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Original Article

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Extractive Spectrophotometric Determination of Miconazole Nitrate in Pure and Pharmaceutical Preparation

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Abstract

A simple extractive spectrophotometric method has been developed for the estimation of miconazole nitrate in both pure and pharmaceutical preparation. The method is based on the formation of ion-pair complex of the drug with acidic dye bromothymol blue (BTB) in acidic condition, followed by its extraction in organic solvent (chloroform). The absorbance was measured against the corresponding blank. The maximum absorbance was found at 434 nm. Linear calibration graph was obtained in the concentration range 1-35 μg ml-1. The method was validated statistically. Recovery studies gave satisfactory results indicating that none of common additives and excipients interfere the assay method. The proposed methods were found to be simple, accurate and reproducible and their performance in analysis of cream formulation was successfully examined.

Keywords: Miconazole nitrate, Extractive spectrophotometry, Bromothymol blue, Ion-pair complex

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1. Introduction

Miconazole nitrate (MIZ), or 1-[2,4-dichloro-(b-(2,4-dichlorobenzyloxy) phenethyl] imidazole, is an antibacterial of the class of imidazole1. MIZ is a commonly used imidazole compound that for effective treatment of cutaneous mycoses. The compound is also an effective agent against severe pulmonary fungal disease which occurs frequently in immune suppressed patients and in patients with terminal cancer2. Miconazole nitrate is a broad-spectrum antifungal agent that has been extensively applied in management of dermal, buccal and vagunal candidiase3. It is applied in the form of a 2.0% cream or powder in treating the infection of nails and skin4. Several analytical procedures have been proposed for the quantification of miconazole in pharmaceuticals and in biological fluids including HPLC5-11, GC12-14, LC15-18, LLE19, 20, SPE17,18,20,21, spectrometry4, voltametry22,23 and spectrophotometry24-26. Despite the availability of this wide spectrum of experimental procedures, however, alternative simple and cost effective methods are needed to the determine of this pharmaceutically important antifungal drugs. The official pharmacopoeias (USP and BP) describe non-aqueous titration of drug in the drug bulk in the presence of suitable indicator27,28. The BP recommends using the UV spectrophotometric assay to measure miconazole nitrate in cream and the USP suggests HPLC assay for pharmaceutical preparations.

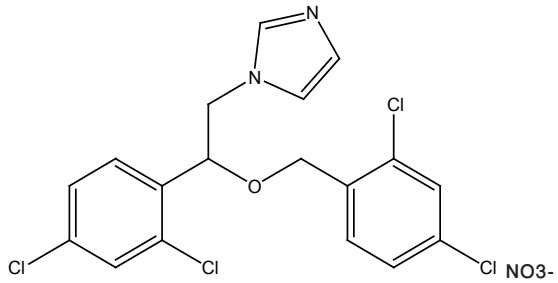

Figure 1. Chemical structure of miconazole nitrate

2.Methods

Experimental Procedure

Pharmaceutical grade pure sample miconazole nitrate and cream 2 (wt%) were obtained from Behvazan Pharmaceutical Company (Rasht industrial city, Iran). All of the chemicals used in this study were of analytical reagent grade from Merck unless otherwise being specified. Freshly prepared solutions were always employed. Standard buffer solution (pH 2-7) was prepared by dissolving 1.28 g potassium hydrogen phthalate in water and completed to 50 ml with water and adjusting pH by addition of 0.2 M hydrochloric acid. Bromothymol blue (BTB 1 M), was prepared in distilled water.

Apparatus

UV - Visible spectrophotometer shimadzu (2100) fitted with 1.0 cm matched quartz cell, was used. Operating conditions scan varied between 200 -700 nm. The pH value of all buffers was adjusted using a Metrohm 825 pH meter.

Preparation Methodology

Standard solution of drug

A stock standard solution (100 μg/ml) was prepared by dissolving miconazole nitrate in 10 ml of 0.05 HCL and filtered then further diluted with the same solvent as appropriate.

Recommended procedure

One ml of standard solution containing appropriate amounts of miconazole nitrate was pipetted in to 50 ml separatory funnel containing 2 ml of BTB and 2 ml phthalate buffer of pH 5.5 and the solution was mixed well. Five ml chloroform was then added to the solution and the solution was shaken for about 5 minutes. The solution was allowed to stand for clear separation of the two phases. The absorbance of the chloroform layer was measured against a reagent blank at 434 nm.

Sample preparation

A weighed portion of the miconazole cream

equivalent to 25 mg of the drug was shaken and gently heated in ethanol until it was completely dissolved and filtered. The resulting clear solution was diluted to the mark with ethanol in a 50 ml volumetric flask. Appropriate dilution of the solution with water was prepared subsequently and the recommended procedure was followed. The method of standard addition was also employed.

4. Results and discussion

Spectral characteristics

The absorption spectra of the ion-pair complex which was formed between miconazole nitrate and BTB was measured within the range of 350-600 nm against the blank solution (Figure 2). The ion-pair complex showed the maximum absorbance at 434 nm.

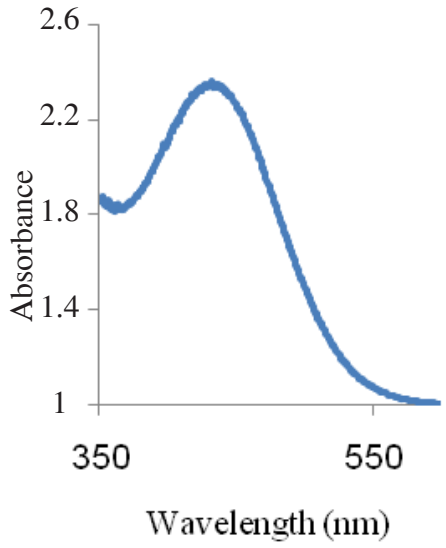

Figure 2. Absorption spectra of MIZ– BTB ion-pair complex in chloroform selecting the extracting solvents

Optimaization of variables

Optimum conditions necessary for rapid and quantitative formation of colored ion-pair complex with maximum stability and sensitivity were established by a number of preliminary experiments.

Effect of pH

The effect of pH was studied by extracting the colored complex in the presence of various buffers such as phthalate, phosphate and phosphate-citrate. Potassium hydrogen phthalate –NaOH buffer of choice did not interfere and gave the highest sensitivity for complex formation and extraction. Different pHs (2-7) were tested and the absorbance reading of the miconazole-BTB ion-pair was examined (Figure 3). The maximum color intensity was observed in the pH 5.5. Thus, a pH of 5.5 was used for further studies.

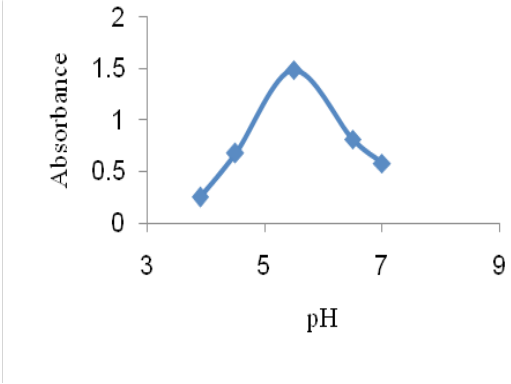

Figure 3. Effect of pH of potassium phthalate buffer solution on the ion-pair complex

The effect of several organic solvents, toluene, acetonitrile, 1,2-dichloromethane and chloroform were examined for effective extraction of the colored species from aqueous phase. Chloroform was preferred for its selective and quantitative extraction. Table 1 shows that the colored ion-pair is extractable only in three chlorinated solvents, chloroform, dichloromethane(DCM) and 1,2-dichloroethane(DCE) solution. Chloroform with higher molar absorptivity

of the complex was used as an organic phase throughout this work. The optimum volume of the organic phase was also studied. The maximum absorbance was achieved by using 5 ml of chloroform during a single stage extraction.

Table 1. Effect of different organic solvents on the extraction efficiency of the ion-pair

Solvent	color of organic layer	Absorbance
Toluen	no color	-
Acetonitrile	no color	-
1,2-dichloroetan	yellow	0.238
Dichlorometan	yellow	0.298
Chloroform	yellow	0.583

Effect of BTB volume

The influence of the volume of BTB solution on the extraction of miconazole nitrate was studied (Figure 4). As seen, maximum extraction occurs when the volume of reagent added is 3 ml.

Stoichiometric relationship

The stoichiometric ratio of the drug to dye in the colored solution was determined using the molar ratio method (Figure 5). The result indicated that 1:1 (drug:dye) ion-pair is formed through the electrostatic attraction between positive protonated MIN+ and BTB-.

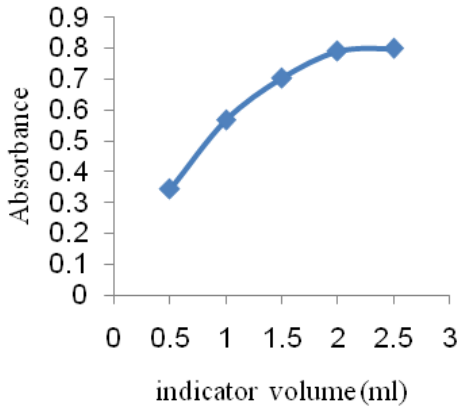

Figure 4. Effect of the volume of BTB on the absorbance of ion pair complex

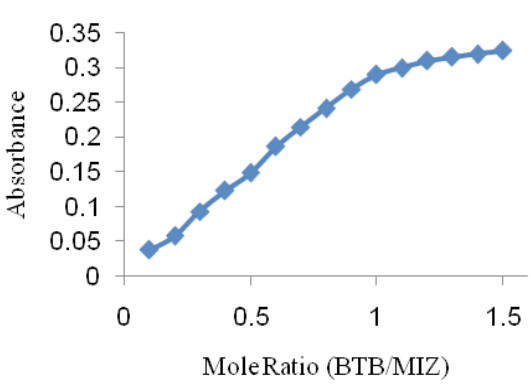

Figure 5. Mole ratio method plot of MIZ-BTB ion-pair complex

Analytical Data

Under the optimized experimental condition, calibration curve was constructed by plotting the absorbance at λmax against the concentration of miconazole nitrate. The results are summarized in Table 2.

Table 2. Optical and regression characteristics

Parameters	**Results**
λ_{max} (nm)	434
Beer's law limit (µg/mL)	1-35
Molar absorptivity (L/mol.cm)	2.57×10^{4}
LOD (mol/L)	2.17×10^{-7}
LOQ (mol/L)	2.81×10^{-7}
Linear regression equation y=mc+b	
Slope (m)	0.0496
Intercept (b)	0.1272
Correlation coefficient (R^2)	0.9956

The high molar absorptivity of the resulting colored complexes indicate the high sensitivity of the method.

Method validation

Samples of pure miconazole nitrate at different concentration were prepared and tested using procedures in two replicates. The complete set of validation assays was performed. The results are given in Table 3.

Table 3. Evaluation of accuracy and precision for the proposed method

Amount taken (µg/ml)	Amount found (µg/ml)	RE(%)	RSD(%)[a]	Recovery(%)
13	12.95	-0.340	2.06	99.7
23	22.98	-0.086	0.99	99.9

a. Avarage of four determinations

Application to the pharmaceutical dosage forms.

The proposed method has been successfully applied to determine miconazole nitrate in pharmaceutical preparations. The ingredient in cream did not interfere in three experiments. The result is described in Table 4.

The validity of the proposed method for

Table 4. Results of determination of ketoconazole in the formulation: Determination of Drug in Cream

Sample	Labeled	Found	Recovery(%) ± SD
Cream	2% (wt%drug)	2.02%	101.4 ± 2.5

analysis of the drug was examined by determining miconazole nitrate in cream as procedure described. The results are summarized in Table 5. The percent recoveries indicate good accuracy and independence of the matrix effect over absorbance measurements. The

Table 5. Assay results of miconazole nitrate in pharmaceutical preparations

Sample	Nominal Amount	Found*,Proposed Method	Official Method	%Recovery by Proposed Method
Cream	2%(wt%drug)	2.02%	2.06%	98.08 ±0.67

*Average±standard deviation of 3 determinations. Theoretical values at 95% confidence limit: F=39 and t=2.7.

performance of the method against the official BP method was evaluated by calculating the t-test and F-test. At confidence limit of 95%, statistical analysis for the cream revealed no significant difference between the performances of the two methods.

Effect of interference

The effect of some commonly available species of pharmaceutical preparations and biological fluids on the ion-pair extraction of the miconazole nitrate was investigated. As shown in Table 6, none of these species

Table 6. Tolerance limits of some excipients in determination of miconazole nitrate

Interference	Tolerance limit (μg/ml)
Starch	100
glucose	500
sucrose	1000
sodium chloride	100

interfered in determining of miconazole nitrate.

5. Conclusion

An important advantage of the extractive spectrophotometric method is that it can be applied for in determining individual compounds in a multi-component mixture. Unlike the gas chromatography and HPLC procedures, the used instrument is simple and cost effective. The proposed method can be used to determine the miconazole nitrate in cream. The method is rapid and simple and offers great sensitivity and accuracy. The proposed method uses of simple reagents, obtainable by an ordinary analytical laboratory and does not need use of any complex apparatus.

6. Acknowledgments

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7. References

Erk N, Aluta ML. Spectrophotometric resolution of metronidazole and miconazole nitrate in ovules using ratio spectra derivative spectrophotometry and RP-LC. J Pharm Biomed Anal. 2001; 25(1):115-22.

Walsh LP, Kuratko CN, Stocco DM. Econazole and miconazole inhibit steroidogenesis and disrupt steroidogenic acute regulatory (StAR) protein expression post-transcriptionally. J Steroid Biochem & Molecular Biology 2000; 75(4-5):229-36.

Nafee NA, Ismail FA, Boraie NA, Mortada LM. Mucoadhesive buccal patches of miconazole nitrate: in vitro/in vivo performance and effect of ageing. International J Pharm 2003; 246(1-2):1-4.

Salem AA, Mossa HA, Barsounm BN. Application of nuclear magnetic resonance spectroscopy for quantitative analysis of miconazole, metronidazole and ulfamethoxazole in pharmaceutical and urine samples. J Pharm & Biomed Anal 2006; 41(2):654-61.

De Zan MM, Cámara MS, Robles JC, Kergaravat SV, Goicoechea HC. Development and validation of a simple stability-indicating high performance liquid chromatographic method for the determination of miconazole nitrate in bulk and cream formulations. Talanta 2009; 79(3):762-7.

Selinger K, Matheou D, Hill HM. High-performance liquid chromatographic method for the determination of miconazole in vaginal fluid. J Chromatogr B Biomed Sciences and Applications 1988; 434(1):259-64.

Tyler TA, Genzale JA. Liquid chromatographic determination of miconazole nitrate in creams and suppositories. J Assoc Off Anal Chem 1989; 72(3):442-4.

Pietra AM, Cavrini V, Adrisano V, Gatti R. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations. J Pharm Biomed Anal 1992; 10(10-12):873-9.

Musch G, Massart DL. J. Chromatogr 1986; 1:370.

Bhoir IC, Raman B, Sundaresan M, Bhagwat AM. Separation and Estimation of Diloxanide Furoate and Metronidazole in Solid Dosage Form Using Packed Column Supercritical Fluid Chromatography. Anal Chim Acta. 1997; 354(1-3):123-128.

Yuan Y, Shi L, zheng X, Yuan. J. Zhongguo Yaoxue Zazh 1998; 33:42.

Akay C, Ozkan SA, Senturk Z, Cevheroglu S. Simultaneous determination of metronidazole and miconazole in pharmaceutical dosage form by HPLC. Farmaco 2002; 57(11):953-957.

Neill GP, Davies NW, McLean S. Automated screening procedure using gas chromatography-mass spectrometry for identification of drugs after their extraction from biological samples. J Chromatogr 1991; 565(1-2):207-24.

Kublin E, Kaniewska T. Detrmination of antimycotic substance, derivatives of imidazole, by gas chromatography. Chem Anal 1996; 41:19.

Chiap P, Hubert P, Crommen J. Strategy for the development of automated methods involving dialysis

and trace enrichment as on-line sample preparation for the determination of basic drugs. J Chromatogr A 2002; 948(1-2):151-61.

Hosotsubo H. Quantitative determination of miconzole in human serum by high-performance liquid chromatography. Chromatographia 1988; 25(8):717-720.

Rifai N, Sakamoto M, Law T, Platt O, Mikati M, Armsby CC et al. HPLC measurement, blood distribution, and pharmacokinetics of oral clotrimazole, potentially useful antisickling agent. Clin Chem 1995; 41(3):387-91.

Kobylinska M, Kobylinska K, Sobik B. High-performance liquid chromatographic analysis for the determination of miconazole in human plasma using solid-phase extraction. J Chromatogr 1996; 685(1):191-5.

Selinger K, Matheou D, Hill HM. High-performance liquid chromatographic method for the determination of miconazole in vaginal fluid. J Chromatogr 1988; 434(1):259-64.

Szathmary SC, Luhmann I. Sensitive and automated gas chromatographic method for the determination of miconazol in plasma samples. J Chromatogr. 1988; 425(1):193-6.

Hubert Ph, Chiap P, Moors M, Bourguignon B, Massart DL, Crommen. J Chromatogr A 1994; 665:87.
(22) Wang Z, Liu J, Zhou S. Fenxi Shiyanshi 1998; 17:40.

(23) Yao Z, Hu J, Wu Z, Li Q. Anal. Letter 1998; 31:429.

Wrobel K, de la Garza Rodriguez IM, Lopezde-Alba PL, Lopez-Martinez L. Determination of miconazole in pharmaceutical creams using internal standard and second derivative spectrophotometry. J Pharm Biomed Anal 1999; 20(1-2):99-105.

Khashaba PY, El-Shabouri SR, Emara KM, Mohamed AM. Analysis of some antifungal drugs by spectrophotometric and spectrofluorimetric methods in different pharmaceutical dosage forms. J Pharm Biomed Anal 2000; 22(2):363-76.

Cavrini V, Di Pietra AM, Gatti R. Analysis of miconazole and econazole in pharmaceutical formulations by derivative UV spectroscopy and liquid chromatography (HPLC). J Pharm Biomed Anal 1989; 7(12):1535-43.

United States Pharmacopoeia, 16th edition, Easton, PA 1985;580.

British Pharmacopeia, HM Stationary Office, London 1993;170.