

First-derivative ultraviolet spectrophotometric and high performance liquid chromatographic determination of ketoconazole in pharmaceutical emulsions

Erika Rosa Maria Kedor-Hackmann*, Maria Inês Rocha Miritello Santoro, Anil Kumar Singh, Andréia Cricco Peraro

Department of Pharmacy, Faculty of Pharmaceutical Sciences, University of São Paulo

First-derivative ultraviolet spectrophotometric (Method I) and reversed phase high performance liquid chromatographic (Method II) methods were developed. The validated methods were applied for quantitative determination of ketoconazole in commercial and simulated emulsion formulations. Quantitative first-derivative UV spectrophotometric determinations were made using the zero-crossing method at 257 nm, with methanol as background solvent. Liquid chromatographic analysis was carried out on a LiChrospher® 100 RP-18 (5µm) column. A mixture of triethylamine in methanol (1:500 v/v) and 0.5% ammonium acetate solution (75:25 v/v) was used as mobile phase at a flow rate of 1.0 mL/min with UV detection at 225 nm. The retention time of ketoconazole and terconazole were 3.9 min and 5.9 min, respectively, the later being used as internal standard. Analytical curves were linear within a concentration range from 5.0 to 30.0 µg/mL for Method I and 20.0 to 80.0 µg/mL for Method II, with correlation coefficients of 0.9997 and 0.9981, respectively. The relative standard deviation (RSD) was 0.56% and 0.41% for simulated and commercial emulsion formulations, respectively, using Method I. The corresponding values were 2.13% and 1.25%, respectively, using Method II. The percentage recoveries were above 100% for both methods. The excipients did not interfere in the analysis. The results showed that either method can be used for rapid ketoconazole determination in pharmaceutical emulsions with precision, accuracy and specificity.

Uniterms

- Ketoconazole
- Emulsion
- First-derivative ultraviolet spectrophotometry
- High performance liquid chromatography

*Correspondence:

E. R. M. Kedor-Hackmann
Faculty of Pharmaceutical Sciences
University of São Paulo
Caixa Postal 66083
05315-970 - São Paulo, SP - Brazil
E-mail: ermkedor@usp.br

INTRODUCTION

Ketoconazole is an imidazole derivative with a wide antifungal spectrum and possesses some antibacterial activity (El-Shabouri *et al.*, 1998; Hardman, Limbird, Gilman, 2001; Korolkovas, 1988).

Ketoconazole, [(±)-*cis*-1-acetyl-4-(4-{[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxy} phenyl) piperazine] (Figure 1), is widely used in the treatment of dermal and systemic mycoses. Ketoconazole presents advantage over other imidazole derivatives in sustaining adequate blood levels following oral

administration (Abounassif, El-Shazly, 1989; Hardman, Limbird, Gilman, 2001; Korolkovas, 1988).

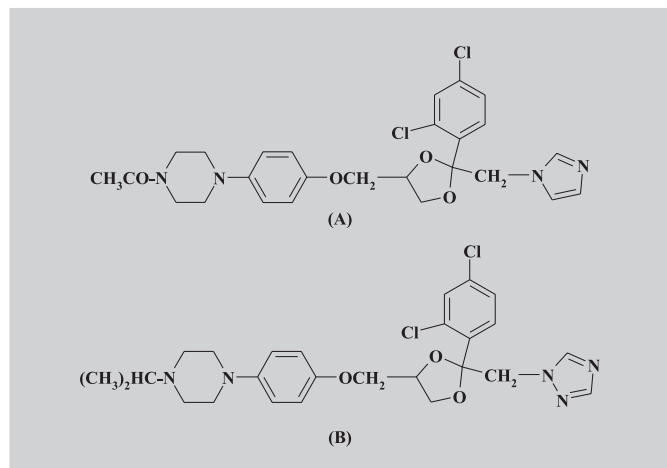


FIGURE 1 – Chemical structures of ketoconazole (A) and terconazole (B).

Ketoconazole commercially available in several dosage forms for oral and topical administration. Through the oral route, it could provoke hepatotoxicity and interfere with the metabolism of testosterone (Abounassif, El-Shazly, 1989; El-Shanawany *et al.*, 1997; Katsambas *et al.*, 1989; Korolkovas, 1988; Low, Wangboonskul, 1999; Roychowdhury, Das, 1996). Topical ketoconazole formulations have been found to be effective in controlling seborrhoeic dermatitis without producing significant adverse reactions (Katsambas *et al.*, 1989; Roychowdhury, Das, 1996). It is active against *pityrosporum* yeasts both “in vitro” and “in vivo” (El-Shabouri *et al.*, 1998).

Several analytical methods have been developed for quantitative determination of ketoconazole, among them visible spectrophotometry (Abdel-Gawad, 1997), ultraviolet (UV) spectrophotometry (El-Shabouri *et al.*, 1998; Kedor-Hackmann, Nery, Santoro, 1994), spectrofluorimetry (El-Shanawany *et al.*, 1997), thin-layer chromatography (Roychowdhury, Das, 1996), supercritical fluid chromatography with UV detection (Ashraf-Khorassani, Levy, 1995), capillary electrophoresis with diode array detection (Arranz *et al.*, 2000), high performance liquid chromatography (HPLC) using different detection modes such as UV (Al-Meshal, 1989; Abdel-Moety *et al.*, 2002; Heyden *et al.*, 2002), diode array (Koves, 1995) and electrochemical detection (Hoffman *et al.*, 1988), and stripping voltametric and polarographic techniques (Arranz *et al.*, 2003). However, no reference was found using first-derivative UV spectrophotometric determination of ketoconazole in emulsions.

The official compendia (British Pharmacopoeia, 2000; United States Pharmacopoeia, 2005) describe

analytical methods for quantitative determination of ketoconazole in tablets and oral suspension, however no validated method was found for its quantitative determination in pharmaceutical emulsions.

The objective of this work was to develop sensitive and efficient analytical methods for quantitative determination of ketoconazole in pharmaceutical emulsions. Two methods were developed and validated for this purpose, using first-derivative UV spectrophotometry (Method I) and HPLC (Method II).

MATERIAL AND METHODS

Instrumentation

A Beckman model DU-70 spectrophotometer with quartz cells of 1 cm path length connected to an Epson-Fx 850 printer was used. The HPLC separations were made on a system comprised of a solvent delivery pump (model 480-C), a variable UV detector set at 225 nm connected to an integrator (model CG-200) (Instrumentos Científicos CG Ltda, São Paulo, Brasil). The system was equipped with a manual Rheodyne® 7125 injection valve, fitted with a 20 μ L loop. The analytical column was a LiChrospher® 100 RP-18 (5 μ m) (125 x 4 mm) in a LiChroCART® (Merck®, Darmstadt, Germany). A Digimed® pH meter (model T-901) and a Thornton® sonicator (model T-14) were also used.

Reference substances, reagents and solutions

Ketoconazole (99.5%) and terconazole (99.2%) active pharmaceutical ingredients were donated by pharmaceutical firms and were used as reference standards without further purification. All reagents and solvents were of analytical grade. The solvents used in the mobile phase were HPLC grade. Distilled water was purified using a Milli-Q® Plus System (Millipore®, Milford, MA, USA).

Two sample matrixes were selected for analysis. The simulated emulsion sample (sample 1) was prepared in our laboratory according to the formula given in Table I. The commercial sample was obtained from a local pharmaceutical firm (sample 2).

METHODS

First-derivative UV Spectrophotometry (Method I)

Methanol solutions in a concentration range from 5.0 to 30.0 μ g/mL were prepared to obtain the analytical curve. Absorption intensities were measured at 257 nm

using methanol as blank, scanning velocity was 300 nm/min and quartz cells of 1 cm path length were used.

An amount equivalent to 25.0 mg of ketoconazole standard was accurately weighed and transferred to a 100 mL volumetric flask. After solubilization in methanol with sonication, the volume was completed with same solvent to obtain a solution containing 250.0 µg of ketoconazole/mL. Six aliquots of this solution from 0.5 to 3.0 mL were transferred to 25 mL volumetric flasks. The volume was completed with methanol and the final concentrations of the solutions were 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0 µg/mL of ketoconazole in respective volumetric flasks. Absorptions of these solutions were determined at 257 nm, using methanol as blank.

For specificity determination of the method, an amount equivalent to 10.0 mg of standard ketoconazole was accurately weighed and transferred to a 100 mL volumetric flask and the volume was completed with methanol. An aliquot of 5.0 mL was transferred to a 25 mL volumetric flask and volume completed with the same solvent to obtain a solution containing 20.0 µg of ketoconazole/mL. An amount of emulsion (500 mg) equivalent to 10 mg of ketoconazole (sample 2) and 500 mg of placebo emulsion (emulsion without ketoconazole) were separately weighed and transferred to three 100 mL volumetric flasks. After adding 80 mL of methanol to each flask, the resulting solutions were sonicated during 15 min, and the volumes completed with same solvent. The solutions were filtered and 5.0 mL aliquots were transferred to 25 mL volumetric flasks and the volumes completed with methanol. These solutions were analyzed by proposed Method I using methanol as blank.

The precision of the method was determined by analyzing three separate standard solutions and ten aliquots of ketoconazole emulsion samples (sample 1 and 2), containing 20.0 µg/mL of ketoconazole each.

To perform the recovery tests an amount of 25.0 mg of ketoconazole standard was weighed and transferred to a 100 mL volumetric flask. The volume was completed with methanol. An amount of 1.25 g of sample 2, equivalent to 25.0 mg of ketoconazole was weighed and transferred to a 100 mL volumetric flask. The drug was solubilized in methanol by sonication for 15 min and volume was completed with the same solvent. The final concentrations of both solutions were 250.0 µg of ketoconazole/mL.

Appropriate volumes of standard solutions were used to spike sample solutions at three concentration levels. The spiked sample solutions were analyzed using the proposed method.

High Performance Liquid Chromatography (Method II)

The mobile phase was constituted of a mixture of triethylamine in methanol (1:500 v/v) and ammonium acetate solution in water (1:200w/v), 75:25 v/v. The analytical column was LiChrospher® 100 RP-18 (5µm) (125x4 mm) in a LiChroCART® (Merck® Darmstadt, Germany). All analyses were done under isocratic conditions, at a flow rate of 1.0 mL/min and at ambient temperature (24 ± 2 °C), with UV detection at 225 nm.

An amount of 10.0 mg of ketoconazole standard was accurately weighed and transferred to a 100 mL volumetric flask. After solubilization in methanol with sonication, the volume was completed with same

TABLE I - Description of simulated pharmaceutical emulsion sample and commercial emulsion sample selected for analyses

Components	Simulated sample (Sample 1)	Commercial sample (Sample 2)*
Aqueous phase (A)	%w/w	
Propyleneglycol	5.00	
Disodium edetate	0.20	
Purified water q.s.p	100.00	
Oil phase (B)	% w/w	Containing 20.0 mg of ketoconazole per gram of emulsion
Polawax®	15.00	
Mineral oil	5.00	
Butylhydroxytoluene	0.05	
Phase (C)	% w/w	
Chemynol®	0.50	
Ketoconazole	2.00	

* Excipients not disclosed by firm.

TABLE II - Analytical curve data of ketoconazole in pharmaceutical emulsions

Statistical parameters	Method I	Method II
Concentration range ($\mu\text{g/mL}$)	5.0-30.0	20.0-80.0
Regression equation	$y=2.1 \times 10^{-4} x + 1.5 \times 10^{-3}$	$y = 6.1 \times 10^{-2} x + 2.3 \times 10^{-2}$
Correlation coefficient (r)	0.9997	0.9981

solvent to obtain a solution containing 100.0 μg of ketoconazole/mL. An amount of 25.0 mg of terconazole (internal standard) was weighed and transferred to a 100 mL volumetric flask. After the solubilization, in methanol with sonication, the volume was completed with same solvent. The final concentration was 250.0 μg of terconazole/mL.

Seven aliquots of ketoconazole standard solution from 2.0 to 8.0 mL were transferred to 10 mL volumetric flasks and a 2.0 mL aliquot of internal standard was also added to each flask. The volume was completed with mobile phase and the final concentrations of the solutions were 20.0, 30.0, 40.0, 50.0, 60.0, 70.0 and 80.0 μg of ketoconazole/mL, along with 50.0 μg of terconazole/mL. These solutions were analyzed in duplicate and the analytical curve was constructed by plotting concentration of ketoconazole versus corresponding area ratios of ketoconazole and internal standard.

The specificity of the method was analyzed using standard ketoconazole, sample 2 and placebo solutions.

An amount of 10.0 mg of standard ketoconazole was transferred to a 100 mL volumetric flask and volume completed with methanol. An aliquot of 4.0 mL was transferred to a 10 mL volumetric flask, 2.0 mL of internal standard solution (250.0 μg of terconazole/mL) was also added and the volume completed with methanol. The final concentrations were 40.0 μg of ketoconazole and 50.0 μg of terconazole/mL of solution. An amount of sample 2 (1.0 g), equivalent to 20.0 mg of ketoconazole was accurately weighed and transferred to a 100 mL volumetric flask, 80 mL of methanol was added and the solution sonicated for 15 min. The volume was completed with methanol and resultant solution filtered through Whatman filter paper no. 1. An aliquot of 2.0 mL of filtrate was transferred to a 10 mL volumetric flask. An aliquot of 2.0 mL of internal standard (250.0 $\mu\text{g/mL}$) was also added to same flask and the volume was completed with the mobile phase. The final concentrations were 40.0 μg of ketoconazole and 50.0 μg of terconazole/mL of solution. An amount of 1.0 g of placebo was weighed and submitted to same treatment without addition of the internal standard.

In order to calculate the precision of the method three solutions of standard ketoconazole were prepared as

described above. The final concentration of resultant solution was 40.0 μg of ketoconazole and 50.0 μg of terconazole/mL. Ten solutions of ketoconazole emulsion (sample 1 and 2) were prepared containing 40.0 μg of ketoconazole and 50.0 μg of terconazole/mL.

To perform the recovery tests 25.0 mg of ketoconazole standard were weighed and transferred to a 100 mL volumetric flask. After solubilization, the volume was completed with methanol to obtain a solution containing 250.0 μg of ketoconazole/mL. An amount of 1.0 g of sample 2 was weighed and transferred to a 100 mL volumetric flask. After the addition of 80 mL of methanol the solution was sonicated during 15 min. The volume was completed with methanol and the solution filtered. The final concentration was 250.0 μg of ketoconazole/mL.

Appropriate volumes of standard and sample solutions were transferred to 25 mL volumetric flasks and analyzed using the proposed method. In all cases, an aliquot of 5.0 mL of terconazole solution containing 250.0 $\mu\text{g/mL}$ was also added.

RESULTS AND DISCUSSION

First-derivative UV Spectrophotometry (Method I)

The first-derivative UV spectrum of ketoconazole, in a range from 211 nm to 295 nm, presented a maximum absorption peak at 257 nm, without any interference from excipients. The method was validated at 257 nm. The analytical curve was constructed in a concentration range from 5.0 to 30.0 μg of ketoconazole/mL. The linearity of the curve was evaluated by linear regression analyses of responses. The standard curve showed linearity within the concentration range studied with a correlation coefficient of 0.9997 ($y=2.1 \times 10^{-4} x + 1.5 \times 10^{-3}$). The precision of the method was reported as relative standard deviation (RSD) (Table III). The accuracy of the proposed method was confirmed by the obtained results (Table IV). Near 100% recovery of ketoconazole from the matrix proves the accuracy of the proposed method. Different excipients used in the formulations (Table I) did not interfere in the analyses using the proposed first-derivative UV spectrophotometric method (Figure 2).

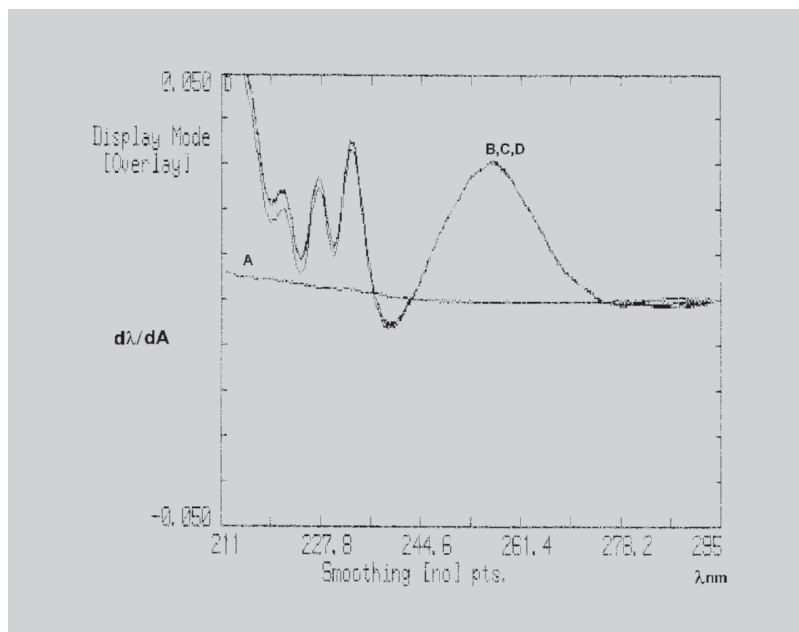


FIGURE 2 – First-derivative ultraviolet absorption spectra in methanol: (A) placebo, (B) placebo with 20.0 $\mu\text{g/mL}$ ketoconazole standard, (C) ketoconazole standard, 20.0 $\mu\text{g/mL}$ and (D) ketoconazole sample, 20.0 $\mu\text{g/mL}$.

High Performance Liquid Chromatography (Method II)

In the preliminary studies several mobile phases were tested. The optimum and efficient separations were obtained when diisopropylamine was substituted by triethylamine (United States Pharmacopeia, 2005). The chromatograms of standard ketoconazole and

terconazole can be observed in Figure 3B and those of sample can be observed in Figure 3C. A base line separation of ketoconazole and terconazole peaks was obtained within approximately 6.0 min. The shorter elution time makes the method especially useful for routine analysis of ketoconazole in pharmaceutical formulations. Analytical curve was constructed in a

TABLE III - Statistical data and results obtained in the determination of ketoconazole contained in pharmaceutical emulsions

Statistical data	Method I		Method II	
	1	2	1	2
Sample				
RSD (%)	0.56	0.41	2.13	1.25
Amount declared (mg/g)	20.00	20.00	20.00	20.00
Amount found (mg/g)	20.30	20.55	21.76	19.65
Confidence limit ($p=95\%$)	101.49 ± 0.40	102.76 ± 0.30	108.79 ± 1.66	98.23 ± 0.87

TABLE IV - Results obtained in the recovery of ketoconazole standard solution added to sample 2 and analyzed by the proposed methods

Sample	Method I			Method II		
	Concentration (mg/mL)		Recovery (%)	Concentration (mg/mL)		Recovery (%)
	Added	Found		Added	Found	
2	10.0	10.3	103.4	10.0	10.0	100.0
	15.0	15.0	100.0	20.0	20.0	100.0
	20.0	19.4	97.0	30.0	30.4	101.3

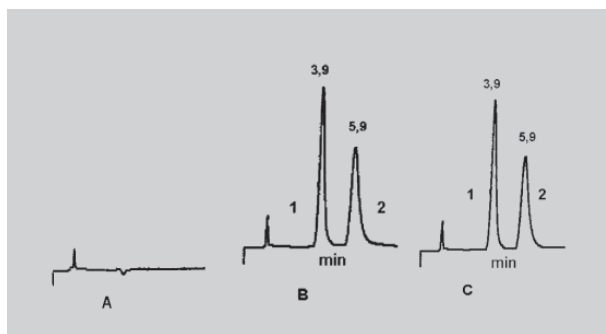


FIGURE 3 – Chromatograms of: (A) placebo; (B) ketoconazole standard, 20.0 $\mu\text{g/mL}$ (1) and terconazole internal standard, 50.0 $\mu\text{g/mL}$ (2); (C) sample (sample 2) containing 20.0 $\mu\text{g/mL}$ ketoconazole (1) and terconazole internal standard, 50.0 $\mu\text{g/mL}$ (2). Chromatographic conditions: mobile phase: triethylamine in methanol (1:500) and ammonium acetate solution in water (1:200), 75:25 (v/v), flow rate 1.0 mL/min and UV detection at 225 nm; LiChrospher[®] 100 RP-18 (5 μm) (125x4 mm) column in a LiChroCART[®], room temperature (24 ± 2 °C).

concentration range from 20.0 to 80.0 $\mu\text{g/mL}$. The linearity of the curve was evaluated by linear regression analysis of responses. The standard curve showed linearity within the concentration range studied with correlation coefficient of 0.9981 ($y = 6.1 \times 10^{-2}x + 2.3 \times 10^{-2}$). The precision of the method reported as RSD can be observed in Table III. The accuracy of the HPLC method was confirmed by the results showed in the Table IV.

The excipients of the emulsion samples did not interfere in either ketoconazole quantitation or in the internal standard peak (Figures 3A and 3C).

The validation data and sample analyses substantiate the precision, accuracy, specificity and efficiency of the two proposed methods in the determination of ketoconazole in pharmaceutical emulsions.

CONCLUSIONS

Analytical curves were linear within a concentration range from 5.0 to 30.0 $\mu\text{g/mL}$ for Method I and 20.0 to 80.0 $\mu\text{g/mL}$ for Method II, with correlation coefficients of 0.9997 and 0.9981, respectively.

The proposed first-derivative ultraviolet spectrophotometric (Method I) and reversed phase high performance liquid chromatographic methods (Method II) presented good precision as is evident from intraday repeatability data. The relative standard deviation (RSD) was 0.56% and 0.41% for simulated and commercial emulsion formulations, respectively, using

Method I. The corresponding values were 2.13% and 1.25%, respectively, using Method II. The emulsion samples containing ketoconazole can be analyzed with more precision using proposed Method I, though RSD values are acceptable in both cases.

The percentage recovery was 100.1% for the Method I and 100.4% for the Method II and methods could be considered accurate.

The specificity data show that the emulsion excipients do not interfere in the analyses. In this case, the first-derivative zero-crossing spectrophotometric method presents a unique advantage that it permits determination of ketoconazole in pharmaceutical emulsions even in the presence of potential interfering excipients.

Based on these observations it was concluded that the proposed methods can be applied for rapid ketoconazole determination in pharmaceutical emulsions with precision and accuracy.

RESUMO

Determinação do cetoconazol em emulsões por espectrofotometria no ultravioleta por derivada de primeira ordem e cromatografia líquida de alta eficiência

Foram desenvolvidos e padronizados métodos por espectrofotometria no ultravioleta (UV) por derivada de primeira ordem (Método I) e cromatografia líquida de alta eficiência (CLAE) (Método II) para a determinação quantitativa de cetoconazol em formulações farmacêuticas sob a forma de emulsão obtida no comércio e formulada em laboratório. A espectrofotometria no UV por derivada de primeira ordem foi padronizada usando-se o método do zero pico a 257 nm, utilizando metanol como solvente. A cromatografia líquida foi realizada empregando-se uma coluna LiChrospher[®] 100 RP-18 (5 μm). A fase móvel utilizada foi a mistura de trietilamina em metanol (1:500) e solução de acetato de amônio em água (1:200) na proporção de 75:25 v/v, com vazão de 1 mL/min e detecção no UV de 225 nm. O tempo de retenção do cetoconazol foi de 3,9 min e do terconazol de 5,9 min, este último utilizado com padrão interno. As curvas analíticas mostraram linearidade dentro das concentrações de 5,0 a 30,0 mg/mL para o Método I e 20,0 a 80,0 mg/mL para o Método II, com coeficientes de correlação linear de 0,9997 e 0,9981, respectivamente. O desvio padrão relativo (DPR) foi de 0,56% e 0,41% para a amostra simulada e comercial, respectivamente, empregando-se o Método I. Para o Método II, os valores foram de

2,13% e 1,25%, respectivamente. A porcentagem de recuperação foi de 100,1% para o Método I e 100,4% para o Método II. Os excipientes não interferiram nas análises. Os resultados mostraram que os dois métodos podem ser usados para a determinação rápida de cetoconazol em formulações de emulsões com precisão, exatidão e especificidade.

UNITERMOS: Cetoconazol. Emulsão. Espectrofotometria no ultravioleta por derivada de primeira ordem. Cromatografia líquida de alta eficiência.

REFERENCES

- ABDEL-GAWAD, F.M. Investigation of ketoconazole-iron (III) complex and its spectrophotometric determination in pharmaceutical preparations. *Farmaco*, Pavia, v. 52, p. 119-122, 1997.
- ABDEL-MOETY, E.M.; KHATTAB, F.I.; KELANI, K.M.; ABOUAL-ALAMEIN, A.M. Chromatography determination of clotrimazole, ketoconazole and fluconazole in pharmaceutical formulations. *Farmaco*, Pavia, v. 57, p. 931-938, 2002.
- ABOUNASSIF, M.A.; EL-SHAZLY, B.E.D.M. D₁-Diferential potentiometric and ¹H-NMR spectrometric determinations of ketoconazole and its formulations. *Anal. Lett.*, New York, v. 22, p. 2233-2247, 1989.
- AL-MESHAL, M.A. Determination of ketoconazole in plasma and dosage forms by high-performance liquid chromatography and a microbiological method. *Anal. Lett.*, New York, v. 22, p. 2249-2263, 1989.
- ARRANZ, A.; ECHEVARRIA, C.; MOREDA, J.A.; CID, A.; ARRANZ, J.F. Capillary zone electrophoretic separation and determination of imidazolic antifungal drugs. *J. Chromatogr. A*, Amsterdam, v. 871, p. 399-402, 2000.
- ARRANZ, P.; ARRANZ, A.; MOREDA, J.M.; CID, A.; ARRANZ, J.F. Stripping voltammetric and polarographic techniques for the determination of antifungal ketoconazole on the mercury electrode. *J. Pharm. Biom. Anal.*, New York, v. 33, p. 589-596, 2003.
- ASHRAF-KHORASSANI, M.; LEVY, J.M. Addition of modifier in supercritical fluid chromatography using a microbore reciprocating pump. *Chromatographia*, New York, v. 40, p. 78-84, 1995.
- BRITISH PHARMACOPOEIA: vol 1. London: Her Majesty's Stationery Office, 2000. p. 208.
- EL-SHABOURI, S.R.; EMARA, K.M.; KASHABA, P.Y.; MOHAMED, A.M. Charge-transfer complexation for spectrophotometric assay of certain Imidazole antifungal drugs. *Anal. Lett.*, New York, v. 31, p. 1367-1385, 1998.
- EL-SHANAWANY, A.A.; EL-BAYOUMI, A.; EL-SADEK, M.E.; EL-SATTAR, A.A. Synchronous spectrofluorimetric determination of famotidine, fluconazole and ketoconazole in bulk powder and in pharmaceutical dosage forms. *Spectrosc. Lett.*, New York, v. 30, p. 25-46, 1997.
- HARDMAN, J.G.; LIMBIRD, L.E.; GILMAN, A.G., (eds) *Goodman and Gilman's the pharmacological basis of therapeutics*. 10 ed., McGraw-Hill, New York, 2001, p. 1301-1302.
- HEYDEN, V.Y.; NGUYET, A.N.M.; DETAEVERNIER, M.R.; MASSART, D.L.; PLAIZIER-VERCAMMEN, J. Simultaneous determination of ketoconazole and formaldehyde in a shampoo: liquid chromatography method development and validation. *J. Chromatogr. A*, Amsterdam, v. 958, p. 191-201, 2002.
- HOFFMAN, D.W.; JONES-KING, K.L.; RAVARIS, C.L.; EDKINS, R.D. Electrochemical detection for high-performance liquid chromatography of ketoconazole in plasma and saliva. *Anal. Biochem.*, New York, v. 172, p. 495-498, 1988.
- KATSAMBAS, A.; ANTONIOU, C.H.; FRANGOULI, E.; AVGERINO, G.; MICHAILIDIS, D.; STRATIGOS, J. A double-blind trial of treatment of seborrhoeic dermatitis with 2% ketoconazole cream compared with 1% hydrocortisone cream. *Br. J. Dermatol.*, Malden, v. 121, p. 353-357, 1989.
- KEDOR-HACKMANN, E.R.M.; NERY, M.M.F.; SANTORO, M.I.R.M. Determination of ketoconazole in pharmaceutical preparations by ultraviolet spectrophotometry and high performance liquid chromatography. *Anal. Lett.*, New York, v. 27, p. 363-376, 1994.
- KOROLKOVAS, A. *Essentials of medicinal chemistry*, 2ed. New York, 1988. p. 680-699.

- KOVES, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology. *J. Chromatogr. A*, Amsterdam, v. 692, p. 103-119, 1995.
- LOW, A.S.; WANGBOONSKUL, J. An HPLC assay for the determination of ketoconazole in common pharmaceutical preparations. *Analyst*, Amsterdam, v. 124, p. 1589-1593, 1999.
- UNITED STATES PHARMACOPEIA. 28ed. Rockville, United States Pharmacopeial Convention, 2005. p. 1098-1099.
- ROYCHOWDHURY, U.; DAS, S.K. Rapid identification and quantification of clotrimazole, miconazole and ketoconazole in pharmaceuticals creams and ointments by thin-layer chromatography-densitometry. *J. AOAC Int.*, Gaithersburg, v. 79, p. 656-659, 1996.

Recebido para publicação em 19 de janeiro de 2005.

Aceito para publicação em 15 de fevereiro de 2006.