

Quality evaluation and dissolution profile of hydrochlorothiazide tablets available in Salvador-BA, Brazil

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SUMMARY

Introduction: hydrochlorothiazide is a diuretic drug indicated for the continuous treatment of hypertension. The drug is presented as a medicine in the form of tablets containing only one active agent or combined in formulations with concentrations of 12.5, 25 and 50 mg. The 25 mg dosage is the most widely used in medical therapy. Therefore, considering the incidence of hypertension and prolonged use of the drug, it is necessary to determine the quality of hydrochlorothiazide tablets available on public and private pharmacies of Salvador, Bahia. Complaints regarding the compromised quality of the products stored in drugstores are reported very often. **Material and Methods:** following the drug monograph, and consequently the pharmacopoeial methods, this study aimed to carry out quality control, physicochemical and microbiological testing of medicines containing hydrochlorothiazide, including the trade name (reference - R), generic (G) and similar (S) medicine, which is provided by **National Health Service** (aka SUS - *Sistema Único de Saúde*). **Results:** it was observed that the samples of the medicines: R, G and S samples showed complies with the pharmacopoeial tests, except for the assay of generic which content presented out of specification.

Keywords: Hydrochlorothiazide; quality control; monograph; tablets.

RESUMEN

Evaluación de calidad y perfil de disolución de tabletas de hidroclorotiazida disponibles en Salvador-BA, Brazil

Introducción: la hidroclorotiazida es un fármaco diurético indicado para el tratamiento continuo de la hipertensión. El fármaco se presenta como medicamento en forma de pastillas que contienen un solo principio activo o combinados en formulaciones con concentraciones de 12,5, 25 y 50 mg. La dosis de 25 mg es la más utilizada en terapia médica. Por lo tanto, considerando la incidencia de hipertensión arterial y el uso prolongado del medicamento, es necesario determinar la calidad de las pastillas de hidroclorotiazida disponibles en las farmacias públicas y privadas de Salvador, Bahía. Las quejas sobre la calidad comprometida de los productos almacenados en las farmacias se informan con mucha frecuencia. **Material y Métodos:** siguiendo la monografía de medicamentos y, en consecuencia, los métodos de la farmacopea, este estudio tuvo como objetivo realizar controles de calidad, pruebas fisicoquímicas y microbiológicas de medicamentos que contienen hidroclorotiazida, incluido el nombre comercial (referencia - R), genérico (G) y medicamento similar (S), que es proporcionado por el Servicio Nacional de Salud (también conocido como SUS - Sistema Único de Saúde). **Resultados:** se observó que las muestras de los medicamentos: muestras R, G y S cumplieron con las pruebas de la farmacopea, excepto el ensayo de genéricos cuyo contenido presentó fuera de especificación.

Palabras clave: Hidroclorotiazida; control de calidad; monografía; pastillas.

RESUMO

Avaliação da qualidade e perfil de dissolução de comprimidos de hidroclorotiazida disponíveis em Salvador-BA, Brasil

Introdução: a hidroclorotiazida é um fármaco diurético indicado para o tratamento contínuo da hipertensão. O fármaco se apresenta como medicamento em forma de comprimidos contendo um único princípio ativo ou combinados em formulações com concentrações de 12,5, 25 e 50 mg. A dose de 25 mg é a mais utilizada na terapia médica. Portanto, considerando a incidência de hipertensão arterial e o uso prolongado do medicamento, é necessário determinar a qualidade dos comprimidos de hidroclorotiazida disponíveis nas farmácias públicas e privadas de Salvador, Bahia. Reclamações sobre a qualidade comprometida dos produtos

armazenados nas farmácias são muito frequentes. **Material e Métodos:** seguindo a monografia do medicamento e, conseqüentemente, os métodos da farmacopeia, este estudo teve como objetivo realizar o controle de qualidade, testes físico-químicos e microbiológicos de medicamentos contendo hidroclorotiazida, incluindo o medicamento comercial (referência - R), genérico (G) e similar (S), que é fornecido pelo Serviço Nacional de Saúde (também conhecido como SUS - Sistema Único de Saúde). **Resultados:** observou-se que as amostras de medicamentos: amostras R, G e S atenderam aos testes de farmacopeia, exceto o teste do genérico cujo conteúdo estava fora da especificação.

Palavras-chave: Hidroclorotiazida; controle de qualidade; monografia; comprimidos.

INTRODUCTION

Systemic Arterial hypertension (SAH) is a chronic disease and a multifactorial clinical condition. SAH is characterized by sustained elevation of blood pressure (≥ 140 and 90 mmHg). It is often associated with metabolic disorders, functional and/or structural alterations of target organs, and it is aggravated by the presence of other risk factors such as dyslipidemia, obesity, glucose intolerance and diabetes [1].

Data from the World Health Organization (WHO) indicates there are about 1.13 billion people worldwide with hypertension [2]. In Brazil, it is estimated that the pathology affects 23.9%, corresponding to 38.1 million people [3], besides being more than 65% of the elderly (≥ 60 yo) in the country [1].

SAH is a major cause of premature death worldwide, with upwards of 1 in 4 men and 1 in 5 women [2]. In Brazil, around 389 thousand people die daily due to hypertension [4].

One of the drugs of first choice for the treatment of SAH, heart failure and edema of a variety of etiologies is hydrochlorothiazide (HCTZ) [1]. This drug is a moderate-acting diuretic, acting on the initial portion of the distal tubule, blocking sodium, chlorine and water reabsorption [5].

HCTZ, chemically known as 2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-3,4-dihydro-, 1,1-dioxide; 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, is a white or almost white, crystalline powder (Figure 1). It presents molecular formula $C_7H_8ClN_3O_4S_2$, Chemical Abstracts Service (CAS) registry number [58-93-5] and relative molecular mass equal to 297.7. HCTZ is very

slightly soluble in water, soluble in acetone, sparingly soluble in ethanol (96 per cent). It dissolves in dilute solutions of alkali hydroxides and it shows polymorphism [6-8].

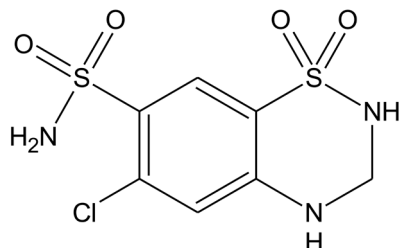


Figure 1. Hydrochlorothiazide chemical structure

This drug is listed in the National List of Essential Drugs (RENAME), in the concentrations of 12.5 mg and 25 mg [9] and it is part of the drugs that must be offered continuously to the **National Health Service** [10]. Commercially, HCTZ is available as uncoated tablets. The medicine is found in Brazilian market at concentrations of 50 mg, 25 mg and 12.5 mg and it may be associated with other drugs.

HCTZ tablets have good oral bioavailability (65 to 75%), with an onset of effect between 1 and 2 hours after administration, peaks in about 4 hours and lasts about 6 to 12 hours; the excretion is urinary [11].

Many factors can compromise the quality of medicines, such as the use of improper-quality of raw material and/or packaging material, the use of irregular manufacturing processes, and factors that may affect its storage and transport stability. In this context, to prevent possible deviations, quality control tests are mandatory.

The objective of this study was to evaluate the quality of hydrochlorothiazide 25 mg tablets obtained in Salvador-BA, in order to verify if the conditions of identity, content, purity, efficacy and harmlessness were attended and maintained in the post-commercialization.

Material and Methods

The reference standard was a Pharmaceutical Secondary Standard (PSS), HCTZ, batch no. C01-20101011, with 100.1% content, kindly provided by NCQ-UNIFAL-MG. The samples of HCTZ uncoated tablets, 25 mg (label claim), were identified as R (reference), G (generic) and S (similar medicine) (n=90 units each). All HCTZ tablets were acquired in commercial and public pharmacies in Salvador-Bahia, in 2018. For ethical reasons, the names of the medicine manufacturers were not informed.

According to the medicine leaflets, in addition to hydrochlorothiazide, R was composed by lactose monohydrate, corn starch, pregelatinized corn starch and magnesium stearate; G by lactose monohydrate, corn starch, pregelatinized corn starch and magnesium stearate; and S were constituted by sodium lauryl sulfate, sodium croscarmellose, sodium starch glycolate, lactose, microcrystalline cellulose, stearate magnesium and silicon dioxide.

The experimental design of this research was carried out with the samples within the expiration date and based on the monographs of HCTZ active pharmaceutical ingredient (API) and/or tablets, from the Brazilian [6], European [7] and American [8] Pharmacopoeias.

All solvents were at least analytical grade, and the following were used: purified water, sodium hydroxide - NaOH (Neon), culture media (Isofar) - *Soybean Casein Digest Broth* (TSB), Soyabean Casein Digest Agar (Tryptone Soya Agar - TSA), Sabouraud-Dextrose Agar (SDA), MacConkey Agar, Xylose Lysine Deoxycholate Agar (XLD), Bismuth Sulfite (BS) Agar, Mannitol Salt Phenol Red Agar (MSA), Cetrimide Agar and Rappaport Vassiliadis Salmonella (RVS) Enrichment Broth (Merck). To biochemical characterization were used Triple Sugar Iron (TSI) Agar; Simmons' Citrate Agar; Sulfur, Indole and Motility (SIM) Agar and Methyl Red (MR) test.

The equipments used were analytical balance (Ohaus, Explorer model), ultrasound (Cristófoli), disintegrator (Nova Ética, model 300), UV/Vis spectrophotometer (Shimadzu, model UV-1800), dissolution tests system (Nova Ética, model 300-1).

Standard Solution: Amount of 30.0 mg of HCTZ PSS were weighted and dissolved in 50 mL of 0.1 M sodium hydroxide. Posterior dilutions were performed with water as solvent.

Test Solution: Twenty tablets of each HCTZ sample (R, G and S) were weighed and pulverized. Amount equivalent to 30.0 mg were dissolved in 50 mL of 0.1 M sodium hydroxide and dilute to 100.0 mL with the same solvent. Test solutions were homogenized and filtered. Posterior dilutions were performed with water as solvent.

Identification by UV absorption spectrophotometry: identification tests were performed as described in the individual HCTZ API monographs [6, 8]. The samples were compared to PSS and also evaluated in spectral range from 250 to 350 nm. The HCTZ tablets complies with the test if similar spectra pattern are obtained and/or absorption maxima at 273 nm and 323 nm are found with absorbance ratio (A_{273}/A_{323}) equal 5.4 to 5.7.

Weight determination: Twenty HCTZ tablets were used. Each unit was weighed on analytical balance, and the mean weight was then calculated. To the specification of this test, up to two out-of-range units were tolerated in relation to the mean weight, and no unit could be above or below twice the percentages indicated [6, 8-12].

Hardness test: Ten units of each sample (R, G, S) were submitted to the equipment and the force needed to disrupt them by crushing were measured. All results were expressed in newtons (N).

Friability test: Twenty HCTZ tablets were carefully dedusted prior to testing. The samples were accurately weighed and placed in a friabilometer drum. The drum was than rotated 100 times (25 rpm/min), and after all, removed the tablets. Removing any loose dust from the tablets as before, the tablets were again accurately weighed. Friability are represented by the difference between tablets initial mass and final one. HCTZ tablets with a loss of less than 1.5% weight are considered acceptable. If cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, the samples fail the test [6]. A maximum loss of mass (obtained from a single test or from the mean of 3 tests) not greater than 1.5 per cent is considered acceptable for most products.

Disintegration tests: Six HCTZ tablets were placed in each 6 tube of the basket-rack assembly. The immersion fluid was water, maintained at $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ in 1-liter beaker. The equipment was activated up to complete tablets disintegration, with a specified time limit of 30 minutes. The requirements of the test are met if all dosage units tested have disintegrated [6, 8-12].

Uniformity of dosage units: Content uniformity was determined by analyzing the individual amount of the active substance in 10 HCTZ tablets taken at random. The evaluation were performed by spectrophotometric assay in concentration of $10\text{ }\mu\text{g}\cdot\text{mL}^{-1}$, at the wavelength of 273 nm, using 0.1 M NaOH and water as solvent. The value of acceptance was calculated [6, 8]. The pharmaceutical product complies Uniformity of dosage test if the Acceptance Value calculated for the 10 units tested is not greater than L1 ($L1 = 15.0$).

Dissolution Studies: Dissolution testing was performed using apparatus 1 (basket) at rate of 100 rpm and 900 mL of 0.1 M hydrochloric acid (HCl) (dissolution medium). The medium was kept at temperature of $37 \pm 0.5\text{ }^{\circ}\text{C}$ [6, 8]. Aliquots of 10.0 mL were manually sampled at 30 minutes [6]. In addition, dissolution profiles were obtained: aliquots of 10.0 mL were removed at 5, 10, 15, 20, 30, 40, 50, and 60 minutes. In the study with multiple sampling times, the aliquots withdrawn were replaced with equal volumes of fresh dissolution medium at $37\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$. The collected aliquots were filtered and transferred to amber vials for subsequent evaluation by UV spectrophotometry

at a wavelength of 272 nm, using 0.1 M HCl as solvent [6, 8]. The absorbance values were converted to concentrations by comparison to PSS values. The percentage of HCTZ released by time were also calculated considering the amount of drug removed in each aliquot and the dilution caused by the replacement of dissolution medium in dissolution profile studies. The requirements are met if not less than 60% (Q) of the labeled amount of hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) is dissolved in 30 minutes [6].

Microbiological Examination of Non-Sterile Products

All microbiological tests were carried out under aseptic conditions designed to avoid extrinsic microbial contamination of the HCTZ product.

Microbial enumeration test (Total Aerobic Microbial Count – TAMC and Total Combined Yeasts/Moulds Count - TYMC): the estimative number of viable microorganisms were performed by plate count method. From each sample - R, G and S, the equivalent of 5 g of HCTZ were weighed and transferred to erlenmeyer containing 45 mL of TSB (1:10). After 10 minutes homogenization, 1 mL of the suspension were placed into a set of 4 sterile Petri dishes. Then, 15 mL of TSA and SDA were added in duplicate in the dishes. The TSA plates were incubated at 30-35 °C for 2 days and plates containing SDA were maintained at 20-25 °C for 7 days [6, 8-12]. After incubation, the arithmetic mean per culture medium of the counts were evaluated and the number of colonies forming units (CFU) per gram of HCTZ per sample were calculated. Only plates with less than 250 for TAMC and 50 for TYMC were considered. Samples comply with the test if they present a maximum of 10^3 CFU/g for aerobic bacteria and 10^2 CFU/g for yeasts/mould [6, 8-12].

Test for Specified Microorganisms: in order to better understand the microorganisms that can contaminate pharmaceutical forms and trigger health problems were performed tests for *Escherichia coli*, *Salmonella sp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [6, 8-12]. The first phase of the test was carried out in a 1:10 dilution non-selective enrichment, performed from the same suspension previous described in TSB, mixed and incubated at 30-35 °C for 24h. The second phase, selective, was performed as described below for each pathogen:

Escherichia coli - from non-selective enrichment, an aliquot of 0.1 mL was taken and transferred to 10 mL of MacConkey Broth and incubated at 42-44 °C for 24 hours. Subculture for pathogen isolation and selection was performed by streaking technique in MacConkey Agar, followed by incubation at 30-35 °C for 24 hours.

Salmonella sp. - after sample preparation and pre-incubation, were transferred 0.1 mL of TSB to 10 mL of RVS and incubated at 30-35 °C for 24 h. Subculture for isolation

and selection was carried out on plates containing XLD Agar, and BS Agar incubated at 30-35 °C for 24 hours.

Pseudomonas aeruginosa - from first phase was inoculated by surface streaking method a suitable amount on a plate of Cetrimide Agar and incubated at 30-35 °C for 24 hours.

Staphylococcus aureus - from sample preparation and pre-incubation, subculture was proceeded for isolation and selection. It was performed on a plate of mannitol salt agar and incubated at 30-35 °C for 24 hours. For interpretation: the presence of growth of colonies leads to identification tests by their typical morphological aspect. In this case, a set of biochemical tests are carried out for each pathogen. Pharmacopoeia stat the product complies with the test if no colonies are present or if the identification tests are negative [6, 8-12].

Biochemical Identification Tests were performed according to Brazilian [6], European [12] and American [8] pharmacopoeias, using TSI; Simmons' Citrate and SIM Agars and MR test.

Assay: The powder amount equivalent to 15 mg of HCTZ was weighed from crushed tablets and solubilized in 0.1 M NaOH with final concentration of 300 $\mu\text{g}\cdot\text{mL}^{-1}$. This solution was homogenized, filtered and diluted with water to a concentration of 10.0 $\mu\text{g}\cdot\text{mL}^{-1}$. A further evaluation of PSS solutions was also performed at concentration of 10.0 $\mu\text{g}\cdot\text{mL}^{-1}$. All evaluations were carried out in triplicate, measuring solutions absorbance at 273 nm. Water was used for zero adjustment. The drug content was calculated comparing the results, as specified by the Brazilian Pharmacopoeia [6]. The Brazilian Pharmacopoeia the specification for this test is at least 93.0% and at most 107.0% of the declared amount of HCTZ [6]. In United States Pharmacopoeia HCTZ tablets should contain not less than 90.0% and not more than 110.0% of the labeled amount of $\text{C}_7\text{H}_8\text{ClN}_3\text{O}_4\text{S}_2$.

Results and discussion

Tablets are solid preparations representing more than 80% of all pharmaceutical forms used currently [13]. They are obtained by compressing uniform volumes of particles or by another suitable manufacturing technique, and are intended for oral administration.

In the manufacture of tablets, evaluations are accomplished to ensure they possess a suitable quality. However, problems in the packaging, storage and distribution of tablets may happen. Despite this, post-commercialization tests are rare. Therefore, appropriated evaluations should be taken to assess the quality and its maintenance.

The HCTZ tablets were subjected to physical-chemical and microbiological tests to identification; weight determination, friability, disintegration, uniformity of dosage units; dissolution; microbial enumeration and specified microorganisms research; and assay. For each sample: reference, generic and similar, the following results were obtained.

The HCTZ tablets evaluated in this study were all white, straight and solid cylinders. The end surfaces of which tablet were flat and the edges were beveled. They have break-marks in one side and were uncoated tablets with diameters of 7.0 mm.

Besides to these non-pharmacopoeial characteristics, identification tests were performed. Spectra obtained from the three HCTZ samples followed same spectral pattern of PSS, with absorbance ranging in UV region and maximum absorption at 273 and 323 nm, as specified, allowing confirmation of the HCTZ presence in the R, G and S drug tablets (Figure 2). Additionally, absorbance ratios were calculated and the results shown average value equal to 5.4.

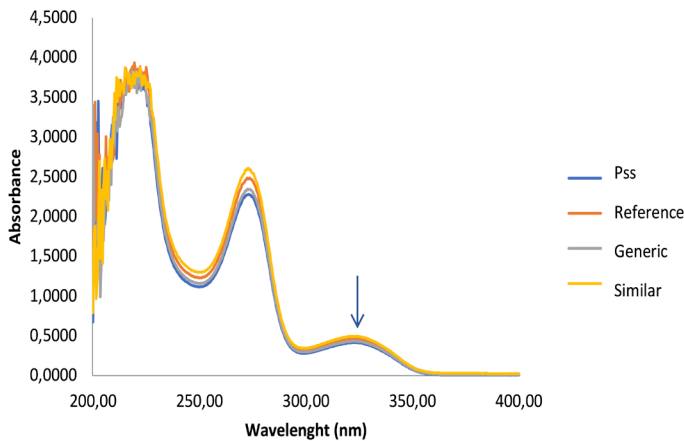


Figure 2. Spectra overlap of HCTZ PSS and samples

Regarding weight determination, all bath samples evaluated presented the mean weight within the acceptable range established by Pharmacopoeias (7.5%) [6, 8-12]. For the R medicine, the average weight determined was 110.1 mg, with a relative standard deviation (RSD) of 1.54%. For G tablets the average weight was 122.4 mg with RSD of 1.99%. For S units the average weight was 122.5 mg with 1.26% RSD (Figure). From the results, it can also be said that there was a good mass uniformity in the tablets, as the relative standard deviation (RSD) was low (less than 2.0%) for all samples.

Weight determination is a simple test but with significant importance for quality control. Since tablet mass and active ingredient amount are directly proportional, very large variations in weight may lead to incorrect doses. Consequently, it may promote absence of expected therapeutic action, in case of lower dosage, and side effects and overdose, in case of higher dosage [14]. Likewise, it is important to observe the minimum variation between the tablets weight. This criteria represents homogeneity of manufacturing. As in the case of this work the samples analyzed remained within the limits and presented relative standard deviations very low. However, the isolated study of this parameter is not indicative of dose regularity between the tablets and, for this purpose, the unit dose uniformity test has to be performed.

The mechanical strength of tablets can be assessed in two main ways, hardness (resistance to crushing of tablets or tablet breaking force) and friability, since this pharmaceutical form must present a certain resistance to not break, crack or detach powders during production, storage and/or transportation [6, 15].

The results obtained by resistance to crushing of tablets were 101.2; 34.0 and 83.5 N, respectively, from R, G and S HCTZ tablets. Although tablet breaking force is an informational test, the results presented the mean minimum value of the force needed to crush each sample.

Friability test is a measurement of tablet friability. It is a supplementary test to assess physical strength of medicine, usually analyzed together with tablet breaking force [12]. The friability is a test that observes particles detachment of product, allowing to determine the resistance of the tablets to abrasion, when subjected to the mechanical action of specific apparatus [6]. This test is fundamental during the production process, as it allows the necessary adjustments to ensure a good tablet cohesion.

By analyzing the data obtained in the present research, it can be observed that the mass loss after the test were 0.28% for R, 0.05% for G and 0.06% for S tablets. Although the mass loss of the reference drug is higher than other samples, the percentage remains within the acceptable upper limit of 1.5% [6]. So the results founded corroborate the adequate manufacture of the tablets under analysis.

Disintegration test is provided to determine whether tablets disintegrate within the prescribed time when placed in a liquid medium under the experimental conditions [12]. It is, therefore, a time-dependent process that occurs under the action of a disintegrating agent [13].

For the purposes of this test, disintegration does not imply complete dissolution of the unit or even of its active constituent. Complete disintegration is defined as that state

in which any residue of the unit remaining on the screen of the test apparatus is a soft mass having no palpably firm core [6, 12].

In cases where disintegration does not occur within the specified maximum time of 30 min for HCTZ tablets [6, 16], there is a risk that the unit will be disposed of unchanged, without absorption of the active ingredient and therefore without expected therapeutic effect.

In this study, R samples were disintegrated in 1 min 28 s, G in 7 min 29 s and S in 1 min 12 s. It can be observed that all samples complied the requirement disintegrating in less than 30 minutes. However, the generic drug presented a time of 7 min 29 s, while the other two samples are below 1 min 30 s. Once R and G presents qualitatively the same excipients, may the compression force have used influenced these tablets disintegration.

Uniformity of dosage units was performed by uniformity of content of single-dose preparations, based on the assay of the individual contents of HCTZ in ten units. It was determined whether the individual contents were within limits set with reference to the average content of the sample [6, 12].

The values found (Table 1) were then replaced in the formula: $AV = |98.5 - \bar{X}| + ks$, for the Acceptance Value (AV) calculation, wherein: \bar{X} corresponds to the average of the individual contents of 10 tablets; K is the acceptability constant equal to 2.4 ($n=10$); and S is the standard deviation of dosing [6]. Performing the calculations, the AV were 10.1 for R, 15.0 for G and 12.3 for S HCTZ samples. Thus, despite the borderline value for G tablets all samples passed the test.

Table 1. Results from Uniformity of dosage units

Tablet #	ASSAY - CONTENT (%)		
	Reference (R)	Generic (G)	Similar (S)
1	96.52	88.91	90.92
2	91.98	87.39	94.75
3	91.59	89.75	93.65
4	96.33	86.21	98.39
5	99.09	87.05	93.29
6	94.94	85.87	96.93
7	95.93	88.23	95.84
8	96.72	91.78	87.28
9	96.13	91.44	93.11
10	90.40	91.44	93.11
Average	94.96	88.81	93.11

(Continúa)

Tablet #	ASSAY – CONTENT (%)		
	Reference (R)	Generic (G)	Similar (S)
RSD (%)	2,89	2,50	3,33
s	2,74	2,22	3,13
AV	10.1	15.0	12.3

From a clinical point of view, it is important for therapy that the variation between the doses present in the medicines keep minimal, since the lack of uniformity may lead to incorrect bioavailability and, consequently, to therapeutic failure or exacerbation of effects.

Dissolution tests is provided to determine compliance with the dissolution requirements for solid dosage forms administered orally. The Brazilian [6] and United States [8] Pharmacopoeias recommend that not less than 60% (Q) of the labeled amount of HCTZ must be dissolved within 30 and 60 minutes, respectively. Since the percentages released in these two times were 90.04 and 99.05 for R, 93.46 and 99.00 for G and 82.31 and 95.23 for S, all samples comply the official compendia recommendations.

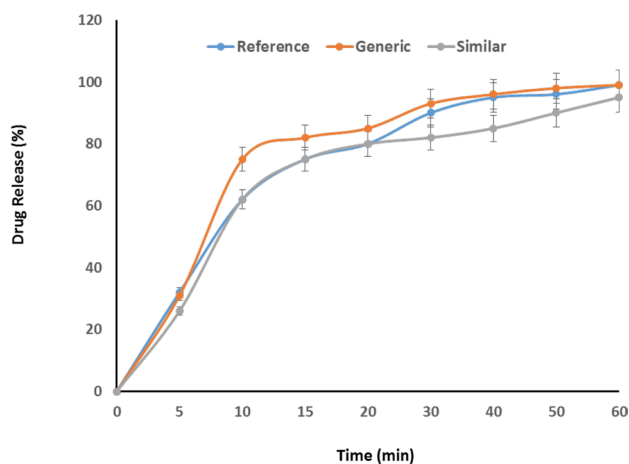


Figure 3. Dissolution profiles of HCTZ tablets.

On the other hand, as can be seen in Figure 3, the levels (the mean values \pm standard deviations) of HCTZ in the dissolution medium for R, G and S samples multipoint analysis followed different patterns. Between 5 and 30 minutes, the G sample showed greater HCTZ release. After 30 min, the dissolution profiles of R and G tablets superimposed, indicating similar dissolution up to the end of the test. S tablets presented analogous dissolution profile of R, until 20 minutes, after that the HCTZ release was lower than those observed for the other samples, but still adequate, considering therapeutics purpose.

Clearly, the dissolution profile reveals the quality of medicines in a broader sense, giving evidence of their bioavailability. In fact, since hydrochlorothiazide is very poorly soluble in water, technological problems or those related to the formulation can compromise the dissolution profile and, consequently, the bioavailability, thereby the study of dissolution is essential.

Microbiological tests are an important part of quality control evaluation many times suppressed for non-sterile pharmaceutical products. However, microbiological contamination frequently changes pharmaceutical product quality, in addition to may interfere in its stability, which consequently creates problems in drug therapy. Moreover, microbial interference may transform APIs and excipients to less or more potent or chemically inactive forms. So, contamination by microorganisms, mainly at the storage and distribution stages may hinder the consumer safety [6, 17].

All data, achieved by means of compendial microbiological and biochemical analyses as described in the methods, have been analyzed in context to the recommended microbial limits and the user safety.

The microbial enumeration test allow quantitative register of mesophilic bacteria and fungi that may grow under aerobic conditions. It are designed primarily to determine whether HCTZ tablets samples complies with the established specification for non-sterile pharmaceutical products.

Count the number of total microorganisms - after the incubation period, the CFU were verified. Growth was observed for the three samples analyzed. All HCTZ samples grew within the limits established of 1000 CFU/g for total aerobic bacteria and 100 CFU/g for molds and yeasts [6, 8-12], however, it was observed that G sample presented the highest and borderline average of contamination molds and yeasts (100 CFU/g), which may cause risks to the product and patients, once it may compromise its physical-chemical stability and facilitate infections and health problems [18].

To identify specified microorganisms the samples were streaked on surface of selective media for selection and isolation of *E. coli*, *Salmonella sp.*, *P. aeruginosa* and *S. aureus*.

In the inoculations performed on Cetrimide Agar to identify the presence of *P. aeruginosa*, only S sample showed bacterial growth in selection and isolation phases (Figures 5A and 5C), but none of the positivity characteristics for this bacterium could be observed. According to Brown and Lowbury [19], the growth on Cetrimide Agar is considered positive for *P. aeruginosa* when it is possible to observe colony with blue pigmentation, characteristic of the pyocyanin production, or blue-green pigmentation surrounding the colonies, indicative of the production of fluorescein or both pigments.

Subsequent biochemical tests were performed on isolated colonies. Negative results from Cetrimide Agar with R sample were observed.

The selection and isolation of *E. coli* on MacConkey Agar for S and R samples showed intense growth of rounded and reddish colonies, indicating the probable presence of this microorganism in those medicaments. The colonies were further submitted to biochemical tests for confirmation.

The G HCTZ sample showed no growth on Cetrimide and MacConkey Agar, which allows to conclude that *P. aeruginosa* and *E. coli* were absent in these medicines.

Regarding the tests performed to investigate the presence of Salmonella sp. no growth was observed by the analyzed samples, on both XLD and BS Agar.

In the research for *S. aureus* on MSA, G sample presented not only growth, but also a very characteristic aspects of this pathogen - unctuous yellow colonies and changed the color of agar, which switched from red to yellow [19]. The reason for this color change is that *S. aureus* has the ability to ferment mannitol, producing an acid, which changes the indicator color [6, 20].

Biochemical identification tests were carried out with the objective to increase efficient microbiological control and improve assurance for the quality of pharmaceutical products [12]. It were carried out on TSI Agar; on Simmons' Citrate Agar; SIM Agar and by MR test.

Biochemical characterization of an unknown microorganism is the classical approach to identification [12] and were performed on all colonies isolated from HCTZ R, G and S samples. The results obtained are shown in Table 2.

The negative results from indole (SIM) and MR for the suspected colonies of *E. coli* exclude their presence in the samples analyzed in this work. The tests performed on the HCTZ S sample show the probable presence of fermenter microorganisms of the genera *Enterobacter* or *Serratia*, while the reactions to the reference drug did not allow identifying the probable genus.

From the suspicious growth of *S. aureus* in the HCTZ G sample, the coagulase test was performed, which was negative, discarding the presence of *S. aureus* in this sample.

Although all samples have complied the specifications determined by the Pharmacopoeias [6, 8-12], the presence of microorganisms in drugs, even below pharmacopoeial limits, is extremely undesirable. Viable microorganisms can promote the formulation degradation through their enzymes or even noticeable changes that can cause infec-

Table 2. Results from biochemical tests

Samples	Media					MR
	TSI	Simmons' Citrate	SIM			
			S	I	M	
S from MacConkey Agar	+ growth, +gas, acid reaction throughout the medium	+	-	-	+	-
R from MacConkey agar	+growth, - gas, acid reaction at base and alkaline on sloping surface	-	-	-	+	-
S from Cetrimide Agar	+growth, + gas, acid reaction at base and alkaline on sloping surface	+	-	-	+	-

Legend: TSI: Triple Sugar Iron Agar; Sulfur, Indole and Motility (SIM) Agar and Methyl Red (MR) test; +: positive result; -: negative result.

tious processes in patients with compromised immune system, elderly and children [21]. Therefore, it can be stated that the efficacy and safety of medicines are directly related to their physicochemical and microbiological quality.

Assay - HCTZ was measured in the tablets samples by UV spectrophotometric method, at 273 nm, using 0.1 M NaOH and water as solvents. Despite the pharmacopoeial indication that assays should be performed in concentration of $15 \mu\text{g}\cdot\text{mL}^{-1}$, in practice (Table 3), it was observed that this concentration is at the limit of Lambert-Beer Law and would not guarantee the correct correlation between absorbance and content [22].

Table 3. PSS concentrations and absorbances obtained.

Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	Absorbance
1	0.057
5	0.278
10	0.594
15	0.826
20	1.120

Thus, a working concentration of $10 \mu\text{g}\cdot\text{mL}^{-1}$ was used to perform the assay. The samples were evaluated in triplicate and the following absorbance values were obtained (Table 4).

Table 4. Results from assay of R, G and S samples

Sample	R	G	S	PSS
Absorbance	0.583	0.515	0.561	0.589
	0.588	0.510	0.546	0.597
	0.586	0.532	0.563	0.594
Average	0.586	0.519	0.557	0.593
RSD (%)	0.35	1.81	1.36	0.56
Content (%)	98.81	87.56	93.91	100.1

Legend: R: reference; G: generic; S: similar sample; PSS: Pharmaceutical secondary standard; RSD: relative standard deviation.

The Brazilian Pharmacopoeia (2019) [8] establishes a minimum of 93.0% and a maximum of 107.0% of the declared quantity of HCTZ. The following assay results were found: 98.81% for R samples, 87.56% for G medicines and 93.91% for S tablets. Generic samples were the below stated by the Brazilian Pharmacopoeia, and the results still remains without acceptable limits when evaluated by the US Pharmacopoeia [16], that established a range of 90.0 to 110.0%.

Analyzing the dosing values obtained by the unit dose uniformity test, Table 1, which followed the same analytical procedure, it can be observed that even with slightly higher average, 88.8% content, none of the assayed tablets from G samples has reached the minimum limit set for the content. Thus, the assays found in the unit dose uniformity test match the results found in this test and disapprove the generic drug for its content.

Stability tests should be additionally made to evaluate loss of HCTZ active substance in the storage and distribution stages. To make that possible, stability indicating analytical method should be developed and validated to fully assess the quality of HCTZ tablets.

CONCLUSIONS

In this work, results obtained from quality control methods for pharmaceutical application in HCTZ tablets were described. For each method, the basic principle was stated and the methods were discussed along with any critical aspects to be considered. Potential uses that may be envisaged based on the principles of the methods concerned were given, but it is not intended to suggest that such applications have been exhaustive contemplated.

It is the intention of this paper to give a general vision of the importance of quality evaluation in pos-commercial to guarantee pharmaceutical quality control.

The information herein may be used, therefore, as an alert for health managers, in the sense of the importance of maintaining the ideal conditions of storage and transport, as well as for the population, so that they keep up the proper conditions of conditioning.

Also, this paper this article draws attention to the need for development and validation stability indicative analytical methods for monitoring the quality of HCTZ tablets.

According to the results obtained it can be concluded that all samples analyzed have the active ingredient hydrochlorothiazide. In general, all the samples presented good performance in the physical-chemical tests, with approval in the tests of weight determination, uniformity of dosage units, friability and disintegration. However, the assay of Generic tablets showed a non-compliance, presenting a content below that recommended by the Pharmacopoeias.

For the microbiological tests, it was observed that all samples had UFC/g count within the established limits for aerobic bacteria and molds and yeasts. Regarding the tests carried out to investigate pathogenic microorganisms, despite the growth of Reference and Similar samples in MacConkey media, biochemical tests excluded the presence of *Escherichia coli* and indicated the presence of microorganisms of the genus *Enterobacter* or *Serratia* for the Similar drug.

Quality control is an important health promotion tool. The conducting tests during and after the production and marketing process is essential to ensure that medicinal products have and maintain the characteristics necessary to ensure their safety and effectiveness. Quality evaluations of drugs, such as the one carried out in this study, corroborate in this field since they are carried out without conflicts of interest.

Finally, this work demonstrates the relevance of Quality Control, because the medicines containing hydrochlorothiazide available on the market comply (in part) with pharmacopoeial requirements to safely and effectively.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

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