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Spectrophotometric resolution of metronidazole and miconazole nitrate in ovules using ratio spectra derivative spectrophotometry and RP-LC

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Abstract

Metronidazole and miconazole nitrate in ovules was determined by ratio spectra derivative spectrophotometry and by high-performance liquid chromatography (HPLC). The first method depends on ratio spectra first derivative spectrophotometry, by utilizing the linear relationship between substances concentration and ratio spectra first derivative peak amplitude. The ratio first derivative amplitudes at 242.6 ($^1DD_{242.6}$), 274.2 ($^1DD_{274.2}$), 261.8 ($^1DD_{261.8}$), 273.5 ($^1DD_{273.5}$) and 281.5 ($^1DD_{281.5}$) nm were selected for the assay of metronidazole and miconazole nitrate, respectively. The second method is based on high-performance liquid chromatography on a reversed-phase column using a mobile phase of methanol–water–phosphoric acid (30:70:0.20 v/v) (pH 2.8) with programmable detection at 220.0 nm. The minimum concentration detectable by HPLC was 0.9 μg ml^{-1} for metronidazole and 0.3 μg ml^{-1} for miconazole nitrate and by ratio derivative spectrophotometry 4.0 μg ml^{-1} for metronidazole and 0.5 μg ml^{-1} for miconazole nitrate. The proposed procedures were successfully applied to the simultaneous determination of metronidazole and miconazole nitrate in ovules with a high percentage of recovery, good accuracy and precision.

Keywords: Metronidazole; Miconazole nitrate; Simultaneous determination; High-performance liquid chromatography; Ratio-spectra derivative spectrophotometry; Ovules

1. Introduction

Metronidazole, or 1-(β-hydroxyethyl)-2-methyl-5-nitroimidazole, is active against a wide variety of anaerobic protozoal parasites and anaerobic bacteria. Miconazole nitrate, or 1-[2,4-dichloro-(β-(2,4-dichlorobenzyloxy)phenethyl] imidazole, is an antibacterial of the class of imidazole. The two drugs are used in association in the treatment of antiprotozoal and antibacterial. So far no method available has been described for the simultaneous determination of these compounds in pharmaceutical forms.

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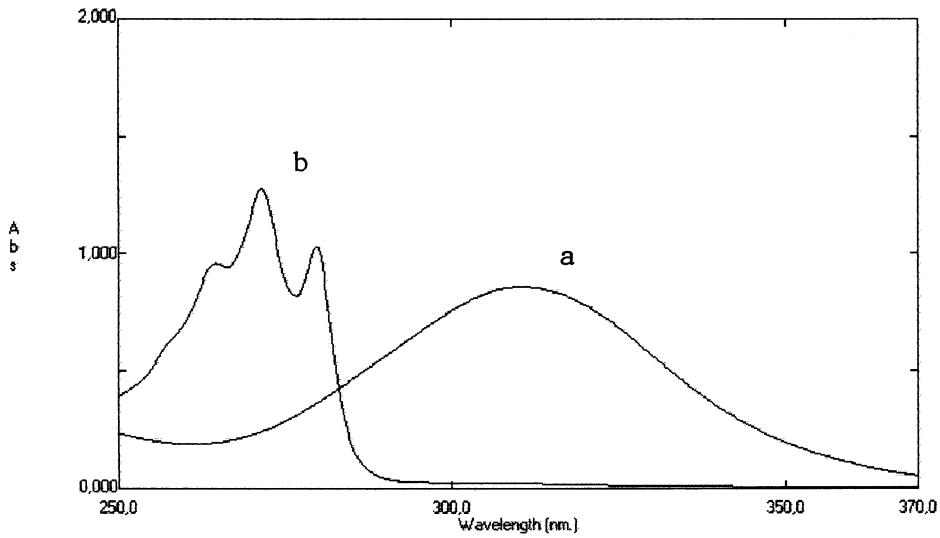

Fig. 1. Zero-order absorption spectra of (a) metronidazole (22.0 μg ml^{-1}); (b) miconazole nitrate (15.0 μg ml^{-1}) in methanol.

Some methods have been reported for the quantitative determination of metronidazole and miconazole nitrate individually or in combination with other drugs, including gas chromatography [1], voltammetry [2,3], high performance liquid chromatography [4–11] and spectrophotometry [12–23].

The ratio spectra derivative method was originally developed by Blanco et al. [24] and subsequently modified by Salinas et al [25]. This method is based on use of the first derivative of the ratio of the spectra and successfully applied to spectroscopic data [26,27]. The absorption spectrum of the mixture is obtained and divided by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph. This method can be applied for resolving binary mixtures of metronidazole–miconazole nitrate.

This paper reports a simple and fast method for the simultaneous quantitation of the two drugs in ovules by ratio spectra derivative spectrophotometry and HPLC providing accurate and precise results.

2. Experimental

2.1. Instrumentation

A double beam, Shimadzu 1601 spectrophotometer model with a fixed slit width (2 nm) connected to an IBM-PC computer loaded with a Lexmark printer was used for all the absorbance signals and treatment of data.

The assays were performed with an HPLC system consisting of a JASCO model PU-980 pump and JASCO UV-975 UV/VIS detector. Samples were injected with a 7725 Rheodyne injector system with a 20-μl sample loop. The detector was set at 220 nm (0.02 a.u.f.s) and peak areas were integrated automatically by computer using the Borwin software programme.

Other apparatus used included a Radiometer NEL pH 890 digital pH meter equipped with a combined glass–calomel electrode and ultrasound generator.

2.2. Chemicals

Metronidazole and miconazole nitrate were obtained from Embil Pharm. Ind. Analytical grade phosphoric acid, double distilled water, and HPLC grade methanol were used.

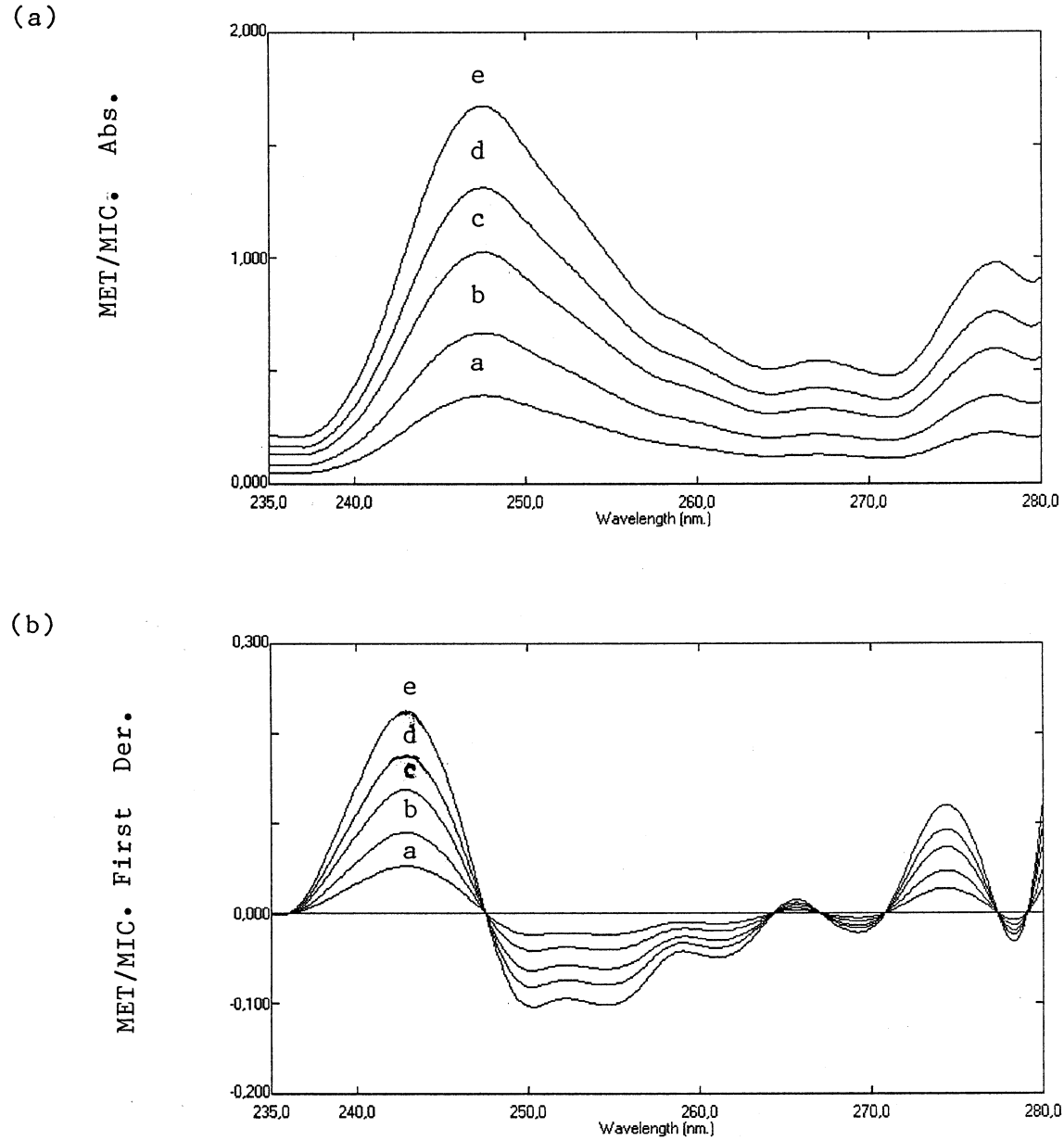

Fig. 2. Ratio spectra (a) and first derivative of the spectra (b) metronidazole for (a) 6.0 μg ml^{-1}, (b) 10.0 μg ml^{-1}, (c) 14.0 μg ml^{-1}, (d) 18.0 μg ml^{-1}, (e) 22.0 μg ml^{-1}, where 9.0 μg ml^{-1} miconazole nitrate was used as divisor in methanol.

2.3. Pharmaceutical preparation

A commercial pharmaceutical preparation, NEO-PENOTRAN® ovule (produced by Embil Pharm. Ind., Turkey), was assayed, batch no: 4578578, containing 500.0 mg of metronidazole and 100.0 mg of miconazole nitrate per ovule.

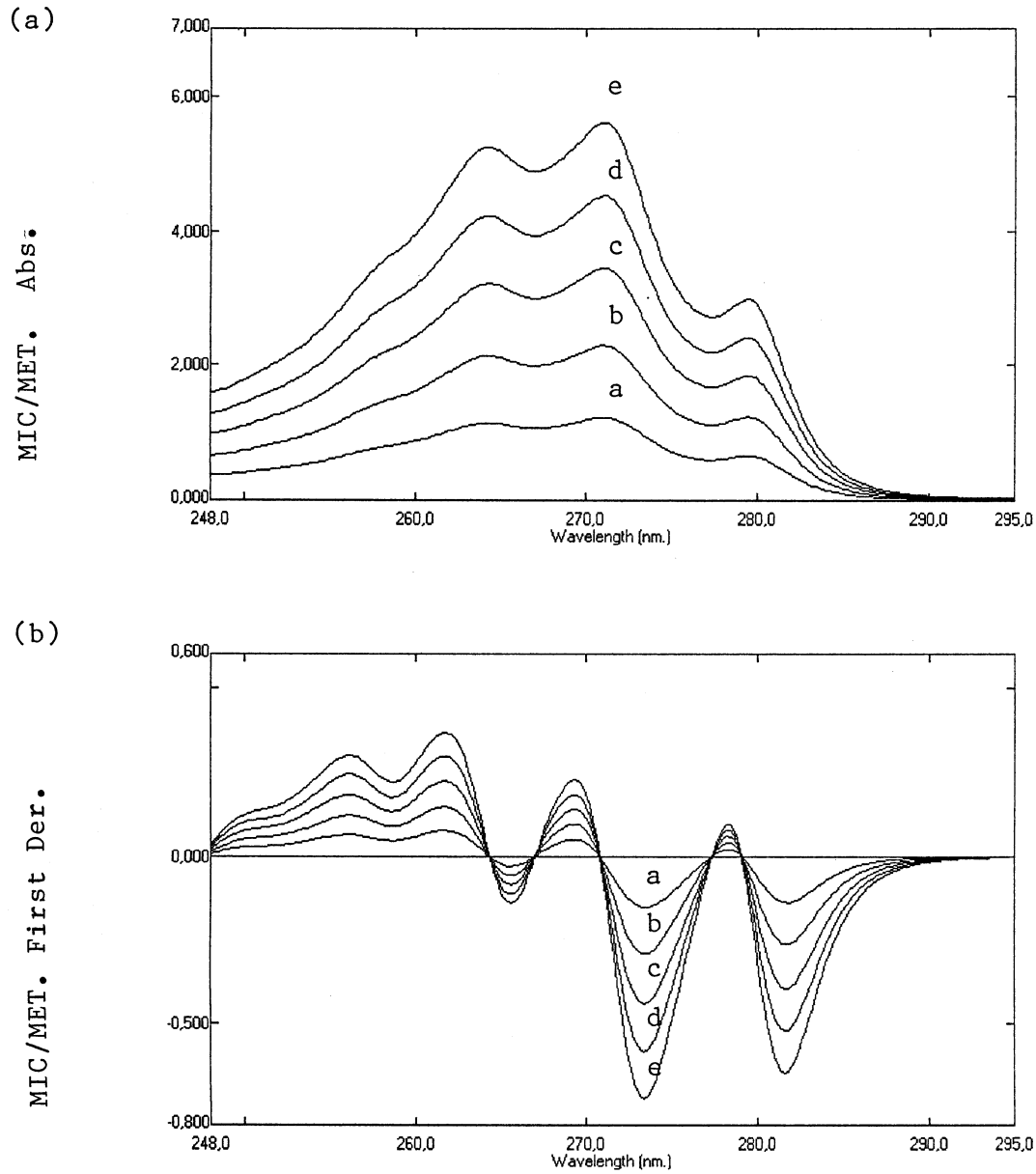

Fig. 3. Ratio spectra (a) and first derivative of the spectra (b) miconazole nitrate for (a) 3.0 μg ml^{-1}, (b) 6.0 μg ml^{-1}, (c) 9.0 μg ml^{-1}, (d) 12.0 μg ml^{-1}, (e) 15.0 μg ml^{-1}, where 14.0 μg ml^{-1} metronidazole was used as divisor in methanol.

2.4. UV measurements

The absorption spectra of the solutions prepared at different concentrations of metronidazole and of its binary mixtures with miconazole nitrate were recorded and divided by the spectrum of the standard solution of miconazole nitrate (9.0 μg ml^{-1} in methanol). The first derivatives of the ratio spectra were calculated with $\Delta\lambda = 8$ nm. In the binary mixtures, the concentration of metronidazole was determined by measuring the first derivative signals at 242.6 ($^1DD_{242.6}$) and 274.2

Table 1
Regression analysis of metronidazole and miconazole nitrate standard solution using ratio first-derivative spectrophotometry

Sample (μg ml^{-1})		Regression equation[b]	Correlation coefficient	SE[c]	
Met[a]	Mic[a]			Slope	Intercept
6.0–22.0	–	$^1DD_{242.6} = 2.4\times10^{-3}C + 3.0\times10^{-4}$	0.9999	1.6×10^{-7}	3.2×10^{-6}
6.0–22.0	–	$^1DD_{274.2} = 4.7\times10^{-3}C + 8.6\times10^{-4}$	0.9986	4.8×10^{-6}	8.9×10^{-5}
–	3.0–15.0	$^1DD_{261.8} = 3.7\times10^{-4}C + 2.3\times10^{-5}$	0.9981	5.2×10^{-5}	4.9×10^{-5}
–	3.0–15.0	$^1DD_{273.5} = -1.8\times10^{-3}C - 2.4\times10^{-4}$	0.9999	4.3×10^{-7}	9.1×10^{-6}
–	3.0–15.0	$^1DD_{281.5} = -2.8\times10^{-4}C - 9.5\times10^{-4}$	0.9999	7.8×10^{-8}	1.1×10^{-5}
5.0–26.0	–	$HPLC = 1.4\times10^{-4}C + 3.8\times10^{-5}$	0.9999	3.3×10^{-7}	1.4×10^{-6}
–	0.7–18.0	$HPLC = 5.9\times10^{-4}C + 7.4\times10^{-5}$	0.9986	8.4×10^{-6}	4.1×10^{-6}

[a] Met, metronidazole; Mic, miconazole nitrate.
[b] Amplitude of the ratio first-derivative signals versus amount of drug (μg ml^{-1}).
[c] Standard error of slope and intercept.

($^1DD_{274.2}$) nm for the metronidazole–miconazole nitrate mixtures, respectively.

A similar procedure was followed for the different concentrations of miconazole nitrate when metronidazole was 14.0 μg ml^{-1}. In the same way as describe above, the content of acetaminophen, and analgin was determined by selecting the first derivative of the ratio spectrum in the range 248.0–295.0 and measuring the signals at 261.8 ($^1DD_{261.8}$), 273.5 ($^1DD_{273.5}$) and 281.5 ($^1DD_{281.5}$) nm, respectively.

Metronidazole and miconazole nitrate are soluble in methanol and their solutions were found to be stable for 2 h at least.

2.5. Standards solutions and calibration graphs for spectrophotometric measurements

Stock solutions were prepared by dissolving metronidazole and miconazole nitrate in methanol to obtain a concentration of 1.0 mg ml^{-1} for each compound. The standard solutions were prepared by dilution of the stock solutions in methanol to reach concentration ranges 6.0–22.0 and 3.0–15.0 μg ml^{-1} for metronidazole and miconazole nitrate, respectively.

2.6. Chromatographic conditions

The eluting medium consisting of methanol–water–phosphoric acid (30:70:0.20 v/v) was prepared and degassed by bubbling helium gas for 5 min, prior to use. Column equilibrium with the eluting solvent was established by pumping the mobile phase at a rate of 0.3 ml min^{-1} overnight. The injection volume was 20 μl with the flow rate set at 1.2 ml min^{-1} during analysis. All determinations were performed at ambient temperature

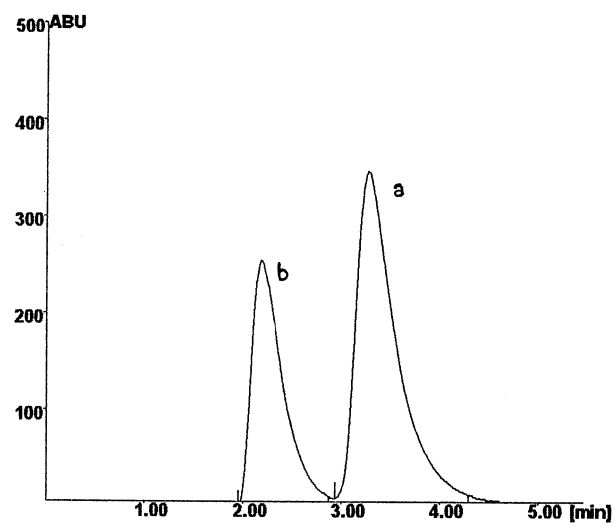

Fig. 4. A typical chromatogram of (a) Neo-Penotran® ovule metronidazole and (b) miconazole nitrate.

Table 2
Assay results for synthetic mixtures and pharmaceutical preparations using ratio first-derivative spectrophotometry and high performance liquid chromatography

Sample	Recovery (%, mean ± S.D.)[a]				
	Metronidazole		Miconazole nitrate		
	$^1DD_{242\cdot6}$	$^1DD_{274.2}$	$^1DD_{261.8}$	$^1DD_{273.5}$	$^1DD_{281.5}$
Ratio Spectra First Derivative Spectrophotometry					
Synthetic mixtures	99.0 ± 0.79	98.1 ± 2.36	98.8 ± 1.93	98.8 ± 0.29	99.5 ± 1.24
Commercial ovules[b]	99.1 ± 2.88	98.9 ± 1.65	99.8 ± 2.61	98.8 ± 0.98	97.9 ± 1.98
High Performance Liquid Chromatography					
	Metronidazole		Miconazole nitrate		
Synthetic mixtures	99.8 ± 0.99		99.8 ± 1.14		
Commercial ovules[b]	99.9 ± 1.06		99.2 ± 2.31		

[a] Mean and relative standard deviation for five determinations; percentage recovery from the label claim amount.
[b] Neo-Penotran® ovules are the product of Embil Pharm. Ind., Turkey; each ovule was labeled to contain 500.0 and 100.0 mg of metronidazole and miconazole nitrate, respectively.

(25°C) using Hichrom C_{18}, reverse phase column (10 μm, 200 × 4.9 mm i.d.). The column effluent was monitored at 220.0 nm which represents the wavelength of maximum absorbancy of metronidazole, and miconazole nitrate.

2.7. Standards solutions and calibration graphs for chromatographic procedure

Standard solutions of metronidazole and miconazole nitrate containing concentration ranges of 5.0–26.0 and 0.7–18.0 μg ml^{-1}, respectively, were prepared in the mobile phase. Triplicate 20-μl injections were made for each solution and the peak area ratio of each drug was plotted against the corresponding concentration to obtain the calibration graph.

2.8. Sample preparation

Five ovules were placed in a dish, gently heated on a steam bath until melted, then stirred, cooled while being stirred and weighed. The equivalent of the content of about one ovule was accurately weighed and transferred to a 100-ml calibrated flask, in methanol for UV method and in mobile phase for HPLC method and mixed well. The flasks were made up to volume with the same solvent. The HPLC samples were filtered through a 0.45-μm membrane filter, then further diluted to suit the calibration graphs for the ratio first derivative spectrophotometric measurements.

3. Results and discussion

3.1. Ratio spectra derivative spectrophotometry

Since both metronidazole and miconazole nitrate dissolve much better in methanol than water, methanol was used first as the solvent for the spectrophotometric method. The stability of working solutions of metronidazole and miconazole nitrate was studied by recording their absorption spectra. At first these spectra were measured. No changes in the spectra were observed for 1 day, therefore standard and sample solutions containing metronidazole and miconazole nitrate were prepared freshly every day and they were kept in amber-colored or aluminium foil-wrapped volumetric flasks.

Fig. 1 shows the absorption spectra of metronidazole and miconazole nitrate. The absorption spectra of the two components strongly overlap.

This spectral overlapping was sufficient to demonstrate the resolving power of the proposed method. Fig. 2(a,b) shows the ratio spectra of different metronidazole standards (spectra divided by the spectrum of a 9.0 μg ml^{-1} miconazole nitrate solution) and their first derivatives. The ratio first-derivative amplitudes at 242.6 ($^1DD_{242.6}$)[1] and 274.2 ($^1DD_{274.2}$) nm corresponding to two maximum wavelengths are proportional to the metronidazole concentration. On the other hand, for determining the miconazole nitrate, an analogous procedure was followed. Fig. 3(a,b) shows the ratio spectra of different standards of miconazole nitrate and their first derivatives. The concentration of the divisor metronidazole was 14.0 μg ml^{-1}, the first derivative was made with $\Delta\lambda = 8$ nm and the derivative ratio spectra were smoothed with 15 experimental points. Calibration graphs were made from the maximum at 261.8 ($^1DD_{261.8}$) nm and two minimum at 281.5 ($^1DD_{281.5}$) nm and 273.5 ($^1DD_{273.5}$) nm wavelengths. The concentration range for Beer's law compliance was 6.0–22.0 μg ml^{-1} for metronidazole and 3.0–15.0 μg ml^{-1} for miconazole nitrate. Table 1 summarizes the statistical data for the calibration graphs in the determination of metronidazole and miconazole nitrate.

Recovery studies were performed on the synthetic mixtures prepared by adding accurately weighed amounts of drugs in our laboratory. Mean recovery was found to be (99.0–98.1%) for metronidazole and 98.8% for miconazole nitrate in binary mixtures. The limit of detection (LOD) and the limit of quantification (LOQ) of metronidazole and miconazole nitrate were calculated. The detection limits (LOD) were 4.0 μg ml^{-1} for metronidazole and 0.5 μg ml^{-1} for miconazole nitrate while the quantification limits (LOQ) were 7.0 μg ml^{-1} for metronidazole and 3.4 μg ml^{-1} for miconazole nitrate.

[1] order derivative$^{\text{Derivative Divided}}_{\text{wavelength measure}}$.

3.2. HPLC methods

Various mobile phase systems were prepared and used for chromatographic separation, but the proposed mobile phase comprising methanol–water–phosphoric acid (30:70:0.20 v/v) gave a better resolution and sensitivity for metronidazole and miconazole nitrate. The mobile phase composition was optimised. Under the described conditions the analyte peaks were well defined, resolved and free from tailing. The elution order was metronidazole (t_r: 3.26 min) and miconazole nitrate (t_r: 2.17 min) at a flow rate of 1.2 ml min^{-1} (Fig. 4). The optimum wavelength for detection was 220.0 nm at which good detector response was obtained.

Linearity was obtained for metronidazole in the concentration range of 5.0–26.0 μg ml^{-1} and miconazole nitrate in the concentration range 0.7–18.0 μg ml^{-1} (Table 1). The regression curve was calculated by the least-squares method and the correlation coefficients were 0.9999 for metronidazole and 0.9986 for miconazole nitrate, indicating good linearity. Recovery studies were performed on the synthetic mixtures prepared by adding accurately weighed amounts of drugs in our laboratory. Mean recovery was found to be 99.8% for metronidazole and 99.8% for miconazole nitrate in binary mixtures. Five replicate determinations at different concentration levels were carried out to test the precision of the methods.

The detection limits (LOD) were 0.9 μg ml^{-1} for metronidazole and 0.3 μg ml^{-1} for miconazole nitrate while the quantification limits (LOQ) were 2.7 μg ml^{-1} for metronidazole and 1.4 μg ml^{-1} for miconazole nitrate.

The proposed methods were applied to the analysis of commercial preparations of metronidazole and miconazole nitrate and laboratory prepared mixtures and the results are presented in Table 2. The relative standard deviations were found to be less than 2.88%, indicating reasonable repeatability of the proposed methods.

Spectrophotometric readings showed no interference by the common excipients placed in the ovule formulations (Witepsol H15 is a mixture of mono-, di- and triglycerides of saturated fatty acids containing a maximum of 15 hydroxyl groups, cocoa butter, glycerine and gelatine).

4. Conclusions

The ratio derivative spectrophotometry and HPLC methods described were found to be reproducible and accurate in the analysis of metronidazole and miconazole nitrate mixtures in commercial formulations without the interference from each drug or ovule excipients. The HPLC method was shown to be a versatile reference method and may offer advantages over ratio derivative spectrophotometry for the selective determination of the two intact drugs in a variety of matrices, but is also more expensive. In general, all the proposed methods can be used for the routine analysis of metronidazole and miconazole nitrate in bulk and ovule dosage forms.

References

[1] E. Kublin, T. Kaniewska, Chem. Anal. 41 (1996) 19.
[2] Z. Wang, J. Liu, S. Zhou, Fenxi Shiyanshi 17 (1998) 40.
[3] Z. Yao, J. Hu, Z. Wu, Q. Li, Anal. Lett. 31 (1998) 429.
[4] J. Han, H. Zeng, H. Tang, Yaowu Fenxi Zazhi 17 (1997) 9.
[5] S.V. Raj, S.U. Kapadia, A.P. Argekar, Indian Drugs 34 (1997) 585.
[6] A.P. Argekar, S.J. Shah, Indian Drugs 30 (1997) 520.
[7] A.P. Argekar, S.J. Shah, Indian Drugs 35 (1998) 71.
[8] D.R. Nadkarni, R.N. Merchant, M. Sundaresan, A.M. Bhagwat, Indian Drugs 34 (1997) 393.
[9] I.C. Bhoir, B. Raman, M. Sundaresan, A.M. Ashok, Anal. Chim. Acta 354 (1997) 123.
[10] Y. Yuan, L. Shi, X. zheng, J. Yuan, Zhongguo Yaoxue Zazhi 33 (1998) 42.
[11] V.R. Bari, U.J. Dhorda, M. Sundarsan, Anal. Chim. Acta 376 (1998) 221.
[12] P. Parimoo, C.V.N. Prasad, R. Vineeth, J. Pharm. Biomed. Anal. 14 (1996) 389.
[13] A.S. Amin, Anal. Lett. 30 (1997) 2503.
[14] M.Y. Mohamed, A.E. El-Gendy, M. El-Bardicy, Spectrosc. Lett. 29 (1996) 299.
[15] R. Paliwal, D.K. Jain, P. Trivedi, Indian Drugs 35 (1998) 165.
[16] R. Paliwal, D.K. Jain, R.S. Gaud, P. Trivedi, Indian J. Pharm. Sci. 60 (1998) 140.
[17] K. Wrobel, K. Wrobel, I.M. De La Garza Rodriguez, P.L. Lopez-De-Alba, L. Lopez-Martinez, J. Pharm. Biomed. Anal. 20 (1999) 99.
[18] X. Guo, Q. Wu, J. Yu, K. Dong, C. Chen, Guangdong Yaoxueyuan Xuebao 14 (1998) 33.
[19] H. Wang, Zhongguo Yiyuan Yaoxue Zazhi 18 (1998) 544.
[20] S. Fu, D. Chen, Huaxi Yaoxue Zazhi 13 (1998) 114.
[21] S. Zhang, H. Li, D. Zhang, Q. Zhou, Guangpaxue Yu Guangpu Fenxi 18 (1998) 488.
[22] D. Basu, K.K. Mahalanabis, B. Roy, Anal. Chim. Acta 249 (1991) 349.
[23] T. Das, C. Das, S. Ray, Indian J. Pharm. Sci. 54 (1992) 111.
[24] M. Blanco, J. Gene, H. Iturriaga, S. Maspoch, J. Riba, Talanta 34 (1987) 987.
[25] J.J. Berzas, C. Guibertean, F. Salinas, Talanta 39 (1992) 547.
[26] J.J. Berzas Nevado, C. Guilberteau abanillas, Anal. Lett. 23 (1990) 2077.
[27] R. Corbella Tena, M.A. Rodriquez Delgado, M.J. Sanchez, F. Garcia Montelongo, Talanta 44 (1997) 673.