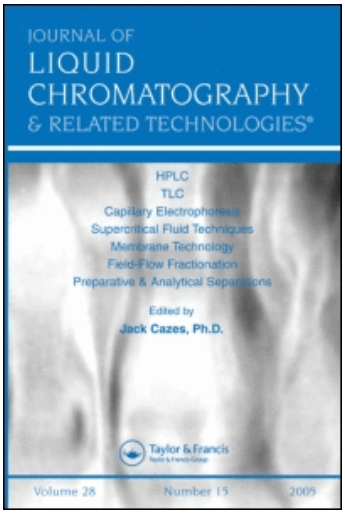

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STABILITY-INDICATING CZE METHOD AND STRESS DEGRADATION STUDIES OF NITAZOXANIDE

Marcelo Donadel Malesuik,[1] Heloísa Mazotti Lopes Gonçalves,[1] Clésio Soldatelli Paim,[1] André Jablonski,[2] Elfrides Eva Scherman Schapoval,[1] and Martin Steppe[1]

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□ *A new, simple, and effective stability-indicating CZE method was developed and validated for the determination of nitazoxanide in pharmaceutical formulations, using nimesulide as an internal standard. The optimum separation was carried out on a fused silica capillary (48.5 cm × 75 μm i.d., effective length 40 cm) maintained at 25°C, and a running electrolyte containing sodium acetate buffer (pH 5.2; 30 mM)-acetonitrile (80:20, v/v). The injections of the samples were performed using the pressure mode at 50 mbar for 5 s, with detection at 360 nm using a photo-diode array detector. The method was suitably validated for specificity, linearity, precision, accuracy, limit of detection and quantitation, and robustness. The high sensitivity of the method was proven with the limit of detection (0.05 μg mL^{-1}) and quantitation (0.2 μg mL^{-1}). The stability-indicating capability of the method was proven using stress conditions (acid and basic hydrolysis, oxidation, and photolysis). The proposed method was successfully applied for the determination of nitazoxanide in coated tablets and oral suspension powder.*

Keywords capillary zone electrophoresis, nitazoxanide, stability-indicating method, stress degradation studies

INTRODUCTION

According to the World Health Organization intestinal parasitic infections are among the most common infections and disease in humans worldwide and are a significant cause of morbidity and mortality. Nitazoxanide (NTZ, Figure 1) is a new broad spectrum antiparasitic drug agent. It is a new nitrothiazole benzamide compound (2-acetyloxyl-N-(5-nitro-2-thiazolyl)

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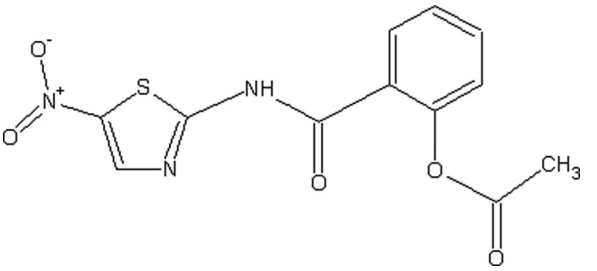

FIGURE 1 Chemical structure of nitazoxanide.

benzamide) notable for its activity in treating both intestinal protozoal and helminthic infections.[1] It was first described in 1975 by Jean Francois Rossignol and was initially developed as a veterinary antihelminthic with activity against intestinal nematodes, cestodes, and trematodes. In Humans, NTZ was approved by the US Food and Drug Administration (FDA) in 2002.[2] It is the first agent with proven efficacy for the treatment of cryptosporidiosis and giardiasis in children and adults. Its precise mechanism of action is unknown, but studies have shown that NTZ inhibits pyruvate ferredoxin oxireductase (PFOR) enzyme dependent electron transfer reactions essential to anaerobic energy metabolism in these organisms,[1,2]

NTZ is currently available in coated tablets (500 mg) and powder for oral suspension (100 mg/5 mL). An official method for determination of this drug in oral formulation has not been described yet. Literature concerning the quantitative determination of NTZ is relatively limited. However, there are studies describing the determination of NTZ and metabolites in biological fluids by liquid chromatography (LC)[3] and LC-MS.[4,5] Recently, visible spectrophotometrics,[6] high performance thin layer chromatography (HPTLC)[7] and LC methods for the estimation of NTZ in bulk and pharmaceuticals formulations were developed.[8–10]

Capillary electrophoresis (CE) has emerged as a powerful analytical technique for pharmaceutical analysis, with distinct applicability toward the separation of compounds of different chemical character, size, and structural features. In pharmaceutical analysis, this separation technique is well established and shows many advantages, such as simple instrumentation, good efficacy, precision, and rapid analysis.[11,12] In some fields, CE is currently displacing LC due to its many advantageous features, such as extremely high efficiency, high resolution, rapid analysis, small consumption of sample and reagents, and reduced cost of the operation, because no packed column, pumps or mobile phase gradient are used. However, at the present moment there is no stability-indicating CZE method published for the determination of NTZ. A stability-indicating method detects the changes in the pertinent properties of the drug substance and

measures the active ingredients and/or its degradation products without interference from other degradation products, excipients, or other potential impurities.[13–16]

The aim of the present study was to develop and validate a simple, rapid, and stability-indicating CZE method for the determination of NTZ in coated tablets and powder for oral suspension. We also present, here, the comparison between these results and the ones obtained from the LC analysis.

EXPERIMENTAL

Chemicals and Reagents

NTZ used as reference substance (assigned purity, 99.53%) was kindly supplied by Shin Yang–Hangzhou Shinyang Samwoo Fine Chemical CO. (Ningbo, China), and nimesulide (internal standard, IS) was purchased from Brazilian Pharmacopoeia. According to guidelines for drugs quality control the use of reference substance with checked purity is necessary.[17,18] The NTZ standard used was analyzed by analytical techniques such as: LC-mass spectrometry, differential scanning calorimetric (DSC), infrared absorption spectroscopy, and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. No impurities were found.

Nixoran® (manufactured by Roemmers, Buenos Aires, Argentina) coated tablets for oral administration (500 mg per tablet, excipients: maize starch, pregelatinized starch, hydroxypropyl methylcellulose, sodium starch glycollate, talc, magnesium stearate, triacetin, iron oxide yellow, titanium dioxide, polyethylene glycol 6000) and Alinia® (manufactured by Romark Laboratories, Tampa, FL, USA) powder for oral suspension (100 mg/5 mL, excipients: sodium benzoate, sucrose, xanthan gum, microcrystalline cellulose and carboxymethylcellulose sodium, anhydrous citric acid, sodium citrate dihydrate, acacia gum, sugar syrup, FD&C Red #40 and natural strawberry flavoring) were purchased. All chemicals used were of pharmaceutical or special analytical grade. All solutions were degassed by ultrasonication and filtered through a membrane diameter of 0.45 μm.

CZE Apparatus and Conditions

The CE instrument model HP^{3D} was purchased from Hewlett-Packard (Waldbrom, Germany) and consisted of a photodiode array (PDA) detector, automatic injector, and temperature control system. The apparatus were connected to a personal computer equipped with a HP Chemstation CE software (rev. A.06.03 Hewlett-Packard) used for instrument control,

data acquisition, and data analysis. At the beginning of each working day, the capillary was conditioned by rinsing with 0.1 M sodium hydroxide for 15 min, followed by water for 15 min, and then with running electrolyte for 30 min. The electrolyte solution was prepared and filtered on the analysis day. To achieve high migration time and reproducibility among injections, the capillary was conditioned with 0.1 M sodium hydroxide (1.5 min), water (1.5 min), and a running buffer (2 min). All experiments were carried out on a fused silica capillary (Agilent, Waldbronn, Germany) with 75 μm i.d. and 48.5 cm of total length (effective length 40 cm), thermostatized at 25°C, and detected at 360 nm using a PDA detector. Samples were injected using the pressure mode for 5 s at 50 mbar and a constant voltage of 25 kV (current about 45 μA) was applied during the analysis. The runing electrolyte consisted of a mixture of 30 mM sodium acetate buffer at pH 5.2 (adjusted by the addition of glacial acetic acid)-acetonitrile (80:20, *v*/*v*).

Preparation of Reference Substance Solution

Standard stock solutions of NTZ and nimesulide (IS) were prepared in acetonitrile. A stock solution of 100 $\mu g\,mL^{-1}$ NTZ reference substance was prepared in a volumetric flask by dissolving 10 mg of the drug in 100 mL of acetonitrile. Aliquots of 3 mL of this solution and 2 mL of the IS solution (200 $\mu g\,mL^{-1}$) were transferred into a 10 mL volumetric flask and the same solvent was added to complete the volume in order to give a final concentration of 30 $\mu g\,mL^{-1}$ (NTZ) and 40 $\mu g\,mL^{-1}$ (IS).

Preparation of Sample Solution

Twenty tablets were weighed and crushed to a fine powder. An accurately weighed amount of tablet powder and oral suspension powder equivalent to 10 mg of NTZ were transferred to a 100 mL volumetric flask with 50 mL of acetonitrile and sonicated for 10 min, followed by adding the same solvent to complete the volume. After filtration, 3 mL of this solution and 2 mL of the IS solution were transferred into 10 mL volumetric flask. The same solvent was added to complete the volume in order to give a final concentration of 30 $\mu g\,mL^{-1}$ (NTZ) and 40 $\mu g\,mL^{-1}$ (IS).

System Suitability Test

A system suitability test of the CZE system was performed before each validation run using five replicate injections of a standard solution.

Theoretical plates, tailing factor, resolution, and injection repeatability were determined.

Degradation Studies

In order to establish if the proposed method is stability-indicating, the pure active pharmaceutical ingredient of NTZ was stressed under different conditions to conduct forced degradation studies.[13] The drug is poorly soluble in ethanol and practically insoluble in water, but freely soluble in acetonitrile. The NTZ solutions for acid hydrolysis were prepared by dissolving the drug in a small volume of acetonitrile (5%, *v*/*v*) and then diluted with aqueous hydrochloric acid to achieve a concentration of $1\,mg\,mL^{-1}$. The acid hydrolysis was performed in 0.1 M HCl at 80°C for 1 h, after the sample was cooled at room temperature and neutralized. The study in alkaline condition was carried out in 0.01 M NaOH at room temperature (24 ± 2°C) for 2 h and neutralized. An aliquot of each solution was diluted with acetonitrile to give a final concentration of $30\,\mu g\,mL^{-1}$.

The oxidative reaction was performed by dissolving NTZ in a small volume of acetonitrile (5%, *v*/*v*) and then diluted with 3% H_2O_2 ($1\,mg\,mL^{-1}$) at room temperature. An aliquot of this solution was diluted in acetonitrile to give a final concentration of $30\,\mu g\,mL^{-1}$.

The stress degradation study in direct UV radiation (245 nm) was performed, exposing the NTZ solution in acetonitrile ($1\,mg\,mL^{-1}$) for 15 min at room temperature in a photostability chamber provided with mirrors. The distance between the lamp and the samples was 10 cm. After, this solution was diluted to $30\,\mu g\,mL^{-1}$ in acetonitrile. Samples submitted to identical conditions, but protected from light, were used as a control.

The peak purity test performed by photodiode array detection was useful to show that the analyte's chromatographic peak did not contain more than one substance.

Validation of the Method

The developed analytical method was validated according to ICH guidelines[17] and USP requirements.[18]

Linearity

Linearity was determined by constructing three calibration curves, each one with five calibration points of NTZ. A stock solution ($100\,\mu g\,mL^{-1}$) of NTZ was prepared in acetonitrile and aliquots were transferred to volumetric flasks to obtain the final concentration of 10, 20, 30, 40, and

50 μg mL^{-1}. In all cases, 40 μg mL^{-1} of nimesulide were added as internal standard. Three replicate injections of each reference substance solution were made to verify the repeatability of the detector response. The peak area ratio of NTZ and IS was used for plotting the graph, and the linearity was evaluated by the least square regression analysis.

Specificity

Forced degradation studies were performed to evaluate the stability-indicating power of the proposed method. The interference of the excipients of the pharmaceutical formulations was determined by the injection of a sample containing only placebo (in-house mixture of tablet and oral suspension powder excipients) and a sample containing placebo added with NTZ at a concentration of 30 μg mL^{-1} (40 μg mL^{-1} of nimesulide was added as internal standard). Then, the specificity of the method was established by determining the peak purity of NTZ in the degraded samples using a PDA detector.

Precision

The precision of the method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision) and was expressed as the relative standard deviation (RSD%) of a measurement series. The repeatability was examined by six evaluations of the same concentration of NTZ (30 μg mL^{-1} and IS), on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on three different days (inter-days) and also by other analyst performing the analysis in the same laboratory (between-analysts).

Accuracy

This parameter was determined by the recovery test, which consisted of adding known amounts of reference substance to the samples solutions at a concentration of 30 μg mL^{-1} (tablets and powder for oral suspension). Aliquots of 1.0, 2.0, and 3.0 mL of a NTZ standard solution (concentration of 40 μg mL^{-1}) were transferred to 10 mL volumetric flasks of samples solutions, respectively (4, 8, and 12 μg, respectively), prepared as described above and nimesulide was added as internal standard. Each solution was prepared in triplicate.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at the LOQ level by injecting three individual preparations of NTZ and the relative standard deviation of the absolute area of the peak was evaluated.

Robustness

The robustness was determined by analyzing the same samples under different conditions of the parameters method, such as: buffer pH and concentration, capillary temperature, voltage applied, and percentage of acetonitrile in the electrolyte. To assess the stability of NTZ, the samples were maintained at 4–8°C for 48 h, and also placed inside the autosampler at room temperature for 48 h.

LC Apparatus and Conditions

The LC system consisted of a Shimadzu LC-10AD$_{VP}$ pump, a SPD-M10A$_{VP}$ Diode Array Detector, a SCL-10A$_{VP}$ system controller, CTO-10AC$_{VP}$ column oven, SIL-10AD$_{VP}$ auto injector, and a degasser module DGU-14A. Data were acquired and processed by the Shimadzu Class-VP® V 6.14 software program (Shimadzu, Kyoto, Japan). The LC method previously developed and validated by our research group was used for comparison with the proposed CE method.[9] The column utilized was a Phenomenex® (Torrance, CA, USA) Synergi Fusion C_{18} column (250 mm × 4.6 mm, i.d., 4 μm particle size) coupled to a C_{18} guard column (4.0 mm × 3.0 mm, i.d., 4 μm). The Shimadzu LC system was operated isocratically at 25°C using a mobile phase of *o*-phosphoric acid 0.1% (v/v) pH 6.0 (adjusted by addition of triethylamine): acetonitrile (45:55, *v*/*v*), run at a flow rate of 1.0 mL min^{-1}, and using PDA detection at 240 nm. The injection volume was 20 μL of a solution containing 30 μg mL^{-1} for both the reference substance and the samples. The quantitation was performed using the absolute area of the peak.

RESULTS AND DISCUSSION

Development and Optimization of the Electophoretic Conditions

The experimental conditions were chosen after testing the different parameters that influence CE analysis, such as the dissolution medium,

temperature, applied voltage, buffer concentration, and pH. To develop the CZE method, the influences of these parameters on migration time, peak symmetry, resolution, baseline noise, and electric current were optimized. Because of the relative instability of this drug under strong acid (pH below 3.0) and basic (pH at least 8.0) conditions, acidic buffers were tried to prevent its possible instability. Several running buffers such as phosphate (pH 3.5–6.0), citrate (pH 3.5–6.0), and acetate (pH 3.5–5.5) were tested for CZE analysis. The results demonstrated that NTZ was easily separated from IS in this pH range and the migration times of these compounds were decreased with decreasing pH. Taking into consideration different parameters (migration time, resolution, symmetry, etc.), the best results were obtained in acetate buffer. The effect of the buffer pH was investigated within the range of 3.5–5.5 at 30 mM acetate buffer concentration. Considering both resolution and migration time, pH 5.2 was selected as optimum.

Buffer concentration also has a significant effect on the separation performance through its influence on the electroosmotic flow (EOF) and on the capillary current. The acetate buffer in the concentration range 10–50 mM was evaluated. The 30 mM acetate buffer was selected for its low current and for generating no significant increase on the migration time. The effects of the organic modifiers acetonitrile or methanol, in the concentration range of 5–25% (v/v), were also evaluated. Appreciable improvement was observed on the baseline when acetonitrile (20%) was added.

The influence of the temperature on the analysis was investigated at 20, 25, and 30°C, showing that an increase of the capillary temperature results in a decrease of the migration time and an increase of the current. A temperature of 25°C was chosen due to the short run time, peak symmetry and acceptable current.

The effect of the applied voltage on the separation was studied through changes from 15 to 30 kV. As expected, increasing the applied voltage increases the EOF, leading to shorter analysis time and higher efficiencies. However, higher voltages also exhibited higher currents and increased joule's heating. The potential of 25 kV yielded a short analysis time with an acceptable current (about 45 μA).

The drug has two maximum absorptions: at 240 and 360 nm. For the method detection 360 nm was applied, because of its high molar absorptivity at the conditions used above.

System Suitability Test

The system suitability test is an integrated part of the analytical method and it ascertains the suitability and effectiveness of the operating system. It

was also carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using five replicate injections of a reference substance solution containing $30\,\mu g\,mL^{-1}$ of NTZ and $40\,\mu g\,mL^{-1}$ of IS (Table 1). These parameters values were satisfactory in accordance with the literature.[19] The tests ensure that the CZE system generates trustful results. Thus, it was established that the CZE system and procedure are capable of providing acceptable quality data.

Degradation Behavior

The forced degradation studies were conducted to evaluate the stability-indicating capability and selectivity of the proposed method using the NTZ reference substance. When submitted to acidic, basic, oxidative, and photolytic conditions the eletropherograms showed a significant decrease of the NTZ area and one additional peak at 6.38 min, in all cases (Figure 2).

The electropherogram peak purity tool was applied to verify NTZ and IS peaks, showing that they were 100% pure in all cases. Thus, it was confirmed that there was no interference of any other substance at the drug and IS migration time. Besides, it was observed that the NTZ peak presents appropriate resolution and selectivity in relation to the degradation products.

As can be observed, the proposed method succeeded in separating the drug from the degradation products. Furthermore, the drug can be evaluated both qualitatively and quantitatively in the presence of degradation products. The resolution of all degradation products allows qualitative and quantitative evaluation of the degradation products, which is relevant when assessing toxic products. The results indicated that the method is stability-indicating.

Analyzing the diode array spectrum (200–500 nm) of each degradation product verified that the products DPp, DPb, DPa, and DPo have the same spectrum and migration time. Therefore, it suggests that they are of the

TABLE 1 Results of the System Suitability Test

Parameter	Nimesulid (IS)[a]	Nitazoxanide[a]
Teoretical plates	70470	87548
Tailing factor	1.5	1.2
Resolution	–	6.0
Migration time (min)	3.55 (RSD = 0.2%)	3.90 (RSD = 0.7%)
The peak area ratio of NTZ to IS (Injection repeatability)	2.15 (RSD = 0.3%)	

[a]Mean of five replicates.

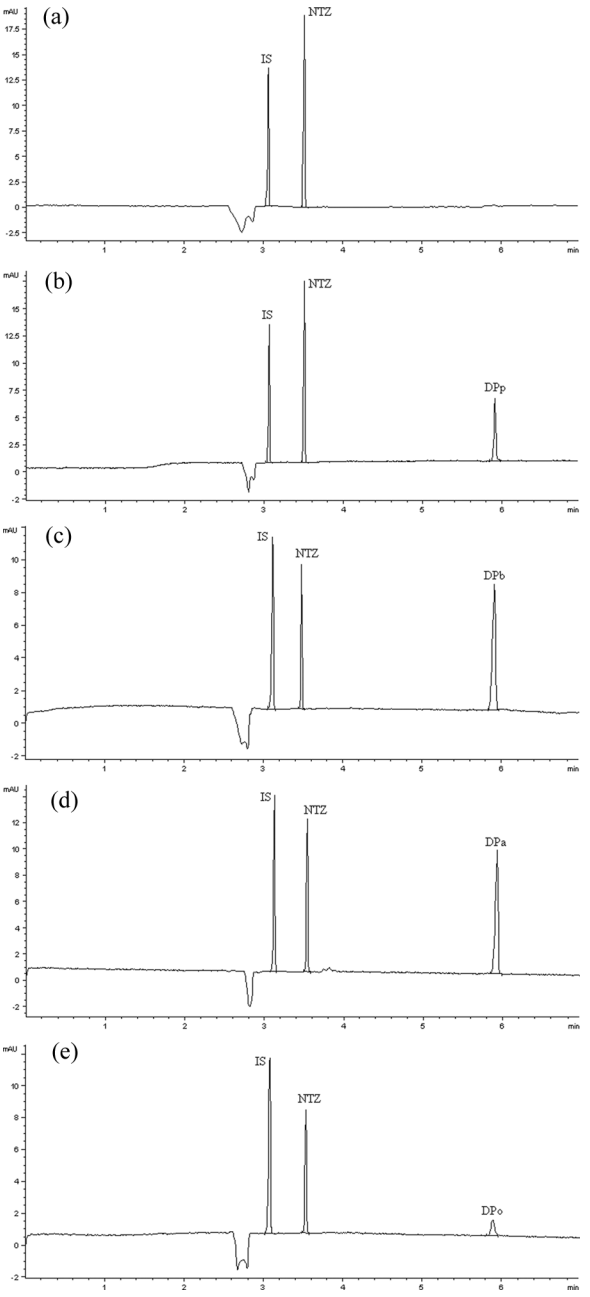

FIGURE 2 (a) Typical electropherogram for 30.0 μg mL^{-1} NTZ and 40.0 μg mL^{-1} IS solution in the experimental selected conditions; (b) IS, photo-degraded NTZ, and degradation product DPp; (c) IS, basic-degraded NTZ, and degradation product DPb; (d) IS, acid-degraded NTZ, and degradation product DPa; (e) IS, oxidative-degraded NTZ, and degradation product Dpo.

same substance, but the identification of these products was not yet performed.

The results obtained from the degradation studies show that the drug is susceptible to the hydrolysis, oxidation, and photolysis. Thus, one must be careful when manipulating the drug.

Validation of the Method

The validation testifies that the procedure is suitable for the intended purpose. The ICH and USP 31 guidelines describe the analytical parameters that should be evaluated in a method validation. The method type and its intended use determine which parameters should be evaluated. It is the analyst's responsibility to select the parameters considered relevant for each method.[20] The analytical method was validated for parameters such as linearity, specificity, precision, accuracy, limit of quantification, limit of detection, and robustness. Nimesulide was used as an internal standard to compensate injection errors and minor fluctuations on the migration time, thus improving the reproducibility of the CZE method.

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves constructed for NTZ were linear in the 10–50 $\mu g\,mL^{-1}$ range. The representative linear equation was: $y = 0.0728(\pm 0.00054)\,x - 0.0268(\pm 0.01804)$ and the correlation coefficient ($r = 0.9999$) was highly significant. The validity of the assay was verified by ANOVA (SAS 6.11 for windows, SAS Institute Inc., Cary, NC, USA), which demonstrated significant linear regression:

$$F_{\text{calculated}} = 7201.41 > F_{\text{critical}} = 10.04; \quad p < 0.05$$

and no significant linearity deviation:

$$F_{\text{calculated}} = 0.40 < F_{\text{critical}} = 3.71; \quad p < 0.05$$

The specificity test demonstrated that there was no interference on the drug and on the IS peaks. No interferences from the stress testing studies, diluents, impurities, and excipients were observed, indicating a high degree of the method specificity for determination of NTZ for both pharmaceutical formulations. The tests with the photodiode array detector showed that there was no coeluting peak interfering in the analysis of NTZ and IS.

Precision was determined by studying the repeatability and intermediate precision. The experimental values obtained for the determination of NTZ in samples are presented in Table 2. The assay precision results (RSD%) were 1.3, 1.6, and 1.4% for coated tablets and 1.0, 0.8, and 1.3%

TABLE 2 Precision Results of CE Assay of Nitazoxanide in Tablets and Powder for Oral Suspension

Dosage Forms	Intra-Day Precision			Inter-Day Precision[b]
	Day 1 ($n=6$) Mean ± RSD%	Day 2 ($n=6$) Mean ± RSD%	Day 3 ($n=6$)[a] Mean ± RSD%	Mean ± RSD%
Tablets	99.14 ± 1.3	98.66 ± 1.6	100.79 ± 1.4	99.53 ± 1.1
Powder for oral suspension	98.4 ± 1.0	99.4 ± 0.8	99.3 ± 1.3	99.03 ± 0.6

[a]Different analyst.
[b]Data expressed as mean of three days.

for oral suspension powder. The variability of the results was low with RSD values of less than 1.7% to intra-day, and the values of inter-day were 1.1% for coated tablets and 0.6% for oral suspension powder. The found RSD values for the analytical method were within the acceptable range, indicating that this method has excellent repeatability and intermediate precision.

The excellent mean percentage recovery values, around 100%, and their low relative standard deviation values (RSD $\leq$ 1.0%) were satisfactory. At each level of the NTZ concentration three determinations were performed. The mean recovery was 100.48% (RSD = 0.7%) for coated tablets and 100.58% (RSD = 0.8%) in oral suspension powder. These results revealed that any small change in the solutions drug concentration could be accurately determined by the proposed analytical method.

The LOD of NTZ was achieved at 0.05 $\mu g\,mL^{-1}$ for 20 µL injection volume. The LOQ of NTZ was achieved at 0.2 $\mu g\,mL^{-1}$. The concentration of 0.2 $\mu g\,mL^{-1}$ was accurately and reliably measured. The precision at the LOQ concentrations of NTZ was 1.0% (RSD%). With such findings in LOD and LOQ, we prove that the method is highly sensible.

The results and the experimental range of the evaluated variables in the robustness assessment are given in Table 3, with the optimized conditions. Analyses were carried out in triplicate and only one parameter was changed in the experiments at a time. There were no important changes in the CZE pattern when the small modifications were made in the experimental conditions, showing, then, that the method is robust. The solutions were also stable during the study period.

Comparison Between CZE and LC Methods

In spite of the fact that quality control analysis of pharmaceuticals is currently performed predominantly using LC, the number of CE instrumentation is increasing in the pharmaceutical laboratories, because it offers a real and attractive alternative to LC. The CE method developed and validated in this study was compared with the previous LC method

TABLE 3 Capillary Electrophoresis Conditions and Investigated Range During Robustness Testing

Variable	Range Investigated	Nitazoxanide (%)[a]	
		Tablets	Powder for Oral Suspension
Buffer pH	5.1	100.79	99.80
	5.3	98.35	98.95
Buffer concentration (mM)	28	99.02	98.64
	30	101.90	99.19
Temperature (°C)	20	99.97	100.23
	30	99.79	100.81
Voltage (kV)	23	100.85	99.19
	27	101.68	98.48
Percent acetonitrile	15	99.46	101.82
	25	101.58	100.75
Solution stability	Autosampler/48 h	100.83	101.05
	4–8°C/48 h	99.40	100.75
Normal conditions	–	99.21	99.47

[a]Each value is the mean of three analysis.

developed by our group. Both methods have some advantages and some drawbacks. However, LC is limited by expensive chromatographic columns and by the consumption of relatively high amounts of chemicals. Nevertheless, CE can offer benefits in terms of providing a lower operating cost, because it requires only few milliliters of running electrolyte, and it decreases the waste generation. Furthermore, CE separation efficiency is greater when sample volumes are very small. The required capillary and electrolyte cost for a specific number of analyses is evidently lower than in LC analysis.

The comparison between results of NTZ quantitative analysis in tablets and oral suspension powder were performed by statistical analysis. The Student's *t*-test was applied and did not reveal any significance between the experimental values obtained for the sample analysis by the two methods. The calculated *t*-values were found to be less than the critical *t*-values at 5% significance level. The developed and validated methods provided similar results for NTZ quantitation. Then, the proposed method can be applied directly and easily to the oral pharmaceutical preparations of NTZ.

CONCLUSIONS

A simple and reliable stability-indicating CZE method was developed and validated for the determination of NTZ in pharmaceutical formulations. The method proved to be specific, selective, linear, precise, accurate, and sensitive, and it offers a simple, fast, and inexpensive way for the drug determination. The developed method is also stability indicating and can

be used for assessing the stability of nitazoxanide in bulk drugs, tablets and oral suspension powder. Therefore, the proposed method was successfully applied as an alternative for the quantitative analysis of NTZ in pharmaceutical formulations, representing an improvement for the quality control, and also contributing to assure the therapeutic efficacy of the drug.

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