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Determination of miconazole in pharmaceutical creams using internal standard and second derivative spectrophotometry

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Abstract

A simple method is proposed for miconazole determination in pharmaceutical creams, based on extraction and second derivative spectrophotometry. In the presence of sodium lauryl sulfate (0.5%) and sulphuric acid (0.4 mol l^{-1}), the miconazole and internal standard (IS) (methylene blue) were extracted to 100 μl of methylene chloride. The organic phase was evaporated in the nitrogen stream and the dry residue was dissolved in methanol (1.5 ml). The analytical signal was obtained as the ratio between second derivative absorbances measured at 236.9 nm (miconazole) and at 663.2 nm (IS). The use of IS in such multi-stage procedure enabled quite good analytical performance in calibration range 50.0–400 mg l^{-1}: linear correlation coefficient 0.9995, precision (measured as CV for ten replicates) at 50.0 mg l^{-1} and at 400 mg l^{-1} of miconazole was 1.5 and 0.5% respectively. Four commercial pharmaceutical creams were analyzed and the results obtained were in good agreement with the results obtained by reversed-phase high performance liquid chromatography (HPLC).

Keywords: Miconazole; Internal standard; Derivative spectrophotometry; Pharmaceutical creams

1. Introduction

Miconazole is a drug of wide antifungal spectrum. It is administered by the troche dosage form or by the intravenous infusion in the treatment of severe systemic fungal infections, or applied as a 2% cream or powder in infections of nails and skin [1].

Several analytical procedures have been proposed for the quantification of miconazole in pharmaceuticals [2–5] and in biological fluids [6–9]. For clinical samples, high performance liquid chromatography (HPLC) was used. Before introduction on the ODS column, the samples were deproteinized with acetonitrile [8]. Separation of miconazole was also achieved by liquid–liquid extraction [7] and solid phase extraction [6]. Szathmary et al. determined miconazole in human plasma using a multi-stage separation/preconcen-

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tration procedure coupled with gas chromatography [9].

High performance liquid chromatography was also applied for the determination of miconazole in pharmaceuticals [3–5]. However, relatively high content of the drug in pharmaceutical creams (2%) calls for the use of a simple and less sensitive procedure. The commercial formulations often contain the mixture of miconazole with metronidazole, clotrimazole, econazole, etc. [1], thus two or more drugs have to be quantified in the sample [4,10,11]. For such an analytical task, derivative spectrophotometry seems to be a good alternative [2,4]. Cavrini et al. determined miconazole and metronidazole in pharmaceutical formulations by derivative spectrophotometry (second and third order spectra) and by HPLC [4]. Satisfactory resolution of the two drugs was achieved by derivative spectrophotometry and a good agreement between the results obtained using the two procedures was reported.

The application of derivatization procedure for UV-Vis spectra enables multicomponent analysis in spectrophotometry. On the other hand, the serious limitation of separation/preconcentration, or a many fold dilution procedure, is the risk of analytical errors which can cause the poor analytical performance of the method. The above mentioned facts encouraged us to introduce the method of internal standard (IS) in the multistage spectrophotometric procedures [12,13]. Considering the general requirements for an IS in different analytical techniques [14], in spectrophotometry the following parameters should be taken into account: (i) similar chemical properties of the IS and the analyte (polarity, ion-pair or complex formation, etc., depending on the procedure used); (ii) no interactions between the IS and the analyte; (iii) low absorbance of the IS in the region of the absorption band of the analyte; (iv) high absorbance of the IS in the region of low analyte absorption (in the UV-Vis region); and (v) IS should not be a natural component of the sample. We found that, using the appropriate IS, errors due to sample dilution [12] and due to preconcentration procedure [13] were reduced if taking the ratio of analyte to IS signals obtained from derivative spectra.

The intent of the present work was to develop a new extraction–spectrophotometric procedure for reliable determination of miconazole in pharmaceutical creams using IS and derivative spectrophotometry. The results obtained were compared with the results of reversed-phase HPLC.

2. Experimental

2.1. Apparatus

A Spectronic 3000 Diode Array Milton Roy spectrophotometer was used (resolution 0.35 nm) coupled to a 486 PC User Data version 2.01 (Milton Roy Inst. Co.) software was used for acquisition, storage and manipulation of spectral data. All data treatment was carried out using a Hewlett-Packard Vectra 486/66 VL microcomputer equipped with the Grams/386 TM software package, version 3.01A (Galactic Ind. Co., Salem, MA).

A Hewlett-Packard (Waldrom, Germany) Series 1050 high performance liquid chromatograph with a multiple wavelength spectrophotometric detector and ChemStation was used. A Waters μBondapak C18 column (3.9 × 300 mm) was used at room temperature.

2.2. Reagents

The solvents were of HPLC-grade quality and all others chemicals were of analytical-reagent grade. A stock solution of miconazole nitrate (1000 mg l^{-1}) was prepared by dissolving the Sigma reagent in ethanol. Methylene blue (IS) was from Aldrich: 1000 mg l^{-1} aqueous solution was prepared. The aqueous solutions of sodium lauryl sulfate (SDS, 5%) was prepared from Sigma reagent. Sulphuric acid (1 mol l^{-1}) was prepared by diluting the concentrated acid (ASC reagent, Sigma) with deionized water. A buffer solution, pH 2.5, was prepared from 15 mmol l^{-1} triethylamine (Aldrich) by adjusting the pH with a mixture of 2.0 mol l^{-1} phosphoric acid (J.T. Baker) and 0.5 mol l^{-1} sulphuric acid. The 1000 mg l^{-1} solutions of butylated hydroxytoluene (BTH)

(Sigma) and butylated hydroxyanisole (BHA) (Sigma) were prepared in methanol. Methylene chloride, acetonitrile and methanol were purchased from J.T. Baker Chemicals (USA). Deionized water (Labconco, USA) was used throughout.

Cream placebo was prepared in the laboratory from the following Sigma reagents: 1.6 g of cetyl alcohol, 4.7 g of stearyl alcohol, 1.1 g of stearic acid, 0.1 g of propyl paraben, 2.4 g of Tween 61, 2.5 g of Tween 60, 0.8 g of Carbopol 940, 0.8 g of triethanolamine, 7.4 g of myristic acid propyl ester and 76.8 g of deionized water.

Parmaceuticals analyzed were: (1) Dactarin (miconazole nitrate in cream), Janssen Pharmaceutica, S.A. de C.V., Mexico; (2) Neomicol (miconazole nitrate in cream), MEDIX, S.A. de C.V., Mexico; (3) Aloid (miconazole nitrate in cream), Cilag de Mexico, S.A. de C.V., Mexico; and (4) Fungiquim (miconazole nitrate in cream), Química y Farmacia, S.A. de C.V., Mexico.

2.3. Procedures

2.3.1. Spectrophotometric procedure

For calibration, varying volumes of stock miconazole solution (0, 0.25, 0.50, 1.0, 1.5, and 2.0 ml) were introduced to test tubes and diluted with ethanol to 2 ml. Then, 2 ml of sulphuric acid (1 mol l^{-1}), 0.5 ml of sodium lauryl sulfate (5%), 0.5 ml of methylene blue (1000 mg l^{-1}, IS) were added. For extraction, a volume of 100 μl was taken from each tube and transferred to Eppendorf tube (1.5 ml), then 100 μl of methylene chloride was added. After extraction and centrifugation ($3000 \times g$, 1 min), about 50 μl of organic phase was taken, and evaporated in a nitrogen stream [13]. In order to remove traces of methylene chloride, the dry residue was dissolved in 200 μl of methanol, solvent was evaporated and the residue was again dissolved in methanol and evaporated. Finally, the dry residue was dissolved in 1.5 ml of methanol and absorption spectra were recorded in the wavelength range 200–800 nm against methanol as a blank. The obtained spectra were smoothed through 35 experimental points and second derivative spectra were calculated ($\Delta\lambda = 12$ nm) using the Savitzky–Golay procedure [15]. The zero-crossing technique was used and the analytical signal was evaluated as the ratio between second derivative absorbances at 236.9 nm (miconazole) and at 663.2 nm (IS). In parallel, calibration was carried out with solutions containing chemical matrix of real samples matched by addition of cream placebo. For each calibration solution, 800 mg of placebo was dissolved in 40 ml of ethanol (60°C, 30 min), the obtained solutions were filtered, the appropriate volumes of miconazole standard solution was added and the volume was brought up to 50 ml in the calibration flasks.

Pharmaceutical formulations were analyzed following the procedure for calibration samples: 800 mg of cream samples were taken for analysis ($n = 5$, samples from one pharmaceutical container).

2.3.2. HPLC procedure

The liquid-chromatographic method described elsewhere [16] was used with some modifications. For calibration, a series of miconazole solutions (containing 0, 2.0, 5.0, 10.0, 20.0, and 40.0 mg l^{-1}) was prepared by diluting the stock miconazole standard with acetonitrile: buffer solution (7:3 v:v, pH 2.5) in the calibration flasks (volume 50 ml). Cream samples (800 mg) were dissolved in 40 ml of ethanol (60°C, 30 min), filtered and the volume was brought up to 50 ml in the calibration flasks. The obtained solutions were 10 fold diluted with mobile phase and filtered. A volume of 20 μl was introduced on to the column and isocratic elution was carried out at a flow rate 1.5 ml min^{-1} with the mobile phase containing 70% acetonitrile and buffer solution (pH 2.5). Spectrophotometric detection was at 225 nm and the peak area was taken as the measurement mode.

3. Results and discussion

3.1. Extraction-spectrophotometric procedure

Considering the complex composition of cream samples, the separation of miconazole (nitrate salt of 1-[2,4-Dichloro-β-([2,4-dichlorobenzyl]oxy)-phenethyl] imidazole) was first carried out. The

extraction to methylene chloride was achieved by ion-pair formation with lauryl sulfate in the presence of sulphuric acid (to assure better yield of ion-pair formation [17]). To avoid excessive dilution of the sample, only 100 μl of the solution was taken and extraction was carried out with 100 μl of methylene chloride. After extraction, about 50 μl of the organic phase was taken for analysis. Due to high absorption of methylene chloride up to 235 nm [18], the solvent was eliminated by evaporation in the nitrogen stream and the residue was dissolved in methanol. Using such a procedure scheme (details in Procedures), the calibration range obtained (50.0–400 mg l^{-1} as referred to the miconazole concentration in solutions before extraction–evaporation–dissolution) covered the miconazole concentration range expected in the solutions of real samples (about 320 mg l^{-1}). Although the procedure seems to present important advantages (small volumes of organic solvents), there is a risk of analytical errors in every step: extraction, evaporation and dissolution, and due to the use small volumes of volatile solvents. In order to minimize such possible errors, a method of IS was applied.

Taking into account general requirements for IS in spectrophotometry [12], in the proposed analytical procedure IS should form ion-pairs with lauryl sulfate, easily be extracted to methylene chloride, and should present the absorption band possibly in a different spectral region from the analyte. In preliminary experiments it was observed that methylene blue (3,7-bis[-dimethylamino]phenazothionum chloride) complied with these requirements. The extraction of miconazole (400 mg l^{-1}) and methylene blue (100 mg l^{-1}) to methylene chloride was studied in the presence of lauryl sulfate. The increasing extraction yields were observed with the increasing concentration of SDS and, starting from 0.2% SDS, the extraction yields of miconazole and of methylene blue did not depend on SDS concentration up to 2% ($99.2 \pm 0.6\%$ and $99.4 \pm 0.5\%$ respectively). The concentration 0.5% of lauryl sulfate in methanol was finally selected. In Fig. 1 it can be observed that miconazole nitrate presents one spectral band with the maximum at 205 nm and methylene blue presents absorption bands in the wavelength ranges 200–350 and 550–700 nm. In order to improve spectral resolution of the two compounds, derivatization procedure was carried out (second derivative spectra). Using the method of zero-crossing, the analytical signal for miconazole should be obtained in the wavelength region of 220–250 nm, while the analytical signal of IS should be measured in the region 620–700 nm. Second derivative absorption spectra of the miconazole and of the methylene blue in the above mentioned wavelength regions are presented in Fig. 2. It can be observed that $\lambda = 663.2$ nm, corresponding to a minimum of the second derivative spectrum of methylene blue, should de used for IS measurement, as there is no spectral contribution of miconazole at this wavelength (Fig. 2b). The miconazole signal can be measured at $\lambda = 236.9$ nm, where the value of IS second derivative absorbance is zero (Fig. 2a). The analytical signal was evaluated as the ratio between signals measured at 236.9 nm (miconazole) and at 663.2 nm (IS):

$$S = {}^2D_{236.9}/{}^2D_{663.2}$$

In order to control better possible analytical errors, the same procedure was carried out for the calibration and for the real samples (see Procedures). Two sets of solutions were used for cali-

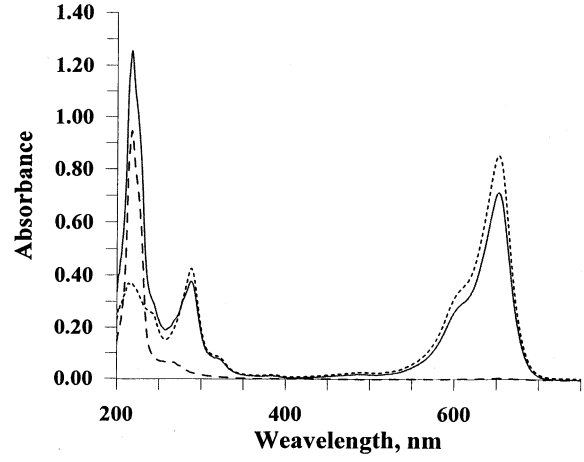

Fig. 1. Zero-order absorption spectra obtained in 0.05% lauryl sulfate in methanol: (– –), miconazole nitrate 5.0 mg l^{-1}; (---), methylene blue, 5.0mg × l^{-1}; (—), miconazole (5.0 mg l^{-1}) + methylene blue (6.0 mg l^{-1}).

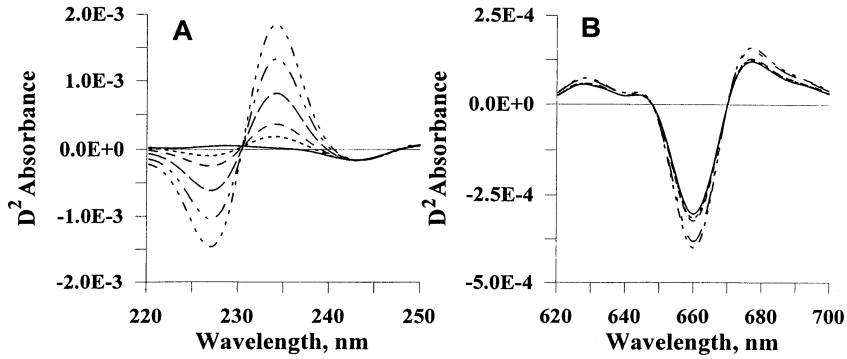

Fig. 2. Second-derivative spectra of the calibration solutions (given concentrations refer to solutions before extraction–evaporation–dissolution procedure) in the two spectral regions: (a) 220–260 nm; (b) 625 –700 nm. (—), methylene blue (IS), 200 mg l^{-1}, (---), IS + miconazole 50.0 mg l^{-1}, (– –), IS + miconazole 100 mg l^{-1}, (— —), IS + miconazole 200 mg l^{-1}, (– -- –), IS + miconazole 300 mg l^{-1}, (– --- –), IS + miconazole 400 mg l^{-1}.

bration: one containing only miconazole and second with addition of cream placebo. The analytical performances were evaluated and the obtained results are presented in Table 1, where good precision of miconazole determination can be observed. The slope and intercept of linear regression obtained for two sets of calibration samples were compared using statistical test t ($\alpha \leq 0.05$). The obtained results indicate that no statistically significant difference existed between these parameters, so we concluded that the presence of cream placebo did not interfere in miconazole determination by the proposed procedure. It should be mentioned that, when not using an IS, the analytical performance of the extraction–spectrophotometric procedure was poorer: the value of linear regression coefficient was lower than 0.95.

Table 1
Analytical characteristics of spectrophotometric procedure for miconazole in the calibration range 50.0–400 mg l^{-1} using two sets of calibration solutions[a]

Parameter	Calibration set (1)	Calibration set (2)
Linear regression equation	$S = -0.00532$, c -0.0153	$S = -0.00526$, c -0.0124
Regression coefficient	0.9995	0.9995
$CV_{50.0\ mg/l}$ (%)	1.5	1.6
$CV_{400\ mg/l}$ (%)	0.5	0.7

[a] Calibration set (1), miconazole + IS in ethanol, Calibration set (2), miconazole + IS + cream placebo in ethanol. $S = {}^2D_{236.9\ nm}/{}^2D_{663.2\ nm}$. c, miconazole concentration in calibration sample (before the procedure), mg l^{-1}. CV, variation coefficient evaluated for ten replicates.

3.2. HPLC procedure

The chromatographic procedure used in this work was based on the method described by Tyler and Genzale [16]. These authors used ion-pair HPLC (sodium salt of 1-octanesulfonic acid as the conter-ion) and the mobile phase was buffered with triethylamine/phosphoric acid. We observed that, at pH 2.5 and in the absence of conter-ion, the retention time of miconazole on the μBondapak C18 column was $t_{ret} = 3.5$ min Using such conditions, there was no interference from the components of placebo (detection at 225 nm). It was also verified that the elution peak of miconazole was resolved from the elution peaks of butylated hydroxyanisole (BHA, $t_{ret} = 2.6$ min) and butylated hydroxytoluene (BHT, $t_{ret} = 4.9$ min), the two commonly used antioxidants (Fig. 3). In order to buffer the mobile phase at pH 2.5, a mixture of sulphuric acid and phosphoric acids (molar ratio 1:4) was used with triethylamine. Doing so, the concentration of acids in mobile phase was lowered as compared with the procedure in which only phosphoric acid was used [16]. In the calibration range 0–40 mg l^{-1} the coeffi-

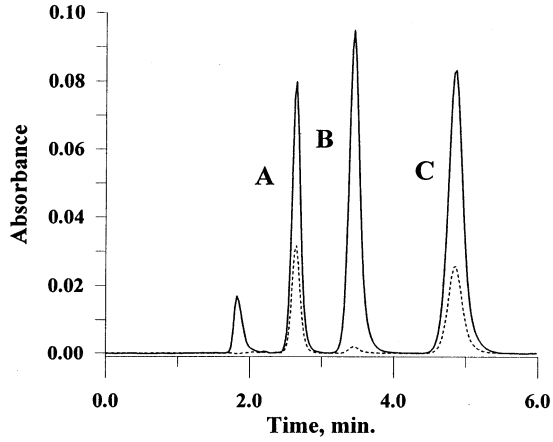

Fig. 3. Typical chromatogram obtained for a solution containing: miconazole, 20 mg l^{-1} (B), BHA, 20 mg l^{-1} (A) and BTH, 40 mg l^{-1} (C). UV-Vis detection at (—) 225 and (---) 280 nm.

cient of linear correlation was 0.9999, precision (measured as CV for ten replicates) at 3.0 mg l^{-1} and at 25 mg l^{-1} of miconazole was 2.2 and 0.5%, respectively.

3.3. Analytical application

Miconazole was determined in four commercial pharmaceutical creams by the proposed extraction–spectrophotometric procedure and by HPLC. The results obtained are presented in Table 2, where excellent agreement between the two procedures can be observed. The analysis of variances was carried out (ANOVA test) and no statistically significant difference was found between the results obtained using the two procedures ($\alpha \leq 0.05$).

Table 2
Analytical results for miconazole determination in pharmaceutical creams by extraction-spectrophotometric procedure and by HPLC[a]

Pharmaceutical cream	Mean value of miconazole content ± SD (%)	
	HPLC	Extraction –spectrophotometry
Dactarin	2.12 ± 0.05	2.15 ± 0.01
Neomicol	2.01 ± 0.03	1.95 ± 0.01
Aloid	2.06 ± 0.04	2.10 ± 0.04
Fungiquim	2.17 ± 0.01	2.19 ± 0.04

[a] Mean value obtained for five replicates of the sample taken from one container.

3.4. Conclusions

In this work, methylene blue was applied as IS for extraction–spectrophotometric determination of miconazole in pharmaceutical creams. It was shown that, in the proposed multi-stage procedure, the application of IS enabled good precision of the results. It was also shown that the presence of other cream components (placebo) did not affect the miconazole determination and the solutions of miconazole + IS can be used for calibration. The proposed procedure was applied for the determination of miconazole in four pharmaceutical creams and the obtained results were in good agreement with those obtained by HPLC procedure (ANOVA test, $\alpha \leq 0.05$).

In conclusion, the proposed extraction–spectrophotometric procedure assured a good precision and accuracy of miconazole determination in pharmaceutical creams, although no rigorous protocol was required and small volumes of volatile organic solvents were used.

Acknowledgements

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