A Validated RP-HPLC Method for the Estimation of Ornidazole in Pharmaceutical Dosage Forms

Ornidazol'ün Farmasötik Dozaj Formlarından Valide Edilmiş RP-HPLC Metodu ile Belirlenmesi

Research Article

Senem Şanlı[1,*], Tuğrul Yıldırım[2], Bediha Akmeşe[2], Nurullah Şanlı[1]
[1]Department of Chemistry, Faculty of Science and Arts, Uşak University, Uşak, Turkey
[2]Department of Chemistry, Faculty of Science and Arts, Hitit University, Çorum, Turkey

ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay has been developed and validated for assay of Ornidazole in tablet formulations. Tinidazole was used as an internal standard. These compounds are separated well on a X-Terra (Waters Corp., Milford, MA, USA) RP-18 column by using a mobile phase consisting of a mixture of acetonitrile : water (30:70; v/v) adjusted to pH 3.00 with 10 mM o-phosphoric acid at a flow rate of 1.0 mL min^{-1}. The method was validated as per the ICH guidelines.

Key Words
Ornidazole, RP-LC, Validation, Pharmaceutical Dosage Forms.

ÖZET

Ornidazol için yeni, kolay, seçici, kesin ve doğru, izokratik ters faz sıvı kromatografik metot geliştirilmiş ve tablet formülasyonlarından miktar tayini için valide edilmiştir. Tinidazol iç standart olarak kullanılmıştır. Bu bileşikler için ayırma, X-Terra (Waters Corp., Milford, MA, USA) RP-18 kolonu ve mobil faz olarak 10 mM o-fosforik asit içeren pH 3'e ayarlanmış (30:70 v/v) asetonitril-su karışımı kullanılarak 1.0 mL $dakika^{-1}$ akış hızında gerçekleştirilmiştir. Metot ICH kurallarına göre valide edilmiştir.

Anahtar Kelimler
Ornidazol, RP-LC, Validasyon, Farmasötik Doz Formları

Article History: Received April 03, 2012; Revised June 27, 2012; Accepted May 3, 2012; Avaliable Online: August 17, 2012.

Correspondence to: Senem Şanlı, Department of Chemistry, Faculty of Science and Arts, Uşak University, Uşak, Turkey

Tel: +90 276 221 21 34 Fax: +90 276 221 21 35 E-Mail:senem.sanli@usak.edu.tr

INTRODUCTION

Ornidazole (ONZ), a nitroimidazole derivative is used in the treatment of a wide range of infections like hepatic and intestinal amoebiasis, giardiasis, trichomoniasis of urogenital tract and bacterial vaginosis. It is also used in the treatment and prophylaxis of susceptible anaerobic infections. It is chemically [1-(2-hydroxy-3-chloropropyl)-2-methyl-5-nitro-imidazole] [1].

ONZ is not official in any Pharmacopoeia. Literature survey reveals that ONZ is estimated by several analytical methods in pharmaceutical dosage forms. The techniques including spectrophotometry [2-4], high performance liquid chromatographic (LC) methods [5-8] and electrochemical methods [9] have also been reported for this drug. But the present described method has the advantage of faster elution time and hence less time-consuming one.

In LC methods, precision and accuracy can often be enhanced by the use of an appropriate internal standard, which also serves to correct for fluctuations in the detector response. One of the main reasons for using an internal standard is for samples requiring significant pretreatment or preparation. Generally, sample preparation steps including reaction, filtration, precipitation and extraction may cause unexpected results because of the sample losses. Ideally, an internal standard should display similar physico-chemical properties to the analytes. Due to choosing tinidazole (TNZ) as an internal standard, it showed a shorter retention time with better peak shape and better resolution from the investigated compounds peak.

We are here with reporting precise and accurate RP-HPLC method developed and validated for rapid assay of ONZ in Pharmaceutical Dosage forms. Also, this paper focuses mainly on the effect of percentage of acetonitrile (ACN) on the chromatographic behavior of ONZ in several ACN-water mixtures, 25, 30, 35% (***v/v***), in order to predict the optimum conditions for its separation to TNZ by RP-LC.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals were used without further purification. Reference sample of Ornidazole was obtained from DEVA Pharm. Ind. and tinidazole was supplied from Pfizer Pharm. Ind. Acetonitrile (HPLC grade) was obtained from Merck (Merck, Darmstadt, Germany). Ortho-phosphoric acid (min. 85%) was purchased from Riedel (Riedel-de Haen Germany). Biteral® tablets (DEVA Pharm. Ind.) were procured from the local pharmacy. Water, with conductivity lower than 0.05 μ/Scm was obtained with a Zeneer Power I (Human Corp. Korea)

Apparatus

The HPLC analysis was carried out on a Shimadzu class LC system with a pump (LC-20 AD), DAD detector system (SPD-M 20A) and column oven (CTO 20 AC). This equipment has a degasser system (DGU 20 A). X-Terra RP-18 column (250

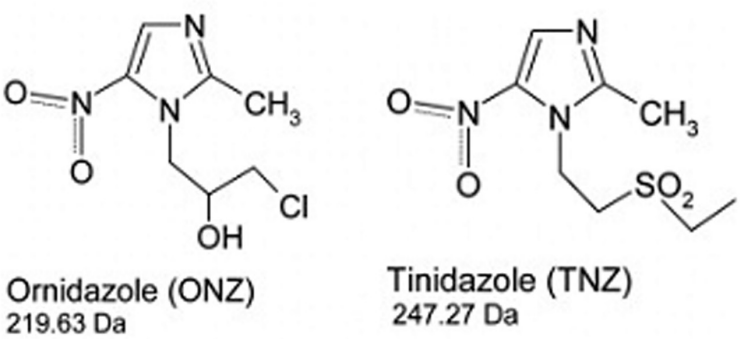

Figure 1. Structural formula of the studied compounds with abbreviations and molecular weights

mm × 4.60 mm ID, 5 μm) was used at 30°C. Mettler Toledo MA 235 pH/ion analyzer with combined glass electrode was used for pH measurements.

The pH values of the mobile phases were measured against a 0.05 mol kg^{-1} potassium hydrogen phtalate solution as primary reference standard solution, dissolved in the appropriate ACN–water medium. This standard was selected because their pH values at different ACN percentages (up to 70% (v/v)) were already known. The use of organic solvent–water mixtures requires the correct measurements of pH in these media. Measurements are performed in a similar way to those performed in water using IUPAC standardization rules [10, 11].

Procedures

Throughout this study, the mobile phases assayed were ACN–water at 25, 30, and 35% ***v/v***, containing 10 mM o-phosphoric acid. The pH of the mobile phase was adjusted to 3.0 by the addition of 1 M sodium hydroxide. The flow rate was maintained at 1.0 mL min^{-1}. For each drug, the retention time values, t_R, were determined from three separate injections for each mobile phase composition and pH considered. The column was pre-conditioned for at least 1 h at low flow-rate (0.5 mL/min) with mobile phase at the corresponding pH before the first injection. For each compound, the retention time values, t_R, were determined from three separate injections for each mobile phase composition and pH considered. Capacity factors were calculated as $k = (t_R - t_0)/t_0$. The dead time (t_0) was measured by injecting uracil solution (0.1% ***w/v*** in water) which was established for each mobile phase composition. The UV detection wavelength was 305 nm. Samples and standard solutions were also filtered through a 0.22 μm nylon membrane and injected using a 20 μL injection loop.

Preparation of Standard Solutions

Stock solution (100 μg mL^{-1}) of drugs was prepared in the acetonitrile. ONZ and TNZ are light sensitive compounds thus all solutions were protected from light and were used within 24 h to avoid decomposition.

The concentration of ONZ was varied in the range 0.5-12 μg mL^{-1}, respectively, and the concentration of tinidazole (IS) was maintained at a constant level of 4.0 μg mL^{-1}. The calibration curves for LC analysis were constructed by plotting the ratio of the peak area of the drug to that of internal standard against the drug concentration.

Analysis of Tablets

Ten tablets were weighed and finely powdered. An accurate weight of the powder equivalent to one tablet content was weighed, transferred into a 100 mL calibrated flask, diluted with methanol, stirred for about 10 min and then completed to volume with the same solution. This solution was filtered and the filtrate was collected in a clean flask. After filtration, appropriate solutions were prepared by taking suitable aliquots of clear filtrate solution. After the addition of the constant amount of IS (4.0 μg mL^{-1}), the solutions were diluted with mobile phase, in order to obtain a final solution. The contents amount of ONZ were calculated from the corresponding regression equations.

To verify the accuracy of the method, recovery experiments were performed after the addition of a known amounts of pure drugs to pre-analyzed tablets. Known amounts of the pure drugs and at a constant level of IS were added to the tablet formulation and the mixtures were analyzed. The percent recovery was calculated by comparing the concentration obtained from spiked samples with the actual added concentration. Thus, the effect of common excipients in tablet formulation on chromatograms (e.g., tailing, broadening) and spectrums was investigated. Recovery experiments from tablets also showed the reliability and suitability of the method.

RESULTS AND DISCUSSION

Drug analysis is undertaken during various phases of pharmaceutical development, such as formulation and stability studies, quality control and pharmacological testing in animals and humans. All these investigations require reliable and validated analytical methods in order to assay drugs in pharmaceutical formulations.

X Terra RP-18 column (250 × 4.60 mm ID × 5 μ) maintained at 30°C was used for the separation and the method validated for the simultaneous determination of ONZ in tablet dosage form. This stationary phase extended pH stability and was thermally more stable and more efficient than classical silica-based packing. The composition, pH and the flow rate of the mobile phase were changed to optimize the separation conditions and the main related substances of the two compounds of interest. The concentration of organic solvent in mobile phase was one of the most important factors in RPLC. Three mobile phase systems (25:75, 30:70 and 35:65, v/v) were prepared and used to provide an appropriate chromatographic separation.

The experimental region was selected in such way that the capacity factors of ONZ and TNZ would stay within the limits 1 < k < 10. These limits were provided in 25:75 ACN - water binary mixtures. But, chromatographic retention times in 25:75 ACN-water binary mixtures were very long. According to the experimental results, it was obvious that relatively small changes of organic modifier content had a great influence on the separation.

The retention on column depends on the percentage of ionized and non-ionized species of each compound. The most dramatic pH-related changes in retention occur at pH values within pK_a ± 1.5. The ionization value helps in selecting the pH of the buffer in the mobile phase. The pK_a value of ONZ was found as 2.6 [12]. The pH of the mobile phase has always been adjusted to be 3.0 with 10 mM orthophosphoric acid, which is optimum pH with best peak asymmetry and retention values. The column temperature was set between 25 and 30°C. Higher temperature has been shown to minimize the tailing of the studied compounds by accelerating the rate of interaction with the stationary phase.

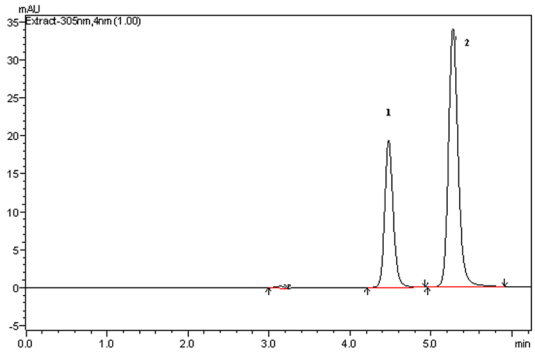

Figure 2. Chromatogram of a standard mixture of TNZ (4 μg/mL) (1) and ONZ (4 μg/mL) (2)

Mobile phases containing the organic modifier ACN have been shown to improve peak performance for studied compounds compared to those based on methanol. 30 °C was selected due to shorter analysis time and improved peak shapes. Finally, the mobile phase ACN : water 30 : 70 (***v/v***) with 10 mM H_3PO_4 (pH 3.0) at a flow rate of 1.0 mL min^{-1} was chosen as the most suitable carrier for LC analysis. The proposed LC method provides a simple procedure for the simultaneous analysis of ONZ and TNZ in drug formulations by DAD detection at 305 nm.

The most widely used criterion for the optimization of RP-LC is the resolution between peak pairs. In terms of fundamental chromatographic parameters the resolution, R_s, is affected by three independent variables:

$$Rs = \frac{1}{4}\sqrt{N}\,\frac{\alpha - 1}{\alpha}\,\frac{k_2}{k_2 + 1} \qquad (1)$$

where N is the number of theoretical plates. The selectivity factors for adjacent solute pairs were calculated in the usual way. Although the selectivity term is generally regarded as the most important in RPLC, full attention must be given to all of the terms in resolution in Eq. (1).

Table 1. The capacity factors, selectivity and separation factor values for the studied compounds

Compounds	ACN %	k_1	k_2	α	α -1/ α	$k_2/1+k_2$	$1/4\sqrt{N}$	R_s
TNZ/ONZ	25	1.101	1.569	1.425	0.298	0.611	96	4.375
TNZ/ONZ	30	0.841	1.170	1.391	0.281	0.539	92	3.492
TNZ/ONZ	35	0.614	0.825	1.344	0.256	0.452	86	2.493

Table 2. System suitability parameters of studied compounds

Parameters	TNZ	ONZ	Recommended value
Retention time (min)	5.123	6.262	-
Tailing factor (T)	1.183	1.178	≤ 2
Capacity factor	0.841	1.170	≥ 1
Resolution (R)	-	3.492	>2
Theoretical plates (N)	7513	8486	>2000
RSD% of retention time	0.012	0.013	≤ 1

After performing the experiments, R_s values were calculated for all peak pairs according to eq. (1). The chromatographic data obtained is illustrated in Table 1.

Using the conditions described above, a satisfactory chromatographic peak resolution was obtained in a short analysis time (< 6 min.) as seen in Figure 2. These compounds were clearly separated and their corresponding peaks were sharply developed.

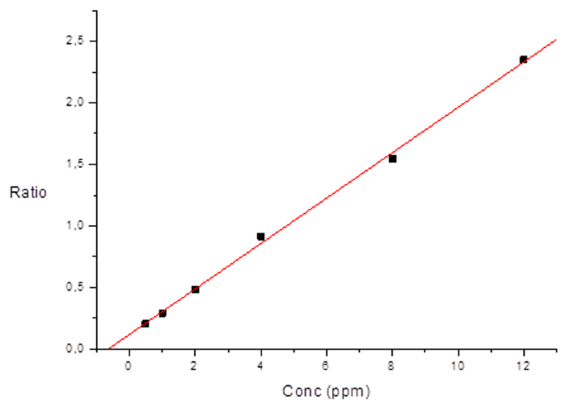

Figure 3. Calibration graph of ONZ taken under optimum conditions

Table 3. Statistical evaluation of the calibration data of ONZ by LC

Linearity Range (μg.mL^{-1})	0.5-12
Slope	0.185
Intercept	0.117
Correlation Coefficient	0.999
SE of slope	0.004
SE of intercept	0.024
Limit of Detection (μg.mL^{-1})	0.121
Limit of Quantification (μg.mL^{-1})	0.367

* Each value is the mean of five experiments.

System suitability tests are an integral part of HPLC method development. The USP Pharmacopoeia (24th) suggests that system suitability tests be performed prior to analysis [13]. The parameters include capacity factor, theoretical plate number, retention time, asymmetry factor, selectivity and RSD% of peak height or area for repetitive injections. System suitability tests were carried out on freshly prepared standard stock solutions of studied compounds. The results are shown in Table 2.

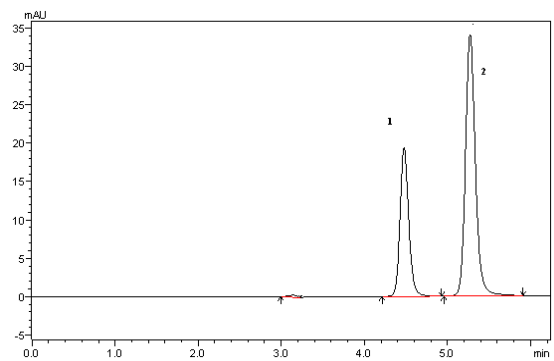

Figure 4. Chromatogram of tablet dosage forms (Biteral®),

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 0.5-12 μg ml^{-1} for ONZ. Peak area ratios of ONZ to the IS were plotted versus their respective concentrations in the mobile phase and linear regression analysis performed on the resultant curves. The regressions of the plots were computed by least square regression method. The linearity of the calibration plots was confirmed by the high value of the correlation coefficients for both compounds. Correlation coefficients were higher than 0.999 (Table 3). The linearity graph was shown in Figure 3. The low SE values of the slope and the intercept show the precision of the

Table 4. Summary of repeatability (intra-day) and reproducibility (inter-day) precision data for ONZ (n=5)

Compound	Concentration (μg ml $^{-1}$)	Intra-day(a)	Inter-day(b)
		Mean Recovery* % ± RSD %	Mean Recovery* % ± RSD %
ORN	1	99.805 ± 0.235	99.935 ± 0.320
	8	99.969 ± 0.523	100.161 ± 0.197

Table 5. Results of the assay and the recovery analysis of ONZ in pharmaceutical dosage forms

Compound	Labeled claim (mg)	Amount found (mg)[a]	RSD %	Added (mg)	Found (mg)[a]	Recovery %	RSD % of recovery
ONZ	250	249.706	0.359	250	249.923	99.962	0.153

proposed method. The LOQ was determined as the lowest amount of analyte. The LOD and LOQ were calculated from the following equations and using the standard deviation (s) of response and the slope (m) of the corresponding calibration curve [14-17].

LOD = 3.3 s/m; LOQ = 10 s/m (2)

Within calibration curves, two different concentrations were prepared in both media and assayed with related calibration curves to determine within-day and between-day variability. The within-day and between-day precision, accuracy, and reproducibility were determined as the RSD% and mean value (Table4).

The proposed HPLC method was applied to the analysis of tablets and the results obtained are summarized in Table 5. This method can be used successfully in the presence of drug without prior separation of the excipients. Each tablet contains the active ingredients of 250 mg of ONZ and the inactive ingredients as the excipients. Figure 3 shows a typical chromatogram obtained follow by analysis of ONZ in tablets Biteral® with IS. As shown in Figure 3, the substances were eluted, forming well shaped, symmetrical single peaks, well separated from the solvent front. No interfering peaks were obtained in the chromatogram due to tablet excipients so the removal of the excipients before the analysis was unnecessary. The utility of all of the proposed methods was verified by means of replicate estimations of pharmaceutical preparations and results obtained were evaluated statistically. The quantities found were in conformity with the values claimed by the manufacturer.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of ONZ in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. High percentages of recovery show that the method is free from the interferences of the commonly used excipients and additives in the formulations of the drug. It would be of interest for quality control and clinical monitoring laboratories to evaluate the product performance of a combined tablet dosage forms.

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