
44. L. Gottfried. No proof that biofilm bacteria are causing dermal filler granulomas. *American Journal of Biomedical Science & Research*, **4**(1)**,** 17–22 (2019). Doi: https://doi.org/10.34297/ajbsr.2019.04.000749
45. V.J. Hassid. Soft-tissue filler complications: the important role of biofilms. *Plastic Reconstructive Surgery*, **126**(5), 1801–1802 (2010). Doi: https://doi.org/10.1097/prs.0b013e3181ef93b5
46. R.J. Rohrich, G. Monheit, A.T. Nguyen, S.A. Brown & S. Fagien. Soft-tissue filler complications: the important role of biofilms. *Plastic Reconstructive Surgery*, **125**(4), 1250–1256 (2010). Doi: https://doi.org/10.1097/prs.0b013e3181cb4620
47. M. Alhede, Ö. Er, S. Eickhardt, K. Kragh, M. Alhede, L.D. Christensen, *et al.* Bacterial biofilm formation and treatment in soft tissue fillers. *Pathogens and Disease*, **70**(3), 339–346 (2014). Doi: https://doi.org/10.1111/2049-632x.12139
48. K. Beer & R. Avelar. Relationship between delayed reactions to dermal fillers and biofilms: facts and considerations. *Dermatologic Surgery*, **40**(11), 1175–1179 (2014). Doi: https://doi.org/10.1097/01.dss.0000452646.76270.53
49. M.M. Draelos & Z.D. Draelos. The biofilm, injectables, and cosmetic dermatology. *Journal of Cosmetic Dermatology*, **12**(4), 245–246 (2013). Doi: https://doi.org/10.1111/jocd.12069
50. N. Doshi & S. Mitragotri. Macrophages recognize size and shape of their targets. *PLoS One*, **5**(4), e10051 (2010). Doi: https://doi.org/10.1371/journal.pone.0010051
51. C.K. Hee & D.J. Messina. *In vitro* inflammatory and immune response to uncrosslinked hyaluronic acid (HA) and HA fillers. *Journal of Immunology and Regenerative Medicine*, **17**, 100065 (2022). Doi: https://doi.org/10.1016/j.regen.2022.100065
52. J.M. Cyphert, C.S. Trempus & S. Garantziotis. Size matters: Molecular weight specificity of hyaluronan effects in cell biology. *International Journal of Cell Biology*, **2015**, 563818 (2015). Doi: https://doi.org/10.1155/2015/563818
53. X. Jia, M. Shi, Q. Wang, J. Hui, J.H. Shofaro, R. Erkhembayar, M. Hui, C. Gao & M.A. Gantumur. Anti-inflammatory effects of the 35kDa hyaluronic acid fragment (B-HA/HA35). *Journal of Inflammation Research*, **16**, 209–224 (2023). Doi: https://doi.org/10.2147/jir.s393495
54. A. Owczarczyk-Saczonek, N. Zdanowska, E. Wygonowska & W. Placek. The immunogenicity of hyaluronic fillers and its consequences. *Clinical, Cosmetic and Investigational Dermatology*, **14**, 921–934 (2021). Doi: https://doi.org/10.2147/ccid.s316352
55. A. Owczarczyk-Saczonek & K. De Boulle. Hyaluronic acid fillers and ASIA syndrome: Case studies. *Clinical, Cosmetic and Investigational Dermatology*, **16**, 2763—2771 (2023). Doi: https://doi.org/10.2147/ccid.s419716
56. H. Witmanowski & K. Błochowiak. Another face of dermal fillers. *Advances in Dermatology and Allergology*, **37**(5), 651–659 (2020). Doi: https://doi.org/10.5114/ada.2019.82859
57. I. Heydenrych, K. De Boulle, K.M. Kapoor & D. Bertossi. The 10-Point Plan 2021: Updated concepts for improved procedural safety during facial filler treatments. *Clinical, Cosmetic and Investigational Dermatology*, **14**, 1601–1602 (2021). Doi: https://doi.org/10.2147/ccid.s346299
58. S.G. Fabi, S. Desyatnikova & S.H. Dayan. Prevention and management of dermal filler complications: A review. *Facial Plastic Surgery & Aesthetic Medicine*, **27**(2), 120–124 (2024). Doi: https://doi.org/10.1089/fpsam.2024.0020
59. M. de Maio. MD Codes™: A methodological approach to facial aesthetic treatment with injectable hyaluronic acid fillers. *Aesthetic Plastic Surgery*, **45**(2), 690–709 (2021). Doi: https://doi.org/10.1007/s00266-020-01762-7
60. Z. Diwan, S. Trikha, S. Etemad-Shahidi, S. Virmani, C. Denning, Y. Al-Mukhtar, C. Rennie, A. Penny, Y. Jamali & N.C. Edwards-Parrish. Case series and review on managing abscesses secondary to hyaluronic acid soft tissue fillers with recommended management guidelines. *The Journal of Clinical and Aesthetic Dermatology*, **13**(11), 37–43 (2020). URL: https://pubmed.ncbi.nlm.nih.gov/33282102/

61. N. Corduff, L. Juniarti, T.S. Lim, F. Lin, K. Mariwalla, T. Pavicic, *et al.* Current practices in hyaluronic acid dermal filler treatment in Asia Pacific and practical approaches to achieving safe and natural-looking results. *Clinical, Cosmetic and Investigational Dermatology*, **15**, 1213–1223 (2022). Doi: https://doi.org/10.2147/ccid.s363583
62. D.K. Funt. Treatment of delayed-onset inflammatory reactions to hyaluronic acid filler: An algorithmic approach. *Plastic and Reconstructive Surgery - Global Open*, **10**(6), e4362 (2022). Doi: https://doi.org/10.1097/gox.0000000000004362
63. M.R. Taha & S.K. Tyring. A review of the role and treatment of biofilms in skin disorders. *Skin Therapy Letters*, **29**(6), 6-9 (2024). URL: https://www.skintherapyletter.com/acne/treatment-of-biofilms-in-skin-disorders/
64. X. Kang, X. Yang, Y. He, C. Guo, Y. Li, H. Ji, Y. Qin & L. Wu. Strategies and materials for the prevention and treatment of biofilms. *Materials Today Bio*, **23**, 100827 (2023). Doi: https://doi.org/10.1016/j.mtbio.2023.100827
65. D.I. Dumitraşcu & A.V. Georgescu. The management of biofilm formation after hyaluronic acid gel filler injections: a review. *Clujul Medical*, **86**(3), 192–195 (2013). URL: https://pubmed.ncbi.nlm.nih.gov/26527945/
66. A.-G. Nicola, M.O. Pricop & B. Ramos-Medina. Clinical management with high-frequency ultrasound of recurrent submental abscess formation after filler placement: Bacterial contamination or immune-mediated adverse event? *Cureus*, **16**(4), e58878 (2024). Doi: https://doi.org/10.7759/cureus.58878
67. S.J. Almushayt. The role of ultrasound in facial hyaluronic acid dermal filler injections - A review article. *Clinical Imaging*, **119**, 110413 (2025). Doi: https://doi.org/10.1016/j.clinimag.2025.110413
68. M. Kamei. Treatments for central retinal vein occlusion. There is no established standard of care for CRVO. Anti-VEGF therapy may become a first-line choice in the near future. *Retina Today*, **Jan/Feb 2013**, 34-36 (2013). URL: https://retinatoday.com/articles/2013-jan/treatments-for-central-retinal-vein-occlusion https://assets.bmctoday.net/retinatoday/pdfs/0113RT_Global%20Perspectives.pdf
69. B.S. Biesman, J.B. Green, R. George, C. Jacob, M. Palm, D.H. Jones, *et al.* A multicenter, randomized, evaluator-blinded study to examine the safety and effectiveness of hyaluronic acid filler in the correction of infraorbital hollows. *Aesthetic Surgery Journal*, **44**(9), 1001–1013 (2024). Doi: https://doi.org/10.1093/asj/sjae073
70. N. Fakih-Gomez, C. Muñoz-Gonzalez, C.A. Porcar-Plana, M. Puzo-Bayod & J. Madero. Retrobulbar hyaluronidase in hyaluronic acid-induced ocular vascular occlusion: Efficacy, challenges, and implications for clinical practice. *Aesthetic Plastic Surgery*, **49**(5), 1458–1468 (2025). Doi: https://doi.org/10.1007/s00266-024-04483-3
71. M. Signorini, S. Liew, H. Sundaram, K.L. De Boulle, G.J. Goodman, G. Monheit, Y. Wu, A.R. Trindade de Almeida, A. Swift & A. Vieira-Braz. Global Aesthetics Consensus: Avoidance and management of complications from hyaluronic acid fillers-evidence- and opinion-based review and consensus recommendations. *Plastic and Reconstructive Surgery*, **137**(6), 961e–971e (2016). Doi: https://doi.org/10.1097/prs.0000000000002184
72. G. Zaccaria, A. Dotti, E. Benanti, C. Vigliarolo & L. Vaienti. A treatment algorithm for hyaluronic acid filler related complications of the face. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, **91**, 207–217 (2024). Doi: https://doi.org/10.1016/j.bjps.2024.02.010
73. O. Artzi, J.L. Cohen, J.S. Dover, A. Suwanchinda, T. Pavicic, M. Landau, *et al.* Delayed inflammatory reactions to hyaluronic acid fillers: A literature review and proposed treatment algorithm. *Clinical, Cosmetic and Investigational Dermatology*, **18**(13), 371–378 (2020). Doi: https://doi.org/10.2147/ccid.s247171
74. F. Urdiales-Gálvez, N.E. Delgado, V. Figueiredo, J.V. Lajo-Plaza, M. Mira, A. Moreno, *et al.* Treatment of soft tissue filler complications: Expert consensus recommendations. *Aesthetic Plastic Surgery*, **42**(2), 498–510 (2018). Doi: https://doi.org/10.1007/s00266-017-1063-0
75. W. Baranska-Rybak, J.V. Lajo-Plaza, L. Walker & N. Alizadeh. Late-Onset Reactions after Hyaluronic Acid Dermal Fillers: A consensus recommendation on etiology, prevention and management.

Dermatology and Therapy (Heidelberg), **14**(7), 1767–1785 (2024). Doi: https://doi.org/10.1007/s13555-024-01202-3

76. Z. Diwan, S. Trikha, S. Etemad-Shahidi, N. Parrish & C. Rennie. Evaluation of current literature on complications secondary to lip augmentation following dermal filler injection. *The Journal of Clinical and Aesthetic Dermatology*, **16**(7), 26–33 (2023). URL : https://pubmed.ncbi.nlm.nih.gov/37560504/
77. M. Coppini, V.C.A. Caponio, R. Mauceri, G. Pizzo, N. Mauceri, L. Lo Muzio & G. Campisi. Aesthetic lip filler augmentation is not free of adverse reactions: lack of evidence-based practice from a systematic review. *Frontiers in Oral Health*, **5**, 1495012 (2024). Doi: https://doi.org/10.3389/froh.2024.1495012

CÓMO CITAR ESTE ARTÍCULO

L.E. Castrillón-Rivera, A. Palma-Ramos, J.I. Castañeda-Sánchez & V. Espinosa-Antúnez. ¿Son realmente seguros los rellenos dérmicos? *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 195–214 (2026). https://doi.org/10.15446/rcciquifa.v55n1.125089

Artículo de investigación clínica

Buenas Prácticas de Dispensación y satisfacción del usuario: Evidencia multidimensional en servicios farmacéuticos hospitalarios

Luisa Noa-Yarasca[1a]*, Doriza Sierra Córdova[1b], Bertrán Santiago Trujillo[2c] & Emilio G. Ramírez Roca[1d]

[1] Facultad de Ciencias de la Salud, Universidad Nacional de San Cristóbal de Huamanga, Ayacucho, Perú.
[2] Departamento Académico de Farmacotecnia y Administración Farmacéutica, Facultad de Farmacia y Bioquímica, Universidad Nacional Mayor de San Marcos, Lima, Perú.

*Autora correspondiente.

Correos electrónicos: [a] luisa.noa@unsch.edu.pe; [b] doriza.sierra.20@unsch.edu; [c] bsantiagot@unmsm.edu.pe; [d] emilio.ramirez@unsch.edu.pe

ORCID IDs:
[a] https://orcid.org/0000-0001-9225-6561; [b] https://orcid.org/0009-0002-2779-4712; [c] https://orcid.org/0000-0003-0286-4892; [d] https://orcid.org/0000-0002-9704-1954

Recibido: 2 de septiembre de 2025
Corregido: 24 de diciembre de 2025
Aceptado: 29 de diciembre de 2025

https://doi.org/10.15446/rcciquifa.v55n1.122606

RESUMEN

Antecedentes: La calidad de los servicios farmacéuticos depende tanto de la correcta aplicación de las *Buenas Prácticas de Dispensación (BPD)* como de la percepción de satisfacción de los usuarios, factores cuya interacción aún no ha sido explorada en profundidad en el contexto hospitalario. **Objetivo:** Este estudio evaluó la relación entre las BPD y la *Satisfacción del Usuario (SU)* en una farmacia hospitalaria, examinando la validez de sus dimensiones teóricas y sus interrelaciones perceptuales. **Materiales y métodos:** En una muestra de 359 pacientes atendidos en la farmacia externa del Hospital Regional "Jesús Nazareno" (Ayacucho, Perú), se aplicaron cuestionarios validados para medir BPD (17 ítems en cinco dimensiones) y SU (20 ítems en cinco dimensiones). **Resultados:** Los análisis estadísticos confirmaron que los participantes diferenciaron claramente los ítems y dimensiones de ambos instrumentos, respaldando su validez conceptual. Asimismo, se identificaron patrones de afinidad y contraste entre dimensiones, destacando la estrecha asociación de las prácticas técnicas de las BPD —particularmente *entrega e información* y *preparación y selección*— con aspectos emocionales y ambientales de la SU, como *capacidad de respuesta*, *empatía* y *tangibilidad*. La primera función canónica mostró una fuerte correlación multivariada (r = 0,83) entre ambas variables, evidenciando su interdependencia. **Conclusión:** Estos hallazgos confirman que las dimensiones técnicas y humanas de la dispensación son interdependientes y deben ser gestionadas de forma integrada para mejorar la calidad del servicio. La evidencia generada aporta un marco empírico para diseñar intervenciones estratégicas orientadas a fortalecer la eficiencia, seguridad y humanización de la atención farmacéutica hospitalaria.

Palabras clave: Buenas Prácticas de Dispensación; satisfacción del usuario; atención farmacéutica hospitalaria; escalamiento multidimensional; análisis de clústeres; correlación canónica.

SUMMARY

Good Dispensing Practices and User Satisfaction: Multidimensional Insights from Hospital Pharmaceutical Services

Background: The quality of pharmaceutical services depends not only on the effective implementation of *Good Dispensing Practices (GDP)* but also on users' perceptions of satisfaction—two factors whose interaction has been insufficiently explored within hospital settings. **Objective:** This study examined the relationship between GDP and *User Satisfaction (US)* in a hospital pharmacy, assessing the validity of their theoretical dimensions and their perceptual interrelations. **Materials and methods:** A validated questionnaire was administered to 359 patients served at the outpatient pharmacy of the "Jesús Nazareno" Regional Hospital (Ayacucho, Peru), measuring GDP (17 items across five dimensions) and US (20 items across five dimensions). **Results:** Statistical analyses confirmed that participants could clearly distinguish the items and dimensions of both instruments, supporting their conceptual validity. Patterns of affinity and contrast among dimensions emerged, highlighting a strong association between technical aspects of GDP—particularly *delivery and information*, and *preparation and selection*—and the emotional and environmental facets of US, such as *responsiveness*, *empathy*, and *tangibility*. The first canonical function revealed a strong multivariate correlation (r = 0.83) between GDP and US, indicating substantial interdependence. **Conclusion:** These findings confirm that the technical and human dimensions of dispensing are interdependent and require integrated management to enhance pharmaceutical service quality. The results provide an empirical basis for designing strategic interventions to improve the efficiency, safety, and humanization of hospital pharmaceutical services.

Keywords: Good Dispensing Practices; user satisfaction; hospital pharmaceutical care; multidimensional scaling; cluster analysis; canonical correlation.

RESUMO

Boas Práticas de Dispensação e Satisfação do Usuário: Evidências Multidimensionais em Serviços de Farmácia Hospitalar

Contexto: A qualidade dos serviços farmacêuticos depende tanto da correta aplicação das Boas Práticas de Dispensação (BPD) quanto da percepção de satisfação dos usuários, fatores cuja interação ainda não foi completamente explorada no contexto hospitalar. **Objetivo:** Este estudo avaliou a relação entre BPD e Satisfação do Usuário (SU) em uma farmácia hospitalar, examinando a validade de suas dimensões teóricas e suas inter-relações perceptivas. **Materiais e métodos:** Questionários validados foram aplicados a uma amostra de 359 pacientes atendidos na farmácia ambulatorial do Hospital Regional "Jesús Nazareno" (Ayacucho, Peru) para mensurar BPD (17 itens em cinco dimensões) e SU (20 itens em cinco dimensões). **Resultados:** As análises estatísticas confirmaram que os participantes diferenciaram claramente os itens e as dimensões de ambos os instrumentos, corroborando sua validade conceitual. Além disso, foram identificados padrões de afinidade e contraste entre as dimensões, destacando a estreita associação das práticas técnicas das Boas Práticas de Dispensação (BPD) — particularmente a entrega e a informação, e a preparação e a seleção — com os aspectos emocionais e ambientais da experiência do paciente, como a capacidade de resposta, a empatia e a tangibilidade. A primeira função canônica mostrou uma forte correlação multivariada (r=0,83) entre ambas as variáveis, demonstrando sua interdependência. **Conclusão:** Esses achados confirmam que as dimensões técnica e humana da dispensação são interdependentes e devem ser gerenciadas de forma integrada para melhorar a qualidade do serviço. As evidências geradas fornecem uma estrutura empírica para o desenvolvimento de intervenções estratégicas voltadas ao fortalecimento da eficiência, segurança e humanização da assistência farmacêutica hospitalar.

Palavras-chave: Boas Práticas de Dispensação; satisfação do paciente; assistência farmacêutica hospitalar; escalonamento multidimensional; análise de cluster; correlação canônica.

1. INTRODUCCIÓN

En los últimos años, el sistema de salud ha experimentado una transformación progresiva y significativa en la calidad de atención, con un enfoque creciente en el fortalecimiento de los servicios farmacéuticos [1, 2]. En este contexto, las Buenas Prácticas de Dispensación (BPD) de medicamentos se han consolidado como un componente esencial para garantizar el uso racional de los medicamentos y promover resultados terapéuticos óptimos [3, 4]. En el Perú, el Ministerio de Salud (MINSA) ha establecido normas y lineamientos específicos para asegurar que la entrega de medicamentos no solo sea técnicamente correcta, sino también humanamente significativa, promoviendo la satisfacción del usuario como un indicador clave de calidad del servicio [5, 6]. No obstante, aún es limitada la evidencia que explore de manera integrada cómo se estructuran, relacionan y jerarquizan empíricamente las dimensiones técnicas de las BPD y las dimensiones perceptuales de la satisfacción del usuario en contextos hospitalarios.

Las BPD son directrices que permiten garantizar la entrega segura y eficiente de medicamentos, asegurando su calidad, eficacia y uso racional. Las BPD incluyen prácticas como la validación de prescripciones, la identificación del paciente, la conservación adecuada de los fármacos, la educación sobre su uso y la prevención de errores [5, 7, 8]. Estas prácticas se basan en normativas nacionales e internacionales, promoviendo una atención farmacéutica responsable y centrada en la seguridad del paciente [3, 5, 8, 9]. Por otro lado, la *Satisfacción del Usuario* (SU) se refiere al grado de conformidad del paciente con la atención recibida, evaluando la comparación entre sus expectativas y la experiencia real del servicio. Factores como la disponibilidad de medicamentos, la atención del personal, los tiempos de espera y la claridad en la información son determinantes en esta percepción [8, 10, 11]. Una alta satisfacción se asocia con una mayor adherencia al tratamiento y confianza en el sistema de salud, impactando directamente en la calidad de la atención [10, 12]. Sin embargo, la mayoría de estudios analiza ambas variables de forma independiente, sin profundizar en los patrones de alineación, u oposición entre sus dimensiones estructurales.

Estudios previos han revelado que la implementación adecuada de las BPD puede influir positivamente en la percepción del usuario, particularmente en lo referido a la calidad de la orientación sobre el uso de medicamentos y la seguridad en el proceso de dispensación [10, 13]. Sin embargo, también se han identificado deficiencias estructurales y organizativas que afectan la percepción del servicio, especialmente cuando existen barreras en la comunicación entre el personal farmacéutico y los usuarios [14]. Otros hallazgos señalan que factores como la edad, el grado de instrucción o el nivel educativo y las características culturales de los usuarios pueden influir en su nivel de satisfacción, lo que sugiere la necesidad de enfoques personalizados en la atención farmacéutica [12, 15]. De manera general, se ha observado una relación positiva entre el cumplimiento de las BPD y la satisfacción del usuario, aunque dicha relación no siempre es homogénea y puede variar según el contexto de implementación [10, 16, 17]. Algunos estudios destacan que la calidad del servicio farmacéutico no depende únicamente de la entrega del medicamento, sino también de la calidad del proceso comunicacional, el entorno físico y la percepción de profesionalismo del personal [10, 14]. No obstante, son escasos los trabajos que examinan simultáneamente la estructura dimensional de ambas variables y su interrelación mediante enfoques multivariados integrados.

En el contexto peruano, investigaciones recientes han presentado resultados diversos. Por un lado, algunos estudios no han encontrado una relación estadísticamente significativa entre el cumplimiento de las Buenas Prácticas de Dispensación (BPD) y la satisfacción del usuario,

a pesar de que los servicios fueron valorados positivamente [18]. Por otro lado, otras investigaciones han demostrado una asociación directa y significativa entre estos factores, destacando que el cumplimiento técnico de las BPD influye notablemente en la percepción de calidad del servicio [19]. Adicionalmente, se ha identificado que variables como la disponibilidad de recursos, la organización interna y la formación del personal tienen un impacto relevante en la experiencia del usuario [20, 21]. Sin embargo, aún no se dispone de evidencia que permita comprender cómo se organizan empíricamente estas dimensiones.

A pesar de los avances normativos promovidos por las instituciones gubernamentales y los esfuerzos académicos liderados por universidades e instituciones de investigación, la implementación efectiva de las BPD enfrenta desafíos persistentes. Estos obstáculos pueden derivar en errores en la medicación, baja adherencia terapéutica y una menor satisfacción del usuario, aspectos que afectan tanto la calidad del servicio como los resultados en salud [15, 17]. Por lo tanto, resulta imperativo profundizar en el análisis de la relación entre las BPD y la percepción de los usuarios sobre la calidad de la atención farmacéutica, especialmente en el ámbito hospitalario [14]. En esta línea, resulta clave no solo medir ambas variables, sino también comprender su configuración estructural y las relaciones de proximidad y divergencia entre sus dimensiones. Este estudio se justifica en la necesidad de fortalecer los servicios farmacéuticos mediante la evaluación sistemática de las BPD como componente técnico esencial y su impacto en la satisfacción del usuario. Entender esta relación permitirá identificar áreas críticas de mejora y diseñar estrategias basadas en evidencia que optimicen la atención farmacéutica, tanto a nivel institucional como nacional.

El objetivo principal de esta investigación es examinar la relación entre el cumplimiento de las BPD y la satisfacción del usuario en la farmacia externa hospitalaria. Para ello, el estudio toma como población a pacientes del Hospital Regional "Jesús Nazareno" de Ayacucho, Perú, empleando un enfoque cuantitativo basado en instrumentos de medición validados dentro de los lineamientos del Ministerio de Salud (MINSA) del Perú. Las BPD son evaluadas a través de 17 ítems organizados en cinco dimensiones: *Recepción y validación* de la prescripción médica, *Análisis e interpretación*, *Preparación y selección*, *Registro* y *entrega e Información*. Por su parte, la satisfacción del usuario se midió con 20 ítems agrupados en las dimensiones de *fiabilidad*, *capacidad de respuesta*, *seguridad*, *empatía* y *tangibilidad*. Asimismo, el estudio se orienta a identificar la estructura empírica, el peso relativo y los patrones de alineación o contraposición entre dichas dimensiones.

En este marco, se plantean los siguientes objetivos específicos: *i*) Evaluar si los participantes diferencian adecuadamente entre los ítems de los instrumentos de medición sobre BPD y satisfacción del usuario; *ii*) Determinar si los participantes reconocen las dimensiones teóricas propuestas para las variables BPD y satisfacción del usuario; *iii*) Evaluar la consistencia entre la estructura dimensional propuesta por el Ministerio de Salud del Perú y la agrupación empírica de los ítems que componen los instrumentos de medición de BPD y satisfacción del usuario; *iv*) Identificar patrones de proximidad o divergencia entre las dimensiones teóricas de las BPD y de la satisfacción del usuario, y *v*) Analizar qué dimensiones de las variables BPD y satisfacción del usuario tienen mayor influencia en sus respectivas variables latentes y explorar el tipo de relación existente entre ambas.

Esta investigación busca generar evidencia robusta que contribuya a la mejora de la calidad de los servicios farmacéuticos, respondiendo a las demandas de un sistema de salud en constante evolución.

2. METODOLOGÍA

Se realizó un estudio cuantitativo, observacional, transversal y correlacional, desarrollado en concordancia con los principios éticos establecidos por el Ministerio de Salud del Perú, los cuales promueven el respeto a la dignidad, la autonomía, la equidad y la confidencialidad del paciente en la atención sanitaria [22, 23]. La recolección de datos para la contrastación de las hipótesis se realizó en las instalaciones del Hospital Regional "Jesús Nazareno" de Ayacucho, Perú, entre octubre de 2024 y marzo de 2025.

2.1 Área de estudio y contexto institucional

El estudio se desarrolló en el Hospital Regional "Jesús Nazareno" de Ayacucho, Perú, establecimiento categorizado como II-E según la Norma Técnica de Salud NTS N.° 021-MINSA/DGSP-V.03 [6, 24], correspondiente a un hospital de segundo nivel de complejidad. Este cuenta con capacidad resolutiva en especialidades clínicas básicas y subespecializadas, así como servicios de apoyo diagnóstico como imagenología, patología clínica, farmacia y nutrición [25]. El hospital brinda atención ambulatoria, de emergencia y hospitalización, atendiendo a una población diversa en edad, género y condiciones clínicas. Asimismo, recibe usuarios del sistema público y privado, con cobertura sanitaria total o parcial según las políticas nacionales vigentes.

El servicio farmacéutico hospitalario está conformado por químicos farmacéuticos y técnicos en farmacia, responsables de la dispensación, almacenamiento, gestión de inventarios, farmacovigilancia y promoción del uso racional y responsable de medicamentos. Es personal estimado incluye un farmacéutico jefe, uno a dos farmacéuticos por turno, personal asistencial según demanda y entre tres y cinco técnicos por turno, además de apoyo en gestión y almacén. Las áreas de dispensación están regidas bajo estándares normativos del Ministerio de Salud [5, 6] para promover una atención segura y de calidad.

2.2 Población y muestra (Participantes)

La población objetivo del estudio fueron usuarios atendidos en el servicio de consulta externa (farmacia ambulatoria) del establecimiento de primer nivel de atención del Hospital. En total, 359 participantes completaron voluntariamente el cuestionario del estudio. Para determinar el tamaño de la muestra, se consideró una población total de 5280 usuarios atendidos en el servicio de farmacia externa del hospital durante el período de estudio. El cálculo se realizó utilizando la fórmula para poblaciones finitas, con un nivel de confianza del 95% ($Z = 1,96$), un margen de error del 5% ($E = 0,05$) y una proporción esperada de respuesta positiva del 50% ($p = 0,5$), asumiendo máxima variabilidad. Inicialmente, se calculó un tamaño de muestra para población infinita de 384 participantes y, tras aplicar la corrección para población finita, se obtuvo un tamaño mínimo requerido de aproximadamente 358 participantes. Para asegurar la validez estadística y considerando posibles pérdidas o cuestionarios incompletos, se decidió incluir finalmente a 359 participantes. La selección se realizó mediante muestreo aleatorio simple a la salida del área de dispensación de medicamentos, garantizando que la participación fuera voluntaria y cumpliendo con los criterios de inclusión y exclusión previamente establecidos. Se incluyeron pacientes mayores de 18 años, con capacidad cognitiva y comunicativa suficiente para responder adecuadamente el cuestionario, que otorgaron su consentimiento informado de manera voluntaria. Como criterios de exclusión se consideraron únicamente aquellos que, cumpliendo con los criterios de inclusión, presentaban excepciones que impedían la participación: participantes menores de edad, personas con limitaciones cognitivas o

comunicativas que dificultaran la comprensión o respuesta del cuestionario, quienes no otorgaron su consentimiento, y quienes completaron el cuestionario de forma parcial o decidieron no continuar en el estudio.

La muestra estuvo conformada por 149 varones (41,5%) y 210 mujeres (58,5%). Según grupos etarios, se clasificaron en adulto joven (18–39 años): 84 participantes (23,4%), adulto intermedio (40–59 años): 148 participantes (41,2%), y adulto mayor (≥ 60 años): 127 participantes (35,4%). No se recolectó información sobre el nivel de escolaridad ni sobre la gravedad de la patología de los participantes. Esta limitación es reconocida, dado que dichas variables podrían influir en la percepción del servicio farmacéutico, por lo que se recomienda su inclusión en futuras investigaciones.

2.3. Variables del estudio

2.3.1. Buenas Prácticas de Dispensación (BPD)

Las BPD se conceptualizan como un conjunto de procedimientos técnicos y normativos orientados a garantizar la seguridad, calidad y eficacia en la entrega de medicamentos [9]. Esta variable abarca no solo la entrega física de los productos, sino también la validación de la prescripción, la adecuada preparación y selección del medicamento, la información proporcionada al paciente y el registro del proceso. Las BPD se midieron a través de cinco dimensiones: *i*) ***recepción y validación*** de la prescripción médica, que evalúa la autenticidad, legibilidad e integridad de la prescripción, así como la identificación del paciente; *ii*) ***análisis e interpretación***, que comprende la revisión clínica de la prescripción y la pertinencia terapéutica del tratamiento; *iii*) ***preparación y selección***, que implica la selección correcta de los medicamentos siguiendo normas de higiene y control de calidad, se verifica, por ejemplo, que el envase cumpla con la normativa vigente como fecha de vencimiento, lote, etiquetado; *iv*) ***registro***, que documenta la dispensación para asegurar la trazabilidad y control de inventario; y *v*) ***entrega e información***, que contempla la orientación clara al paciente sobre el uso, efectos adversos, interacciones de los medicamentos y condiciones de almacenamiento; y

2.3.2. Satisfacción del Usuario

La satisfacción del usuario (SU) constituye un indicador esencial para evaluar la calidad de los servicios de salud, reflejando la medida en que las expectativas de los pacientes se alinean con su experiencia real en la atención recibida [22]. Este constructo integra tanto la percepción subjetiva del paciente como aspectos objetivos relacionados con la calidad técnica y humana de los servicios prestados. La SU no solo permite identificar deficiencias en los procesos de atención, sino que también se asocia directamente con la adherencia al tratamiento, los resultados en salud y la continuidad en la utilización de los servicios sanitarios.

La satisfacción del usuario toma como referencia el modelo SERVQUAL, ampliamente utilizado para medir la calidad del servicio a partir de la brecha entre las expectativas y las percepciones del usuario [26]. Este modelo contempla cinco dimensiones fundamentales que capturan diferentes aspectos de la experiencia del paciente. *i*) ***Fiabilidad***, que refleja la capacidad del personal para cumplir de manera consistente y precisa con los compromisos adquiridos, transmitiendo confianza al usuario sobre la exactitud y calidad del servicio. *ii*) ***Capacidad de respuesta***, que evalúa la disposición y rapidez del personal para atender las necesidades del paciente, incluyendo la gestión de quejas o solicitudes especiales, lo que contribuye a la percepción de eficiencia. *iii*) ***Seguridad***, que abarca tanto la competencia técnica del personal como la garantía de confidencialidad y la sensación de protección física y emocional durante la atención. *iv*) la ***empatía***, que pone de relieve la capacidad del personal para brindar una

atención individualizada, mostrando sensibilidad y comprensión hacia las necesidades emocionales y físicas de los pacientes. Finalmente, *v*) ***Tangibilidad***, que evalúa las condiciones físicas del entorno asistencial, incluyendo la limpieza, el estado de las instalaciones, la disponibilidad y apariencia del equipo, así como la presentación personal del personal de salud.

2.4. Normativas y fundamentos éticos

El presente estudio se desarrolló conforme a las disposiciones éticas y legales vigentes en el Perú para la protección de la salud pública y la regulación de los productos farmacéuticos. La Ley General de Salud N.º 26842 constituye el marco normativo fundamental que garantiza el derecho a la protección de la salud, promueve el bienestar de la población y regula el cumplimiento de las disposiciones sanitarias [25]. Complementariamente, la Ley N.º 29459 establece el marco regulador para el manejo responsable de productos farmacéuticos, dispositivos médicos y productos sanitarios, definiendo principios y exigencias orientados a su adecuada gestión [9]. En el ámbito específico de la dispensación, la Resolución Ministerial N.º 013-2009/MINSA norma el uso racional de medicamentos, asegurando la calidad de los productos y la correcta ejecución de las Buenas Prácticas de Dispensación [5, 9]. Asimismo, la Resolución Ministerial N.º 539-2016/MINSA fortalece los sistemas de farmacovigilancia y tecnovigilancia, promoviendo la seguridad en el uso de medicamentos y dispositivos médicos a través de mecanismos de monitoreo, reporte y evaluación de riesgos [27]. Este conjunto normativo proporciona el fundamento legal que orienta y sustenta la implementación de prácticas seguras y eficaces en los servicios farmacéuticos.

2.5. Instrumento de Medición

Las dimensiones de las Buenas Prácticas de Dispensación (BPD) se evaluaron mediante un cuestionario de 17 ítems (Tabla 1), empleando la escala tipo Likert, en el cual 1 representó la mínima valoración y 7 la máxima. Este instrumento, diseñado y validado (confiabilidad interna, α = 0,90) por Briceño-Rodríguez [28] fue elegido por su alineación con las dimensiones establecidas por el MINSA [9], que no provee un cuestionario específico para su medición. En el presente estudio, el instrumento fue nuevamente sometido a validación. La validez de contenido fue evaluada mediante juicio de expertos y el coeficiente V de Aiken, alcanzando un valor de 0,97. La confiabilidad interna, estimada mediante el coeficiente alfa de Cronbach, fue de 0,90, lo que confirma una adecuada consistencia interna del instrumento.

La satisfacción del usuario (SU) se evaluó mediante un cuestionario de 20 ítems (Tabla 2), empleando la misma escala tipo Likert, donde 1 correspondió a la mínima valoración y 7 a la máxima. Se utilizó la versión adaptada por Briceño-Rodríguez [28], basada en las dimensiones oficiales establecidas por el MINSA [22], con modificaciones para optimizar la claridad y relevancia de los ítems. En su validación original, este instrumento reportó una alta confiabilidad (α = 0,98). En el presente trabajo, el cuestionario fue nuevamente validado, obteniéndose un coeficiente V de Aiken de 0,98 para la validez de contenido y un alfa de Cronbach de 0,98, confirmando su consistencia interna y adecuación para la población estudiada.

Tabla 1. Instrumento de medición de las buenas prácticas de dispensación con escala tipo Likert, en el cual 1 representó la mínima valoración y 7 la máxima.

ITEM	CODIGO	PREGUNTA	CALIFICACION						
			1	2	3	4	5	6	7
Recepción y Validación									
1	D1	¿El profesional farmacéutico le solicitó su receta al inicio de la atención de manera adecuada?							
2	D2	¿El profesional farmacéutico revisó minuciosamente sus datos como nombre del paciente, diagnóstico, datos del médico presentes en la receta?							
Análisis e Interpretación									
3	D3	¿Cómo fue la lectura de sus medicamentos prescritos en la receta, si lograron entender el nombre y sus abreviaturas correctamente?							
4	D4	¿La interpretación de la receta fue rápida, sin inconvenientes, sin esperar mucho tiempo?							
5	D5	¿El profesional farmacéutico conoce sobre medicamentos y no tuvo que recurrir a otros profesionales para consultar por los productos?							
6	D6	¿La información que brindaron acerca de medicamentos alternativos es clara y entendible?							
Preparación y Selección									
7	D7	¿Las condiciones en las que encuentra sus medicamentos que compro son buenas?							
8	D8	¿Cómo califica el cuidado que tienen al momento de seleccionar el medicamento correcto según la receta?							
9	D9	¿El tiempo que se tomaron para verificar los productos y evitar errores fue el necesario?							
Registro									
10	D10	¿Las anotaciones de dosificación al dorso de su receta se dieron por parte del profesional, para su mejor entendimiento?							
11	D11	¿La información adicional que el profesional anotó al dorso de su receta, la considera útil para su tratamiento?							
12	D12	¿El profesional farmacéutico realizó un registro detallado y entendible de todos los medicamentos y cantidades que usted compró?							
Entrega e Informacion									
13	D13	¿Al momento de la entrega el personal verificó cuidadosamente el producto, con la receta y la boleta de compra?							
14	D14	¿El profesional farmacéutico le brindo información sobre dosis, reacciones con oros medicamentos, o sobre alguna molestia que le produce el medicamento?							
15	D15	¿Le brindaron información entendible de alguna recomendación adicional o cuidados para mejorar el uso de sus medicamentos?							
16	D16	¿El profesional farmacéutico conoce y domina la información de los medicamentos?							
17	D17	¿El profesional farmacéutico resolvió sus dudas o preguntas en cuanto al uso de sus medicamentos?							

Tabla 2. Instrumento de medición de la satisfacción del usuario con escala tipo Likert, en el cual 1 representó la mínima valoración y 7 la máxima.

ITEM	CODIGO	PREGUNTA	CALIFICACION						
			1	2	3	4	5	6	7
Fiabilidad									
1	S1	¿Usted fue atendido (a) sin diferencia alguna en relación a otras personas?							
2	S2	¿Su atención se realizó según el horario publicado en el establecimiento de salud?							
3	S3	¿Su atención se realizó según corresponde y respetando el orden de llegada?							
4	S4	¿Cuándo usted quiso presentar alguna queja o reclamo, el establecimiento contó con mecanismos para atenderlo?							
5	S5	¿Considera útil para su tratamiento la información brindada por el personal farmacéutico?							
6	S6	¿El establecimiento farmacéutico contó con los medicamentos que recetó el médico?							
Capacidad de Respuesta									
7	S7	¿El personal cuenta con conocimiento para absolver sus consultas?							
8	S8	¿El tiempo que usted esperó para ser atendido en el establecimiento fue corto?							
9	S9	¿La atención en el área de dispensación y entrega de medicamentos fue el tiempo necesario?							
10	S10	¿El personal del establecimiento solucionó inmediatamente algún problema o dificultad que usted tuviera?							
Seguridad									
11	S11	¿El personal farmacéutico priorizó atenderlo a usted antes que atender asuntos personales?							
12	S12	¿El profesional farmacéutico que le atendió le inspiró confianza?							
13	S13	¿El profesional farmacéutico que le atendió, le brindó el tiempo suficiente para contestar sus dudas o preguntas?							
14	S14	¿Durante su atención en el establecimiento farmacéutico se respetó su privacidad?							
Empatía									
15	S15	¿El profesional farmacéutico que atiende le trato con amabilidad, respeto y paciencia?							
16	S16	¿Usted comprendió la explicación que le brindó el profesional sobre sus medicamentos y cuidados para su salud?							
17	S17	¿El personal farmacéutico muestra interés en solucionar su problema?							
Tangibilidad									
18	S18	¿Las áreas y espacios son adecuados y muy presentables?							
19	S19	¿El establecimiento farmacéutico conto con los materiales necesarios para su atención?							
20	S20	¿La sal de ventas y mesón se encontraron limpios y contaron con amplios espacios para su comodidad?							

2.6. Procedimiento

La recolección de datos se realizó de forma presencial en el servicio de farmacia externa del Hospital. Se les informó sobre los objetivos del estudio y las condiciones de participación, y se les solicitó la firma del consentimiento informado para asegurar su participación voluntaria y consciente.

Una vez otorgado el consentimiento, los pacientes recibieron instrucciones claras para completar los instrumentos de medición. Se les entregaron los cuestionarios correspondientes a las BPD y Satisfacción del Usuario impresos. Ambos cuestionarios fueron autoadministrados, brindando a cada participante el tiempo necesario para responder con tranquilidad, sin

interferencias externas y en un ambiente adecuado para la comprensión y reflexión sobre los ítems.

2.7. Procesamiento y Análisis de datos

Las respuestas de los cuestionarios fueron ingresadas y verificadas manualmente para garantizar su calidad, luego organizadas en hojas de cálculo y procesadas con herramientas de código abierto. El análisis se realizó en Python (v. 3.10) usando bibliotecas como pandas, scipy, statsmodels, scikit-learn y matplotlib/seaborn. Se aplicaron buenas prácticas de manejo de datos, incluyendo revisión de valores faltantes, detección de atípicos y verificación de consistencia.

Para caracterizar los niveles de las Buenas Prácticas de Dispensación (BPD) y la percepción de Satisfacción del Usuario (SU), se promediaron los puntajes por dimensión utilizando la escala tipo Likert descrita anteriormente. Siguiendo convenciones ampliamente reportadas en la literatura, se establecieron tres niveles de interpretación: para BPD, malo (1–2,99), regular (3–4,99) y bueno (5–7); y para SU, insatisfecho (1–2,99), indiferente (3–4,99) y satisfecho (5–7) [29, 30]. Esta categorización permite una interpretación comparativa consistente entre dimensiones y variables, facilitando un análisis coherente de la percepción del servicio farmacéutico.

Para evaluar diferencias significativas entre los ítems y dimensiones de cada variable, se aplicó la prueba no paramétrica de Friedman, adecuada para datos ordinales y dependientes, al constituir una alternativa robusta al ANOVA de medidas repetidas [31, 32]. Las comparaciones post hoc se realizaron con ajuste de Bonferroni, con el fin de controlar el error tipo I [33]. Además, se llevó a cabo un análisis de conglomerados (cluster analysis) empleando el algoritmo *k*-means, con el objetivo de identificar patrones latentes en las respuestas. El número óptimo de clústeres se determinó mediante el método del codo, que detecta el punto de inflexión en la reducción de la varianza intra-clúster [34, 35].

Asimismo, se utilizó escalamiento multidimensional (*Multidimensional Scaling*, MDS) para cada variable, permitiendo visualizar las similitudes entre ítems en un espacio de menor dimensionalidad, lo que facilitó la interpretación de las relaciones estructurales de los constructos [36, 37].

Finalmente, se aplicó un análisis de correlación canónica (CCA) para examinar las asociaciones entre las dimensiones de ambas variables, identificando combinaciones lineales que maximizan la relación entre los dos conjuntos de dimensiones. Esta técnica proporcionó una perspectiva integrada y robusta de las interdependencias entre las BPD y la SU [38].

3. RESULTADOS

Las calificaciones asignadas por los participantes a los ítems de los cuestionarios de BPD y de satisfacción del usuario se muestran en forma de histogramas en la Figura 1 y la Figura 2, respectivamente. Estas distribuciones ofrecen una visión detallada de la calidad percibida en la dispensación de medicamentos y del nivel general de satisfacción con el servicio recibido.

En ambos casos, las calificaciones muestran una notable concentración en el extremo superior de la escala Likert (valores de 6 a 7), lo que sugiere una percepción predominantemente positiva. Para la variable BPD, este patrón indica que los participantes consideraron la información proporcionada como clara y eficaz, aunque las calificaciones más bajas sugieren áreas puntuales susceptibles de mejora. De manera análoga, las respuestas en la variable "Satisfacción del Usuario" reflejan una valoración general favorable del servicio, con algunas calificaciones más bajas que podrían señalar aspectos específicos a fortalecer.

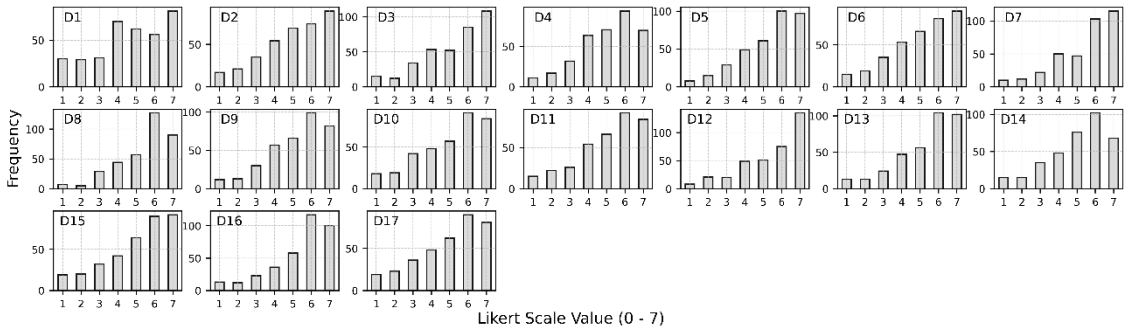


Figura 1. Distribución de las calificaciones otorgadas por los participantes a los 17 ítems correspondientes a la variable de Buenas Prácticas de *Dispensación de medicamentos*.

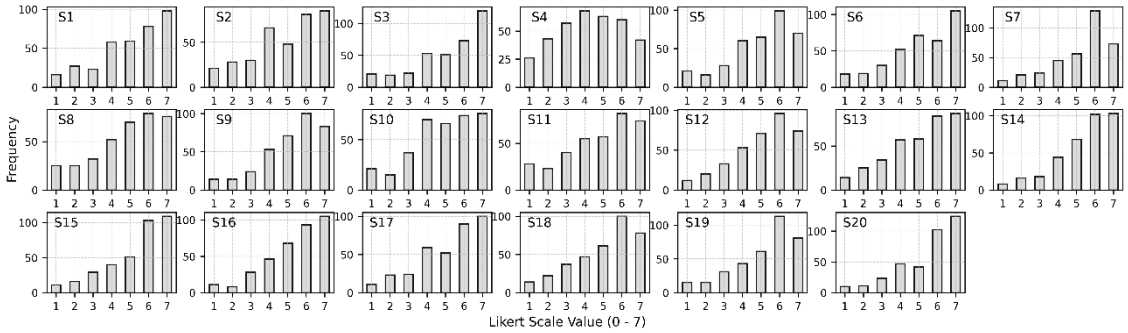


Figura 2. Distribución de las calificaciones otorgadas por los participantes a los 20 ítems correspondientes a la variable de *Satisfacción del usuario.*

Las Tablas 3 y 4 resumen la distribución de los niveles de percepción de las BPD y de la Satisfacción del Usuario por dimensiones. En el caso de las BPD, se observó un predominio consistente del nivel bueno en todas las dimensiones evaluadas, con un promedio global de 64,8%. La dimensión *Preparación y Selección* presentó la mayor proporción de valoración positiva, seguida por *Entrega e Información*, *Registro*, *Análisis e Interpretación* y *Recepción y Validación*. El nivel malo de BPD se mantuvo bajo (promedio 7,7%), mientras que un segmento de usuarios mostró percepción regular (27,5%), indicando áreas con margen de mejora.

De manera similar, la SU mostró un predominio del nivel satisfecho en todas las dimensiones, con un promedio general de 63,1%. La dimensión *Empatía* registró la mayor proporción de usuarios satisfechos, seguida de *Tangibilidad*, *Seguridad*, *Capacidad de Respuesta* y *Fiabilidad*. La insatisfacción se mantuvo baja (8,9%), mientras que un 28,1% de usuarios manifestó percepción indiferente, evidenciando un grupo con valoración intermedia de la experiencia de atención.

En conjunto, estos resultados reflejan una percepción global favorable tanto de las BPD como de la satisfacción de los usuarios, aunque la existencia de segmentos de percepción mala/insatisfecha y regular/indiferente sugiere oportunidades para fortalecer ciertos aspectos del servicio farmacéutico.

Tabla 3. Distribución de niveles de percepción de las BPD por dimensión: malo, regular y bueno (%)

Nivel de BPD	Recepción y Validación	Análisis e Interpretación	Preparación y selección	Registro	Entrega e Información	Promedio
Malo	12,8%	5,3%	4,2%	7,5%	8,6%	7,7%
Regular	31,5%	30,4%	24,5%	26,5%	24,8%	27,5%
Bueno	55,7%	64,3%	71,3%	66,0%	66,6%	64,8%

Tabla 4. Distribución de niveles de percepción de la Satisfacción del Usuario (SU) por dimensión: insatisfecho, indiferente y satisfecho (%)

Nivel de Satisfacción del Usuario	Fiabilidad	Capacidad de Respuesta	Seguridad	Empatía	Tangibilidad	Promedio
Insatisfecho	10,0%	9,7%	9,2%	7,0%	8,4%	8,9%
Indiferente	34,5%	30,1%	29,2%	22,0%	24,5%	28,1%
Satisfecho	55,4%	60,2%	61,6%	71,0%	67,1%	63,1%

3.1. Evaluación de las dimensiones de las variables BPD y SC

La prueba de Friedman mostró diferencias estadísticamente significativas entre las calificaciones otorgadas a los ítems en ambas variables: BPD ($\chi^2(16) = 163{,}50$, $p < 0{,}001$) y Satisfacción del Usuario ($\chi^2(19) = 354{,}10$, $p < 0{,}001$). Estos resultados indican que los participantes evaluaron de manera diferenciada los distintos ítems, lo que sugiere que perciben y priorizan aspectos específicos dentro de cada constructo. Esta diferencia también revela/valida que los ítems están cumpliendo el propósito de medir diferentes percepciones de los participantes. En consecuencia, la significancia de la prueba respalda la necesidad de realizar análisis post hoc para identificar las diferencias específicas entre las dimensiones dentro de cada variable.

En las comparaciones post hoc, con corrección de Bonferroni, se identificaron patrones diferenciados de valoración dentro de cada variable. En el caso de BPD, la dimensión *recepción y validación* difirió significativamente de todas las demás ($p < 0{,}001$), mientras que *preparación y selección* mostró diferencias significativas con *análisis e interpretación*, *registro* y *entrega e información* ($p < 0{,}05$) (Tabla 5). No se observaron diferencias significativas entre *análisis e interpretación*, *registro* y *entrega e información*, lo que sugiere un posible agrupamiento perceptual entre ellas.

En la variable *Satisfacción del Usuario*, la dimensión *fiabilidad* se distinguió significativamente de las demás dimensiones ($p < 0{,}05$), mientras que la dimensión *capacidad de respuesta* presentó diferencias con las dimensiones *empatía* y *tangibilidad* (Tabla 6). La dimensión *seguridad* también difirió significativamente de las dimensiones *empatía* y *tangibilidad* ($p < 0{,}05$), mientras que no se observaron diferencias entre las dimensiones *capacidad de respuesta* y *seguridad* ($p = 0{,}316$), ni entre *empatía* y *tangibilidad* ($p = 0{,}162$).

Estos hallazgos confirman los objetivos específicos 1 y 2, al evidenciar que los participantes distinguen los ítems y reconocen las dimensiones teóricas de las variables *BPD* y *Satisfacción del Usuario*, lo que respalda la validez de las medidas.

Tabla 5. Comparaciones post hoc entre dimensiones de BPD (*p*-valores, prueba de Friedman con corrección de Bonferroni).

Dimensión de BPD	Recepción y validación	Análisis e interpretación	Preparación y selección	Registro	Entrega e información
Recepción y validación	--	< 0,001	< 0,001	< 0,001	< 0,001
Análisis e interpretación		--	< 0,001	0,189	0,628
Preparación y selección			--	0,005	< 0,001
Registro				--	0,378
Entrega e información					--

Tabla 6. Comparaciones post hoc entre dimensiones de "Satisfacción del usuario" (*p*-valores, prueba de Friedman con corrección de Bonferroni).

Dimensión de SU	Fiabilidad	Capacidad de respuesta	Seguridad	Empatía	Tangibilidad
Fiabilidad	--	0,008	0,003	< 0,001	< 0,001
Capacidad de respuesta		--	0,316	< 0,001	< 0,001
Seguridad			--	< 0,001	0,003
Empatía				--	0,162
Tangibilidad					--

3.2. Análisis de clústeres de los ítems de BPD y satisfacción del usuario

El análisis de conglomerados sobre los ítems de BPD evidenció un descenso progresivo de la suma de cuadrados intra-clúster (SCIC) a medida que se incrementaba el número de grupos, lo que refleja una mejora en la cohesión interna (Figura 3a). Sin embargo, esta mejora no alcanzó un punto claro de estabilización, lo que sugiere que la estructura de los datos no presenta agrupamientos naturalmente muy definidos. La mayor reducción de la SCIC se observó al pasar de uno a dos grupos, con una disminución aproximada del 11,5 %, lo que indica un beneficio sustancial en la primera partición. Reducciones importantes de la SCIC también se observan al pasar de dos a cinco grupos. A partir de cinco grupos, las reducciones adicionales fueron menores, indicando un rendimiento decreciente en la mejora de homogeneidad interna. En función de estos resultados, una partición en cinco clústeres parece ofrecer un compromiso adecuado entre parsimonia y capacidad explicativa, coherente además con las cinco dimensiones teóricas previamente establecidas para la BPD.

El análisis de conglomerados de los ítems de satisfacción del usuario mostró un comportamiento similar: una reducción progresiva de la SCIC sin una inflexión marcada que sugiriera un número natural de clústeres. Las disminuciones más notables en la SCIC se produjeron entre uno y dos, tres y cuatro, y cinco y seis clústeres (Figura 3b). Más allá de seis grupos, las mejoras fueron marginales, lo que indica que aumentar el número de clústeres adicionales no aporta beneficios sustanciales a la homogeneidad interna. Así, una solución con cinco clústeres

se considera apropiada y congruente con la estructura dimensional propuesta para la variable satisfacción del usuario.

Este análisis confirma el objetivo específico 3, evidenciando una sólida concordancia entre la estructura dimensional propuesta por el MINSA y la agrupación empírica de los ítems, lo que fortalece la validez teórica y práctica de los instrumentos de *BPD* y *Satisfacción del Usuario*.

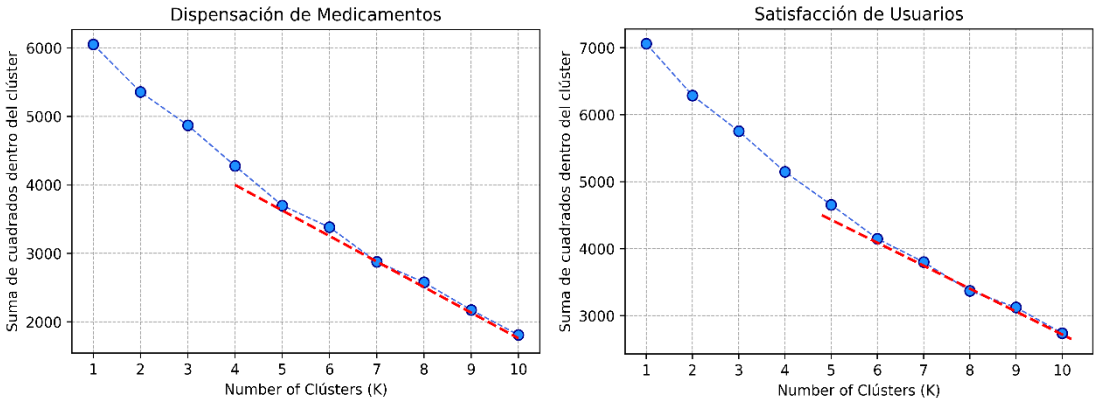


Figura 3. Análisis de agrupamiento (clústeres) de los ítems correspondientes a las variables (a) BPD y (b) Satisfacción de usuarios. Se muestra la evolución de la suma de cuadrados intra-clúster (SCIC) según el número de grupos. En ambos casos, la solución de cinco clústeres resulta consistente con la estructura dimensional teórica de ambas variables.

3.3. Análisis de escalamiento multidimensional

3.3.1. Buenas Prácticas de Dispensación de medicamentos

El análisis de Escalamiento Multidimensional (EMD) ofrece una representación espacial bidimensional de los 17 ítems de las BPD, evidenciando proximidades y oposiciones conceptuales relevantes para la interpretación de los patrones subyacentes. En la configuración espacial, los ítems de la dimensión *recepción y validación* (ítems D1 y D2, Figura 4a y Tabla 1) se ubican en el extremo izquierdo y se opone claramente a los de la dimensión *entrega de información* (ítems: D13 al D17), en el lado derecho. Esta oposición sugiere que las prácticas orientadas a verificar y validar prescripciones difieren marcadamente de las centradas en la orientación al paciente. Esta oposición también podría reflejar la tensión entre procedimientos estandarizados y normativos frente a la interacción con el usuario

Asimismo, la cercanía de los ítems de la dimensión *recepción y validación* con los de *análisis e interpretación* (ítems: D3 a D6) y *preparación y selección* (ítems: D7 a D9) indican la existencia de prácticas complementarias, posiblemente conformando un continuo que abarca desde procedimientos técnicos (*recepción y validación*) hasta acciones de verificación de la prescripción (*análisis e interpretación)* así como del medicamento (*preparación y selección*).

En cambio, la relativa oposición entre *análisis e interpretación* y *registro* sugiere enfoques diferenciados, priorizando uno la pertinencia clínica y otro el control documental y de inventario. Finalmente, los ítems de *preparación y selección* (ítems: D7 a D9), situada en posición central y ligeramente orientada hacia las dimensiones *recepción y validación* y *análisis e interpretación*, parece actuar como un puente integrador, articulando dominios divergentes y reforzando su papel mediador en las prácticas de dispensación. En conjunto, estos patrones espaciales permiten visualizar relaciones estructurales y funcionales entre dimensiones de BPD, ofreciendo información útil para optimizar la coherencia práctica y priorizar intervenciones estratégicas.

3.3.2. Satisfacción del usuario

El EMD aplicado a los 20 ítems de la variable *Satisfacción del Usuario* revela relaciones significativas y patrones interpretativos entre sus dimensiones (Figura 4b). Los ítems de la dimensión *fiabilidad* (S1–S6, excepto S4) se oponen claramente a los de *seguridad* (S11–S14), lo que sugiere un contraste entre la consistencia y exactitud en la prestación del servicio y la percepción de protección y confidencialidad durante la atención. La proximidad de *tangibilidad* (S15–S17) con *empatía* y *seguridad* indica una complementariedad entre la calidad del entorno físico, la sensibilidad del personal hacia el usuario y la sensación de confianza y protección, lo que refuerza la importancia de factores tanto emocionales como ambientales en la percepción de satisfacción. También se observa una oposición entre *capacidad de respuesta* y *tangibilidad*, junto con afinidades entre *capacidad de respuesta* y *seguridad*, lo que podría reflejar la tensión entre rapidez y eficiencia en la atención frente al cuidado del ambiente físico, y su asociación con la competencia técnica y la sensación de resguardo del paciente.

Destaca, además, la alineación direccional de *empatía* y *tangibilidad* hacia el lado derecho del EMD, con *empatía* ocupando una posición más central, lo que sugiere su papel articulador entre las dimensiones emocionales, físicas y técnicas del servicio. Notablemente, el ítem S4, asignado originalmente a *fiabilidad*, se ubica en la región correspondiente a *capacidad de respuesta*, lo que indica que podría estar captando aspectos relacionados con la disposición y prontitud del personal más que con la consistencia del servicio. Este patrón pone de relieve la complejidad y multidimensionalidad de las percepciones de satisfacción, así como la necesidad de revisar ítems potencialmente ambiguos o solapados para optimizar la validez de la medición.

En conjunto, estos resultados responden al objetivo específico 4, al identificar patrones de proximidad y divergencia entre las dimensiones teóricas de las *BPD* y la *Satisfacción del Usuario*.

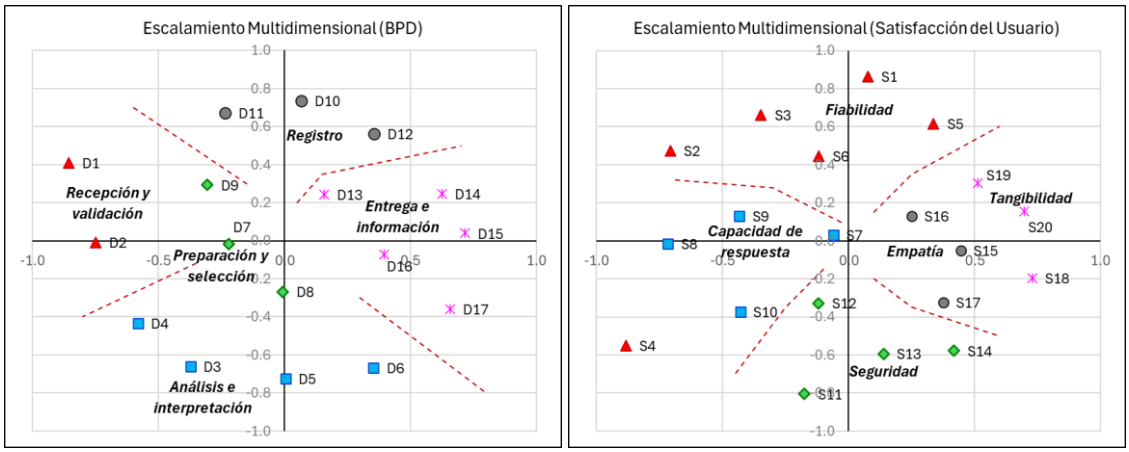


Figura 4. Escalamiento multidimensional (EMD) de los ítems de (a) BPD y (b) SU. La proximidad espacial entre ítems indica similitud perceptual, mientras que la oposición señala contraste conceptual. En (a), por ejemplo, se observa la oposición entre *Registro* y *Análisis e interpretación*. En (b) se evidencia el contraste entre *Fiabilidad* y *Seguridad*, así como la cercanía entre *Empatía*, *Tangibilidad* y *Seguridad*.

3.4. Análisis de correlación canónica

Para explorar la relación multivariada entre las dimensiones de las BPD y la satisfacción del usuario, se aplicó un Análisis de Correlación Canónica (ACC). Este procedimiento generó cinco funciones canónicas; sin embargo, solo la primera fue estadística y prácticamente significativa, con una correlación canónica elevada (r = 0,83). Este valor indica una fuerte relación

multivariada entre las combinaciones lineales de las variables de BPD y satisfacción del usuario. Las funciones canónicas subsiguientes mostraron correlaciones mucho menores (entre 0,191 y 0,012), careciendo de relevancia interpretativa.

La variable canónica del conjunto de BPD estuvo definida principalmente por las dimensiones de *entrega e información* (carga = 0,73) y *preparación y selección* (carga = 0,63) (*Figura 5*), lo que sugiere que prácticas vinculadas a la claridad en la entrega de medicamentos y la organización operativa son los aspectos más influyentes en la percepción global de calidad.

Por su parte, en la satisfacción del usuario, la variable canónica estuvo dominada por las dimensiones de *capacidad de respuesta* (carga = 0,56), *empatía* (carga = 0,56) y *tangibilidad* (carga = 0,50), dimensiones relacionadas con puntualidad, claridad de la información y calidad del trato recibido.

En conjunto, estos resultados indican que los participantes que reportaron percepciones más favorables sobre aspectos clave de la BPD de medicamentos (especialmente los representados por *preparación y selección* y *entrega e información*) también tendieron a reportar una mayor satisfacción con el servicio recibido (particularmente en *capacidad de respuesta*, *empatía* y *tangibilidad*). La fuerte correlación entre las dos variables canónicas (r = 0,83) resalta una relación significativa entre la calidad de las BPD y la satisfacción del usuario.

Estos hallazgos sugieren que mejoras en prácticas específicas de BPD—como la eficiencia, la organización y la comunicación clara—podrían impactar positivamente en la satisfacción del paciente en entornos hospitalarios.

En conjunto, estos hallazgos permiten identificar qué dimensiones de las BPD y de la satisfacción del usuario tienen mayor influencia en sus respectivas variables latentes y evidencian la estrecha relación entre ambas, cumpliendo así con el objetivo planteado de explorar esta interdependencia.

Figura 5. Primera función del Análisis de Correlación Canónica entre las dimensiones de las BPD y la Satisfacción del Usuario. Los valores sobre las flechas representan las cargas canónicas estandarizadas y la flecha central indica la correlación entre ambos constructos (r = 0,83). En BPD destacan *Entrega e información* (0,73) y *Preparación y selección* (0,63), mientras que en SU predominan *Capacidad de respuesta* (0,56), *Empatía* (0,56) y *Tangibilidad* (0,50)

3.5. Configuración de las dimensiones de las variables de BPD de medicamentos y satisfacción de pacientes

A partir de los resultados del escalamiento multidimensional y del análisis de correlación canónica, las dimensiones de las variables BPD de medicamentos y satisfacción del usuario fueron organizadas en un *continuo circular*, ilustrado en la *Figura 6*. Este patrón refleja las simili-

tudes y disimilitudes perceptuales identificadas en las respuestas de los participantes, proporcionando una representación integradora y visualmente intuitiva de las relaciones entre dimensiones.

En esta configuración, las dimensiones ubicadas adyacentes en cada disco reflejan mayor compatibilidad o afinidad perceptual, mientras que las situadas en posiciones opuestas denotan mayor contraste, tensión o conflicto conceptual. Esta representación resalta no solo la estructura interna de cada constructo, sino también las posibles tensiones o sinergias entre componentes, lo que puede orientar intervenciones más específicas.

Además, las dimensiones resaltadas en color negro dentro de los discos corresponden a aquellas que mostraron mayor contribución explicativa en sus respectivas variables, evidenciando su centralidad en la experiencia percibida. La *Figura 6* constituye así una herramienta novedosa para visualizar de forma dinámica las relaciones estructurales y funcionales entre las dimensiones, facilitando tanto la interpretación teórica como su aplicación práctica en la mejora de las BPD y la satisfacción del usuario.

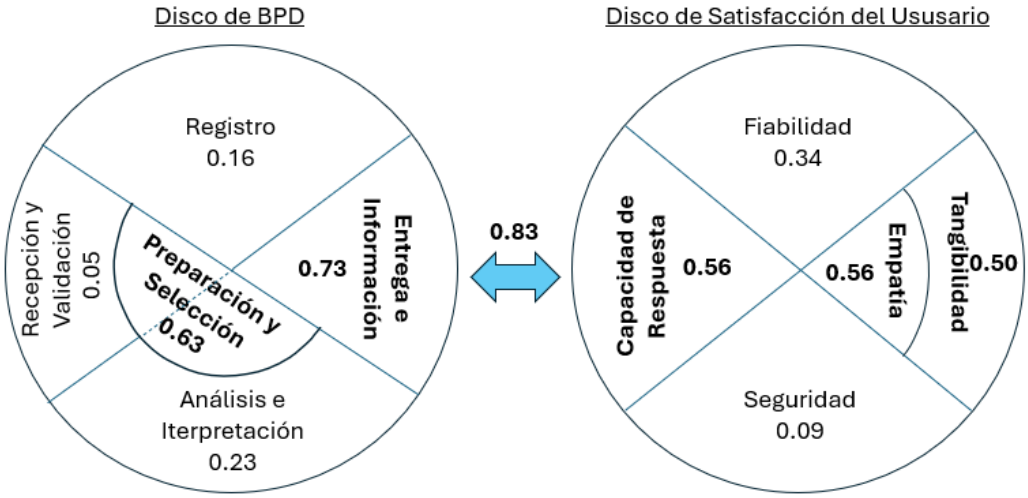


Figura 6. Configuración circular de las dimensiones de BPD (izquierda) y satisfacción del usuario (derecha), derivada del escalamiento multidimensional y la correlación canónica. Las dimensiones adyacentes indican mayor afinidad perceptual, mientras que las opuestas reflejan contraste conceptual. En negro se resaltan las dimensiones con mayor contribución canónica.

4. DISCUSIÓN

Este estudio aporta evidencia empírica relevante sobre la validez de las dimensiones propuestas para las *Buenas Prácticas de Dispensación (BPD)* y la *Satisfacción del Usuario (SU)*, así como sobre la estrecha relación entre ambas. En primer lugar, la diferenciación significativa entre ítems y dimensiones en ambas variables respalda los objetivos específicos 1 y 2, confirmando que los participantes perciben y priorizan aspectos concretos del *servicio de dispensación farmacéutica evaluado a través de sus dimensiones operativas de satisfacción, específicamente las dimensiones de fiabilidad, capacidad de respuesta, seguridad, empatía y tangibilidad*. Este hallazgo es coherente con investigaciones previas que destacan que los usuarios son capaces de identificar claramente tanto las acciones técnicas como los componentes humanos y ambientales del servicio [10, 13].

El análisis de clústeres y de escalamiento multidimensional reveló patrones de oposición y complementariedad entre dimensiones, que no solo reflejan la estructura conceptual plan-

teada por el MINSA, sino también la tensión natural entre componentes normativos y administrativos, por un lado, y aquellos más centrados en el usuario, por otro. Por ejemplo, la oposición entre las dimensiones *recepción y validación* y *entrega e información* en las BPD, o entre *fiabilidad* y *seguridad* en la SU, sugiere que ciertos aspectos de la atención pueden percibirse como prioritarios en contextos distintos. Estas observaciones coinciden con lo reportado por Martins-Cruz *et al.* (2022) [14] y Arteta-Poveda & Palacio-Salgar (2018) [17], quienes señalaron que las prácticas centradas exclusivamente en la normatividad técnica pueden entrar en tensión con aquellas orientadas a la experiencia subjetiva del usuario.

La fuerte asociación multivariada identificada mediante el análisis de correlación canónica entre las dimensiones de BPD y SU ($r = 0.83$) resalta que mejoras en prácticas técnicas —especialmente en *preparación y selección* y *entrega e información*— repercuten directamente en dimensiones clave de satisfacción, como *capacidad de respuesta*, *empatía* y *tangibilidad*. Estos hallazgos refuerzan la evidencia de estudios como los de Toribio-Alva (2022) [19], Seclén-Palacin & Darras (2005) [10] y Briceño-Rodríguez (2020) [28], quienes encontraron una relación positiva entre la calidad técnica de las BPD y la percepción global del servicio. En contraste, Delgado-Diaz & Suxe-León (2023) [18], no hallaron dicha asociación significativa, lo que podría explicarse por diferencias en contextos institucionales o en la implementación de las normativas.

Los resultados del presente estudio sobre satisfacción del usuario son consistentes con la evidencia internacional en servicios farmacéuticos hospitalarios. En estudios realizados en establecimientos de salud de España se han reportado altos niveles de satisfacción asociados al trato directo con el farmacéutico, en concordancia con la dimensión de empatía, que también resultó altamente relevante en este trabajo [39]. De forma concordante, investigaciones desarrolladas en otros contextos europeos identificaron como principales determinantes de la satisfacción del paciente la accesibilidad física del servicio, la capacidad de respuesta, las habilidades de comunicación profesional, la cortesía, la amabilidad y la efectividad de las intervenciones farmacéuticas, dimensiones que se corresponden directamente con la tangibilidad, capacidad de respuesta, empatía y fiabilidad evaluadas en este trabajo [40, 41]. En el presente estudio, estas cuatro dimensiones también se posicionaron como las más influyentes en la satisfacción global del usuario, como se evidencia en la Figura 6.

Por otro lado, la insatisfacción ha sido vinculada a deficiencias en la sala de espera, el mobiliario, los horarios restrictivos y los tiempos prolongados de atención, factores asociados a las dimensiones de tangibilidad y capacidad de respuesta, ambas identificadas como relevantes en nuestro estudio [40, 42]. Hallazgos similares han sido reportados en establecimientos hospitalarios de Ecuador, donde la insatisfacción se vinculó al mal trato personal y a la ineficiente gestión del servicio, aspectos equivalentes a las dimensiones de empatía y fiabilidad en nuestro modelo [43]. En contraste, mientras algunos estudios asociaron la satisfacción con la confidencialidad del acto farmacéutico [30], correspondiente a la dimensión de seguridad, en nuestro estudio esta dimensión resultó menos relevante, marcando una diferencia atribuible al contexto institucional y organizacional.

Las implicaciones de estos hallazgos son tanto teóricas como prácticas. Teóricamente, el estudio contribuye a consolidar el modelo dimensional propuesto por el MINSA, al demostrar que es percibido y validado empíricamente por los usuarios. Prácticamente, los resultados sugieren que intervenciones focalizadas en fortalecer las dimensiones de *entrega e información* y *preparación y selección* en la dispensación pueden tener un efecto positivo notable sobre la satisfacción del usuario, especialmente en aspectos emocionales y ambientales del servicio. La configuración circular de las dimensiones también ofrece una herramienta útil para visualizar

y gestionar las sinergias y tensiones entre componentes, facilitando la toma de decisiones orientadas a la mejora continua.

Entre las limitaciones de este estudio se encuentra su dependencia en autoinformes, que pueden estar sujetos a sesgos de deseabilidad social, y el hecho de haberse realizado en un solo hospital, lo que puede limitar la generalización de los resultados. Por tanto, futuras investigaciones podrían incorporar métodos observacionales, ampliar la muestra a distintos contextos institucionales y explorar la evolución longitudinal de estas percepciones para evaluar el impacto de intervenciones específicas.

Adicionalmente, el presente estudio no se limita a la validación métrica de los instrumentos, sino que propone una lectura estructural e integrada de las dimensiones de las BPD y la satisfacción del usuario, evidenciando patrones de alineación, complementariedad y contraposición que aún no han sido explorados en el contexto del servicio farmacéutico hospitalario. Esta aproximación permite trascender la evaluación descriptiva y aporta un marco analítico para la priorización estratégica de intervenciones en gestión farmacéutica.

En conjunto, este estudio confirma que la calidad percibida de las BPD y la satisfacción del usuario son constructos estrechamente vinculados y multidimensionales, cuya comprensión integral permite no solo validar instrumentos de medición sino también orientar estrategias efectivas para mejorar la experiencia del usuario y la calidad del servicio farmacéutico en el marco de las políticas nacionales de salud.

5. CONCLUSIONES

Este estudio tuvo como objetivo examinar la relación entre las *Buenas Prácticas de Dispensación (BPD)* y la *Satisfacción del Usuario (SU)* en un servicio farmacéutico hospitalario, evaluando la validez de las dimensiones teóricas propuestas y su interrelación perceptual.

Este estudio evidenció que los usuarios diferencian claramente los ítems y las dimensiones teóricas de las *Buenas Prácticas de Dispensación (BPD)* y la *Satisfacción del Usuario (SU)*, validando así la estructura conceptual propuesta por el MINSA. Los análisis de clústeres, escalamiento multidimensional y correlación canónica confirmaron la consistencia interna de ambas variables y la existencia de patrones de proximidad y oposición entre sus dimensiones.

Los hallazgos muestran que las dimensiones técnicas de las BPD, especialmente *entrega e información* y *preparación y selección*, están fuertemente asociadas con aspectos emocionales y ambientales de la SU, como *capacidad de respuesta*, *empatía* y *tangibilidad*. Esto sugiere que la mejora de prácticas técnicas en la dispensación puede tener un impacto positivo en la percepción global del servicio.

La representación circular de las dimensiones proporciona una herramienta integradora para visualizar las relaciones funcionales entre componentes y orientar intervenciones estratégicas. En conjunto, los resultados fortalecen la validez teórica y práctica de los instrumentos utilizados y subrayan la necesidad de abordar simultáneamente la calidad técnica y la experiencia subjetiva del usuario en la atención farmacéutica.

Futuras investigaciones podrían examinar cómo varían estas percepciones a lo largo del tiempo y en respuesta a intervenciones específicas, así como replicar el estudio en otros contextos para aumentar la generalización de los hallazgos.

En definitiva, estos resultados brindan a los gestores una base sólida para orientar decisiones estratégicas que mejoren simultáneamente la calidad técnica y la experiencia del usuario, promoviendo un servicio farmacéutico más seguro, eficiente y humanizado. Adicionalmente, la identificación de patrones de alineación y contraposición entre dimensiones aporta

un enfoque novedoso para la priorización estratégica de la gestión farmacéutica basada en evidencia.

AGRADECIMIENTOS

Agradecemos a los pacientes que participaron en la encuesta del servicio de farmacia externa del Hospital Regional "Jesús Nazareno", así como al hospital por facilitar la realización del estudio. También expresamos nuestro reconocimiento al Dr. Efrain Noa-Yarasca por sus valiosas sugerencias en la revisión del manuscrito y su orientación en el proceso de publicación. Finalmente, agradecemos a la Universidad Nacional San Cristóbal de Huamanga por el respaldo institucional brindado.

CONTRIBUCIONES DE AUTORÍA

LNY: Conceptualización; Metodología; Investigación; Análisis formal; Curación de datos; Redacción del borrador original; Revisión y edición del manuscrito. DSC: Recolección y preprocesamiento de datos. ERR, BST: Revisión crítica del manuscrito. Todos los autores discutieron los resultados y brindaron comentarios críticos sobre el artículo. LNY incorporó las contribuciones de todos los coautores. Todos los autores han leído y aprobado la versión final del manuscrito.

Uso de la inteligencia artificial generativa

Los autores declaran no haber usado herramientas de Inteligencia Artificial (IA) generativa en ninguna fase del desarrollo del estudio ni en la redacción del manuscrito. Todo el material de apoyo que aparece en el texto (figuras o tablas, si así fuera el caso) fue elaborado por los autores, sin requerir apoyo de ninguna herramienta de IA.

CONFLICTOS DE INTERÉS

Los autores declaran que no tienen ningún conflicto de interés.

REFERENCIAS

1. M. Fernández-Rodríguez, F. Martínez-Martínez, D. Iñiguez-Pineda, M. Morales-Flores, M.D. Cabezas-López & J.P. García-Corpas. Farmacias comunitarias de Ecuador y España. Aspectos legales. *Ars Pharmaceutica*, **62**(2), 163–174 (2021). https://doi.org/10.30827/ars.v62i2.16704
2. A. Cunha-Reis, G.A. Castro-Goulart, C.D. Carneiro de Almeida, A. Fonseca-Medeiros, K. Barbosa-Detoni, N. Sernizon-Guimarães & M.A. Parreiras-Martins. Quality management tools applied to drug dispensing in hospital pharmacy: A scoping review. *Research in Social and Administrative Pharmacy*, **19**(4), 582–590 (2023). https://doi.org/10.1016/j.sapharm.2022.11.008
3. Organización Panamericana de la Salud (OPS). *Estándar de Buenas Prácticas de Farmacia Hospitalaria.* Montevideo, Uruguay, 2022; 134 p. URL: https://www.paho.org/es/documentos/estandar-nacional-buenas-practicas-farmacia-hospitalaria
4. L. Schepel, K. Aronpuro, K. Kvarnström, A.-R. Holmström, L. Lehtonen, O. Lapatto-Reiniluoto, R. Laaksonen, K. Carlsson & M. Airaksinen. Strategies for improving medication safety in hospitals: Evolution of clinical pharmacy services. *Research in Social and Administrative Pharmacy*, **15**(7), 873–882 (2019). https://doi.org/10.1016/j.sapharm.2019.02.004

5. República del Perú, Ministerio de Salud (MINSA). *Manual de Buenas Prácticas de Dispensación.* Lima, Perú, 2017; 31 p. URL: https://www.gob.pe/institucion/minsa/informes-publicaciones/280843-manual-de-buenas-practicas-de-dispensacion
6. República del Perú, Ministerio de Salud (MINSA). *Resolución Ministerial N° 677 2005/MINSA: Constitución de la Red Nacional de Establecimientos de Salud que cuenten con Sistema de Dispensación de Medicamentos en Dosis Unitaria.* Lima, Perú, 2005. URL: https://bvs.minsa.gob.pe/local/MINSA/1022_DIGEMID58.PDF
7. P.J. Schneider, C.A. Pedersen & D.J. Scheckelhoff. ASHP national survey of pharmacy practice in hospital settings: Dispensing and administration—2017. *American Journal of Health-System Pharmacy*, **75**(16), 1203–1226 (2018). https://doi.org/10.2146/ajhp180151
8. Organización Mundial de la Salud (OMS). *Buenas Prácticas en Farmacia. Directrices conjuntas FIP/OMS sobre Buenas Prácticas en Farmacia: Estándares para la calidad de los servicios farmacéuticos.* La Haya, 2012; 18 p. URL: https://www.fip.org/file/1479
9. República del Perú, Ministerio de Salud (MINSA). *Resolución Ministerial N.° 013-2009-MINSA. Aprueban el Manual de Buenas Prácticas de Dispensación.* Lima, Perú, 2009; 18 p. URL: https://www.gob.pe/institucion/minsa/normas-legales/247139-013-2009-minsa
10. J. Seclén-Palacin & C. Darras. Satisfacción de usuarios de los servicios de salud: Factores sociodemográficos y de accesibilidad asociados. Perú, 2000. *Anales de la Facultad de Medicina, Universidad Nacional Mayor de San Marcos*, **66**(2), 127–141 (2005). URL: http://www.scielo.org.pe/pdf/afm/v66n2/a07v66n2.pdf
11. M. Al-Zaidan, A.M. Mohammed, M.I.M. Ibrahim, M. Al-Mahmoud, S. Al-Abdulla & M.G. Al-Kuwari. Pharmaceutical care service at primary health care centers: An insight on patient satisfaction. *International Journal of Clinical Practice*, **2022**, 6170062 (2022). https://doi.org/10.1155/2022/6170062
12. M.-C. Chen, C.-L. Hsu & L.-H. Lee. Service quality and customer satisfaction in pharmaceutical logistics: An analysis based on Kano model and Importance-Satisfaction model. *International Journal of Environmental Research and Public Health*, **16**(21), 4091 (2019). https://doi.org/10.3390/ijerph16214091
13. N. Amara, A.Y. Naser & E.O. Taybeh. Patient satisfaction with pharmaceutical services in Jordan: A cross-sectional study. *Jordan Journal of Pharmaceutical Sciences*, **16**(1), 1–10 (2023). https://doi.org/10.35516/jjps.v16i1.1030
14. M. Martins-Cruz, K.O.d.S. Cassaro-Heringer, C. Lyrio, G. Alexandre-Brasil, E. Miranda de Lima, D. Coutinho-Endringer, D. Lenz, A.M. Bartels-Rezende & T. Uggere de Andrade. Pharmaceutical services in primary health care: dissatisfaction among users, managers and pharmacists. *Brazilian Journal of Pharmaceutical Sciences*, **58**, e18849 (2022). https://doi.org/10.1590/s2175-97902020000318849
15. A. Ismail, Y.N. Gan & N. Ahmad. Factors associated with patient satisfaction towards pharmacy services among out-patients attending public health clinics: Questionnaire development and its application. *PLoS One*, **15**(11), e0241082 (2020). https://doi.org/10.1371/journal.pone.0241082
16. P.M. Ortiz-Vargas. *Nivel de satisfacción del usuario externo sobre la calidad de atención en los servicios de salud según encuesta SERVQUAL, en la micro red Villa-Chorrillos en el año 2014.* Trabajo de Grado. Universidad Ricardo Palma, Lima, Perú, 2016; 113 p. URL: https://repositorio.urp.edu.pe/server/api/core/bitstreams/b01a79e0-920b-4198-9ac1-2764ac57a299/content
17. L.E. Arteta-Poveda & K. Palacio-Salgar. Dimensiones para medición de la satisfacción del paciente en centros de urgencia. *Revista de Salud Pública*, **20**(5), 629–636 (2018). https://doi.org/10.15446/rsap.v20n5.61032
18. L. Delgado-Diaz & L.M. Suxe-León. *Satisfacción del usuario y dispensación expendio de medicamentos en los establecimientos farmacéuticos de la urbanización Pastor Sevilla- Villa El Salvador mayo junio 2022.* Trabajo de Grado. Universidad María Auxiliadora, Lima, Perú, 2023; 58 p. URL: https://repositorio.uma.edu.pe/handle/20.500.12970/1727
19. L.P. Toribio-Alva. *Buenas prácticas de dispensación y satisfacción del usuario en un establecimiento farmacéutico, Carhuaz 2022.* Tesis de Maestría. Universidad Cesar Vallejo, Chimbote, Perú, 2022; 89 p. URL: https://hdl.handle.net/20.500.12692/98354

20. D. Quispe-Huanca & N.R. Valeriano-Chávez. *Cumplimiento de las buenas prácticas de dispensación y satisfacción del usuario que acude al servicio de farmacia del Policlínico Essalud Juliaca, setiembre 2021.* Trabajo de Grado. Universidad María Auxiliadora, Lima, Perú, 2022; 62 p. URL: https://hdl.handle.net/20.500.12970/1061
21. C.N. Palomino-Yupari. *Calidad de servicio y satisfacción de los usuarios en la dispensación de medicamentos en el Área de Farmacia de consultorio externo.* Departamento de Farmacia del Hospital Regional de Ayacucho, 2022. Tesis de Maestría. Universidad Nacional de San Cristobal de Huamanga, Ayacucho, Perú, 2022; 85 p. URL: http://repositorio.unsch.edu.pe/handle/UNSCH/5720
22. República del Perú, Ministerio de Salud (MINSA). *Guía Técnica para la Evaluación de la Satisfacción del Usuario Externo en los Establecimientos y Servicios Médicos de Apoyo: R.M. N° 527-2011/MINSA.* Lima, Perú, 2012; 58 p. URL: https://www.gob.pe/institucion/minsa/informes-publicaciones/321674-guia-tecnica-para-la-evaluacion-de-la-satisfaccion-del-usuario-externo-en-los-establecimientos-y-servicios-medicos-de-apoyo-r-m-n-527-2011-minsa
23. L. Noa-Yarasca. *Metodología de la Investigación, Investigación Cuantitativa en Ciencias de la Salud*. Grupo Editorial Amarti S.A.C., Ayacucho, Perú, 2025; 59 p. URL: https://www.researchgate.net/publication/398357880_Metodologia_de_la_Investigacion_Investigacion_Cuantitativa_en_Ciencias_de_la_Salud
24. República del Perú, Ministerio de Salud (MINSA). *Resolución Ministerial N.° 546-2011-MINSA. Aprobar la NTS Nº021-MINSAIDGSP-V.03 Norma Técnica de Salud "Categorías de Establecimientos del Sector Salud" que en documento adjunto forma parte de la presente resolución.* Lima, Perú, 2011; 150 p. URL: https://www.gob.pe/institucion/minsa/normas-legales/243402-546-2011-minsa
25. República del Perú, Ministerio de Salud (MINSA). Ley N.° 26842. Ley General de Salud. Lima, Perú, 1997; 27 p. URL: https://www.gob.pe/institucion/minsa/normas-legales/256661-26842
26. A. Jonkisz, P. Karniej & D. Krasowska. The Servqual method as an assessment tool of the quality of medical services in selected Asian countries. *International Journal of Environmental Research and Public Health*, **19**(13), 7831 (2022). https://doi.org/10.3390/ijerph19137831
27. República del Perú, Ministerio de Salud (MINSA). *Resolución Ministerial N.° 539-2016-MINSA. Aprobar la NTS N° 123-MINSA/DIGEMID-V.01 Norma Técnica de Salud que regula las actividades de Farmacovigilancia y Tecnovigilancia de Productos Farmacéuticos, Dispositivos Médicos y Productos Sanitarios, que forma parte integrante de la presente Resolución Ministerial.* Lima, Perú, 2016; 17 p. URL: https://www.gob.pe/institucion/minsa/normas-legales/192066-539-2016-minsa
28. Y.J. Briceño-Rodríguez. *Dispensación de medicamentos relacionada con satisfacción de usuarios en boticas en el Centro Histórico de Trujillo.* Tesis de Maestría. Universidad Cesar Vallejo, Trujillo, Perú, 2020; 111 p. URL: https://hdl.handle.net/20.500.12692/46941
29. A. Joshi, S. Kale, S. Chandel & D. Pal. Likert scale: Explored and explained. *Current Journal of Applied Science and Technology*, **7**(4), 396–403 (2015). https://doi.org/10.9734/bjast/2015/14975
30. E. Izquierdo-García, Á. Fernández-Ferreiro, M. Campo-Angora, J. M. Ferrari-Piquero & A. Herreros de Tejada. Satisfacción percibida por los pacientes y por el personal auxiliar de farmacia del área de atención a pacientes externos como método de mejora de la calidad. *Revista de Calidad Asistencial*, **26**(3), 161–167 (2011). https://doi.org/10.1016/j.cali.2010.12.002
31. J. Liu & Y. Xu. T-Friedman test: A new statistical test for multiple comparison with an adjustable conservativeness measure. *International Journal of Computational Intelligence Systems*, **15**(1), 29 (2022). https://doi.org/10.1007/s44196-022-00083-8
32. S. Noa-Yarasca & A. López-Flores. Resistencia flexural de cementos resinosos. *Revista Científica Odontológica*, **13**(1), e228 (2025). https://doi.org/10.21142/2523-2754-1301-2025-228
33. R.W. Emerson. Bonferroni correction and Type I error. *Journal of Visual Impairment & Blindness*, **114**(1), 77–78 (2020). https://doi.org/10.1177/0145482x20901378
34. E.S. Dalmaijer, C.L. Nord & D.E. Astle. Statistical power for cluster analysis. *BMC Bioinformatics*, **23**(1), 205 (2022). https://doi.org/10.1186/s12859-022-04675-1
35. T. Ni, M. Qiao, Z. Chen, S. Zhang & H. Zhong. Utility-efficient differentially private K-means clustering based on cluster merging. *Neurocomputing*, **424**, 205–214 (2021). https://doi.org/10.1016/j.neucom.2020.10.051

36. M.C. Hout, M.H. Papesh & S.D. Goldinger. Multidimensional scaling. *WIREs Cognitive Science*, **4**(1), 93–103 (2013). https://doi.org/10.1002/wcs.1203
37. E. Noa-Yarasca, M. Babbar-Sebens, M. Chiou & K.L. Macuga. Influence of presenting uncertainty information on the evaluation of watershed plans by users of an automation-assisted environmental decision support system. *Journal of Cognitive Engineering and Decision Making*, **19**(2), 223–240 (2025). https://doi.org/10.1177/15553434251324537
38. X. Zhuang, Z. Yang & D. Cordes. A technical review of canonical correlation analysis for neuroscience applications. *Human Brain Mapping*, **41**(13), 3807–3833 (2020). https://doi.org/10.1002/hbm.25090
39. J. Urda-Romacho, J.M. Fernandez-Martin, D. Gonzalez-Vaquero, M. Torres-Rodriguez, J. Canto-Mangana & M.A. Castro-Vida. Análisis de las encuestas de satisfacción realizadas en consulta de farmacia hospitalaria. Comparación de resultados. *Alianzas y Tendencias BUAP*, **6**(22), 76–88 (2021). URL: https://www.aytbuap.mx/aytbuap-622/an%C3%A1lisis-de-las-encuestas-de-satisfacci%C3%B3n-realizadas-en-consulta-de-farmaci
40. J.F. Márquez-Peiró & C. Pérez-Peiró. Evaluación de la satisfacción y de la insatisfacción de pacientes atendidos en la unidad de atención farmacéutica a pacientes externos. *Farmacia Hospitalaria*, **32**(2), 71–76 (2008). https://doi.org/10.1016/s1130-6343(08)72817-4
41. R. Antón-Torres, A. Murcia-López, J. Borrás-Blasco, J.F. Navarro-Gracia, A. Navarro-Ruiz & M. González-Delgado. Evaluación de la calidad percibida por los usuarios de una unidad de atención farmacéutica a pacientes externos. *Farmacia Hospitalaria*, **30**(2), 99–104 (2006). https://doi.org/10.1016/s1130-6343(06)73954-x
42. R. Díaz. Satisfacción del paciente: principal motor y centro de los servicios sanitarios. *Revista de Calidad Asistencial*, **17**(1), 22–29 (2002). https://doi.org/10.1016/s1134-282x(02)77470-8
43. M.H. Goya-Salinas & A.M. Lam-Vivanco. Evaluación de la Implementación de un Modelo de Gestión de Calidad en el Servicio de Farmacia Hospitalaria: Estudio de Caso en el Hospital Ángela Loayza de Ollague, Abril 2025. *Vitalia: Revista Científica y Académica*, **6**(2), 976–997 (2025). https://doi.org/10.61368/r.s.d.h.v6i2.657

CÓMO CITAR ESTE ARTÍCULO

L. Noa-Yarasca, D. Sierra-Córdova, B.S. Trujillo & E.G. Ramírez-Roca. Buenas Prácticas de Dispensación y satisfacción del usuario: Evidencia multidimensional en servicios farmacéuticos hospitalarios. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 215–237 (2026). https://doi.org/10.15446/rcciquifa.v55n1.122606

Review article

Recent microfluidic applications in Pharmaceutical Sciences and its potential utility in bottom-up concepts of Quality by Design

Edward Rodríguez[a], Helber Barbosa[b] & Bibiana Vallejo[c]

Departamento de Farmacia, Universidad Nacional de Colombia, sede Bogotá, Cra. 30 No. 45-03 –Edif. 150, Bogotá D. C., Colombia

E-mail addresses: [a] edarodriguezve@unal.edu.co; [b] hdbarbosab@unal.edu.co; [c] bmvallejod@unal.edu.co

ORCID IDs: [a] https://orcid.org/0009-0006-7250-5798; [b] https://orcid.org/0000-0003-0061-5358; [c] https://orcid.org/0000-0001-9390-3855

Received: September 24, 2025
Corrected: December 28, 2025
Accepted: December 30, 2025

https://doi.org/10.15446/rcciquifa.v55n1.122915

SUMMARY

Introduction: Microfluidic science has significantly permeated biomedical sector since the 1990s, today they have potential application in quality-based design of pharmaceutical products using bottom-up approaches. **Methodology:** In this article, a review of some articles published from 2019 to 2025 is made to show the utility that microfluidics can have in each stage of medicines development. To demonstrate the capabilities of micro systems some examples are summarized, highlighting the qualities that may be attractive from the Quality by Design (QbD) methodology point of view; In order to segment the information, the topics are classified into four groups: medical diagnosis, production of drug delivery systems, drug discovery and organ-on-a-chip technology. **Results:** Since microfluidics make it possible to produce different types of nanostructured formulations and control their final properties, when increasing the conceptual basis on the mechanisms of diseases and evaluate the effect of drugs when exposed to human cells, it is possible to spread its potential in an integral way to all development phases of pharmaceutical products.

Keywords: Microfluidics; medical diagnostics; drug delivery system; drug discovery; Organ-on-a-chip; Quality by Design; bottom-up approach.

RESUMEN

Aplicaciones recientes de microfluidos en las ciencias farmacéuticas y su utilidad potencial en conceptos "abajo-hacia-arriba" asociados a la Calidad Desde el Diseño

Introducción: La ciencia de microfluidos ha permeado de forma importante al sector biomédico desde 1990, hoy en día tienen aplicación potencial en el diseño de productos farmacéuticos basado en la calidad, mediante el enfoque de abajo hacia arriba. **Metodología:** En este artículo se hace una revisión de algunos artículos publicados desde 2019 hasta 2025 con la finalidad de mostrar la utilidad que pueden tener los microfluidos en cada una de las etapas del desarrollo de medicamentos. Para demostrar las capacidades que tienen las microplataformas se resumen algunos ejemplos destacando las cualidades que pueden ser atractivas desde el punto de vista de la metodología de Calidad desde el Diseño (QbD). Con la finalidad

de segmentar la información, se clasifican los temas dentro de cuatro grupos: diagnósticos médicos, producción de sistemas de liberación de fármacos, descubrimiento de fármacos y tecnología de órgano-en-chip. **Resultados:** Dado que los microfluidos permiten producir diferentes tipos de formulaciones nanoestructuradas y controlar sus propiedades finales, al incrementar las bases conceptuales sobre los mecanismos de las enfermedades y evaluar el efecto de los medicamentos al exponerlos a células humanas, es posible extender su uso de forma integral a todas las etapas del diseño de productos farmacéuticos.

Palabras clave: Microfluidos; diagnósticos médicos; sistemas de liberación de fármacos; descubrimiento de fármacos; Órganos-en-Chips; Calidad desde el Diseño; aproximación "abajo-hacia-arriba".

RESUMO

Aplicações recentes da microfluídica nas Ciências Farmacêuticas e sua potencial utilidade em conceitos bottom-up de Qualidade por Design

Introdução: A ciência da microfluídica permeou significativamente o setor biomédico desde a década de 1990 e, atualmente, apresenta potencial aplicação no design baseado na qualidade de produtos farmacêuticos, utilizando abordagens bottom-up. **Metodologia:** Este artigo apresenta uma revisão de artigos publicados entre 2019 e 2025 para demonstrar a utilidade da microfluídica em cada etapa do desenvolvimento de medicamentos. Para ilustrar as capacidades dos microssistemas, alguns exemplos são resumidos, destacando as qualidades que podem ser atrativas sob a perspectiva da metodologia de Qualidade por Design (QbD). Para segmentar as informações, os tópicos são classificados em quatro grupos: diagnóstico médico, produção de sistemas de liberação de fármacos, descoberta de fármacos e tecnologia de órgãos em chip. **Resultados:** Como a microfluídica possibilita a produção de diferentes tipos de formulações nanoestruturadas e o controle de suas propriedades finais, ao ampliar a base conceitual sobre os mecanismos das doenças e avaliar o efeito de fármacos quando expostos a células humanas, é possível estender seu potencial de forma integral a todas as fases de desenvolvimento de produtos farmacêuticos.

Palavras-chave: Microfluídica; diagnóstico médico; sistema de liberação de fármacos; descoberta de fármacos; órgão-em-um-chip; Qualidade por Design; abordagem bottom-up.

1. INTRODUCTION

Microfluidics are defined as the discipline responsible for studying fluid behaviors when they circulate in confined spaces at microscopic scales; this branch of the fluid mechanics had origin in the 1980s thanks to the advances in miniaturization for micro electro-mechanical systems (MEMS) which spread to another areas of knowledge (chemistry, biology, medicine) since 1990s [1]. In the last decades this field has permeated toward different topics in pharmaceutical sciences which, in the current article, are classified into four general groups: Medical diagnosis, production of micro/nano platforms, drug discovery, and organ-on-a-chip (OoC) technology.

Since microfluidic science approaches are given at submillimeter scale, it could be a powerful tool for products design by means of bottom-up theories. Those concepts imply a thorough understanding of the micro and nanoscale events influence on the product desired property; it is believed that a systematic integration of multiple disciplines to model complex phenomena will lead to translating the molecular processes into phenomenological equations to create and control functionalities through the manufacturing process. This group of concepts is framed in what is described in the literature as *le Genie du triple "processus–produits–procédé"* (the triplet molecular processes–product–process engineering) [2]

Some recent proposals in scientific literature mention the importance of applying emergent concepts into general and accepted methodologies for drug design and development that is the case of QbD which has been accepted by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) since 2009.

It is mentioned in the article written by Colombo *et al.* [3] the concern about high failure rates in nanopharmaceutical research and development due to the incipient bottom-up strategies for products manufacture which lead to an inappropriate control of quality attributes. In that order of ideas whether one study microscopic phenomena throughout micrometric technology (microfluidics, particularly), it is possible to improve designs, process control, and parameter predictability. In his words there are "emerging opportunities in the synergistic implementation of QbD strategies and microfluidic production in contemporary development and manufacturing of nanopharmaceuticals".

The foundations of *Quality by Design* (QbD), based on knowledge and risk management, support the development of systems that incorporate microfluidic studies. The creation of functional microstructures—grounded in the understanding of material properties at the microscale, the identification of critical quality attributes (CQAs), and the definition of manufacturing processes based on critical process parameters (CPPs) in the microscale represent a challenge of establishing a robust design space. This approach enables the development of functional products through a bottom-up design strategy, where desired properties emerge from the structure and behavior at the microscale.

As a consequence of the microfluidics unique features such as short analysis time, low reagents consumption, accelerated mass and heat transfer, parametric control, profitability, possibility of integrating complementary technology *in situ*, ability to incorporate advanced cell cultures, and so on, it has demonstrated potential to improve lifecycle in products development, medical diagnosis efficiency, safety and toxicity characterization, drug-cells and cell-cell interaction comprehension, disease mechanistic understanding and human response predictability. All these qualities make it a powerful tool that could contribute significantly to medicines development with the aid of QbD because the aforementioned methodology is based on concepts that seek to integrate knowledge about safety, efficacy, bioavailability, stability, process engineering, risk assessments, and control strategies with the purpose of materializing high-quality products supported on solid basis.

Based on the need to publicize the importance of microfluidics in the pharmaceutical world, in this article are discussed some microfluidic applications in pharmaceutical sciences shown in papers from 2019 to 2025 with the purpose of evidencing its potential as a support tool for the design of biologically active products into the QbD and bottom-up frames.

2. MICROFLUIDICS IN MEDICAL DIAGNOSIS

Microfluidics technology can be applied for the early diagnosis of different diseases and hard threatening conditions, for example, malignant tumors or nosocomial infections. Microfluidic devices have sparked interest in the scientific community due to their advantages over laboratory conventional methodologies for samples analysis. Miniaturized systems provide high sensibility, short analysis times, are less prone to human errors, and they could be economically feasible since the amount of reagents and samples are lower [4].

For diseases diagnostics, researchers have explored the possibility of developing high sensibility and accuracy biosensors with the purpose of perform biochemical analysis with the same reliability of a laboratory assay [5]. A series of sensors have been elaborated with the aid of microfluidics technologies, which allow their application in a wide variety of diagnostic

assays [6, 7]; among the physical phenomena, optic, electronics, microwaves and radiofrequencies are the most used in sensors [5]. The following discusses several scientific articles aimed at demonstrating the potential and possibilities that microfluidics offers in disease diagnosis:

Lu *et al.* [8] were able to craft a microfluidic device with the potential to early diagnose cholangiocarcinoma. According to the authors, one of the drawbacks related to the diagnosis of this disease is the variety of biological material that can lead to wrong results or even false positives. In those cases, microfluidic platforms can increase interactions between molecules and the intrinsic processes in biochemical analysis could be automated. The microfluidic chip designed by Lu *et al.* consisted in three layers of polydimethylsiloxane (PDMS) and one layer of polycarbonate (PC) coated with PDMS, linked together by a plasma oxygen treatment. The design of the device was conceived to carry out extraction, isolation, and cancer cells staining; to do so, it was necessary to integrate micro elements such as micropumps, micromixers, reservoirs, microvalves, and microchambers with reagents. In general, the working principle of the microdevice falls into four steps: (1) bile fluid incubation, (2) isolation of tumor cells by magnetic pearls coated with affinity reagents, (3) washing and removal of unbounded cells, (4) Addition of antibodies and a special reagent for the cell staining. The microfluidic chip developed by Lu *et al.* is shown in Figure 1:

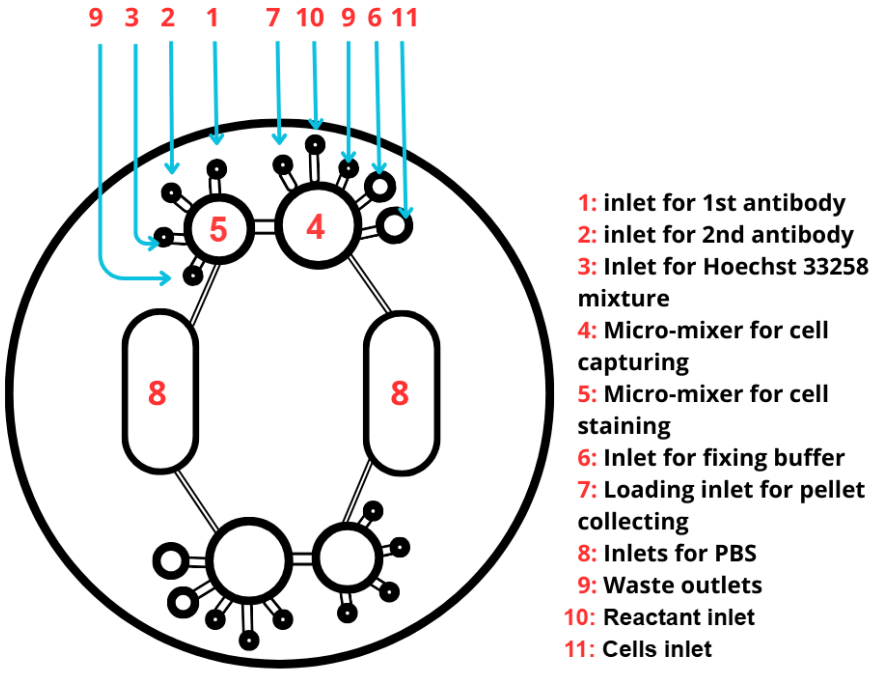


Figure 1. Graphical representation of the microchip for cholangiocarcinoma diagnostic. Adapted from Ref. [8].

In addition, Maji *et al.* [9] demonstrated that a microfluidic device has the potential to be used in platelet loss diagnostic during a traumatic hemorrhage. This research is relevant because a fast platelet count and their effect in the hemostatic process is valuable if it is necessary to implement strategies to mitigate trauma-induced coagulopathies. Despite there are laboratory assays to monitor patient's health, access to appropriate instrumentation is not always possible and thus it could not be carried out the corresponding analysis. In that order of ideas, it would be useful to have a miniaturized system which integrates analytical techniques to track patients' health rapidly. The microfluidic chip (shown in figure 2) developed by Maji *et al.* is

based on qualitative measurements by dielectric spectroscopy; this technique allows the quantification of a material relative dielectric permittivity, a useful property to detect cellular and non-cellular abnormalities in hemostatic processes. Results obtained by Maji *et al.* show that analytical determinations by dielectric spectroscopy have potential to provide information about hemostatic imbalances. In general, the analysis of blood composition is of interest for various biomedical applications and disease diagnostics; for this reason, Ma *et al.* [10] developed a microdevice capable of separating cellular components from blood plasma using acoustic radiation.

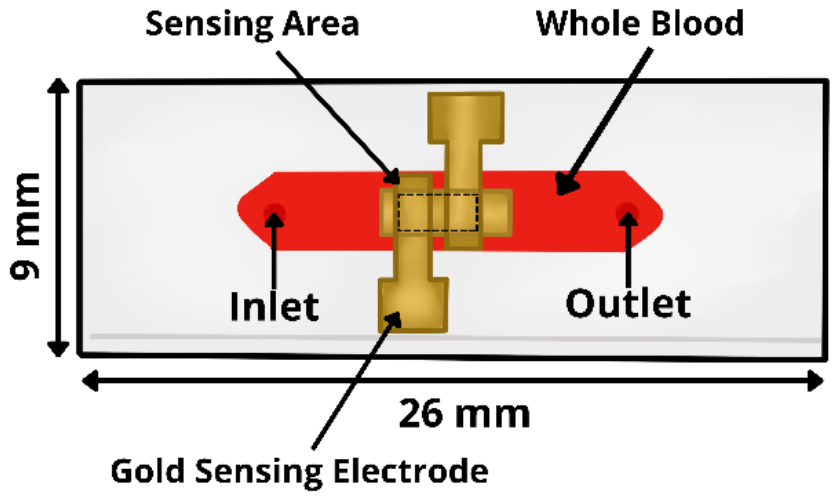


Figure 2. Prototype of microfluidic device for monitoring platelet loss during traumatic hemorrhages.

The study conducted by Fang *et al.* [11] demonstrated the advantages of microfluidic systems in fast physiopathology diagnostics. In that research a microfluidic based device was developed to detect sepsis, a potentially mortal disease derived from the immune response towards bacterial, fungal, or viral infections. This condition requires a rapid and accurate diagnostic with the purpose of provide drugs in a timely manner. According to Fang *et al.*, a conventional sepsis diagnostic needs a blood culture which takes 48 to 72 hours, followed by a bacterial identification assay. To solve this problem, researchers proposed the integration of microfluidic elements which allow separation of microorganisms from blood and a specific bacterial isolation. To achieve the desired diagnostic, a two-chip system was developed; the first is responsible of sample pretreatment, inside their microchannels is found a micropillar structure which deviate particles larger than 3 - 4 μm; the second chip is constituted of special microchambers loaded with magnetic particles coated by mannose-binding lectin. The microparticles and the bacterial isolated sample are transferred to a micromixer, later the bacteria – pearl complex is recollected by a magnet. Experiments developed in the study suggest that microsystems have potential to diagnose sepsis in a few hours.

Microfluidics have been also applied in research projects derived from the COVID-19 pandemic. Kim *et al.* [12] developed an innovative method to detect SARS-CoV-2 by using a mesh-based system which contain microfluidic pores. The working principle of that device can be summarized in the following manner: There are two chambers connected by a microchannel. In one of them is loaded the sample; the second is sealed by a rubber stopper. Due to pressure effects, the sample will not be transferred to the second chamber. Once the stopper is perforated, there are two possibilities: (1) the sample flow towards the empty chamber or (2) the sample will keep static. Since there is a conjugated mesh which can form a DNA hydrogel, the

sample behavior depends on the presence of the pathogen. If the pathogen is present in the sample, the hydrogel will clog the membrane pores and subsequently, the liquid stays static in the first chamber. Otherwise, the liquid will be transferred to the second reservoir. When operating conditions are optimized, it is possible to reach a detection limit of 3 aM with an analysis time of 15 minutes or a detection limit of 30 aM with an analysis time of 5 minutes. A more recent study related to the detection of this disease is presented by Nguyen *et al.* [13]. In this article, a microfluidic platform was developed that integrates electronic elements and a series of microreactors, allowing remote manipulation of fluid control, temperature control, fluorescence detection, and data analysis. This device is essentially a micro-laboratory aimed at maximizing the automation of procedures required for disease detection based on the reverse transcriptase-loop-mediated amplification (RT-LAMP) assay.

Other researchers like Zhu *et al.* [14] have worked in novel technology development which can be applied in microsystems with the aim of diagnose potential mortal diseases for healthcare workers. Their proposal is a "lightweight and flexible multiplexing harmonic transponder sensor, which allows for rapid, *in situ* detection of at least two types of liquid samples" [14]. In summary, the devised microdevice consists of a transceiver which transmits a constant intensity frequency to a multiplexed harmonic sensor. The signal is received by an antenna, and the intensity is modulated through the sample analyzed within the microchannels. Received signals can be retransmitted to a specific receptor such a cellphone. Radiofrequency-based technology has the capacity of monitoring liquid properties rapidly, without direct contact, and in noisy environments.

Microfluidics have also paved the way to new methodologies in cancer research. According to Silva *et al.* [15] "microfluidic devices have emerged as promising tools for research and applications in oncology, because the manipulation of small volumes of liquid is ideal for tumor cell sorting from biofluids or liquid cultures, and the development of 2D and 3D cell culture models for cancer cell, allows the migration/metastasis studies and drug screening, as well as the design of sophisticated drug delivery systems". In microfluidic cancer diagnostic field, circulant tumor cell analysis is a popular and well accepted technique because it have demonstrated to be useful in understanding, diagnose and treat different types of cancer [15, 16].

To identify cancer cells, it is required to use cell manipulation techniques which are based on microfluidic devices; among those techniques it is found the cell isolation. Tavakoli *et al.* [16] state that "single cell isolation is crucial to single-cell analysis in order to better understand the variations from cell to cell, which can provide valuable information for diagnostics and other biomedical applications". In that order of ideas, microfluidic technologies have a relevant role in producing platforms which could manipulate cells precisely by entrapment, transport, and cell delivery. In scientific literature, studies have been reported about a few entrapment methodologies such as mechanical traps and microfluidic droplets. Below are discussed the methodologies and some related examples.

Mechanical traps are based on the use of microstructures such as microwells, microreservoirs, microvalves, among others. In a microsystem intended for the diagnosis of cancer, combinations of these microstructures and variations in geometry, size, and configuration can be found, resulting in a wide variety of studies that show different yields in the cell isolation process. To mention a few examples, we have the study by Tu *et al.* [17] in which the capture of cells is carried out in a microsystem that contains triangular microwells; Rho *et al.* [18] designed a V-type valve to improve the handling of particles of different sizes in microdevices, proving to have potential application in single cell analysis; Armbrecht *et al.* [19] developed a microfluidic chip capable of capturing, monitoring, and analyzing single cells. In this study,

hydraulic traps were used that allow static cells to be kept in a specific position within the microdevice, thus facilitating automated cell analysis.

Droplet-based cell capture technologies consist in the formation of compartments (represented by droplets) that can isolate cells individually for subsequent storage, identification, and transport [16]. Isolation using microdroplet technology has been commonly performed by applying T-type microstructures where the dispersion of two phases immiscible with each other is made; however, the need to form droplets of specific sizes and characteristics has implied the development of microplatforms that use external actuation techniques, including electrical, magnetic, optical, thermal controls, among others [16].

Isolation of cancer cells in microsystems can also be carried out by applying knowledge in antibody-antigen interactions and targeting epithelial cell adhesion molecules because this surface antigen is overexposed in almost all types of cancer [15]. In this sense, the investigations that use this theoretical foundation of antibody-antigen interactions focus on the functionalization of micro-devices with different types of antibodies that bind to the specific antigens of the cells of interest. Some examples in the scientific literature include the functionalization of microsystems for the detection of lung cancer [20], pancreatic cancer [21], colon cancer [22], breast cancer [23], ovarian cancer [24], and gastric cancer [25].

The application of microfluidic science in cancer diagnosis has gained increasing interest in recent years; this can be demonstrated by searching in Scopus database the keywords “Microfluidics” and “Cancer diagnostic” (see Figure 3).

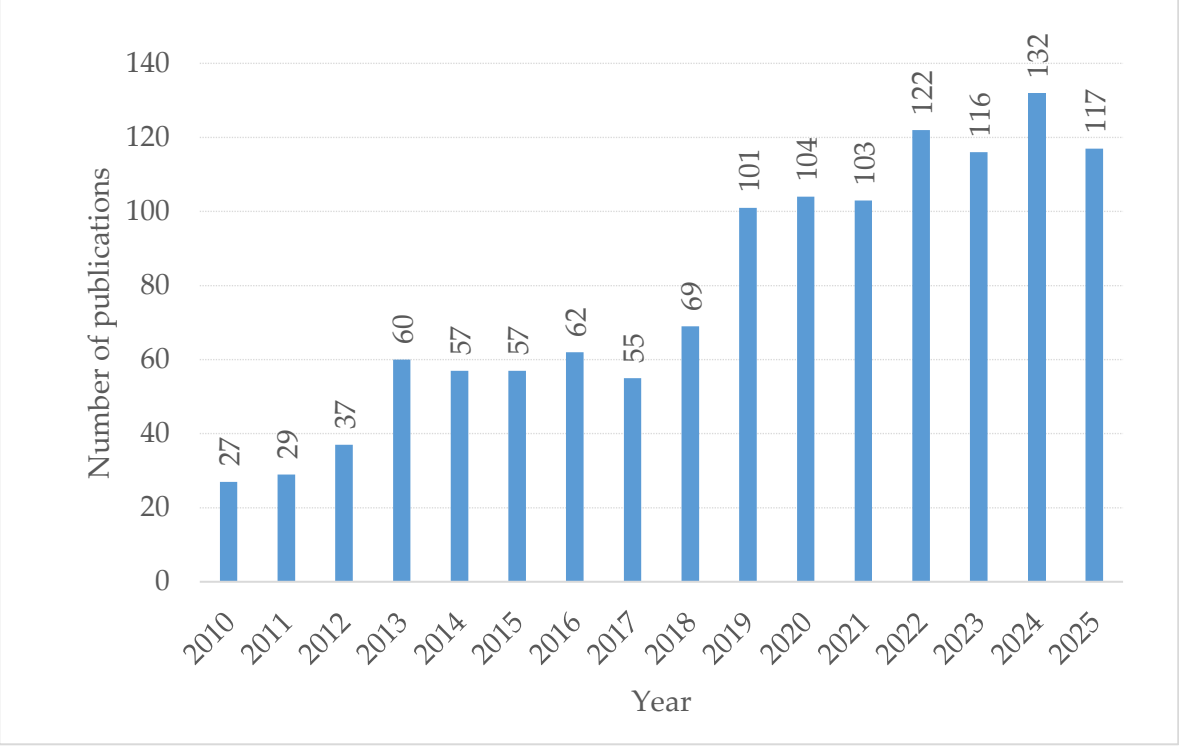


Figure 3. Search results in Scopus database, using the keywords "Microfluidics" and "Cancer diagnostic". The data was obtained in September 2025.

Research related to cancer diagnosis is based on the study of biomarkers such as circulating cells, cell-free DNA and extracellular vesicles; Additionally, there is a wide variety of methodologies based on microfluidics that allow the isolation of these biomarkers, including immunoaffinity, filtration, acoustofluids, viscoelastic flow, and electrokinetics [26]. Some of these methodologies are discussed in depth in review articles published along the last years, such is

the case of the document published by Yang *et al.* [27]. This paper also discusses other methods for diseases detection caused by viruses and bacteria through the use of microsystems made of materials such as PDMS (polydimethyl siloxane), paper or other polymeric materials, and incorporating technological elements such as sensors that can be connected to smartphones to carry out diagnoses with a certain level of speed.

The potential of microfluidics in the field of medical diagnostics lies in its ability to provide deeper insights into the underlying mechanisms of various diseases. This understanding can be leveraged to design more effective therapeutic strategies. As a result, microfluidics supports the development of better-informed pharmaceuticals and enables the identification of critical quality attributes that must be addressed to meet patient expectations and regulatory standards.

3. MICROFLUIDICS IN CONTROLLED RELEASE SYSTEMS

In recent scientific literature, a broad variety of studies can be found about microfluidic chips with different structures such as microchannels, microvalves, micromixers and other microscopic elements that allow the production of micro and nanoplatforms (for pharmaceutical and cosmetic applications) with well-defined physicochemical properties. In the following paragraphs, some newest applications of microfluidics to obtain pharmaceutical and cosmetic formulations will be discussed, as well as some relevant conceptual aspects in each case.

One advantage of microfluidic technology compared to conventional mass production methods is the ability to continuously produce formulations with precisely controlled morphological characteristics. However, it should be considered that obtaining nanoparticles in reduced spaces can cause clogging and failure of the microelements incorporated into the system. For that reason, Bolze *et al.* [28] mention that the use of active mixers (that is, those that involve the addition of power from external sources) in microsystems can reduce the clogging probability.

Among the variety of active mixers (rotors, magnetic or electric fields, acoustic waves, etc.), the authors of the article selected ultrasound as the external energy source to enhance the continuous production of trimyristin lipid nanoparticles by the nanoprecipitation method. In this investigation, experiments were carried out to verify the ultrasound effect in nanoparticles production and it was found that when mixing the fluids (solvent, antisolvent and an auxiliary current whose purpose is to improve mixing efficiency) without ultrasound, a supersaturated solution remains in contact with the internal walls of the microchip, which favors precipitation and as a consequence of this, obstructions occur within the microchannels, thus generating leaks in the seals before having completed 5 minutes of operation. When the system is subjected to ultrasonication, it is possible to carry out the production of lipid particles from 4 to 7 hours until the appearance of leaks in the system seals. Bolze *et al.* [28] suggest that ultrasonication has a cleaning action thanks to a combined effect of cavitation and inertial effects that are caused by rapid movements within the microchip. Finally, it is worth highlighting the fact that in this article the effect of the frequency of acoustic waves on particle size and polydispersity was not thoroughly evaluated, therefore, it is expected a study that incorporates these conceptual aspects. A schematic of the microfluidic chip used by Bolze *et al.* [28] is shown in Figure 4:

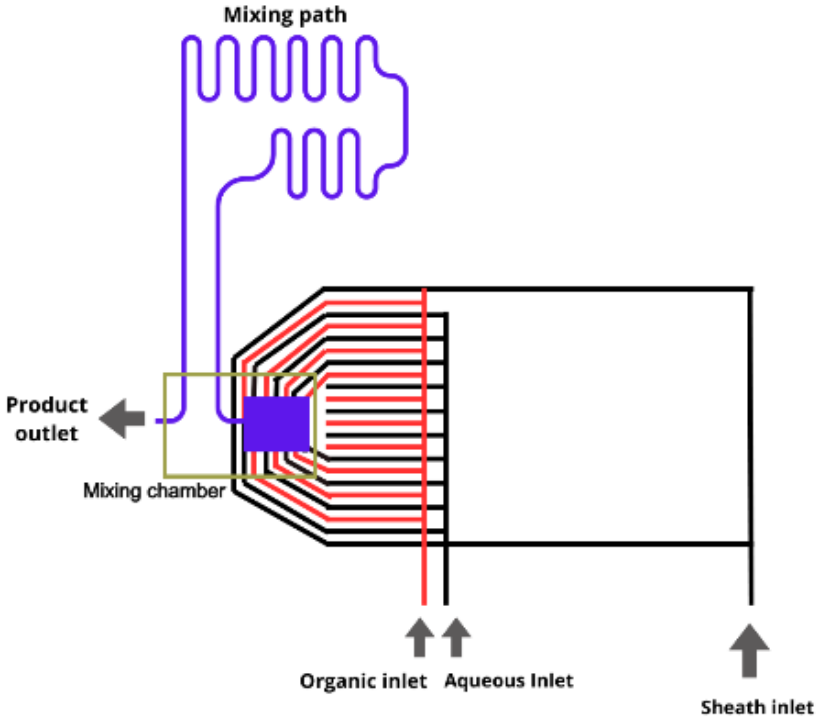


Figure 4. Schematic of the chip configuration used by Bolze *et al.* [28].

Other research that uses microfluidic science for the continuous production of pharmaceutical formulations is made by Patil *et al.* [29]. In this study, luliconazole-loaded microemulsions were obtained and the process parameters that significantly influence droplet size and polydispersity were identified, which in turn determine reproducibility, stability, and effectiveness in *ex vivo* permeation assays. The experiments carried out by the authors allowed us to compare the droplet sizes obtained when processing by a batch methodology and by microfluidics (a schematic of the chip structure developed by the authors is shown in Figure 5). Unlike the article by Bolze *et al.* [28], in this case passive micromixers are used, that is, geometries and microelements that do not require an external energy source to favor the fluids mixing process. Nonetheless, the two studies agree that the precise control of the internal mixing of the raw materials is decisive in the quality characteristics of the product and that, for this reason, traditional batch production methods fail in terms of reproducibility, stability, and consistency in the half-life of the formulation. For this case study, the authors found that, for the operation on the microchip, the factors that most influence the quality of the microemulsion are the concentration of the oily phase, concentration of surfactant and substances flows; it was possible to study the effect of these factors on the droplet size through an experimental design. The results of the investigation showed that the batch process produces formulations with inconsistent oil phase drop sizes, while using the microdevice achieves a uniform dispersion of the oil phase due to the efficiency of the mixing process; a well mixing process in turn ensures that there is an adequate amount of surfactant on the surface of the droplets, thus preventing coalescence. Furthermore, the droplet sizes that are produced by microfluidic technology imply a greater permeation area of luliconazole through the skin. Some final comments made by Patil *et al.* [29] highlight the potential of microfluidics as a technology that offers feasibility for scaling microemulsion production processes and for accelerating screening of excipients in order to expedite the formulation of micro/nanoplatforms.

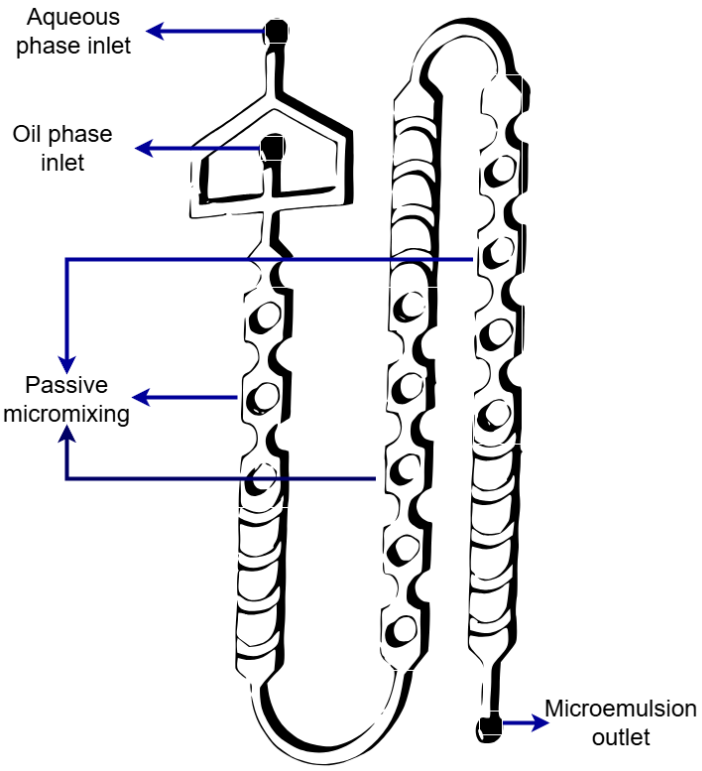


Figure 5. Schematic of the microchip used by Patil *et al.* [29].

Authors such as Mares *et al.* [30] confirm the usefulness of microfluidic science to control morphological aspects of pharmaceutical formulations. In this work, poly(lactic-co-glycolic) acid (PLGA) and polyethylene glycol (PEG)-based nanoparticles are produced using the manual mixing method and nanoprecipitation on a microchip method (the microdevice schematic is shown in Figure 6). First of all, it is worth mentioning that amphiphilic block copolymers such as PLGA have good biocompatibility and biodegradability, which makes them safe candidates for the formulation of systems aimed at treating conditions in humans [31]. PEG, for its part, works as a stabilizer and decreases the formation of the crown protein after the administration of the pharmaceutical form [32]. Conceptually, it should be considered that the formation of nanoparticles involves 3 stages which are nucleation, growth, and aggregation; if the mixing processes are inefficient, the nucleation process will be slow, and larger particles will be generated. On the other hand, if the mixing is too fast, the aggregation occurs in such a way that many nuclei will be formed. The microchip used in this study considers three input currents (two lateral and one central) whose arrangements and flows allow controlling the process of diffusion and formation of the nanoparticles. As results of this investigation, it was obtained that the particle sizes are dependent on the mixing of the solvent and antisolvent phase; the higher the antisolvent flow, the smaller the particle size. It is estimated that the particle sizes that can be produced by the nanoprecipitation method in the microdevice range from 44 nm to 97 nm depending on the solvent-antisolvent flow ratio and the size distribution obtained is narrow and unimodal. Moreover, with the manual mixing method, sizes ranging from 71 nm to 89 nm can be obtained depending on the proportions of solvent–antisolvent and it is expected sparse size distributions which tend to be bimodal. The intellectual product of this research allows us to affirm that by applying a nanoparticle production methodology based on microfluidic science, the final size of the particles can be controlled without having to alter the characteristics of the polymer, and consequently, the properties of the final product.

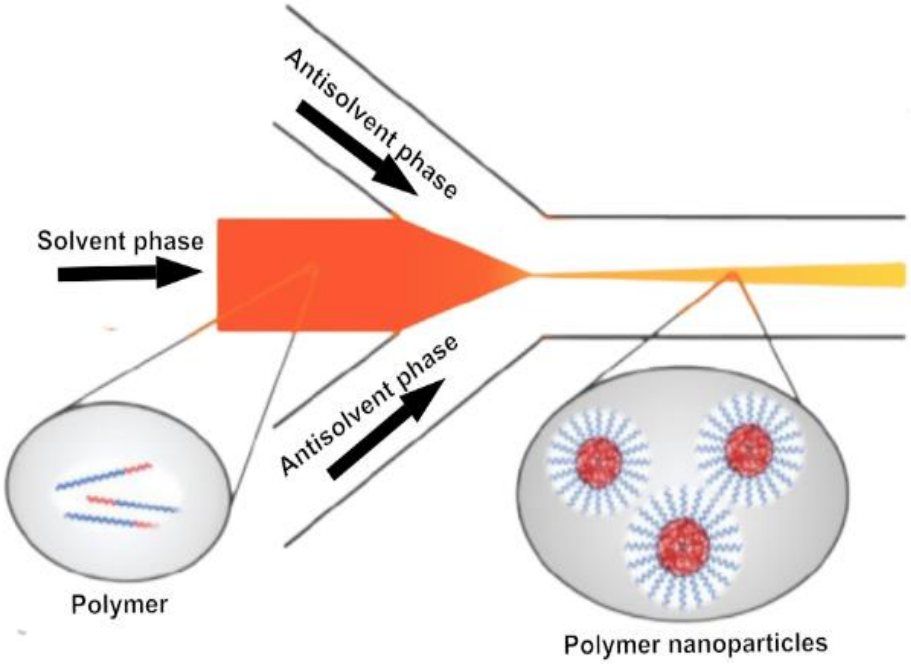


Figure 6. Diagram of the microchip used in the study by Mares *et al.* [30].

Other authors have explored slightly more precise methodologies for the control of final product quality characteristics, such is the case of Loy *et al.* [33]. In this research, the design of a modular platform for the operation of microfluidics is made. The first module is the control module or *Raspberry Pi module*, whose purpose is to execute scripts to control the pumps and the fraction collected in the second module, which corresponds to the *collection module*; the third module refers to the power supply of the microchip and is made up of the *programmable syringe pumps*; Finally, there is a *formulation module* (the scheme of the modular platform is shown in Figure 7). With this platform, polyplexes are formulated from siRNA (small interfering RNA) and oligomers; the electrostatic interaction between the cationic groups of polymers or oligomers with the negative charges of nucleic acids favor the formation of nanoparticles [34]. By using the previously described modular system, it was possible to obtain polyplexes with hydrodynamic diameters between 52.5 and 141 nm by modifying the oligomer/siRNA ratio, and polydispersity indices in the range of 0.03 and 0.114. The production platform proposed by Loy *et al.* [33] implies greater control and reproducibility of the formulations because each production cycle follows the same instructions provided by the software; In addition, this modular methodology can be useful for process scaling because, if necessary, the modules can be easily replaced by other more efficient components.

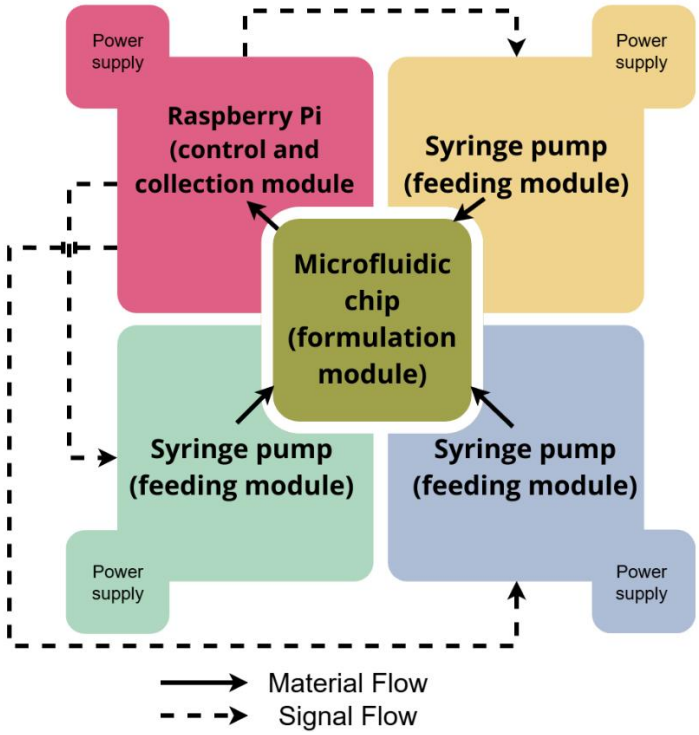


Figure 7. Diagram of the modular system used by Loy *et al.* [33].

In this research field on the automation of processes that can be carried out in microfluidic systems, there is also the study by Egorov *et al.* [35]. They discuss the integration of robotics and artificial intelligence (AI) in the synthesis of liposomes and polymeric drug delivery systems. According to the authors, due to the recent COVID-19 pandemic, the demand for robotic experimentation has increased; this has not only allowed samples to be analyzed safely but has also contributed to the minimization of human errors and fast formulations preparation. The use of these novel technologies is justified by the fact that in pharmaceutical forms manufacture, changes in process parameters and compositions can alter the properties of the final product, thus hindering the possibility of obtaining reproducible results [36, 37]. If areas of knowledge such as microfluidic science, robotics and machine learning are integrated into production processes, it is possible to increase understanding of structure-property relationships and interactions with tissues and cells. Despite the advantages of the approach explained above, there are challenges that must be addressed by the scientific community to get the most out of it, contribute to nanotechnology growth and pave paths to improve research in personalized medicine; among them are: "the limitation of data sets to report the results of machine learning and the change in the structure of chemical laboratories in order to open them up to students and experts from numerous fields of knowledge". A workflow scheme with the robotics – microfluidics – AI approach is shown in Figure 8:

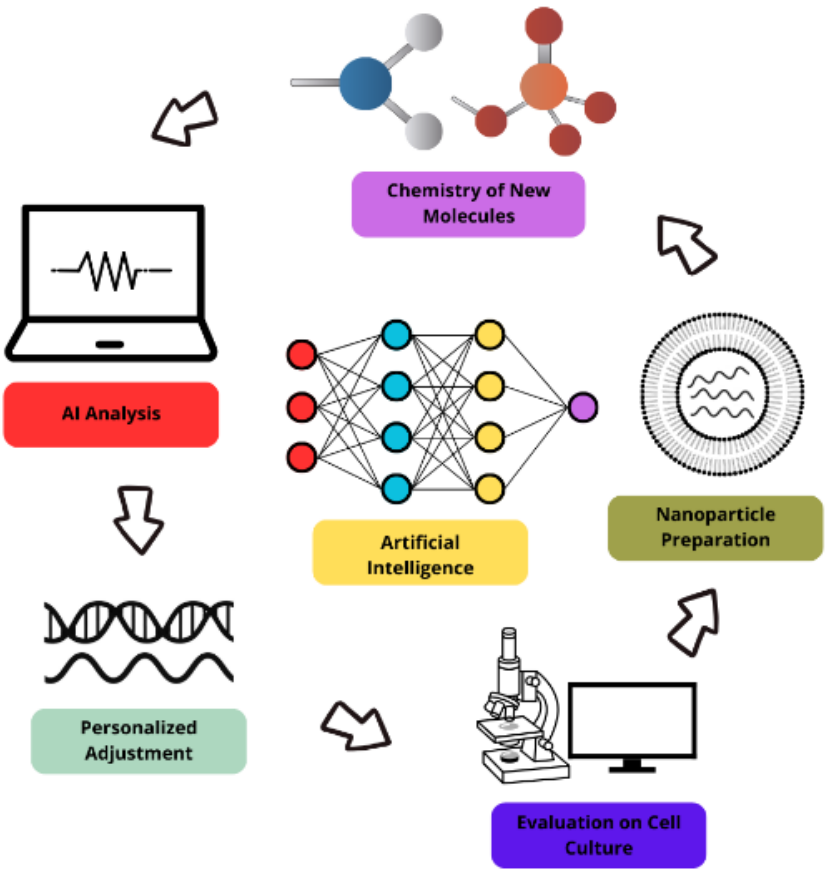


Figure 8. Workflow through the robotic approach - microfluidics - AI, proposed by Egorov *et al.* [35].

Microfluidics has been useful to produce controlled release systems for drugs and cosmetic active ingredients, because as previously mentioned, the advantages associated with this manufacturing method involve obtaining micro/nano platforms with precisely controlled properties and high reproducibility. This, added to an adequate selection of excipients, leads to obtaining formulations that respond to stimuli, which are profitable and/or that have good biocompatibility. These characteristics together make it feasible to apply different pharmaceutical forms (produced by microfluidic technology) in clinical trials [38]. To cite some examples, there is the study by Baby *et al.* [39] in which polymeric nanoparticles sensitive to pH are produced, loaded with curcumin, with potential application for the treatment of some types of cancer; This article has as particularities the spatial configuration of the microchip (it is in 3D and not in 2D, as usual, which contributes to a higher productivity and better control of the particle sizes) and that, depending on the operating conditions, it can be encapsulated different amounts of the active ingredient, managing to load up to 57% curcumin in the polymer system.

Lari *et al.* [40] for their part, carried out the production of nanoparticles of carboxymethyl chitosan crosslinked with $CaCl_2$ for the encapsulation of metformin hydrochloride in order to develop a treatment for type II diabetes. Thanks to the biocompatibility of the excipients used, the production method, and the release characteristics of the system, it was possible to demonstrate that the charged particles (synthesized by means of microfluidic technology) were more effective for the treatment of diabetes than metformin, conventionally administered.

Other excipients that are considered attractive for the development of controlled release systems, and that have been processed in microfluidic chips, are polylactic acid [41] and polysaccharides such as pullulan, mannan, hyaluronic acid, chitosan, alginate, dextran, among others. The latter are characterized by being biocompatible and biodegradable and, mainly, they are used for the production of nanogels that can encapsulate active principles by physical or chemical processes which can be carried out inside a microfluidic chip [42].

In the study made by Tiboni *et al.* [43], they propose the use of the 3D fused deposition methodology to economically produce a microchip whose purpose is to manipulate small volumes of raw materials to obtain polymeric nanoparticles and liposomes loaded with cannabidiol. In this research, the use of Computational Fluid Dynamics simulations allowed to evaluate the mixing potential of the microsystem proposed by the authors; this demonstrates the importance of supporting the methodological part of the investigations with simulations, since these facilitate optimizing designs and reducing experimentation times.

The integration of microfluidic technologies in the production of controlled release systems facilitates the systematic evaluation of various excipients and component mixtures that interact with the active ingredients of a formulation. This approach enables the rapid definition of design spaces by providing a clear understanding of how processing variables influence critical quality attributes. Moreover, process miniaturization allows for precise control over design parameters, ensuring the functional properties of the resulting pharmaceuticals or cosmetic products. Furthermore, the use of Design of Experiments (DoE) in building robust design spaces, as established by *Quality by Design* (QbD), has attracted significant interest in the development of commercially viable microfluidic systems. This bottom-up approach strengthens both product and process performance, paving the way for a successful scale-up phase aimed at large-scale manufacturing.

4. MICROFLUIDICS IN DRUG DISCOVERY

Microfluidic science has utility in any of the stages of new drug discovery and even in the development of pharmaceuticals; according to Kang *et al.* [44] these are: (1) target selection, (2) identification and optimization of the hit, (3) preclinical studies, (4) clinical trials, (5) manufacturing and (6) development of the final product. Some particular applications are based on high-throughput screening, toxicity assessments, and drug – cell cultures interaction studies [45-48]. There are a considerable number of microfluidic-based methodologies that seek to provide solutions to the drawbacks found in the conventional development cycle of a biologically active system. For example, when chemical synthesis is a critical step in obtaining new drugs, microscopic methods offer an advantage because heat and mass transfer is accelerated and, additionally, precise control can be achieved when dosing reagents; this translates into better yields and shorter residence times [49]. Studies such as the made by Torabinia *et al.* [49] have used the principle of dielectric wettability (which is based on the modification of the wettability of surfaces by applying electric fields) to carry out reaction, neutralization, evaporation and crystallization processes. Within the microdevice designed by the researchers there are reservoirs of the different solvents and reagents required for the reaction; In addition, there are sections of the chip where the transformation and separation stages are carried out. A schematic of the microsystem is shown in Figure 9. Another experimental activity developed in this study contemplates the optimization of the operating conditions and the voltages used for the manipulation of fluids. Once the working conditions in the microchip were optimized, yields ranging from 20.6% to 48.8% were obtained, depending on the initial concentration of

the reagent used in the study (benzoic acid). This research provides interesting bases for what could be the development of fully automated microplatforms.

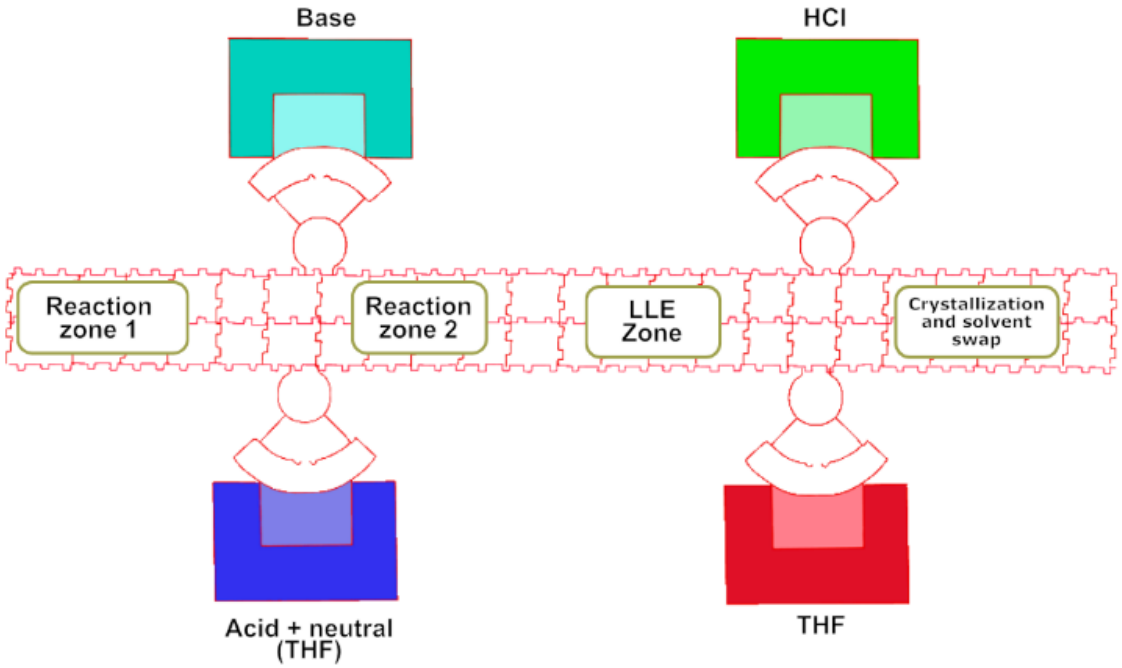


Figure 9. Scheme of the microchip for organic synthesis. Adapted from Torabinia *et al.* [49].

Within the area of high-resolution screening, microfluidic science has played a relevant role in recent years when it comes to searching for molecules with antifungal, antibiotic, and anti-cancer activity, mainly. Researchers such as Matilla [50] make clear the need to incorporate elements of microfluidic science in order to facilitate the search for molecules with antibiotic properties because miniaturization "facilitates the cultivation of microorganisms under a great variety of experimental conditions, the analysis of the microbial biodiversity of clinical and environmental samples, the screening of combinations of drugs to analyze their effectiveness against microorganisms resistant to multiple drugs, the analysis of interactions between biological molecules or the identification of enzymes with activities of interest".

High-resolution screening for drug discovery has been used in research such as the proposed by Oberpaul *et al.* [51]. Because the miniaturization of systems facilitates the manipulation of fluids, the study researchers were able to separate and cultivate strains of microorganisms extracted from soils by generating droplets on a microchip and using a methodology called FACS (Fluorescence-Activated Cell Sorting). Besides, these microfluidic elements were coupled with a methodology for the discovery of natural products guided by their bioactivity (bioguided fractionation using UHPLC-QTOF-MS in combination with molecular networking). This integration of tools for cell separation and evaluation of natural products allowed them to study the effect of more than 6,000 extracts against human pathogens and agricultural pests.

The discovery of drugs with anticancer activity has advanced considerably thanks to the implementation of three-dimensional cell cultures in microscopic systems. Conventional two-dimensional approaches can fail because they do not faithfully recreate the microenvironment with which cancer cells interact. According to Langhans [47], advances in three-dimensional cell culture have "provided opportunities to identify new targets and focus on microenvironmental factors that contribute to progression, drug response, and drug resistance". In this type of study, a wide variety of methodologies can be used to study the interactions of drugs with

the cells that are intended to be investigated; In essence, three-dimensional culture systems are carried out in three types of models (1) anchorage-dependent, (2) anchorage-independent and (3) hybrids of the two previous ones. One of the most widely used methodologies within the anchorage-dependent model is the cell growth in microfluidic devices. There is a wide variety of knowledge about three-dimensional cell culture and its correlation with microfluidics. In fact, Langhans states that this field of research has grown exponentially in the last 15 years. For this reason, it is recommended to refer to her review article for more details.

Nonetheless, the possibility of recreating physiological conditions with sufficient precision and detail by means of microfluidic devices has opened the doors to drug screening for the discovery of new therapies for already known diseases. Some examples are summarized below: in the article by Mistretta *et al.* [46], the potential of a microfluidic platform for the simultaneous experimental evaluation of multiple compounds on a study model was demonstrated. This model aimed to study the phenotypic change of single-cell, as reducing cell-to-cell variation can make bacterial populations vulnerable. Other articles have combined the development of organ-on-a-chip platforms with drug screening. These types of research are highly correlated because simulating realistic microenvironments makes it possible to understand mechanisms and accurately evaluate the effect of drugs on different conditions. For this reason, microsystems have been developed to facilitate drug discovery aimed at the treatment of cancer [52], neurodegenerative diseases [53], cardiovascular diseases [54], obesity [55], and even pain treatment through the understanding of neuronal signaling [56].

Microfluidic platforms have also had an important role in the discovery of drugs with antifungal activity. The search for molecules with these properties is of interest whether one takes into consideration that some types of infections caused by fungi can be fatal, there are few classes of compounds capable of treating this type of conditions, some drugs have considerable toxicity and even those can interact with other molecules of therapeutic action [57]. Microdevices have been used for high-resolution screening, particularly to observe the behavior of cells and biofilms cultured in hydrogels; this has provided relevant knowledge because according to Willaert [57] "a better understanding of fungal biofilms provides new opportunities for the development of agents and novel strategies that are urgently needed". Furthermore, microscopic systems have also been used to perform Antifungal Susceptibility Tests (AST) because this type of approximation is faster, more precise, and less costly; many of the studies carried out are based on the observation of cells (confined in volumes of the order of microns) by means of microscopy or fluorescence to obtain organisms resistance profiles; in this way it is possible to prescribe a patient with an effective drug [57].

Several studies focused on microfluidic systems in the pharmaceutical field—particularly for space applications—have integrated the *Quality by Design* (QbD) approach. These investigations address the functionality of cells and microorganisms under conditions of microgravity and space radiation, emphasizing the need for comprehensive knowledge generation and robust risk assessment. This is essential to ensure the reliable performance of microfluidic systems in environments with gravitational forces that differ from those on earth [58].

This module demonstrated the potential of applying microfluidic technologies to the evaluation of various active molecules, significantly reducing research and development times while fostering robust knowledge management. By assessing different drugs and their interactions with cultured human cells, it is possible to accurately determine the toxicity of pharmaceutical compounds, ensuring that active ingredient concentrations remain within safe thresholds. This contributes to the definition of safe design spaces for pharmaceutical products.

5. ORGAN-ON-A-CHIP TECHNOLOGY

Among the wide variety of biomedical methodologies for understanding, modeling, monitoring, and predicting drug responses, Organ-on-a-Chip (OoC) has shown potential since its conception in the early 21st century. OoC technology consists of a submillimeter *in vitro* platform designed to simulate specific functions of tissues or organs by cell cultures and microfluidics which allow to resemble real microenvironment conditions and biomechanical stimuli that surrounds the study system [59].

OoC platforms have attributes that make it stand out from other technologies. The most remarkable features are listed as: *i*) In the case of animal models, besides the ethical aspects, it has been demonstrated that they cannot equalize some human functions and responses due to differences in organs structure and cells complexity. These limitations imply, for example, lack of pathogenesis similarity compared to humans (which reduce their application in viral infections research) [60]; impossibility of using animal models in some neurodegenerative diseases researches owing to their less complex brain structure and cognitive functions [61]; and failure in resemble visual acuity due to lack of trichromacy [62]. All the challenges mentioned above have been addressed from the perspective of OoC technology. *ii*) Unlike classical two-dimensional cell cultures, OcC can simulate microenvironments and its alterations during disease, and has greater ability to assess drug responses in complex systems [63]. *iii*) It is estimated that OoC technology has an impact on different cost drivers in R&D process. A quantitative study made by Franzen *et al.* [64], showed that implementation of OoC could: reduce the cycle length in lead optimization stage, increase success rates at preclinical and phase II stages, and reduce considerably the cost at preclinical stage. The aforementioned is reflected numerically in an average reduction between 10% and 26% in R&D costs per new drug and savings of, approximately, 169 million USD to 706 million USD [64]. *iv*) Facilitate high-throughput screening of large drug libraries and its effect on different types of human cells; this could pave the way to personalized prescriptions by understanding the physiological characteristics of each patient. *v*) Represents a potential tool for nanomedicine assessment [65], pharmacokinetics and pharmacodynamics studies, and continuous dynamic monitoring in disease modeling [59]. *vi*) It has demonstrated potential to replicate *in vivo* responses and therefore clinical trials and toxicity studies could be facilitated [66].

The different approaches made through OoC seek to establish simplistic models that provide information about mechanistic aspects of diseases and drug-cells interactions, mainly. For that reason, it is critical to understand the anatomy of the organ to study which involves microstructures, cellular microenvironments and mechanical forces implicated; some key factors are listed as: *i*) In lung-on-a-Chip applications it must be considered air-blood microenvironment as well as mechanical stretching and specific cell types [66]. *ii*) In gut-on-a-Chip studies it is important to mimic peristalsis and the microbial environment [66]. *iii*) Retina-on-a-Chip models face challenges due to the complex 3D physiological structure; for that reason it is necessary to evaluate cells characteristics and microphysiology associated to the process of interest [62]. *iv*) In cardiovascular models such as blood-vessel-on-chip and heart-on-chip it is recommended to consider shear stresses, tensile strains, flow type, and mechanical and electrical stimulation since it determines cell morphologies, proliferation and even it is critical in evaluating drug cardiotoxicity [66]. *v*) In kidney models a key concept is related to the constant flow of the glomerular filtrate because it implies continuous shear rates which have an effect on epithelial cells morphology [66]. *vi*) In Organ-on-a-Chip models for female reproductive

systems, researchers have explored elements such as placental barriers, cellular constituents, structural organization and biomechanical microenvironment like blood flow [67, 68].

Without a doubt, the OoC field is significantly vast and for each tissue or organ model, researchers must evaluate and discern among the plethora of micro-conditions that can affect the performance and the ability to predict biological behaviors in the human being. However, a systematic approach for constructing organs-on-a-chip and for analyzing microscopic phenomena has established a tool with the potential to replace conventional methodologies for diseases, and drug interactions assessment.

An example of the stated above is found in the paper by Achberger *et al.* [62]; here it is exposed the importance of OoC technology to simulate the complex stratified retinal tissue. By means of conventional models for drug development and diseases research it is not possible to, simultaneously, recreate crucial elements such as physiological structures, perfusion, constant nutrients supply, biological barriers functions, cell interactions, and mechanical stability. The authors developed a microfluidic platform able to mimic the human retina anatomy *in vitro;* to perform this task, inside the chip top layer was cultured retinal organoids derived from induced pluripotent stem cells (hiPSC), whereas the bottom layer had the function of supplying nutrients, resembling a vasculature perfusion. The retina-on-a-chip model proposed by Achberger *et al*. was tested to study the secretion kinetics and the controlled delivery of compounds to the tissue, the establishment of interactions between segment structures of the retinal organoid and retinal pigment epithelium cells. Moreover, it was possible to assess the retinal functions by evaluating the ability to produce an *in vivo*-like calcium flux and the functionality of the visual cycle was verified.

Once the platform's ability to recreate physiological functions was verified, its potential to carry out drug development and toxicity studies was evaluated by exposing the system to chloroquine and gentamicin. After drug exposure it was quantified the cell viability though a staining method; the results suggest that concentrations above 80 μg/mL of chloroquine and 0.5 mg/mL of gentamicin had an important effect on cell death, which was an expected behavior because there are reports in the literature that confirm the pathological side effects of these drugs on the retina.

In the same way, it is found a wide variety of research from 2019 to 2025 that study the mechanistic of diseases to identify determining physiological aspects that can be the starting point for the development of helpful treatments. As examples, there are investigations dedicated to study the effect of ambient fine particles on carcinogenic development [69], prediabetic hyperglycemia [70], polycystic kidney disease mechanisms [71], human cardiac development [72] (among a quantity of more than 2500 documents according to Scopus database in the aforementioned data range).

This type of approach, which seeks to understand diseases in depth to find effective and personalized therapies, is a key pivot for the development of drugs based on QbD because they provide a comprehensive view of the patient's response in terms of toxicity, effectiveness, and safety, the latter are essential for the rational application of the methodology and, in combination with the nanomedicine, it is possible to have a parametric control from the manufacture of the formulation to the functionality and biological activity *in vivo*.

Since there are a plethora of papers that can be classified into the "organ-on-a-chip technology" group, it is recommended to delve into specialized review articles on specific topics; if the expectation is to deepen on the importance of OoC mechanical stimuli in diseases assessment see [66]; to explore the usefulness of OoC for nanomedicine evaluation refer to [65], to consult some innovations in disease modeling and drug development in OoC please read [59]. Also, there is almost one review article per organ or tissue in the human body that represents

a challenge from the biological, pharmacological, pharmacodynamic and pharmacokinetic point of view. In fact, of the 5,088 articles published in Scopus between 2019 and 2025 related to OoC technology, almost a third (31%) are review articles.

This group of microfluidic applications is of great interest during the development stages of pharmaceuticals and even cosmetic products, as it enables the cultivation of cells from the patient—or the target individual for a therapy or treatment—on microchips [73]. This approach makes it possible to understand the mechanistic behavior of their cellular response and to design formulations tailored to their specific needs. Understanding microscale interactions is essential to effectively leverage critical process variables, design parameters, and quality attributes within a defined design space, with the goal of developing safe and effective products.

6. PERSPECTIVES

To date, and to the best of our knowledge, the scientific investigations that bring together microfluidic and pharmaceutical sciences have focused on the different stages of medicine design from an isolated perspective and focusing on the elucidation of fundamentals. However, as has been mentioned throughout this article, microfluidic science has applications in each of the phases of product development, therefore, it is expected that in future research it will be possible to have platforms that carry out all design activities in a single platform, namely: producing nanomedicines, characterizing critical parameters in functional performance, and evaluating its toxicity, therapeutic activity and security in order to replace the models that nowadays are considered conventional. Likewise, research that quantitatively demonstrates the advantages of microfluidics over other technologies will be relevant to attract more researchers to the pharmaceutical area and even to cosmetic and phytotherapeutic sectors. Furthermore, with the accelerated development of artificial intelligence, it is likely that the application of such computational tools in the development of microplatforms will become more popular, enabling them to perform many more tasks in a more intuitive and efficient manner.

7. CONCLUSIONS

Microfluidic systems serve as a powerful tool for the entire lifecycle of pharmaceutical products development and enhance the knowledge basis for the effective application of quality-based methodologies. This technology can be used to improve each of the key items that are highlighted in the ICH guidelines since its bottom-up approach allows a precise parametric control and therefore, it is possible to establish effects and correlations between microscopic events, product quality attributes, functionalities, and process variables. Moreover, the latent opportunity to evaluate medicines, drugs and therapies in human cells makes it an ethical and profitable alternative to predict the response of people in the clinical phases.

ACKNOWLEDGEMENTS

We deeply thank the Universidad Nacional de Colombia for being a promoter of knowledge and for enabling the development of this article through partial funding from the project titled “Diseño integrado de un prototipo funcional de dispositivo tipo tapón nasal, para el tratamiento de la epistaxis”, which was a part of the call “UN INNOVA”: Convocatoria de proyectos para el fortalecimiento de la innovación en la universidad nacional de Colombia a partir del desarrollo de prototipos y experiencias piloto 2019–2021”.

AUTHOR CONTRIBUTIONS

Edward Rodriguez led the manuscript writing, conducted the background research, performed the information analysis, and made the necessary adjustments. Helber Barbosa contributed to the supervision of the work and provided critical feedback on the manuscript. Bibiana Vallejo contributed by providing relevant information, supervising the research, reviewing the manuscript, and offering feedback.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

1. P. Tabeling & S. Chen. *Introduction to Microfluidics.* Oxford University Press, Oxford, 2005. https://doi.org/10.1093/oso/9780198568643.001.0001
2. J.-C. Charpentier. Four main objectives for the future of chemical and process engineering mainly concerned by the science and technologies of new materials production. *Chemical Engineering Journal*, **107**(1-3), 3–17 (2005). https://doi.org/10.1016/j.cej.2004.12.004
3. S. Colombo, M. Beck-Broichsitter, J.P. Bøtker, M. Malmsten, J. Rantanen & A. Bohr. Transforming nanomedicine manufacturing toward Quality by Design and microfluidics. *Advanced Drug Delivery Reviews*, **128**, 115–131 (2018). https://doi.org/10.1016/j.addr.2018.04.004
4. S. Ghosh, D. Maity, A. Chowdhury, S.K. Roy & C. Giri. Efficient fault detection and diagnosis of digital microfluidic biochip using multiple electrodes actuation. *2020 IEEE International Test Conference on India (ITC India)*. Bangalore, India, 2020; pp. 1–4. https://doi.org/10.1109/itcindia49857.2020.9171793
5. E. Di Giampaolo & A.D. Natale. A configurable microwave microfluidic sensor for medical diagnosis and chemical analysis. *2019 PhotonIcs & Electromagnetics Research Symposium - Spring (PIERS-Spring).* Rome, Italy, 2019; pp. 4194–4197. https://doi.org/10.1109/piers-spring46901.2019.9017489
6. Z. Ying, L. Qiao, B. Liu, L. Gao & P. Zhang. Development of a microfluidic wearable electrochemical sensor for the non-invasive monitoring of oxidative stress biomarkers in human sweat. *Biosensors and Bioelectronics*, **261**, 116502 (2024). https://doi.org/10.1016/j.bios.2024.116502
7. Z. Izadifar, B. Charrez, M. Almeida, S. Robben, K. Pilobello, J. van der Graaf-Mas, *et al.* Organ chips with integrated multifunctional sensors enable continuous metabolic monitoring at controlled oxygen levels. *Biosensors and Bioelectronics*, **265**, 116683 (2024). https://doi.org/10.1016/j.bios.2024.116683
8. T.-H. Lu, N.-J. Chiang, C.-J. Huang, P. Gopinathan, H.-C. Tu, Y.-C. Tsai, Y.-S. Shan, S.-C. Hung & G.-B. Lee. An integrated microfluidic platform for cholangiocarcinoma diagnosis from clinical bile juice samples by utilizing multiple affinity reagents. *2020 IEEE 15th International Conference on Nano/Micro Engineered and Molecular System (NEMS).* San Diego, (CA), 2020; pp. 261–264. https://doi.org/10.1109/nems50311.2020.9265566
9. D. Maji, S. Pourang, U. D. S. Sekhon, A. S. Gupta, M. A. Suster & P. Mohseni. Toward diagnosis of platelet loss in trauma injury using a microfluidic dielectric sensor. *2019 IEEE Sensors.* Montreal (QC), 2019; pp. 1–4. https://doi.org/10.1109/sensors43011.2019.8956491
10. Z. Ma, J. Xia, N. Upreti, E. David, J. Rufo, Y. Gu, *et al.* An acoustofluidic device for the automated separation of platelet-reduced plasma from whole blood. *Microsystems & Nanoengineering*, **10**(1), 83 (2024). https://doi.org/10.1038/s41378-024-00707-3
11. Y.-L. Fang, W.-B. Lee, C.-H. Wang, C.-C. Chien, H.-L. You, M.S. Lee & G.-B. Lee. An integrated microfluidic system for fast isolation of bacteria in human whole blood for diagnosis of sepsis. *2020*

IEEE 33rd International Conference on Micro Electro Mechanical Systems (MEMS). Vancouver (BC), Canada, 2020; pp. 1014–1017. https://doi.org/10.1109/mems46641.2020.9056344

12. H.-s. Kim, N. Abbas & S. Shin. A rapid diagnosis of SARS-CoV-2 using DNA hydrogel formation on microfluidic pores. *Biosensors and Bioelectronics*, **177**, 113005 (2021). https://doi.org/10.1016/j.bios.2021.113005
13. H.Q. Nguyen, V.D. Nguyen, V.M. Phan & T.S. Seo. A novel point-of-care platform for rapid SARS-CoV-2 detection utilizing an all-in-one 3D-printed microfluidic cartridge and IoT technology», *Sensors and Actuators B: Chemical*, **410**, 135632 (2024). https://doi.org/10.1016/j.snb.2024.135632
14. L. Zhu, H. Huang, M.M.-C. Cheng & P.-Y. Chen. Compact, flexible harmonic transponder sensor with multiplexed sensing capabilities for rapid, contactless microfluidic diagnosis. *IEEE Transactions on Microwave Theory and Techniques*, **68**(11), 4846–4854 (2020). https://doi.org/10.1109/tmtt.2020.3006286
15. A.C.Q. Silva, C. Vilela, H.A. Santos, A.J.D. Silvestre & C.S.R. Freire. Recent trends on the development of systems for cancer diagnosis and treatment by microfluidic technology. *Applied Materials Today*, **18**, 100450 (2020). https://doi.org/10.1016/j.apmt.2019.100450
16. H. Tavakoli, W. Zhou, L. Ma, S. Perez, A. Ibarra, F. Xu, S. Zhan & X. Li. Recent advances in microfluidic platforms for single-cell analysis in cancer biology, diagnosis and therapy. *TrAC Trends in Analytical Chemistry*, **117**, 13–26 (2019). https://doi.org/10.1016/j.trac.2019.05.010
17. C. Tu, B. Huang, J. Zhou, Y. Liang, J. Tian, L. Ji, X. Liang & X. Ye. A microfluidic chip for cell patterning utilizing paired microwells and protein patterns. *Micromachines*, **8**(1), 1 (2016). https://doi.org/10.3390/mi8010001
18. H.S. Rho, Y. Yang, A.T. Hanke, M. Ottens, L.W.M.M. Terstappen & H. Gardeniers. Programmable v-type valve for cell and particle manipulation in microfluidic devices. *Lab on a Chip*, 16(2), 305–311 (2016). https://doi.org/10.1039/c5lc01206f
19. L. Armbrecht, G. Gabernet, F. Kurth, J.A. Hiss, G. Schneider & P.S. Dittrich. Characterisation of anticancer peptides at the single-cell level», *Lab on a Chip*, **17**(17), 2933–2940 (2017). https://doi.org/10.1039/c7lc00505a
20. S. Maheswaran, L.V. Sequist, S. Nagrath, L. Ulkus, B. Brannigan, C.V. Collura, *et al.* Detection of mutations in EGFR in circulating lung-cancer cells. *The New England Journal of Medicine*, **359**(4), 366–377 (2008). https://doi.org/10.1056/nejmoa0800668
21. W. Sheng, O.O. Ogunwobi, T. Chen, J. Zhang, T.J. George, C. Liu & Z.H. Fan. Capture, release and culture of circulating tumor cells from pancreatic cancer patients using an enhanced mixing chip. *Lab on a Chip*, **14**(1), 89–98 (2014). https://doi.org/10.1039/c3lc51017d
22. F.G. Ortega, M.A. Fernández-Baldo, M.J. Serrano, G.A. Messina, J.A. Lorente & J. Raba. Epithelial cancer biomarker EpCAM determination in peripheral blood samples using a microfluidic immunosensor based in silver nanoparticles as platform. *Sensors and Actuators B: Chemical*, **221**, 248–256 (2015). https://doi.org/10.1016/j.snb.2015.06.066
23. H.J. Yoon, A. Shanker, Y. Wang, M. Kozminsky, Q. Jin, N. Palanisamy, *et al.* Tunable thermal-sensitive polymer-graphene oxide composite for efficient capture and release of viable circulating tumor cells. *Advanced Materials*, **28**(24), 4891–4897 (2016). https://doi.org/10.1002/adma.201600658
24. Y. Wu, C. Wang, Y. Guo, Y. Zhang, X. Zhang, P. Wang, *et al.* Small extracellular vesicle-based one-step high-throughput microfluidic platform for epithelial ovarian cancer diagnosis. *Journal of Nanobiotechnology*, **23**(1), 278 (2025). https://doi.org/10.1186/s12951-025-03348-4
25. D. Yu, J. Gu, J. Zhang, M. Wang, R. Ji, C. Feng, H.A. Santos, H. Zhang & X. Zhang. Integrated microfluidic chip for neutrophil extracellular vesicle analysis and gastric cancer diagnosis. *ACS Nano*, **19**(10), 10078–10092 (2025). https://doi.org/10.1021/acsnano.4c16894
26. S. Kuang, N.M. Singh, Y. Wu, Y. Shen, W. Ren, L. Tu, K.-T. Yong & P. Song. Role of microfluidics in accelerating new space missions. *Biomicrofluidics*, **16**(2), 021503 (2022). https://doi.org/10.1063/5.0079819
27. S.-M. Yang, S. Lv, W. Zhang & Y. Cui. Microfluidic point-of-care (POC) devices in early diagnosis: A review of opportunities and challenges. *Sensors*, **22**(4), 1620 (2022). https://doi.org/10.3390/s22041620

28. H. Bolze, J. Riewe, H. Bunjes, A. Dietzel & T.P. Burg. Continuous production of lipid nanoparticles by ultrasound-assisted microfluidic antisolvent precipitation. *Chemical Engineering Technology*, **44**(9), 1641–1650 (2021). https://doi.org/10.1002/ceat.202100149
29. S. Patil, A. Pandit, G. Gaikwad, P. Dandekar & R. Jain. Exploring microfluidic platform technique for continuous production of pharmaceutical microemulsions. *Journal of Pharmaceutical Innovation*, **16**(3), 441–453 (2021). https://doi.org/10.1007/s12247-020-09457-x
30. A.G. Mares, G. Pacassoni, J.S. Marti, S. Pujals & L. Albertazzi. Formulation of tunable size PLGA-PEG nanoparticles for drug delivery using microfluidic technology. *PLoS One*, **16**(6), e0251821 (2021). https://doi.org/10.1371/journal.pone.0251821
31. H.K. Makadia & S.J. Siegel. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers*, **3**(3), 1377–1397 (2011). https://doi.org/10.3390/polym3031377
32. J.S. Suk, Q. Xu, N. Kim, J. Hanes & L.M. Ensign. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced Drug Delivery Reviews*, **99**(Part A), 28–51 (2016). https://doi.org/10.1016/j.addr.2015.09.012
33. D.M. Loy, R. Krzysztoń, U. Lächelt, J.O. Rädler & E. Wagner. Controlling nanoparticle formulation: A low-budget prototype for the automation of a microfluidic platform. *Processes*, **9**(1), 129 (2021). https://doi.org/10.3390/pr9010129
34. C. Vasile (editor). *Polymeric Nanomaterials for Nanotherapeutics.* Micro & nano technologies series. Elsevier, Amsterdam, 2019. https://doi.org/10.1016/c2017-0-00607-9
35. E. Egorov, C. Pieters, H. Korach-Rechtman, J. Shklover & A. Schroeder. Robotics, microfluidics, nanotechnology and AI in the synthesis and evaluation of liposomes and polymeric drug delivery systems. *Drug Delivery and Translational Research*, **11**(2), 345–352 (2021). https://doi.org/10.1007/s13346-021-00929-2
36. H.A. Santos, J. Riikonen, J. Salonen, E. Mäkilä, T. Heikkilä, T. Laaksonen, L. Peltonen, V.-P. Lehto & J. Hirvonen. *In vitro* cytotoxicity of porous silicon microparticles: Effect of the particle concentration, surface chemistry and size. *Acta Biomaterialia*, **6**(7), 2721–2731 (2010). https://doi.org/10.1016/j.actbio.2009.12.043
37. H.S. Leong, K.S. Butler, C.J. Brinker, M. Azzawi, S. Conlan, C. Dufés, *et al.* On the issue of transparency and reproducibility in nanomedicine. *Nature Nanotechnology*, **14**(7), 629–635 (2019). https://doi.org/10.1038/s41565-019-0496-9
38. N. Kamaly, Z. Xiao, P.M. Valencia, A.F. Radovic-Moreno & O.C. Farokhzad. Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. *Chemical Society Reviews*, **41**(7), 2971–3010 (2012). https://doi.org/10.1039/c2cs15344k
39. T. Baby, Y. Liu, G. Yang, D. Chen & C.-X. Zhao. Microfluidic synthesis of curcumin loaded polymer nanoparticles with tunable drug loading and pH-triggered release. *Journal of Colloid and Interface Science*, **594**, 474–484 (2021). https://doi.org/10.1016/j.jcis.2021.03.035
40. A.S. Lari, P. Zahedi, H. Ghourchian & A. Khatibi. Microfluidic-based synthesized carboxymethyl chitosan nanoparticles containing metformin for diabetes therapy: In vitro and in vivo assessments. *Carbohydrate Polymers*, **261**, 117889 (2021). https://doi.org/10.1016/j.carbpol.2021.117889
41. M.N. Abu-Hajleh, A. AL-Samydai & E.A.S. Al-Dujaili. Nano, micro particulate and cosmetic delivery systems of polylactic acid: A mini review. *Journal of Cosmetic Dermatology*, **19**(11), 2805–2811 (2020). https://doi.org/10.1111/jocd.13696
42. N. Zoratto, E. Montanari, M. Viola, J. Wang, T. Coviello, C. Di Meo & P. Matricardi. Strategies to load therapeutics into polysaccharide-based nanogels with a focus on microfluidics: A review. *Carbohydrate Polymers*, **266**, 118119 (2021). https://doi.org/10.1016/j.carbpol.2021.118119
43. M. Tiboni, M. Tiboni, A. Pierro, M. Del Papa, S. Sparaventi, M. Cespi & L. Casettari. Microfluidics for nanomedicines manufacturing: An affordable and low-cost 3D printing approach. *International Journal of Pharmaceutics*, **599**, 120464 (2021). https://doi.org/10.1016/j.ijpharm.2021.120464
44. L. Kang, B.G. Chung, R. Langer & A. Khademhosseini. Microfluidics for drug discovery and development: From target selection to product lifecycle management. *Drug Discovery Today*, **13**(1-2), 1-13 (2008). https://doi.org/10.1016/j.drudis.2007.10.003
45. F. Bonanini, R. Dinkelberg, M. Caro-Torregrosa, N. Kortekaas, T.M.S. Hagens, S. Treillard, D. Kurek, V. van Duinen, P. Vulto & K. Bircsak. A microvascularized in vitro liver model for disease

modeling and drug discovery. *Biofabrication*, **17**(1), 015007 (2025). https://doi.org/10.1088/1758-5090/ad818a

46. M. Mistretta, M. Cimino, P. Campagne, S. Volant, E. Kornobis, O. Hebert, *et al.* Dynamic microfluidic single-cell screening identifies pheno-tuning compounds to potentiate tuberculosis therapy. *Nature Communications*, **15**(1), 4175 (2024). https://doi.org/10.1038/s41467-024-48269-2
47. S.A. Langhans. Using 3D *in vitro* cell culture models in anti-cancer drug discovery. *Expert Opinion on Drug Discovery*, **16**(8), 841–850 (2021). https://doi.org/10.1080/17460441.2021.1912731
48. S. Momtahen, M. Taajobian & y A. Jahanian. Drug discovery applications: A customized digital microfluidic biochip architecture/CAD flow. *IEEE Nanotechnology Magazine*, **13**(5), 25–34 (2019). https://doi.org/10.1109/mnano.2019.2927773
49. M. Torabinia, U.S. Dakarapu, P. Asgari, J. Jeon & H. Moon. Electrowetting-on-dielectric (EWOD) digital microfluidic device for in-line workup in organic reactions: A critical step in the drug discovery work cycle. *Sensors and Actuators B: Chemical*, **330**, 129252 (2021). https://doi.org/10.1016/j.snb.2020.129252
50. M.A. Matilla. Facing crises in the 21st century: microfluidics approaches for antibiotic discovery. *Microbial Biotechnology*, **15**(2), 392–394 (2022). https://doi.org/10.1111/1751-7915.13908
51. M. Oberpaul, S. Brinkmann, M. Marner, S. Mihajlovic, B. Leis, M.A. Patras, *et al.* Combination of high-throughput microfluidics and FACS technologies to leverage the numbers game in natural product discovery. *Microbial Biotechnology*, **15**(2), 415–430 (2022). https://doi.org/10.1111/1751-7915.13872
52. S. Kheiri, I. Yakavets, J. Cruickshank, F. Ahmadi, H.K. Berman, D.W. Cescon, E.W.K. Young & E. Kumacheva. Microfluidic platform for generating and releasing patient-derived cancer organoids with diverse shapes: Insight into shape-dependent tumor growth. *Advanced Materials*, **36**(44), 2410547 (2024). https://doi.org/10.1002/adma.202410547
53. M. Ohbuchi, M. Shibuta, K. Tetsuka, H. Sasaki-Iwaoka, M. Oishi, F. Shimizu & Y. Nagasaka. Modeling of blood–brain barrier (BBB) dysfunction and immune cell migration using human BBB-on-a-chip for drug discovery research. *International Journal of Molecular Sciences*, **25**(12), 6496 (2024). https://doi.org/10.3390/ijms25126496
54. Z. Gao, Z. Du, Y. Hou, K. Hua, P. Tu, X. Ai & Y. Jiang. A microfluidic coculture model for mapping signaling perturbations and precise drug screening against macrophage-mediated dynamic myocardial injury. *Acta Pharmaceutica Sinica B*, **14**(12), 5393–5406 (2024). https://doi.org/10.1016/j.apsb.2024.11.004
55. L.K. Huff, C.M. Amurgis, L.E. Kokai & R.D. Abbott. Optimization and validation of a fat-on-a-chip model for non-invasive therapeutic drug discovery. *Frontiers in Bioengineering and Biotechnology*, **12**, 1404327 (2024). https://doi.org/10.3389/fbioe.2024.1404327
56. G. Kimourtzis & R. Raouf. A microfluidic model of the first sensory synapse for analgesic target discovery. *Molecular Pain*, **20**, 17448069241293286 (2024). https://doi.org/10.1177/17448069241293286
57. R.G. Willaert. Micro- and nanoscale approaches in antifungal drug discovery. *Fermentation*, **4**(2), 43 (2018). https://doi.org/10.3390/fermentation4020043
58. E.S. Nelson. Design principles for microfluidic biomedical diagnostics in space. In: R. Fazel (editor). *Biomedical Engineering - From Theory to Applications.* InTech, London, 2011. https://doi.org/10.5772/21669
59. Z. Li, J. Hui, P. Yang & H. Mao. Microfluidic organ-on-a-chip system for disease modeling and drug development. *Biosensors*, **12**(6), 370 (2022). https://doi.org/10.3390/bios12060370
60. F. Shahabipour, S. Satta, M. Mahmoodi, A. Sun, N.R. de Barros, S. Li, T. Hsiai & N. Ashammakhi. Engineering organ-on-a-chip systems to model viral infections. *Biofabrication*, **15**(2), 022001 (2022). https://doi.org/10.1088/1758-5090/ac6538
61. M.A.M. Jahromi, A. Abdoli, M. Rahmanian, H. Bardania, M. Bayandori, S.M.M. Basri, A. Kalbasi, A.R. Aref, M. Karimi & M.R. Hamblin. Microfluidic brain-on-a-chip: Perspectives for mimicking neural system disorders. *Molecular Neurobiology*, **56**(12), 8489–8512 (2019). https://doi.org/10.1007/s12035-019-01653-2

62. K. Achberger, C. Probst, J. Haderspeck, S. Bolz, J. Rogal, J. Chuchuy, *et al.* Merging organoid and organ-on-a-chip technology to generate complex multi-layer tissue models in a human retina-on-a-chip platform. *eLife*, **8**, e46188 (2019). https://doi.org/10.7554/elife.46188
63. K. Goluba, V. Parfejevs, E. Rostoka, K. Jekabsons, I. Blake, A. Neimane, *et al.* Personalized PDAC chip with functional endothelial barrier for tumour biomarker detection: A platform for precision medicine applications. *Materials Today Bio*, **29**, 101262 (2024). https://doi.org/10.1016/j.mtbio.2024.101262
64. N. Franzen, W.H. van Harten, V.P. Retèl, P. Loskill, J. van den Eijnden-van Raaij & M. IJzerman. Impact of organ-on-a-chip technology on pharmaceutical R&D costs. *Drug Discovery Today*, **24**(9), 1720–1724 (2019). https://doi.org/10.1016/j.drudis.2019.06.003
65. X. Chen, Y.S. Zhang, X. Zhang & C. Liu. Organ-on-a-chip platforms for accelerating the evaluation of nanomedicine. *Bioactive Materials*, **6**(4), 1012–1027 (2021). https://doi.org/10.1016/j.bioactmat.2020.09.022
66. C.L. Thompson, S. Fu, H.K. Heywood, M.M. Knight & S.D. Thorpe. Mechanical stimulation: A crucial element of organ-on-chip models. *Frontiers in Bioengineering and Biotechnology*, **8**, 602646 (2020). https://doi.org/10.3389/fbioe.2020.602646
67. R.E. Young & D.D. Huh. Organ-on-a-chip technology for the study of the female reproductive system. *Advanced Drug Delivery Reviews*, **173**, 461–478 (2021). https://doi.org/10.1016/j.addr.2021.03.010
68. A. Ahvaraki, E. Gheytanchi, E. Behroodi, H. Latifi, F. Vakhshiteh, Z. Bagheri & Z. Madjd. Advanced co-culture 3D breast cancer model to study cell death and nanodrug sensitivity of tumor spheroids. *Biochemical Engineering Journal*, **209**, 109400 (2024). https://doi.org/10.1016/j.bej.2024.109400
69. L. Zheng, Y. Wang, Y. Zhang, Z. Chai, S. Liu, B. Wang, B. Dai & D. Zhang. Investigation of PM2.5-induced carcinogenic effects through mediation of ErbB family based on DNA methylation and transcriptomics analysis by a lung-mimicking microfluidic platform. *Ecotoxicology and Environmental Safety*, **248**, 114318 (2022). https://doi.org/10.1016/j.ecoenv.2022.114318
70. R.Z. Shafagh, S. Youhanna, J. Keulen, J.X. Shen, N. Taebnia, L.C. Preiss, *et al.* Bioengineered pancreas–liver crosstalk in a microfluidic coculture chip identifies human metabolic response signatures in prediabetic hyperglycemia. *Advanced Science* (Weinheim), **9**(34), 2203368 (2022). https://doi.org/10.1002/advs.202203368
71. S.R. Li, R.E. Gulieva, L. Helms, N.M. Cruz, T. Vincent, H. Fu, J. Himmelfarb & B.S. Freedman. Glucose absorption drives cystogenesis in a human organoid-on-chip model of polycystic kidney disease. *Nature Communications*, **13**(1), 7918 (2022). https://doi.org/10.1038/s41467-022-35537-2
72. M. Stiefbold, H. Zhang & L.Q. Wan. Engineered platforms for mimicking cardiac development and drug screening. *Cellular and Molecular Life Sciences*, **81**(1), 197 (2024). https://doi.org/10.1007/s00018-024-05231-1
73. Y. Huang, X. Wu, Y. Xu, N. Yang, P. Xi, Y. Wang, Y. Zhu & X. Chen. Organoids/organs-on-chips towards biomimetic human artificial skin. *Burns & Trauma*, **13**, tkaf029 (2025). https://doi.org/10.1093/burnst/tkaf029

HOW TO CITE THIS ARTICLE

E. Rodríguez, H. Barbosa & B. Vallejo. Recent microfluidic applications in Pharmaceutical Sciences and its potential utility in bottom-up concepts of Quality by Design. *Rev. Colomb. Cienc. Quim. Farm.*, **55**(1), 238–261 (2026). https://doi.org/10.15446/rcciquifa.v55n1.122915

REVISTA COLOMBIANA DE CIENCIAS QUÍMICO FARMACÉUTICAS

NORMAS PARA PUBLICACIÓN

La Revista Colombiana de Ciencias Químico Farmacéuticas es editada por el Departamento de Farmacia de la Universidad Nacional de Colombia (Sede Bogotá), con una periodicidad cuatrimestral y tiene como objetivo publicar artículos originales de diversos tópicos relacionados con las ciencias farmacéuticas, como recursos naturales, atención farmacéutica, evaluación clínica y preclínica, y los relacionados con la industria farmacéutica, la salud y los medicamentos.

Además de artículos completos, la revista acepta revisiones, comunicaciones breves, reportes de caso y cartas al Editor. Las contribuciones pueden enviarse en español, portugués o inglés, en letra Times New Roman, tamaño 12, al correo electrónico rcciquifa_fcbog@.unal.edu.co o a través del sistema OJS en el sitio web: https://revistas.unal.edu.co/index.php/rccquifa.

Todo el material propuesto para publicación será revisado por el Comité Editorial, y luego de su aceptación para evaluación, será sometido a revisión por pares académicos. Las evaluaciones recibidas por el Comité Editorial serán remitidas al autor para que se realicen los ajustes sugeridos. Los autores tendrán un lapso de cuatro semanas para responder cada una de las observaciones, introducir en el texto las modificaciones del caso y retornar el documento corregido al Comité Editorial. Después de que los evaluadores o el Comité Editorial lleven a cabo una segunda revisión del manuscrito, la revista comunicará a los autores la decisión sobre la publicación o no del mismo.

Los manuscritos deberán acompañarse de una carta firmada por todos los autores en la cual se declare que el trabajo es inédito; es decir, que el artículo ni parte de este ha sido publicado o está en vía de publicación en otra revista, y en la que se responsabilizan por la información publicada.

Se entiende claramente que los trabajos enviados no están siendo considerados para su publicación en otros medios.

Criterios para la presentación de manuscritos

- La fuente de los manuscritos debe ser Times New Roman tamaño 12.
- No se admiten notas a pie de página.
- Título, centrado y en minúscula.
- Autor(es) con su respectiva filiación institucional y correo electrónico del autor principal.
- Resumen, Summary y Resumo. Se debe presentar de forma estructurada e incluye la justificación del estudio y los principales hallazgos y conclusiones, debe tener entre 50 y 200 palabras. Se debe incluir el título del artículo y el resumen en inglés (Summary) y portugués (Resumo) independiente del idioma del manuscrito.
- Palabras clave (debajo del Resumen), Keywords (debajo de Summary) y Palavras-chave (debajo de Resumo). De tres a seis palabras en minúscula, excepto la primera, y separadas por punto y coma y espacio. Preferiblemente tomadas del *MeSH Tools for finding Keywords* (https://www.nlm.nih.gov/mesh/authors.html)
- En los manuscritos en español y portugués se empleará la coma (,) como separador decimal, en inglés se utilizará el punto (.).
- Los artículos se dividirán en las siguientes secciones: Introducción, Metodología, Resultados y Discusión, Conclusiones (si las hay), Agradecimientos, Conflicto de intereses y Referencias (Las secciones y subsecciones deben estar numeradas de forma arábiga). Los

títulos deben estar separados por dos espacios en sus partes superior e inferior, centrados y en negrilla. Los detalles acerca de la metodología experimental utilizada deberán ser lo suficientemente claros como para repetir la experimentación.

- Las tablas deben llevar numeración arábiga de acuerdo con el orden de aparición en el texto. El título debe ir en su parte superior y las notas en la parte inferior. En los encabezamientos de las columnas se deben anotar los símbolos de las unidades utilizadas.
- Las fotografías, gráficas, dibujos y esquemas se denominan 'figuras' y deben llevar numeración arábiga de acuerdo con el orden de aparición en el texto. El título debe ir debajo de las figuras.

De otro lado, los artículos relacionados con experimentación con animales deben ajustarse plenamente a los lineamientos éticos trazados por la Organización Mundial de la Salud. Los extractos o fracciones evaluados *in vitro* o *in vivo* deben definirse químicamente, cuando menos en cuanto a la clase de constituyente. El material vegetal deberá estar clasificado botánicamente.

Las abreviaturas de pesos y medidas serán las indicadas por la Farmacopea de los Estados Unidos en su edición oficial o unidades SI. Los datos espectroscópicos se deben presentar de la siguiente manera:

UV λ_{max} (solvente ε) nm (log ε). Ej.: UV λ_{max} (MeOH) 275 (log ε 2,94).

IR ν_{max} (medio) cm^{-1}. Ej.: IR ν_{max} (KBr) 1740, 1720 cm^{-1}.

EM *m*/*z* (% intensidad relativa). Ej.: EM *m*/*z* (%): 340 (M^+, 100), 295 (10), 134 (26),...

RMN ^1H (solvente, frecuencia de registro) δ ppm (integración, multiplicidad, J en Hz, asignación). Ej.: RMN ^1H ($CDCl_3$, 400 MHz) 3,84 (^1H, δ, J = 10,3 Hz, H-30).

RMN ^{13}C (solvente, frecuencia de registro) δ ppm (multiplicidad, asignación). Ej.: RMN ^{13}C ($CDCl_3$, 600 MHz) 16,60 (t, C-12).

Las abreviaturas usadas para describir la multiplicidad de las señales en RMN son: *s* = singlete, *d* = doblete, *t* = triplete, *m* = multiplete, *dd* = doble de dobletes, *ddd* = doble de doble de dobletes.

Las abreviaturas para los solventes y reactivos más comúnmente usados son: EtOH= etanol, MeOH = metanol, $CHCl_3$ = cloroformo, C_6H_6 = benceno, AcOEt = acetato de etilo, EP = éter de petróleo, Me_2CO = acetona, DMSO = dimetilsulfóxido, AcOH = ácido acético.

Referencias en los manuscritos

Las referencias se citarán en el texto con su respectiva numeración. Solo se pueden citar tesis y libros o artículos que hayan sido publicados. Es necesario indicar el número doi o el link de todas las referencias si están disponibles.

Revistas: Iniciales del nombre y apellido completo de todos los autores, título completo del artículo, nombre abreviado o nombre completo de la revista dependiendo si aparece en el *Chemical Abstract* o en índices equivalentes. El nombre completo de la revista debe ir en letra itálica, volumen en negrilla, páginas inicial y final, año entre paréntesis, y doi. Ej.:

1. H.P. Baden, L.A. Goldsmith & B. Fleming. A comparative study of the physicochemical properties of human keratinized tissues. *Biochimica et Biophysica Acta (BBA) - Protein Structure*, **322**(2), 269–278 (1973). https://doi.org/10.1016/0005-2795(73)90303-6

Comunicaciones personales: Iniciales del nombre, apellido completo e institución, seguido por las palabras comunicación personal y el año. Ej.:
2. A.J.M. Leeuwenberg, Agricultural University, Wageningen, Holanda, comunicación personal, 1984.

Libros: Iniciales del nombre y apellido completo de los autores, título del libro en letra itálica, editorial, ciudad, año, volumen y página. Ej.:
3. D.R. Morris. *The Biochemistry of Disease*. Morris et Marton Eds., London, 1981; Vol. 8, p. 223.

Capítulos de libros escritos por varios autores: Iniciales del nombre y apellido completo del autor, título del capítulo, seguido de: En: editores, título del libro en letra itálica, editorial, ciudad, año, volumen, páginas. Ej.:
4. A.D. Elbein & R.J. Molyneux. The chemistry and biochemistry of simple indolizidine and related polyhydroxy alkaloids. En: S.W. Pelletier (editor). *Alkaloids: Chemical and Biological Perspectives*. Wiley, New York, 1987; Vol. 5, pp. 1-54.

Tesis: Iniciales del nombre y apellido completo de los autores, título en cursiva, Tesis de…, institución, ciudad, año, páginas. Ej.:
5. F. Salcedo. *Contribución al estudio de las Cinchonas colombianas*. Tesis de Grado. Universidad del Valle, Cali, 1983; pp. 14-16.

Referencias de Internet: Iniciales del nombre y apellido completo del autor, título del documento en letra itálica, dirección URL y fecha de revisión. Ej.:
6. Lipidat. *Lipid thermotropic phase transition database*. Ohio State University. URL: http://www.lipidat.chemistry.ohio-state.edu. Consultado en septiembre de 2001.

La correspondencia puede enviarse a la siguiente dirección o al correo de la revista:

Comité Editorial
Revista Colombiana de Ciencias Químico-Farmacéuticas
Departamento de Farmacia
Facultad de Ciencias
Universidad Nacional de Colombia
Cra. 30 N.° 45-03, Ed. 450, Of. 310
Fax: 57-1-3165060
Bogotá D. C., Colombia

Correo electrónico: rcciquifa_fcbog@unal.edu.co

Dirección WEB: http://www.revistas.unal.edu.co/index.php/rccquifa