Technological research article / https://doi.org/10.15446/rcciquifa.v53n3.119126

Compatibility study of clonazepam and excipients in solid pharmaceutical formulations

Franciny Aparecida Alves[1], Renan Marcel Bonilha Dezena[2], Sidnei da Silva Beltrão[1], Paulo César Pires Rosa[1,2]*

[1]Faculty of Pharmaceutical Sciences at The State University of Campinas (UNICAMP), Campinas-SP, Brazil.

[2]Department of Pharmacology, Faculty of Medical Sciences at The State University of Campinas (UNICAMP), Campinas-SP, Brazil.

*Corresponding Author: Paulo César Pires Rosa. Faculty of Pharmaceutical Sciences at The State University of Campinas (UNICAMP), Campinas-SP, Brazil. Phone: 5519991158286. Email: paulocpr@unicamp.br. Address: R. Candido Portinari, 200 - Cidade Universitária, Campinas - SP, 13083-871. Orcid Number: 0000-0002-5896-0093.

Received: March 17, 2023
Corrected: April 8, 2024
Accepted: April 15, 2024

Summary

This study has had as main objectives to apply the analysis via fourier-transform infrared spectroscopy (FTIR), thermogravimetry (TG), differential scanning calorimetry (DSC) to verify the degradation and alteration of the functional groups of clonazepam, due to their importance as a characterization tools, and to use a validated method for the quantification of clonazepam, with a new chromatographic analysis with no use of buffer in the mobile phase to be applied in the studies of stability of pharmaceutic formulations and in the analysis of degradation products. The results obtained from the thermal analysis have showed that the studied excipients did not reveal changes in the thermal behavior of the binary mixtures, clonazepam excipient, with no indication of incompatibility. The analytic method developed by high performance liquid chromatography (HPLC) has been shown adequate for quantification of clonazepam and its impurities. The implementation of thermal analysis and the application of new analytic methods have been considered important strategies in the diverse areas of the pharmaceutical industry, providing information that define the technological quality parameters of the products, aiming the development of new formulations.

Keywords: Clonazepam, Fourier-transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC), thermogravimetry (TG), High performance liquid chromatography (HPLC), Compatibility Study.

Resumen

Estudio de compatibilidad del clonazepam y excipientes en formulaciones farmacéuticas sólidas

Este estudio ha tenido como principales objetivos aplicar el análisis mediante espectroscopía infrarroja por transformada de Fourier (FTIR), termogravimetría (TG), calorimetría diferencial de barrido (DSC) para verificar la degradación y alteración de los grupos funcionales del clonazepam, debido a su importancia como herramientas de caracterización, y utilizar un método validado para la cuantificación de clonazepam, con un nuevo análisis cromatográfico sin uso de tampón en la fase móvil para ser aplicado en los estudios de estabilidad de formulaciones farmacéuticas y en el análisis de productos de degradación . Los resultados obtenidos del análisis térmico han mostrado que los excipientes estudiados no revelaron cambios en el comportamiento térmico de las mezclas binarias, excipiente clonazepam, sin indicios de incompatibilidad. El método analítico desarrollado por cromatografía líquida de alta resolución (HPLC) se ha mostrado adecuado para la cuantificación de clonazepam y sus impurezas. La implementación del análisis térmico y la aplicación de nuevos métodos analíticos han sido consideradas estrategias importantes en las diversas áreas de la industria farmacéutica, proporcionando información que define los parámetros tecnológicos de calidad de los productos, visando el desarrollo de nuevas formulaciones.

Palabras clave: Clonazepam, espectroscopia infrarroja por transformada de Fourier (FTIR), calorimetría diferencial de barrido (DSC), termogravimetría (TG), cromatografía líquida de alta resolución (HPLC), estudio de compatibilidad.

Resumo

Estudo de compatibilidade de clonazepam e excipientes em formulações farmacêuticas sólidas

Este estudo teve como principais objetivos aplicar a análise via espectroscopia de infravermelho por transformada de Fourier (FTIR), termogravimetria (TG),

calorimetria exploratória diferencial (DSC) para verificar a degradação e alteração dos grupos funcionais do clonazepam, devido à sua importância como ferramenta de caracterização, e utilizar um método validado para quantificação do clonazepam, com uma nova análise cromatográfica sem uso de tampão na fase móvel para ser aplicada nos estudos de estabilidade de formulações farmacêuticas e na análise de produtos de degradação. Os resultados obtidos na análise térmica mostraram que os excipientes estudados não revelaram alterações no comportamento térmico das misturas binárias, excipiente clonazepam, sem indícios de incompatibilidade. O método analítico desenvolvido por cromatografia líquida de alta eficiência (CLAE) mostrou-se adequado para quantificação do clonazepam e suas impurezas. A implementação de análises térmicas e a aplicação de novos métodos analíticos têm sido consideradas estratégias importantes nas diversas áreas da indústria farmacêutica, fornecendo informações que definem os parâmetros tecnológicos de qualidade dos produtos, visando o desenvolvimento de novas formulações.

Palavras-chave: Clonazepam, Espectroscopia de infravermelho com transformada de Fourier (FTIR), Calorimetria diferencial de varredura (DSC), Termogravimetria (TG), Cromatografia líquida de alta eficiência (HPLC), Estudo de compatibilidade.

Introduction

It is a benzodiazepine derivative widely administered as an anxiolytic, anticonvulsant, muscle relaxant and sedative anesthetic [1-3]. In order for quality control to guarantee the efficacy and safety of the use of a drug, the use of sensitive analytical methods is essential [4-6].

The choice of method to be used in the analysis depends on several factors, such as the nature of the drug, purity and sample amount [7]. In addition, the laboratory conditions and the costs involved in the analysis must be taken into account.

Among the methods for this purpose, spectroscopic, chromatographic and thermal methods stand out [8-10]. In this context, thermal analysis constitutes a group of techniques of great interest in the pharmaceutical area, since it allows obtaining relevant data regarding the thermal behavior of drugs and pharmaceutical ingredients, in a relatively short time, fundamental for the evaluation of possible incompatibilities and for the development of new products [11,12].

The main thermoanalytical techniques applied in this area are differential scanning calorimetry (DSC), differential thermal analysis (DTA) [13, 14]. The application of

thermal analysis for this purpose is recent and has grown significantly in the last ten years [15, 16]. This fact motivates the evaluation of the thermal stability of this drug and drug/excipient compatibility.

The evaluations of drug-excipient interaction, through the compatibility study, were complemented through the technique of high-performance liquid chromatography (HPLC), which can be performed by analyzing the degradation product of a drug [17].

Due to the therapeutic relevance of clonazepam and its wide commercialization, the development of the present work becomes important in order to contribute to the evaluation of stability through the analysis of assay and formation of clonazepam degradation products complementing the study of interactions between clonazepam and excipients through the techniques of differential scanning calorimetry, differential thermal analysis, fourier-transform infrared spectroscopy.

Material and methods

Drug + excipient interaction study

According to Table 1, in the preparation of the binary mixtures (weight ratio of 1:1) the individual mass of each component was weighed, added separately to a porcelain capsule and ground with the drug for 2 minutes.

Table 1. Binary mixtures.

Mixtures	Components
1	Clonazepam + Lactose
2	Clonazepam + Microcrystalline Cellulose
3	Clonazepam + Starch
4	Clonazepam + Magnesium Stearate

Fourier-transform infrared spectroscopy (FTIR)

The sample was analyzed in an infrared equipment, brand Agilent Cary 6300 FT-IR, using a diffuse reflectance module to obtain the spectrum.

The absorption spectra were generated in the region of 4000 to 400 cm^{-1}, in total there were 32 spectra.

Thermogravimetry (TG)

The sample was analyzed in a TG equipment, brand Mettler Toledo, with Software STARe SW, version 10.0.

For this test, the excipients were analyzed alone and in combination with the drug. Before each thermogravimetric test, a blank was performed with the sample pan empty for the evaluated condition.

Conditions for TG: temperature range: 25 to 300 °C; gas: N_2 at 100 mL/min; heating ratio: 10 °C·min^{-1}; pan: platinum

Differential scanning calorimetry (DSC)

Sufficient amount of clonazepam was weighed in an alumina pan, then covered in a DSC equipment, brand Shimadzu, model DSC-60. In addition to clonazepam, the other raw materials and binary mixtures were tested.

Conditions for DSC: temperature range: 25 to 300 °C; gas: N_2 at 100 mL/min; heating ratio: 10 °C·min^{-1}; pan: alumina

High performance liquid chromatography (HPLC)

The HPLC experiments were initiated based on the American Pharmacopoeia, whose mobile phase is constituted by a mixture of phosphate/methanol/tetrahydrofuran buffer in the proportion of 600:520:130 v/v/v.

Due to the high amount of buffer, a new mobile phase was developed, consisting of acetonitrile/water in the proportion 600:400 v/v.

The proportions between the mobile phase constituents, injection volume, mobile phase flow rate and column analysis temperature were evaluated.

The analysis conditions tested were: injection volumes: 5 to 50 μL; column temperatures: 25 to 40 °C; flow rates: 0.8 to 1.5 mL/min; elution modes: isocratic and gradient; wavelength: 254 nm; analytical columns: C8 – 125 × 4.6 mm – 5 μm / C8 – 150 × 4.6 mm – 5 μm / C8 – 250 × 4.6 mm – 5 μm

The column was previously stabilized with the mobile phase for 30 minutes under the respective pre-established conditions.

Before the start of each analysis, a solution containing clonazepam, CR A (related compound A), CR B (related compound B) was injected in order to observe the following parameters: N (number of theoretical plates) ≥ 2000; T (asymmetry) between 0.8 and 1.2; R (resolution) ≥ 2; and the coefficient of variation between injections ≤ 2 %, in order to verify the suitability of the system.

Optimized chromatographic conditions: injection volumes: 30 μL; column temperatures: 25 °C; flow rate: 1.0 mL/min; elution modes: isocratic; wavelength: 254 nm; analytical column: C8 – 250 × 4.6 mm.

Stability Studies

The preparation of the simulated sample of excipients was performed, as described in Table 2. The samples were stored in climatic chambers, under the following conditions of temperature and humidity: 40 ± 2 °C / 75% ± 5% RH and 30 ± 2 °C/ 75% ± 5% RH for 30, 60 and 90 days.

Table 2. Simulated sample of excipients.

Components	Amount (mg)
Clonazepam	2.00
Lactose	121.50
Microcrystalline cellulose	18.70
Starch	20.40
Magnesium stearate	1.70

The prepared solutions were evaluated as described below:

- Clonazepam standard solution: 10.00 mg of clonazepam standard was added and transferred to a 100.0 mL flask. Approximately 5.0 mL of diluent was added and it was taken to ultrasound until complete dissolution. The volume was made up with diluent (0.1 mg/mL).
- Clonazepam stock sample solution 85.00 mg of clonazepam sample was weighed and transferred to a 10.0 mL flask. Approximately 5.0 mL of diluent was added and it was taken to ultrasound until complete dissolution. Make up to volume with diluent (0.1 mg/mL).

The solutions were injected into the chromatograph to observe the possible reduction or increase in the drug assay and/or formation of degradation products.

Results and discussion

Fourier-transform infrared spectroscopy (FTIR)

The spectra of clonazepam in the infrared were shown in Figure 1. The observed peaks are in agreement with the chemical structure of clonazepam, with the identification of the main functional groups in Table 3.

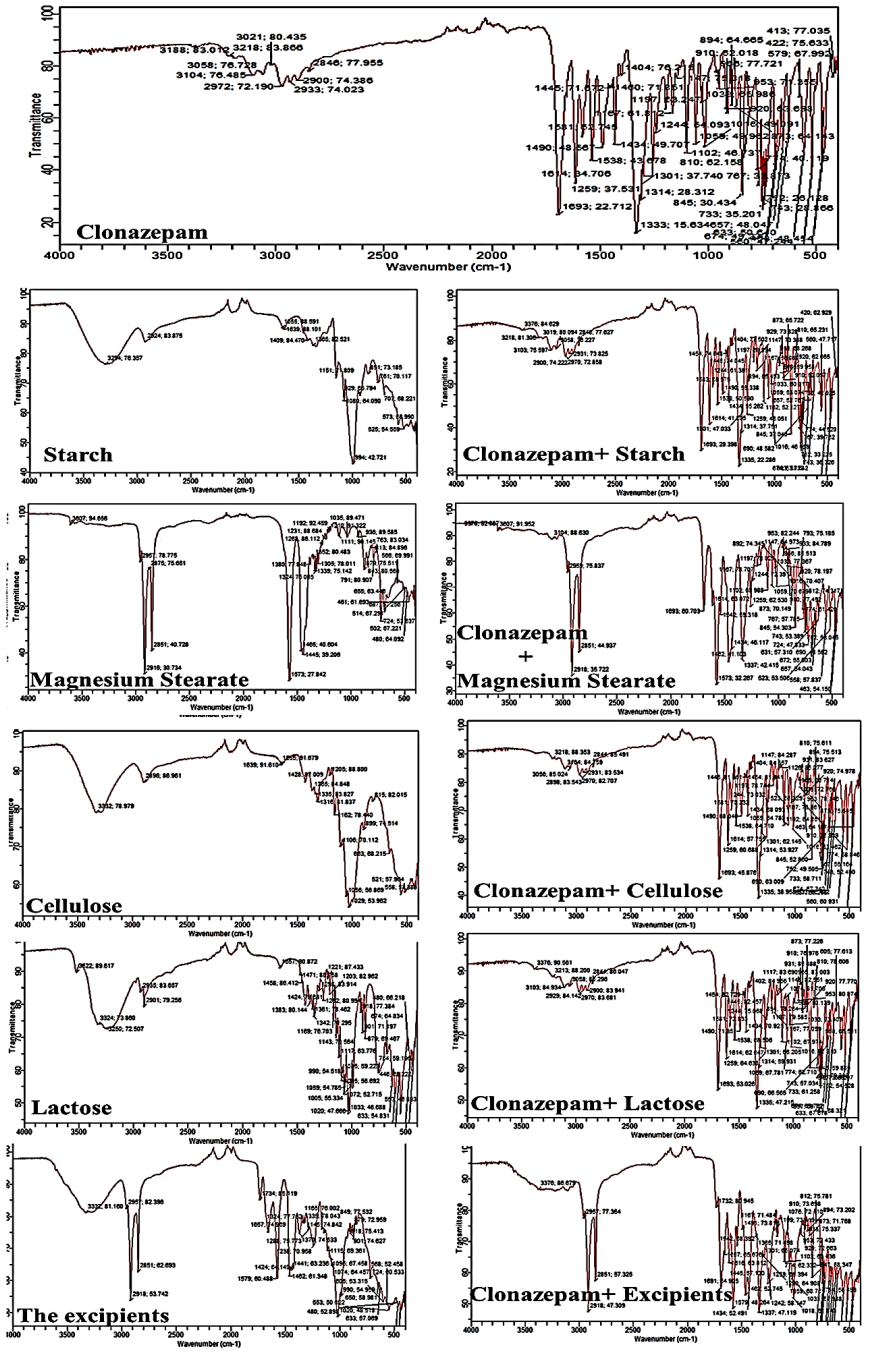

Figure 1. The individual and binary mixtures FTIR spectrum of clonazepam and excipients.

Table 3. Absorption of the main functional groups for Clonazepam.

Absorption range cm-1	Functional Group
1693	C = O deformation, amide
1614	C=N deformation
1538	C=C deformation, aromatic
1490	C=C deformation, aromatic
1445	C=C deformation, aromatic
1333	N-O_2 deformation
752	aromatic ring deformation

The spectrum obtained from the binary mixture between clonazepam and starch, the main identification bands of clonazepam are observed, 1693 cm^{-1}, 1614 cm^{-1}, 1538 cm^{-1}, 1490 cm^{-1}, 1445 cm^{-1}, 1335 cm^{-1} and 752 cm^{-1}. The starch bands are superimposed on the clonazepam bands, but it is possible to observe a widening of the bands close to 3200 cm^{-1} of the O-H bond deformation. The data obtained by FTIR for mixing clonazepam with starch are not indicative of incompatibility.

The spectrum obtained from the binary mixture between clonazepam and magnesium, the main identification bands of clonazepam are observed, 1693 cm^{-1}, 1614 cm^{-1}, 1542 cm^{-1}, 1491 cm^{-1}, 1434 cm^{-1}, 1337 cm^{-1} and 724 cm^{-1}. Magnesium stearate bands are also observed at 2918 cm^{-1} and 2851 cm^{-1}, 1573 cm^{-1}, 1462 cm^{-1}, 1102 cm^{-1}, indicating that there is no incompatibility between these studied inputs.

The spectrum obtained from the binary mixture between clonazepam and microcrystalline cellulose, the main identification bands of clonazepam are observed, 1693 cm^{-1}, 1614 cm^{-1}, 1538 cm^{-1}, 1490 cm^{-1}, 1434 cm^{-1} and 1335 cm^{-1}. The bands that identify cellulose are in overlapping regions with the clonazepam bands, and it is not possible to evaluate them individually. Data obtained by FTIR for mixing clonazepam sodium with cellulose are not indicative of incompatibility.

In the spectrum of the mixture of clonazepam with lactose monohydrate, the clonazepam identification bands are observed at 1693 cm^{-1}, 1614 cm^{-1}, 1538 cm^{-1}, 1490 cm^{-1}, 1434 cm^{-1} and 1335 cm^{-1}. The bands that identify lactose are in overlapping regions with the clonazepam bands, and it is not possible to evaluate them individually. Data obtained by FTIR for blending clonazepam with lactose are not indicative of incompatibility.

The spectrum of the mixture between clonazepam and all the excipients, some of the main identification bands of clonazepam are observed 1691 cm^{-1}, 1616 cm^{-1}, 1542 cm^{-1}, 1491 cm^{-1}, 1434 cm^{-1} and 1337 cm^{-1}. It is also possible to observe some bands of excipients, namely, 2918 cm^{-1} and 2851 cm^{-1} from magnesium stearate.

The main clonazepam bands and bands from the excipients are visualized with minimal shifts compared to the individual spectra. The data obtained by infrared spectroscopy show that the excipients are compatible with clonazepam under the conditions tested, since the similarity of the bands were in agreement with the literature and there was no formation of other bands in the spectrum.

Thermogravimetry (TG)

The clonazepam TG curve, Figure 2, shows only one event in the range of 272.83 °C to 324.54 °C due to decomposition (19.1% of mass loss) with a midpoint at 292.37 °C.

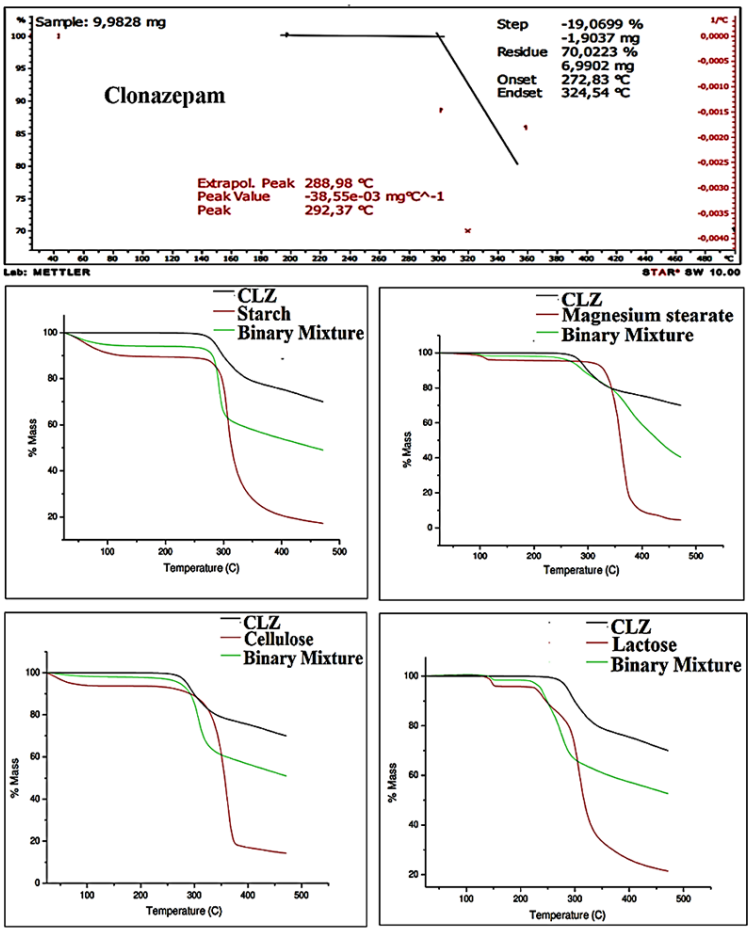

Figure 2. The individual and binary mixtures TG curves of clonazepam and excipients.

Table 4 presents a summary of the data obtained for the individual curves and binary mixtures. It is observed in the TG that the mass loss from the excipients was due to dehydration and decomposition and for clonazepam due to decomposition. The TG curves are similar to the individual curves, not indicating incompatibility.

Table 4. Discussion of results for the TG for Clonazepam and excipients.

Sample	Temperature Range (°C) Onset – Endset	Weight Loss (%)	Event
Clonazepam	272.83 – 324.54	19.1	Decomposition
Starch	35.5 °C – 96.38	9.4	Dehydration
	294.5°C – 322.5	82.6	Decomposition
Clonazepam + Starch	28.9 – 85.11°C	5.7	Dehydration
	283.82 – 300.21	32.3	Decomposition
Magnesium Stearate	99.71 – 146.0	2.48	Dehydration
	343.09 –440.0	22.89	Decomposition
	449.08 – 492.046	0.51	Decomposition
Clonazepam + Magnesium Stearate	93.73 – 109.49	1.39	Dehydration
	268.26 – 297.64	8.65	Decomposition
	353.82 – 415.85	33.46	Decomposition
Microcrystalline Cellulose	28.78 – 71.84	5.80	Dehydration
	337.24 – 371.17	85.58	Decomposition
Clonazepam + Microcrystalline Cellulose	37.7 – 96.02	1.81	Dehydration
	291.42 – 322.31	48.96	Decomposition
Lactose	140.95 – 151.24	5.80	Dehydration
	229.60 – 252.59	6.78	Decomposition
	295.06 – 332.51	52.00	Decomposition
Clonazepam + Lactose	142.75 – 150.39	1.71	Dehydration
	233.380- 253.03	9.41	Decomposition
	268.95 – 291.95	15.39	Decomposition

Differential scanning calorimetry (DSC)

The clonazepam has only one endothermic event, related to melting, with temperature ranges of T_{onset} = 237.38 °C and T_{peak} = 237.36 °C, Figure 3, followed by decomposition according to an exothermic event close to 300 °C.

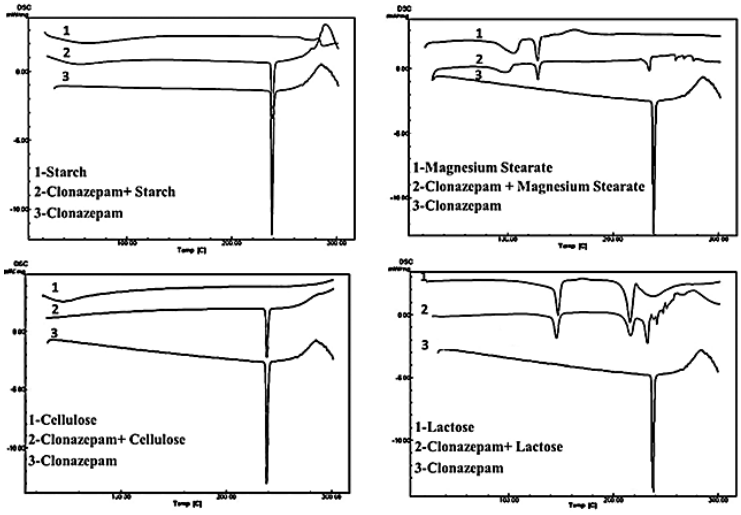

Figure 3. The individual and binary mixtures DSC curves of clonazepam and excipients.

Table 5 presents a summary of the data obtained for the individual and binary mixtures DSC curves (Figure 3). It is observed in the curve of the binary mixtures that the enthalpies for the loss of moisture from the excipients and the melting of clonazepam are proportional to the individual curves. The data indicate that the excipients and clonazepam are compatible under the conditions evaluated.

Table 5. Discussion of results for the DSC for Clonazepam and excipients.

Sample	Temperature (°C) (On set – Peak)	Enthalpy (J/g)	Event
Clonazepam	237.38 - 238.36	113.01	Fusion
Starch	22.8 - 63.29	286.43	Water Loss
Clonazepam + Starch	24.6 - 55.83	110.63	Water Loss
	237.49 - 238.96	53.51	Fusion
Magnesium Stearate	88.77 - 105.82	97.97	Water Loss
	125.35 - 128.90	42.12	Fusion
Clonazepam + Magnesium Stearate	85.55 - 97.97	57.87	Water Loss
	126.92 - 128.93	25.05	Fusion
	231.45 - 234.43	21.42	Fusion (Clonazepam)
Microcrystalline Cellulose	27.68 - 45.19	84.38	Water Loss

(*Continued*)

Table 5. *Continuation.*

Sample	Temperature (°C) (On set – Peak)	Enthalpy (J/g)	Event
Clonazepam + Microcrystalline Cellulose	31.09 - 57.84	2.36	Water Loss
	237.27 - 238.73	53.48	Fusion (Clonazepam)
Lactose	143.31 - 146.99	130.39	Water Loss
	211.62 - 215.88	128.12	Fusion
	222.27 - 237.07	101.20	Decomposition
Clonazepam + Lactose	142.08 - 145.39	51.42	Water Loss
	211.37 - 216.49	54.17	Fusion / Decomposition
	228.96 - 232.61	40.71	Fusion (Clonazepam)

High performance liquid chromatography (HPLC)

In the studies carried out, the data obtained from the thermal analysis were compared with the results of assay and related substances by HPLC (validated method) as shown in Figure 4 and Figure 5, allowing greater security in the interpretation of the data and also enriching the work with a comparative that challenges the efficiency of thermal analysis.

Through the analysis of the chromatograms of the standard solutions, sample, and placebo, it was observed that the proposed method has specificity, since it does not suffer interference from the excipients next to the clonazepam signal. In addition, the observed clonazepam peak purity is 0.99.

Peak purity calculations were performed by comparing the spectrum of the peak apex (reference spectrum, point that presents the highest concentration of the analyte and the best-defined spectrum), with several spectra sampled from the beginning to the end of the clonazepam peak, being collected a spectrum every 400 milliseconds.

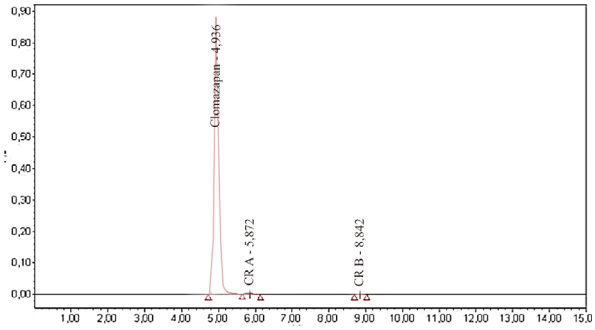

Figure 4. Chromatogram of clonazepam, CR A (related compound A), CR B (related compound B) Standards Solution.

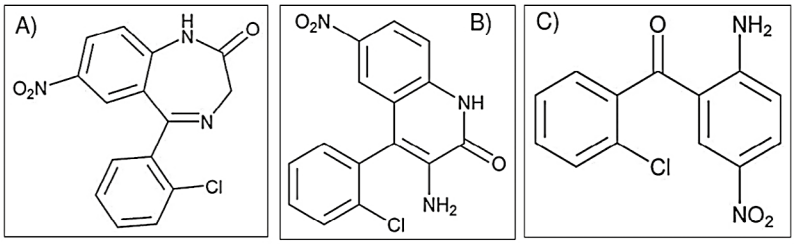

Figure 5. A: molecular structure of clonazepam. **B:** molecular structure of related compound A. **C:** molecular structure of related compound B.

From this total of spectra, an evaluation was carried out, counting all the spectra that presented at least 95% similarity with the reference spectrum and also calculating those that presented less than 95% of similarity.

When subjected to forced degradation, the chromatograms showed a reduction in clonazepam assay, and the formation of degradation peaks was observed in all types of hydrolysis (Figure 6 and Figure 7). When subjected to thermal and photolytic degradation, there was little variation in the assay for both the standard and the sample.

The compiled with the results of the forced degradation study of the standard and of the sample referring to clonazepam are shown respectively in tables 6 and 7.

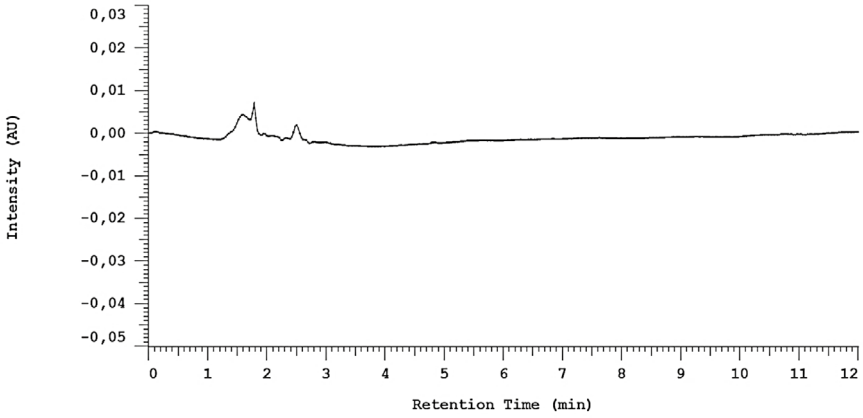

Figure 6. Chromatogram of placebo (Basic Degradation - NaOH 0.1 N).

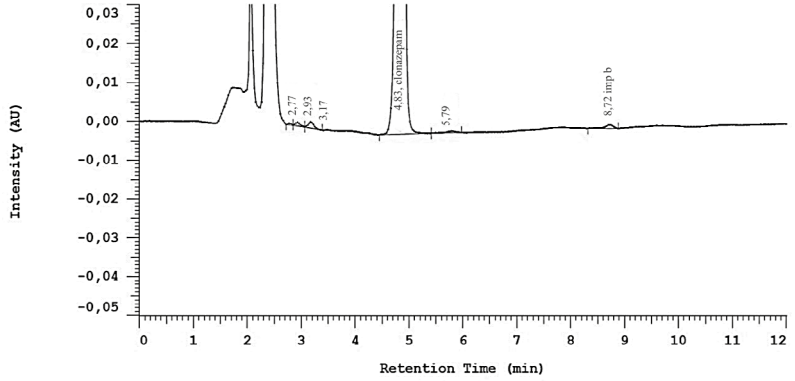

Figure 7. Chromatogram of clonazepam (Basic Degradation - NaOH 0.1 N).

Table 6. Standard forced degradation.

Conditions	Recovery (%) 24 hours	Degradation (%) 24 hours	Recovery (%) 48 hours	Degradation (%) 48 hours
Control Sample	100.0	0.0	100	0.0
Acid Degradation (HCl 0.1 N)	89.5	10.5	79.7	20.3
Basic Degradation (NaOH 0.1 N)	65.3	34.7	48.6	51.4
Oxidative Degradation (H_2O_2 3%)	94.8	5.2	93.8	6.2
Thermal Degradation (60 °C)	102.3	2.3	88.9	11.1
Photolytic Degradation (UV)	99.0	1.0	99.6	0.4

Table 7. Sample forced degradation.

Conditions	Recovery (%) 24 hours	Degradation (%) 24 hours	Recovery (%) 48 hours	Degradation (%) 48 hours
Control Sample	100.0	0.0	100	0.0
Acid Degradation (HCl 0.1 N)	91.6	8.4	79.9	20.1
Basic Degradation (NaOH 0.1 N)	64.8	35.2	48.6	51.4
Oxidative Degradation (H_2O_2 3%)	97.9	2.1	93.8	6.2
Thermal Degradation (60 °C)	103.0	3.0	98.1	1.9
Photolytic Degradation (UV)	99.7	0.3	98.9	1.1

Stability Studies

The samples submitted to temperatures of 30 °C and 40 °C did not show changes in their organoleptic and physical characteristics, when observed macroscopically. The percentage assay obtained at the temperature of 30 °C had minimal variations in the three tested periods. The observation of degradation products in this same condition was only observed in the final period of 90 days.

In samples submitted at a temperature of 40 °C, lower values of assay were observed when compared to the values obtained at a temperature of 30 °C, but without significant variations. Degradation products can be observed in the second evaluated period of 60 days.

The degradation products observed in the accelerated stability study according to Table 8 were also observed in the forced degradation study, without a considerable increase in them. There was no change or variation in the assay results (dosing) above 5% when compared to the initial result in both the 30 °C/75% of relative humidity and in the study at 40 °C/75% of relative humidity as demonstrated in Table 9.

Table 8. Formation of degradation products during the stability study.

Degradations Products	Tested Conditions		
	30 °C / 75% RH	40 °C / 75% RH	
	90 days	60 days	30 days
Related Compound A (RRT≈ 1.20)	x	x	x
Related Compound B (RRT≈1.81)	x	x	x
Unknown impurity (RRT≈0.45)	-	-	x
Unknown impurity (RRT≈0.88)	x	-	x
Unknown impurity (RRT≈0.93)	x	-	x

Table 9. Assay results during the stability study.

Tested Conditions	30°C / 75% RH	40°C / 75% RH
30 days	99%	98%
60 days	99%	97%
90 days	98%	95%

Conclusion

Based on the analyzed dataset, the drug-excipient compatibility performed through the DSC and TG techniques can be considered as the first-choice technique for evaluating compatibilities, due to its speed and versatility.

However, it must be associated with HPLC and FTIR, since these provided quantitative and qualitative information about the active ingredient, respectively. Thermal analysis provided quick results, but difficult to interpret. For this reason, the complementary techniques (FTIR, HPLC) were valid to confirm the results obtained by the thermal analysis and thus generate more reliable results.

According to the results obtained by DSC, TG, FTIR and HPLC, it was possible to identify that there were no significant changes in the isolated drug and in the 1:1 drug/excipient binary mixtures, indicating that there is no interaction between the tested excipients and clonazepam.

The HPLC method developed was optimized to obtain the best conditions for the identification and quantification of clonazepam, offering great advantages in terms of simplicity, since the mobile phase employed is easy and quick to prepare, which allows it to be used routinely in quality control analyses.

Another advantage is the fact that it does not contain salts in its composition, which could damage the column and the chromatographic system, in addition to allowing the identification and reproducible quantitative analysis of clonazepam with analytical reliability.

Through this study it was possible to unify the information about compatibility between clonazepam and excipients, highlighting the importance of these studies for the development of stable formulations.

Acknowledgments

The authors wish to thank the Extension and Research Support Fund (FAEPEX 2015/15) of the University of Campinas (UNICAMP) for the financial support.

Conflict of interest

All authors report that they do not have any conflicts of interest.

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How to cite this article

F.A. Alves, R.M. Bonilha-Dezena, S. da Silva-Beltrão, P.C. Pires-Rosa, Compatibility study of clonazepam and excipients in solid pharmaceutical formulations, *Rev. Colomb. Cienc. Quim. Farm.,* **53**(3), 667-685 (2024). Doi: https://doi.org/10.15446/rcciquifa.v53n3.119126