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Short Communication

Simultaneous determination of metronidazole and miconazole in pharmaceutical dosage forms by RP-HPLC

Cemal Akay [a], Sibel A. Özkan [b,*], Zühre Şentürk [c], Şemsettin Cevheroğlu [d]

[a] *Department of Pharmaceutical Sciences, Gülhane Military Medical Academy, 06018 Ankara, Turkey*
[b] *Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey*
[c] *Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey*
[d] *Turkish Army Drug Factory, 06110 Ankara, Turkey*

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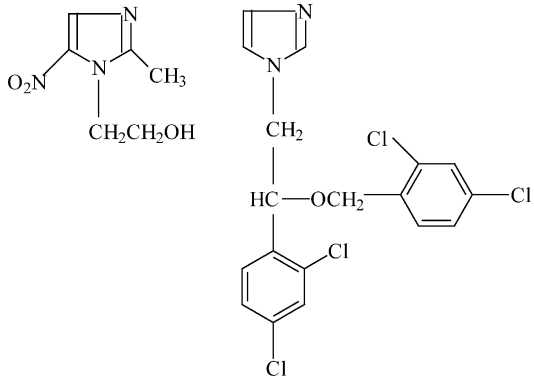
Abstract

A reversed-phase high performance liquid chromatography (RP-HPLC) method with UV detection is described for the simultaneous determination of metronidazole and miconazole in pharmaceutical dosage forms. Chromatography was carried out on a C18 reversed-phase column, using a mixture of methanol–water (40+60, v/v) as a mobile phase, at a flow rate of 1.0 ml min^{-1}. Sulfamethoxazole was used as an internal standard and detection was performed using a diode array detector at 254 nm. The method produced linear responses in the concentration ranges 10–70 and 1–20 μg ml^{-1} with detection limits 0.33 and 0.27 μg ml^{-1} for metronidazole and micanozole, respectively. This procedure was found to be convenient and reproducible for analysis of these drugs in ovule dosage forms.

Keywords: Reversed-phase high performance liquid chromatography; Metronidazole and miconazole; Pharmaceutical dosage forms

1. Introduction

Metronidazole (MTZ) is a 5-nitroimidazole derivative with activity against anaerobic protozoa and bacteria; it also has a radiosensitising effect on hypoxic tumour cells [1,2]. Miconazole, another imidazole derivative, is applied widely as the nitrate salt (MIC), to treat fungal infections [1,2]. The mixture of MIC with MTZ has been marketed in some commercial preparations for anti-protozoal and antibacterial therapy.



Metronidazole Miconazole

Literature survey reveals some methods for the determination of MIC in pharmaceutical preparations or in biological fluids including HPLC [3–7], gas chromatography [8], spectrophotometry [4,9–11], spectroflorimetry [11] and titrimetry [12]. Several methods have also been described for the determination of MTZ either alone or in combination with various drugs, such as spectrophotometry [13–15], HPLC [16–22], electrophoresis [23], and voltammetry [24].

* Corresponding author
E-mail address: ozkan@pharmacy.ankara.edu.tr (S.A. Özkan).

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To our knowledge, only a study [25] has been published recently for the determination of these two drugs from the combined dosage form, based on ratio spectra first-derivative spectrophotometry, and HPLC as a comparison method. However, as no internal standard is used, this reported HPLC procedure for pharmaceutical dosage forms could not be satisfactory for biological fluids, which require complicated and many-steps separation such as extraction and evaporation. There is no official method in any pharmacopoeias for this purpose.

An attempt was therefore made to develop a new, rapid, sensitive, simple and fully validated HPLC procedure using an internal standard for the determination of MTZ and MIC in presence of each other. The utility of the developed method to determine the content of both drugs in commercial ovules was also demonstrated.

2. Experimental

2.1. Apparatus

A liquid chromatographic system consisted of a Waters Isocractic LC pump 510, with an automatic sample injection system (Waters 717 plus Autosampler), equipped with a Waters 996 photodiode array detector. μ-Bondapak C18 reverse phase column packed with 10 μm dimethyl octadecylsilyl bonded amorphous silica (300 mm × 3.9 mm) was used as stationary phase.

2.2. Chemicals and reagents

MTZ and MIC and ovule dosage forms (Neo-Penotran®) were kindly supplied by Embil Pharm. Ind. (Istanbul, Turkey) and internal standard sulfamethoxazole was kindly provided from Fako Drug Inc. (Istanbul, Turkey).

Methanol was of HPLC grade, purchased from Merck (Darmstadt, Germany). All other chemicals were commercial analytical reagent grade. Doubly distilled water was used for preparing mobile phase solutions.

2.3. Chromatographic conditions

The proposed method was conducted using a reversed-phase technique, UV monitoring at 254 nm and sulfamethoxazole as an internal standard. A mixture of methanol–water (40+60, v/v) was used as a mobile phase. The mobile phase was prepared daily, filtered, and sonicated before use, and delivered at a flow rate of 1.0 ml min^{-1}. Fifty microliters of each solutions was injected and chromatograms were recorded.

2.4. Standard stock solution and calibration curves

Stock solutions of 1 mg ml^{-1} of MTZ and MIC were prepared by dissolving in methanol. Standard solutions for HPLC were prepared with mobile phase by varying the concentration of MTZ in the range of 10–70 μg ml^{-1} and MIC in the range of 1.0–20 μg ml^{-1}, maintaining the concentration of sulfamethoxazole (internal standard) at a constant level of 25.0 μg ml^{-1}. Calibration curve for HPLC analysis was obtained by plotting the peak area ratio of the drug to internal standard against the drug concentration.

2.5. Analysis of ovule dosage forms

Five ovules were weighed and melted on a steam bath, and then cooled while being stirred. The equivalent of the content of about one ovule was weighed accurately and transferred to a 100-ml calibrated flask and diluted with methanol, sonicated for 10 min and then completed to the volume with the same solvent. After filtration through a 0.45-μm membran filter, appropriate volume (equivalent to 20 μg ml^{-1} of MTZ and 4 μg ml^{-1} MIC) of the filtered solution was taken in a 10-ml flask. Appropriate amount of internal standard (25 μg ml^{-1}) was added and diluted up to the mark with the mobile phase. The amount of MTZ and MIC per ovule was calculated from the related linear regression equations.

3. Results and discussion

In order to effect the simultaneous analysis of the two component peaks under isocratic conditions, the mixtures of methanol or acetonitrile with water in different combinations were assayed as the mobile phase using C18 packing as stationary phase. Binary mixture of methanol–water in proportion of 40+60 (v/v), proved to be better than the mixture of acetonitrile–water for the separation since the chromatographic peaks were better defined and resolved, and almost free from tailing. Among several flow rates tested (0.5–2 ml min^{-1}), the rate of 1.0 ml min^{-1} was the best with respect to location and resolution of analytical peaks. The structure of sulfamethoxazole is not similar to those of MTZ and MIC. However, it was chosen as the internal standard because it showed better peak shape with better peak location, compared to other potential internal standards such as ornidazole, secnidazole. Using a diode array detector at 254 nm, the above described chromatographic conditions allow a resolution between MIC, MTZ and IS in a reasonable time of 3.35, 4.46 and 5.86 min, respectively.

System suitability test was applied to a representative chromatogram of freshly prepared standard stock solutions of MTZ and MIC, to check various parameters

Table 1
Characteristics of the linear regression analysis of metronidazole (MTZ) and miconazole nitrate (MIC)

	MTZ	MIC
Linearity range [a] (μg ml^{-1})	10–70	1–20
Slope	0.022	0.048
Intercept	0.018	0.002
Correlation coefficient	0.999	0.999
RSD of slope	1.07	1.18
RSD of intercept	0.26	0.41
LOD (μg ml^{-1})	0.33	0.27
LOQ (μg ml^{-1})	1.11	0.89

[a] Data represents five replicate injections of standard solutions.

such as resolution, selectivity and peak tailing. Resolution and selectivity factors for this system were found 2.23 and 1.11, respectively. Tailing factors were obtained as 1.13 for MTZ and 1.33 for MIC. The results are in concurrence with the USP requirements.

The peak area ratios of MTZ and MIC to the internal standard exhibited linear relationship with their concentrations. The characteristics of regression equations and the working concentrations are given in Table 1. The limit of detection (LOD) and limit of quantitation (LOQ) of the procedure are also shown in Table 1, which were calculated according to the $3s/m$ and $10s/m$ criterions, respectively, where s, is the standard deviation of the peak area ratios (four injections) of the sample and m is the slope of the corresponding calibration curve [26].

The intra- and inter-day variations of the method were determined using three replicate injections of four different concentrations, which were prepared and analysed on the same day and on three different days over a period of two weeks, respectively (Table 2). These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs.

Sample solutions analysed after 48 h did not show any appreciable change in assay values.

In order to assess the validity and applicability of the proposed method for assaying each drug in presence of the other, synthetic mixtures with different proportions of the two drugs were prepared and then assayed using the proposed method. (Table 3). The results obtained for the recovery of both drugs showed that the precision was satisfactory.

3.1. Assay in pharmaceutical dosage forms

On the basis of above results, the proposed method was applied to the determination of MTZ and MIC in ovule dosage forms which comprised the binary mixture (500 mg MTZ and 100 mg MIC). Fig. 1 shows a typical chromatogram obtained for the analysis of MTZ and MIC in ovules. The differences between the amount claimed and those measured were very low and the RSD values were within the acceptable windows mentioned by pharmacopoeias. The mean values of 496.2 and 99.4 with RSD% of 0.84 and 0.66 for MTZ and MIC, respectively ($n = 5$).

As the dosage form of MTZ and MIC is not pharmacopoeial yet, the recovery of the procedure was carried out by spiking the already analyzed samples of ovules with the known concentrations of standard MTZ and MIC. The mean percentage recoveries obtained after five repeated experiments were 99.25 and 99.36% with a RSD of 0.34 and 0.47% for MTZ and MIC, respectively, indicating that the results are accurate and precise and there is no interference from the common excipients used in the ovule formulation (Witepsol H15, which is mixture of mono-, di- and triglycerides of saturated fatty acids, cocoa butter, glyserine and gelatine).

4. Conclusion

It can be concluded that the proposed method is sufficiently sensitive and reproducible in the analysis of

Table 2
Intra-day and inter-day precision of metronidazole (MTZ) and miconazole nitrate (MIC) standard solutions

Comp.	Theoretical concentration (μg ml^{-1})	Intraday measured [a] concentration (μg ml^{-1})		Interday measured [b] concentration (μg ml^{-1})	
		Mean	RSD %	Mean	RSD %
MTZ	10	9.89	0.31	9.81	0.81
	15	14.57	0.32	14.50	0.58
	25	24.43	0.14	24.39	0.28
	50	49.46	0.73	49.22	0.57
MIC	5	4.91	0.81	4.83	0.99
	10	9.83	0.34	9.78	0.57
	15	14.73	0.31	14.67	0.51
	20	19.51	0.26	19.35	0.59

[a] Mean values represent three different sample standards for each concentration.
[b] Interday reproducibility was determined from three different runs for each concentration over a 2-week period.

Table 3
Determination of metronidazole (MTZ) and miconazole nitrate (MIC) in laboratory prepared mixtures by RP-HPLC

Added (μg ml^{-1})		Found (μg ml^{-1})		Recovery (%)		Mean recovery [a] (%)		RSD (%)	
MTZ	MIC	MTZ	MIC	MTZ	MIC	MTZ	MIC	MTZ	MIC
10	5	9.81	4.98	98.2	99.6				
15	5	14.88	4.91	99.2	98.2				
20	5	20.04	5.03	100.4	100.6				
30	5	29.56	4.95	98.5	99.0				
50	5	49.20	4.93	98.4	98.6	98.94	99.2	0.91	0.95
20	2	19.68	1.97	98.4	98.5				
20	5	20.07	4.92	100.4	98.4				
20	10	19.84	9.87	99.2	98.7				
20	15	19.68	14.75	98.4	98.3				
20	20	19.97	19.67	99.9	98.4	99.26	98.46	0.90	0.15

[a] Average of five experiments.

MTZ and MIC in pharmaceutical dosage forms within a short analysis time (<6 min). The proposed HPLC method was validated by evaluation of the validation parameters. The LOD, LOQ values, relative standard deviation of slope and intercept, correlation coefficient, within- and between-day reproducibility, resolution, selectivity, tailing and capacity factors for this technique were obtained.

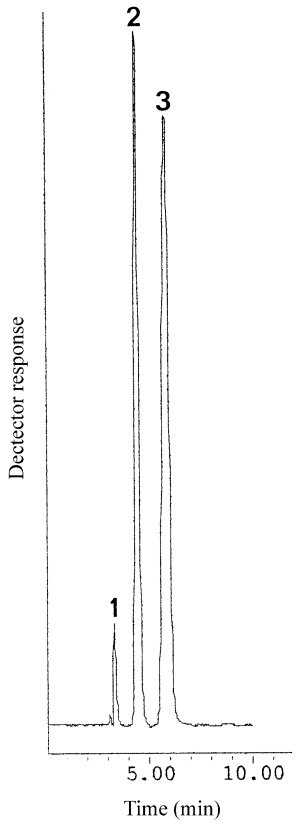

Fig. 1. The chromatogram of a solution of miconazole nitrate (MIC) (**1**) (4 μg ml^{-1}) and metronidazole (MTZ) (**2**) (20 μg ml^{-1}) with sulfamethoxazole (IS) (**3**) (25 μg ml^{-1}). Mobile phase, methanol:water (40+60; v/v), flow rate 1.0 ml min^{-1} and detection wavelength 254 nm.

Assay parameters used in this study reduced tailing for all the peaks and improved the resolution, however, in the case of previously reported HPLC method [25], the reduction in peak symmetry was observed accompanied by peak broading. The methods could not be compared in terms of resolution, validation parameters of the calibration graph, inter- and intra-day precision since these values were not given in reported method. The most considerable difference of this proposed method in comparison to reported HPLC method, is the addition of internal standard, which reduce the expected analytical errors due to the sample dilution and filtration procedure and improve the analytical performance of the method. Sensitivities of the both methods are almost same ($\sim$0.3 μg ml^{-1}) for MIC, whereas the proposed HPLC method (0.33 μg ml^{-1}) is about three times more sensitive than reported HPLC method (0.9 μg ml^{-1}) for MET. The proposed method is also more precise than literature method since the RSD values in ovules were reported as 1.06 and 2.31% for MET and MIC, respectively. It may be due to the presence of internal standard, which enabled good precision of the results.

On the other hand, high separation power of HPLC may be an advantage over spectrophotometry [25] for the analysis of these drugs in a variety of complex matrices. The applicability of proposed method to serum samples is under investigated.

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