

Development and validation of a stability-indicating method for benznidazole and its degradation products by high-performance liquid chromatography

Flávia Patrícia Morais de Medeiros ¹, Talita Atanazio Rosa ², Maria Joanellys dos Santos Lima ² +, Larissa Araújo Rolim, Rosali Maria Ferreira da Silva ², Pedro José Rolim Neto ²

¹ Empresa Brasileira de Hemoderivados e Biotecnologia

² Universidade Federal de Pernambuco

Abstract. Benznidazole (BNZ) is the main trypanocidal drug used for the etiological treatment of Chagas disease. The development of stability-indicating analytical methods is important for monitoring the degradation of the drug and detecting degradation products formed under stress and storage conditions. To develop and validate a stability-indicating analytical method for the determination of BNZ and its degradation products by high-performance liquid chromatography (HPLC). BNZ was subjected to forced degradation under hydrolytic, oxidative, photolytic, and thermal conditions. An HPLC method was developed and optimized for the separation of BNZ and related compounds. The method was evaluated for selectivity, specificity, linearity, precision, accuracy, robustness, detection limit, and quantification limit. The method was subsequently applied to the evaluation of degradation products in BNZ 100-mg tablets subjected to an accelerated stability study. The method showed adequate chromatographic separation of BNZ and the related compounds 2-nitroimidazole and N-benzylchloroacetamide. The analytical response was linear over the range of 4–40 µg/mL, with coefficients of determination of 0.9955, 0.9961, and 0.9985 for 2-nitroimidazole, BNZ, and N-benzylchloroacetamide, respectively. The method showed satisfactory precision and accuracy, as well as robustness to the evaluated variations in chromatographic conditions. BNZ showed greater susceptibility to photodegradation and alkaline hydrolysis, while degradation under acidic, thermal, and oxidative conditions was comparatively lower under the conditions evaluated. Two degradation products were detected in BNZ tablets after accelerated stability testing, with concentrations of 0.156% and 0.200%. The developed HPLC method was suitable for the determination of BNZ and the monitoring of its degradation products under the evaluated conditions, demonstrating its applicability as a stability-indicating analytical method.

Keywords: Benznidazole; Chagas disease; degradation products; forced degradation; high-performance liquid chromatography; stability-indicating method; analytical method validation.

Introduction

Chagas disease is a neglected tropical disease caused by the protozoan *Trypanosoma cruzi*. Although historically endemic to 21 countries in Latin America, population migration and urbanization have contributed to the detection of cases in non-endemic regions, expanding the global public health relevance of the disease. The World Health Organization estimates that approximately 6–7 million people are infected worldwide, with millions of individuals at risk of infection (WHO, 2024).

Benznidazole (BNZ), an antiparasitic nitroimidazole derivative, is one of the main drugs used for the etiological treatment of Chagas disease and has been used clinically since the 1970s. In Brazil, BNZ is available as conventional tablets, including 100-mg tablets manufactured by the

Pharmaceutical Laboratory of the State of Pernambuco (LAFEPE). The drug therefore represents an important pharmaceutical product for the treatment of a neglected disease, particularly in regions where Chagas disease remains endemic.

The quality, safety, and efficacy of pharmaceutical products depend, among other factors, on the chemical stability of their active pharmaceutical ingredients. During storage and use, drug substances may undergo degradation through different pathways, including hydrolysis, oxidation, photolysis, and thermal degradation. The formation of degradation products may affect the quality of the pharmaceutical product and therefore requires appropriate analytical monitoring.

Forced-degradation studies, also known as stress testing, are commonly employed to

investigate the intrinsic stability of drug substances and pharmaceutical products under conditions that promote degradation. These studies are useful for identifying potential degradation pathways and for developing stability-indicating analytical methods capable of distinguishing the active pharmaceutical ingredient from its degradation products (Singh & Bakshi, 2000; Silva *et al.*, 2009).

For BNZ, the development of a stability-indicating analytical method is particularly relevant because degradation products generated under different stress conditions must be adequately separated from the active pharmaceutical ingredient to permit reliable evaluation of drug stability. Previous analytical studies have investigated the determination of BNZ; however, a comprehensive stability-indicating method capable of detecting and quantifying degradation products generated under different forced-degradation conditions remains relevant for quality control purposes.

Therefore, this study aimed to investigate the degradation behavior of BNZ under different

forced-degradation conditions and to develop and validate a stability-indicating HPLC method capable of detecting and quantifying BNZ and its degradation products. The applicability of the proposed method was subsequently evaluated in BNZ 100-mg tablets subjected to accelerated stability conditions.

Materials and methods

BNZ, *N*-benzyl-2-(2-nitroimidazole-1-yl)acetamide, Lot 15 772, as well as the working standard (BNZ 07-1051/000), were supplied by the Pernambuco State Pharmaceutical Laboratory (LAFEPE®) and manufactured by Roche Pharmaceuticals. A second BNZ working standard sample was donated by Nortec® Fine Chemicals, along with related compounds (RCs), synthetic precursors of BNZ: 2-aminoimidazole sulfate (2-AZS), 2-nitroimidazole (2-NIZ), and *N*-benzylchloroacetamide (NBCA) (Figure 1).

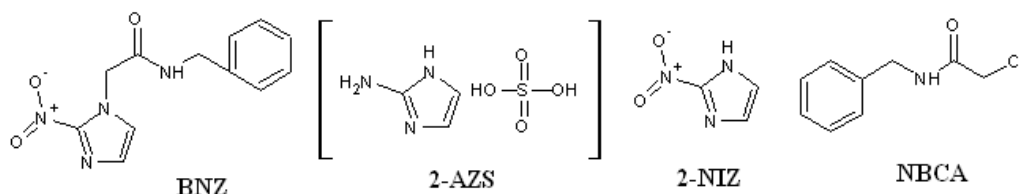


Figure 1. Chemical structures of BNZ and its related compounds used in the development and validation of the analytical method.

Forced degradation studies were conducted under individual stress conditions to evaluate the susceptibility of BNZ to hydrolytic, oxidative, photolytic, and thermal degradation and to support the development of a stability-indicating analytical method.

Hydrolytic degradation studies were conducted using BNZ raw material under acidic, basic, and neutral conditions. Acidic hydrolysis was performed using HCl, basic hydrolysis using NaOH, and neutral hydrolysis using ultrapure water. The samples were maintained at room temperature and protected from light, as outlined in Figure 2. Oxidative degradation was conducted using 3% hydrogen peroxide at room temperature, protected from light, for a maximum period of 30 days.

Solid samples of BNZ were exposed to the same irradiation dose in a thin layer (1 mm) on a Petri dish, under an exposure of 1.2 million lux·h and 200 Wh·m⁻².

In order to develop a stability-indicating analytical method, solid-state BNZ was thermally degraded by exposure to 60 °C in a calibrated oven for a maximum period of 30 days.

BNZ samples were prepared by weighing 10 mg of BNZ raw material into a 50-mL volumetric flask. Initially, 10 mL of acetonitrile was added, and the sample was sonicated for 5 min to promote dissolution. The volume was then made up to 50 mL

with ultrapure water, obtaining a stock solution at a final concentration of 200 µg/mL.

This solution was subsequently diluted with acetonitrile:water (1:4, v/v) to obtain a final concentration of 12 µg/mL.

BNZ standard solutions and related compounds were prepared similarly, using the respective substances to obtain a final concentration of 12 µg/mL.

Once the analytical methodology had been established, a volumetric calibration curve was prepared under the same analytical conditions used for the samples.

A BNZ working standard solution was prepared at a concentration of 200 µg/mL, followed by serial dilutions using the mobile-phase solvent system to obtain the calibration solutions.

The linearity study was performed using six concentration levels: 4, 8, 12, 16, 20, and 40 µg/mL.

Initially, solutions obtained from the degradation studies were analyzed separately by HPLC according to the parameters described by Silva *et al.* (2007), using a C18 column (25 cm × 4.6 mm, 5 µm) as the stationary phase and acetonitrile:water (50:50, v/v) as the mobile phase, at a flow rate of 1.0 mL/min and a column temperature of 30 °C.

As the separation of BNZ and its degradation products was not sufficiently selective

under the initial conditions, modifications to the analytical parameters were evaluated, including mobile-phase composition, pH, flow rate, and column oven temperature.

The optimized method consisted of an isocratic mobile phase of phosphate buffer:acetonitrile (80:20, v/v), adjusted to pH 2.7, at a flow rate of 1.0 mL/min, using a C18 column (25

cm × 4.6 mm, 5 µm) maintained at 40 °C. A diode-array detector (DAD) was used for chromatographic analysis, allowing evaluation of the UV spectra and peak purity of the chromatographic signals.

The optimized method was validated according to the parameters described in ICH Q2(R1) and the applicable Brazilian regulatory requirements for analytical method validation.

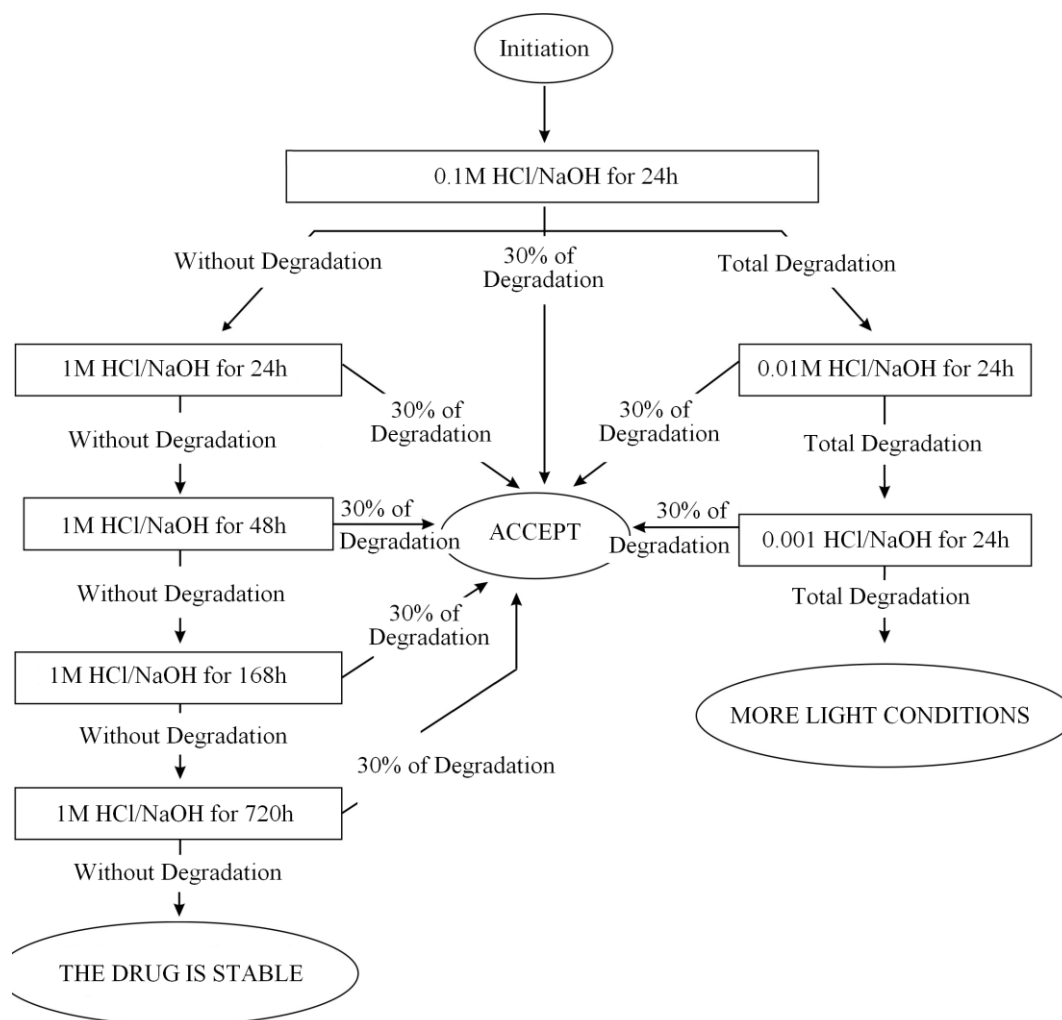


Figure 2. Representation of the methodology used for the hydrolytic degradation of BNZ.

The validation of the analytical method involved the evaluation of robustness, linearity and range, detection limit, quantification limit, precision, accuracy, specificity, and selectivity (ICH Q2(R1), 2005) and considering the current ICH Q2(R2) (2023) guideline. These parameters are also consistent with the analytical method validation requirements established by ANVISA Resolution RDC No. 166/2017, currently applicable in Brazil.

The reliability of the analytical parameters was evaluated using the coefficient of variation (CV%) or relative standard deviation (RSD). For the validation studies, a coefficient of variation of less than 5% was established as the acceptance

criterion. Statistical analyses were performed for each validation parameter.

Chromatographic performance was also evaluated based on resolution, column efficiency, capacity factor, and peak asymmetry, according to USP General Chapter <621>, Chromatography (USP, 2021).

Each analytical run was accompanied by a control calibration curve prepared from a stock solution at concentrations of 8, 12, and 16 µg/mL containing 2-aminoimidazole sulfate, 2-nitroimidazole, BNZ, and *N*-benzylchloroacetamide simultaneously.

Robustness was evaluated by assessing the ability of the analytical method to remain unaffected

by small and deliberate variations in the established analytical parameters. The following variables were evaluated:

- ✓ variation in the manufacturer of dipotassium hydrogen phosphate used as a component of the mobile phase, comparing Carlo Erba® and Sinth® brands;
- ✓ variation in the chromatographic column oven temperature at 39, 40, and 41 °C;
- ✓ variation in the mobile-phase flow rate at 0.99, 1.00, and 1.01 mL/min;
- ✓ evaluation of the stability of the sample solution after 24 h of storage at 2–8 °C.

Linearity was evaluated based on the ability of the analytical method to obtain results directly proportional to the concentration of the analyte within the established range.

Six concentration levels were evaluated: 4, 8, 12, 16, 20, and 40 µg/mL. BNZ was initially prepared in acetonitrile at a concentration of 200 µg/mL and subsequently diluted using the mobile-phase solvent system to obtain the calibration solutions.

Statistical analysis was performed using least-squares linear regression based on the mean response obtained for each concentration. Three independent calibration curves were prepared. The correlation coefficient (*r*) was calculated for each calibration curve.

The detection limit (LOD) was defined as the lowest amount of analyte in a sample that can be detected, but not necessarily quantified, under the established experimental conditions.

The quantification limit (LOQ) was defined as the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy under the established experimental conditions (ICH Q2(R1), 2005).

The LOD and LOQ were estimated from the standard deviation of the intercepts and the mean slope obtained from three independent calibration curves, using factors of 3 and 10, respectively.

Precision was evaluated by determining the degree of agreement among individual measurements obtained from multiple samples of the same homogeneous sample under the established analytical conditions (ICH Q2(R1), 2005).

Repeatability (intra-run precision) was evaluated by six independent determinations at the 100% concentration of the test. The analyses were performed by the same analyst using the same instrumentation under the same experimental conditions.

Intermediate precision (inter-run precision) was evaluated on two different days by different analysts within the same laboratory.

Reproducibility (inter-laboratory precision) was evaluated in two different laboratories, with three determinations performed in each laboratory.

Accuracy was evaluated after establishing linearity and range. BNZ raw material samples were

analyzed at concentrations of 8, 12, and 16 µg/mL, with three determinations performed at each concentration. The results obtained for the BNZ raw material were compared with those obtained using the BNZ working standard under the same analytical conditions.

Specificity was evaluated based on the chromatographic separation of BNZ from its related compounds. The retention time and relative retention time of BNZ and the related compounds 2-nitroimidazole (2-NIZ) and *N*-benzylchloroacetamide (NBCA) were evaluated using solutions containing the respective compounds.

The chromatograms obtained from BNZ samples subjected to different stress conditions were compared with those obtained from non-degraded BNZ samples. The BNZ peak was identified based on its retention time and UV spectrum obtained using the DAD detector. The same procedure was applied to the related compounds used as analytical standards.

Selectivity was evaluated by determining the purity of the chromatographic peaks corresponding to BNZ and its related compounds using a diode-array detector (DAD) over the wavelength range of 200–400 nm.

Peak purity was evaluated using Shimadzu LC Solution® software. The chromatographic results were adjusted for the baseline, and the total purity spectrum was calculated based on the mean of three points.

The selectivity of the method was further assessed by evaluating the chromatographic profiles and mass balance of samples subjected to forced degradation.

To evaluate the degradation products generated during storage, one batch of BNZ 100-mg tablets manufactured by LAFEPE® was subjected to an accelerated stability study at 40 °C ± 2 °C and 75% RH ± 5% for 6 months (Brasil, 2019).

Following the accelerated stability study, the BNZ tablets were analyzed using the validated stability-indicating method to determine the content of BNZ and the degradation products detected in the samples.

The results were evaluated to determine the need for recording, identification, and/or classification of the detected degradation products according to applicable ICH and ANVISA requirements.

Results and discussion

The development of the stability-indicating method was initially investigated using solutions of BNZ subjected to photodegradation. According to the literature, photolysis can be an important degradation pathway for BNZ, resulting in the formation of several degradation products.

Based on previously reported methods by Soares-Sobrinho *et al.* (2006) and Silva *et al.* (2007), two chromatographic conditions were initially evaluated. The first method employed a gradient of acetonitrile and water, with the proportion of

acetonitrile varying from 0 to 100%, over a 100-min run. The second method employed an isocratic phosphate buffer:acetonitrile (80:20, v/v) mobile

phase at pH 2.7, with a 60-min run. The residual BNZ concentrations obtained under both conditions are presented in Table 1.

Table 1. Degradation kinetics of BNZ determined using gradient and isocratic chromatographic methods.

Sample	Time (h)	BNZ remaining (%) – Gradient	BNZ remaining (%) – Isocratic
BNZ raw material	0	100.00	100.00
Solution photodegraded for 1 h	1	66.79	64.86
Solution photodegraded for 2 h	2	41.69	36.65
Solution photodegraded for 3 h	3	22.99	16.36
Solution photodegraded for 4 h	4	8.25	1.21
Solution photodegraded for 5 h	5	8.10	0.60
Solution photodegraded for 6 h	6	7.96	0.63

The results demonstrated a progressive reduction in the BNZ concentration following irradiation under both chromatographic conditions. However, differences were observed between the two methods, particularly after prolonged irradiation. After 5 h of photodegradation, approximately 8% of the initial BNZ concentration was detected using the gradient method, whereas only 0.60% was detected using the isocratic method.

The lower residual BNZ concentration obtained with the isocratic method suggests that the latter provided improved chromatographic separation under the conditions evaluated. This difference may be associated with the co-elution of degradation products or other matrix components under the gradient conditions, which could result in an overestimation of the residual BNZ concentration.

Based on these results, the isocratic method was selected for further optimization and development of the stability-indicating method.

The stability-indicating method was evaluated according to the applicable requirements for analytical method validation. In the present study, robustness, linearity, detection limit, quantification limit, precision, accuracy, specificity, and selectivity were evaluated.

Chromatographic performance parameters were also assessed to verify the suitability of the chromatographic system. The retention time, relative retention time, number of theoretical plates, resolution, asymmetry, and capacity factor obtained for BNZ and its related compounds are presented in Table 2.

The related compounds selected for the study were 2-aminoimidazole sulfate (2-AZS), 2-nitroimidazole (2-NIZ), and *N*-benzylchloroacetamide (NBCA), which are synthetic precursors or structurally related compounds associated with the synthesis of BNZ.

Table 2. Chromatographic parameters obtained for the mixed standard solution of BNZ and its related compounds.

Parameter	2-AZS	2-NIZ	BNZ	NBCA
Retention time (min)	2.473	3.767	20.853	24.427
Relative retention time	0.118	0.180	1.000	1.170
Number of theoretical plates	1,910	4,230	5,593	5,581
Resolution	0.000	5.648	25.370	2.949
Asymmetry	1.139	1.321	1.429	1.453
Capacity factor	0.066	0.624	7.988	9.528

The chromatographic parameters indicated adequate retention and separation of BNZ from 2-NIZ and NBCA. In contrast, 2-AZS presented a very low capacity factor (0.066), with a retention time of 2.473 min, which was close to the column void volume (2.32 min). Therefore, 2-AZS was not considered suitable for evaluation based on chromatographic separation from the solvent front.

Nevertheless, its relative retention time in relation to BNZ could be calculated, and its UV spectrum could be evaluated using the DAD detector. These characteristics were considered useful for the investigation of degradation products.

The chromatogram obtained from the mixed standard solution containing the related compounds selected for method validation is shown in Figure 3.

The robustness of the analytical method was evaluated by introducing small and deliberate variations in column oven temperature, mobile-phase composition related to the manufacturer of

the phosphate salt, mobile-phase flow rate, and sample-solution stability.

The effect of column oven temperature was evaluated at 39, 40, and 41 °C. The results are presented in Table 3. Statistical analysis was performed using one-way analysis of variance (ANOVA). The calculated *F* value (0.025) was lower than the tabulated *F* value (3.682), indicating that no statistically significant difference was detected among the evaluated temperatures at the 95% confidence level.

The influence of the phosphate salt manufacturer was also evaluated by comparing Synth® and Carlo Erba® reagents. Student's *t*-test, assuming unequal variances, resulted in a calculated *t* value of 0.045, which was lower than the tabulated value of 2.228. Therefore, no statistically significant difference was observed between the two manufacturers under the evaluated conditions.

The robustness of the method with respect to mobile-phase flow rate was evaluated at 0.99, 1.00, and 1.01 mL/min. The calculated F value (0.143) was lower than the tabulated F value (3.682), indicating no statistically significant

difference among the evaluated flow rates at the 95% confidence level.

The results obtained for the robustness study are presented in Table 3.

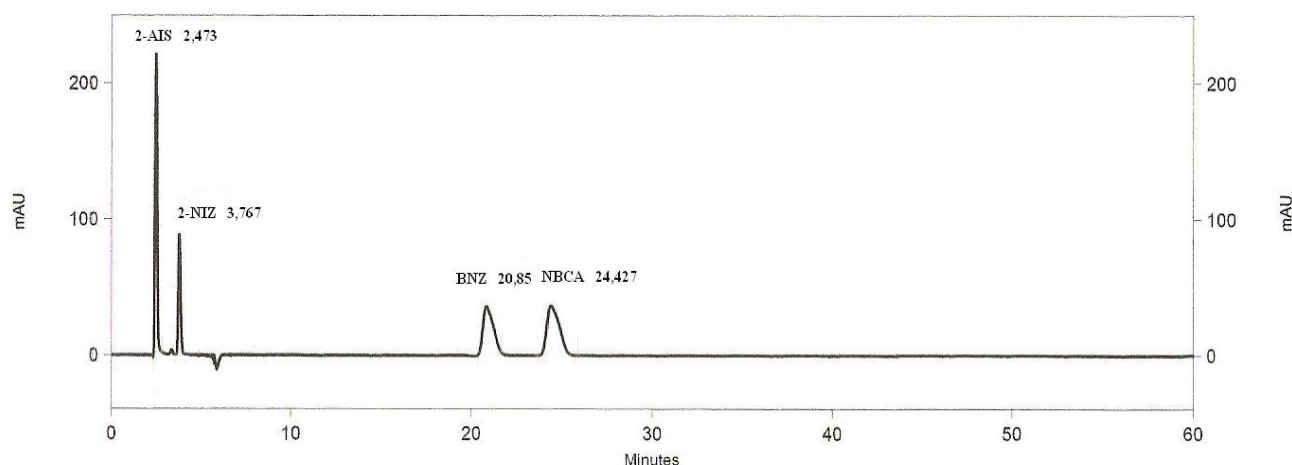


Figure 3. Chromatogram of the mixed standard solution of BNZ and related compounds at 12 µg/mL.

Table 3. Results obtained in the robustness study of the analytical method.

Sample	Temperature			Synth®	Carlo Erba®	Flow		
	39 °C	40 °C	41 °C			0.99 mL/min	1.00 mL/min	1.01 L/min
1	12.32	12.31	12.34	12.31	13.06	12.32	12.31	12.40
2	12.45	12.34	12.35	12.34	12.09	12.31	12.34	12.44
3	12.04	12.01	12.02	12.01	12.16	11.98	12.01	12.07
4	12.81	12.79	12.93	12.79	12.04	12.69	12.79	12.83
5	11.96	11.90	11.85	11.90	12.12	11.84	11.90	11.99
6	11.85	11.79	11.80	11.79	12.25	11.81	11.79	11.87
Mean	12.24	12.19	12.22	12.19	12.29	12.16	12.19	12.26
CV (%)	2.94	3.00	3.45	3.00	3.14	2.82	3.00	2.92

The CV values obtained under all evaluated conditions were below 5%, supporting the reproducibility of the analytical response under the tested variations.

Finally, the stability of the sample solution was evaluated after 24 h of storage at 2–8 °C using four determinations. Student's *t*-test resulted in a calculated *t* value of 1.080, compared with a tabulated value of 2.446 at the 95% confidence level, indicating no statistically significant difference between the evaluated conditions.

Overall, the results support the robustness of the method with respect to the evaluated parameters.

The linearity of the analytical response was evaluated over the concentration range of 4–40 µg/mL for BNZ, 2-NIZ, and NBCA. Three independent calibration curves were prepared, and the data were evaluated by least-squares linear regression. The coefficients of determination (R^2) were 0.9955, 0.9961, and 0.9985 for 2-NIZ, BNZ, and NBCA, respectively. These results indicate a strong linear relationship between concentration and chromatographic response over the evaluated range.

Table 4. Linearity results of the stability-indicating analytical method for BNZ and its related compounds.

Substance	Concentration range (µg/mL)	R^2
2-NIZ	4–40	0.9955
BNZ	4–40	0.9961
NBCA	4–40	0.9985

The detection limit (LOD) and quantification limit (LOQ) were estimated from the calibration data obtained for BNZ and its related compounds. The calculations were based on the standard deviation of the intercepts and the mean slope obtained from

three independent calibration curves, using factors of 3 and 10, respectively. The resulting LOD and LOQ values are presented in Table 5.

The estimated LOD values were 0.2613, 0.03537, and 0.2058 µg/mL for 2-NIZ, BNZ, and

NBCA, respectively. The corresponding LOQ values were 0.8071, 0.1179, and 0.6860 µg/mL.

Among the evaluated compounds, BNZ presented the lowest LOD and LOQ values, indicating a higher analytical response relative to the related compounds under the established chromatographic conditions.

The CV values obtained for the evaluated precision conditions ranged from 1.74% to 3.24%, remaining below the 5% criterion established for the study.

For the comparison between the evaluated precision conditions, the calculated Student's *t* value was 0.505, which was lower than the tabulated value of 2.262 at the 95% confidence level.

These results indicate satisfactory precision under the experimental conditions evaluated.

Precision was evaluated through repeatability and intermediate precision. The results obtained for six determinations are presented in Table 6.

Table 5. Detection and quantification limits of BNZ and its related compounds.

Substance	LOD (µg/mL)	LOQ (µg/mL)
2-NIZ	0.2613	0.8071
BNZ	0.03537	0.1179
NBCA	0.2058	0.6860

Table 6. Results obtained in the precision study of the analytical method.

Sample	Repeatability – Analyst 1, Day 1	Analyst 1, Day 1	Analyst 2, Day 2	Analyst 1, Day 2	Analyst 2, Day 2
1	12.13	10.74	11.70	12.13	12.31
2	12.26	11.58	11.45	12.26	12.34
3	11.83	11.68	11.19	11.83	12.01
4	12.27	11.76	11.86	12.27	12.79
5	12.44	11.58	11.51	12.44	11.90
6	12.04	11.55	11.72	12.04	11.79
Mean	12.16	11.48	11.57	12.16	12.19
CV (%)	1.74	3.24	2.06	1.74	3.00

Accuracy was evaluated at three concentration levels: 8 µg/mL, 12 µg/mL, and 16 µg/mL, with three determinations at each concentration. The coefficients of variation obtained at the low, intermediate, and high concentration levels were 2.46%, 0.83%, and 1.48%, respectively. All CV values were below 5%.

Specificity was evaluated using BNZ samples subjected to different forced-degradation conditions. The chromatograms obtained from stressed samples were compared with those obtained from non-degraded BNZ samples prepared under the same analytical conditions.

The BNZ peak was identified based on its retention time and UV spectrum obtained using the DAD detector. The chromatographic behavior of the related compounds 2-NIZ and NBCA was also evaluated using their respective reference solutions.

The results demonstrated chromatographic separation between BNZ and the evaluated related compounds, supporting the ability of the method to distinguish BNZ from the selected related substances under the established conditions.

Selectivity was further evaluated by determining the purity of the chromatographic peaks corresponding to BNZ and its related compounds using a diode-array detector (DAD) over the wavelength range of 200–400 nm.

Peak purity was assessed using Shimadzu LC Solution® software. The chromatographic data were adjusted for the baseline, and the total purity spectrum was calculated based on the mean of

three points. The reported peak-purity value for BNZ and the evaluated related compounds was 0.9900.

The selectivity assessment, together with the chromatographic separation and DAD spectral evaluation, was used to support the ability of the method to distinguish BNZ from related compounds and degradation products.

The validated stability-indicating method was applied to BNZ samples subjected to different forced-degradation conditions, including acidic and basic hydrolysis, neutral hydrolysis, oxidation, photodegradation, and thermal degradation.

Under the evaluated acidic conditions (1.0 M HCl for 30 days) and thermal conditions (60 °C for 30 days), BNZ showed limited degradation, with a reduction of less than 3% of the initial content.

In contrast, exposure to 0.1 M NaOH resulted in substantially greater degradation. After 30 days, approximately 35% of the initial BNZ content remained.

Under oxidative conditions using 3% hydrogen peroxide, BNZ was relatively stable during the first 7 days, with no degradation greater than 5%. After 30 days, approximately 90% of the initial BNZ concentration remained.

These results indicate that BNZ was more susceptible to alkaline hydrolysis than to the acidic and thermal conditions evaluated. Oxidative degradation occurred more slowly, becoming evident only after prolonged exposure to hydrogen peroxide.

Photodegradation represented the most pronounced degradation pathway among the

conditions investigated. BNZ showed photosensitivity both in solution and in the solid state. Under the irradiation conditions used for the solid-state study, an average degradation of approximately 15% was observed.

In solution, substantially greater degradation was observed during irradiation, with the residual BNZ concentration decreasing to less than 1% after prolonged exposure under the conditions described in Section 3.1.1.

The different degradation profiles demonstrate the importance of using a stability-indicating method capable of separating BNZ from the products formed during stress testing.

The degradation processes generated chromatographic signals with retention characteristics similar to those of some of the related compounds used during method development. However, retention-time similarity alone is insufficient to establish the chemical identity of the degradation products. Therefore, additional structural characterization would be required to identify the degradation products conclusively.

The chromatograms obtained from BNZ subjected to the different stress conditions are presented in Figure 4.

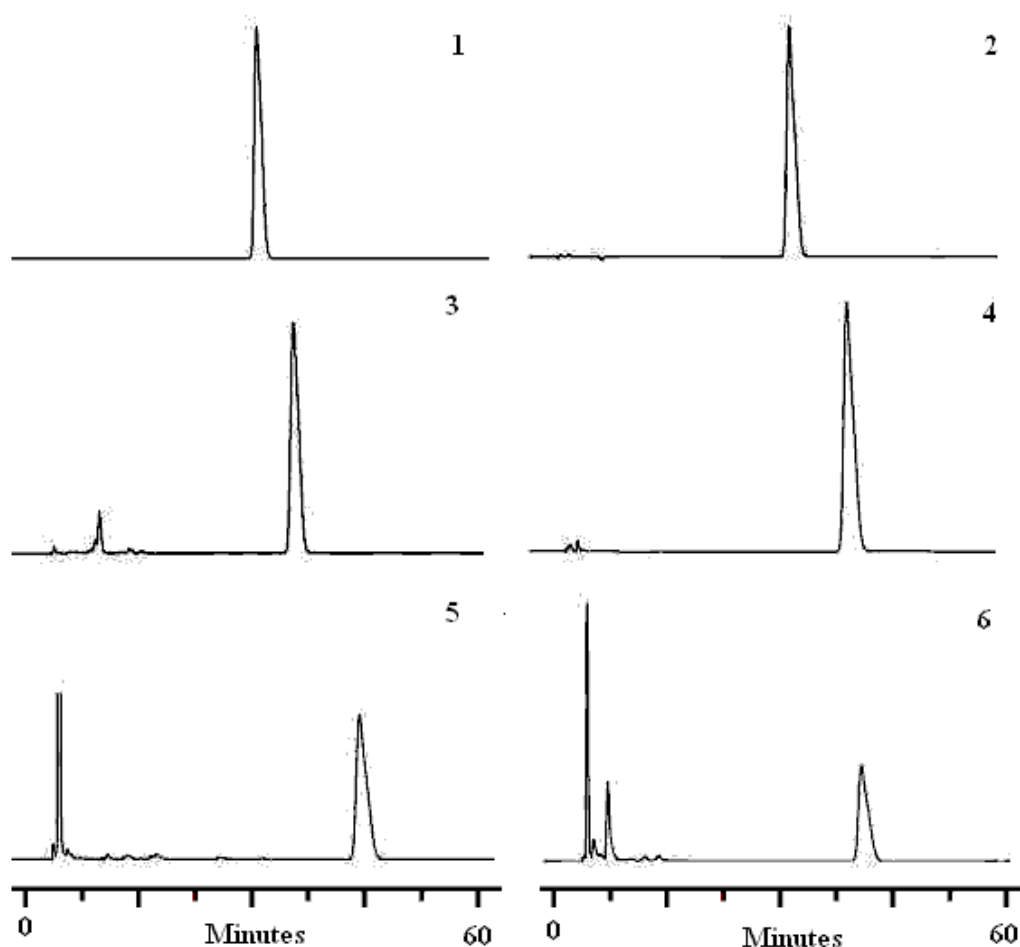


Figure 4. Chromatograms obtained after forced degradation of BNZ. 1 – BNZ standard; 2 – BNZ thermodegradation at 60 °C for 30 days; 3 – solid-state photodegradation of BNZ; 4 – BNZ acid hydrolysis in 1.0 M HCl for 30 days; 5 – BNZ oxidation with 3% H₂O₂ for 30 days; 6 – BNZ basic hydrolysis in 0.1 M NaOH for 30 days.

Following the accelerated stability study, BNZ 100-mg tablets manufactured by LAFEPE® were analyzed using the validated stability-indicating method to evaluate BNZ content and the formation of degradation products.

The tablets were subjected to accelerated stability conditions of 40 °C ± 2 °C and 75% RH ± 5% for 6 months.

The chromatographic analysis revealed two degradation products, designated DP-1 and DP-2,

with retention times of 2.653 and 3.585 min, respectively (Figure 5).

Both degradation products were also detected in chromatograms obtained from samples subjected to oxidative, alkaline hydrolytic, photolytic, and thermal stress conditions. This observation indicates that these chromatographic signals may be associated with degradation pathways occurring under more than one stress condition.

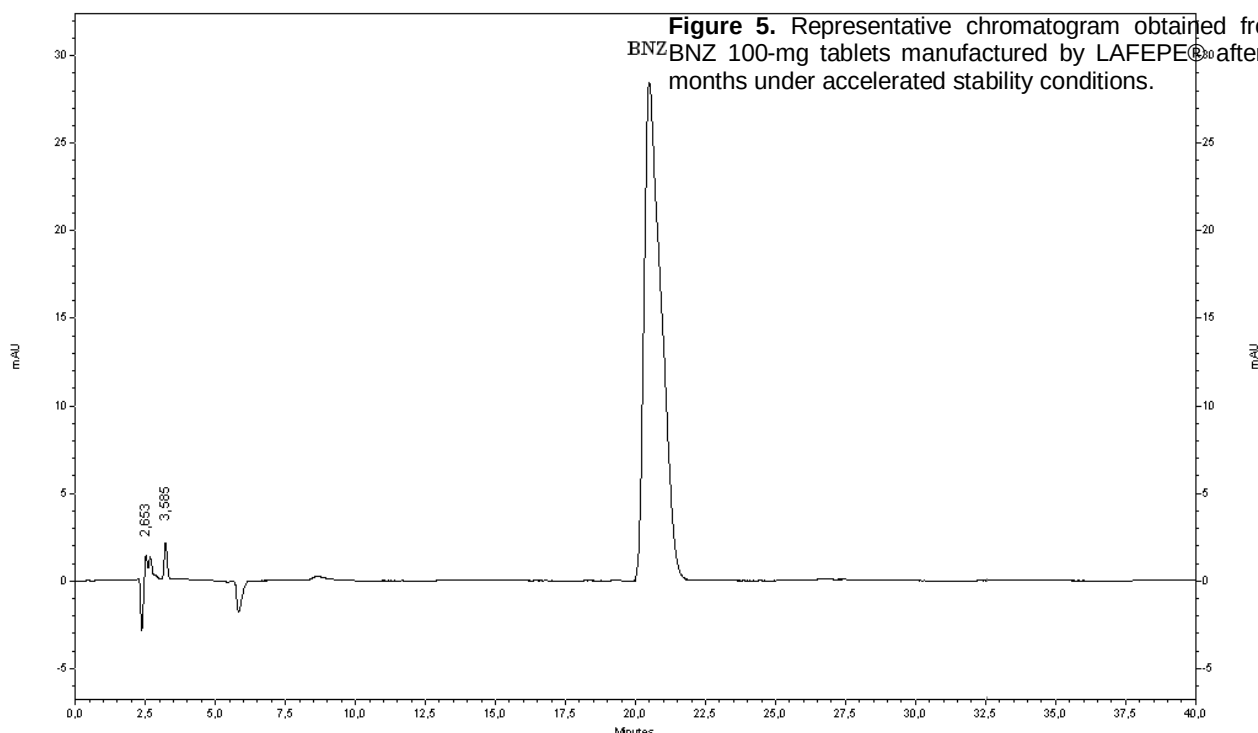


Figure 5. Representative chromatogram obtained from BNZ 100-mg tablets manufactured by LAFEPE[®] after 6 months under accelerated stability conditions.

The degradation products were quantified at 0.156% for DP-1 and 0.200% for DP-2.

Considering the reported daily BNZ dose of 200–300 mg, further investigation of these degradation products may be warranted if their levels reach applicable regulatory reporting or qualification thresholds. Their chemical structures and potential toxicological relevance would need to be established before any definitive conclusion regarding their safety can be made.

The results presented here were obtained from a single batch of BNZ tablets. Therefore, they should be considered preliminary. Evaluation of additional batches would be necessary to determine whether the observed degradation profile is reproducible and representative of the product.

Conclusions

The stability-indicating analytical method developed for BNZ was evaluated according to the analytical validation principles described in ICH Q2(R1) (2005). These parameters are also consistent with the current Brazilian regulatory framework established by ANVISA Resolution RDC No. 166/2017. The method demonstrated satisfactory robustness, linearity, precision, and chromatographic selectivity under the evaluated experimental conditions. The method was also capable of detecting and quantifying BNZ degradation products generated under different forced-degradation conditions.

The forced-degradation studies demonstrated that BNZ was particularly susceptible to photodegradation and alkaline hydrolysis, whereas limited degradation was observed under acidic and thermal conditions. Oxidative degradation with 3% hydrogen peroxide was relatively slow, becoming evident after prolonged exposure.

The application of the method to BNZ 100-mg tablets subjected to accelerated stability conditions resulted in the detection and quantification of two degradation products, DP-1 and DP-2, at concentrations of 0.156% and 0.200%, respectively. These findings demonstrate the applicability of the developed method for monitoring BNZ degradation in the solid dosage form.

Further studies involving additional batches and structural characterization of the degradation products are necessary to confirm their identity and to establish the reproducibility and toxicological relevance of the observed degradation profile.

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