SIMULTANEOUS DETERMINATION OF METRONIDAZOLE AND MICONAZOLE NITRATE IN GEL BY HPTLC

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ABSTRACT

A new, simple, precise, rapid and selective high-performance thin-layer chromatographic (HPTLC) method for the simultaneous quantification of Metronidazole (MTZ) and Miconazole nitrate (MCZ) in gel has been developed. It was performed on silica gel 60 GF_{254} Thin Layer Chromatographic plates using mobile phase comprising of Toluene: Chloroform: Methanol (3.0:2.0:0.6 v/v) and the detection was carried out at 240 nm using densitometer. The retention factors of MTZ and MCZ were 0.34 and 0.55 respectively. Calibration curves were linear in the range of 300-700 ng/spot of MTZ and 600-1400 ng/spot of MCZ both by height and by area. The percent recovery of the drugs from gel carried out by standard addition method was found to be 100.13±1.59 (by height) and 98.92±0.76 (by area) for MTZ and 99.49±1.58 (by height) and 99.63±1.46 (by area) for MCZ indicative of accuracy and precision of simultaneous determination of MTZ and MCZ nitrate.

Keywords: Metronidazole, miconazole nitrate, HPTLC, Gel

INTRODUCTION

Metronidazole (MTZ), or 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-ethanol, is active against a wide variety of anaerobic parasites and anaerobic bacteria. Miconazole nitrate (MCZ), or 1-[2,4-dichloro-β-[(2, 4-dichloro-benzyl)oxy] phenethyl] imidazole, is an anti bacterial of the class of imidazole. The two drugs are used in association in the treatment as antiprotozoal and antibacterial.

Various methods are available for the quantitative determination of MTZ and MCZ nitrate individually or in combination with other drugs, such as gas chromatography (Kublin and Kanjewaska, 1996), voltametry (Yao *et al*., 1998) HPLC (Raj *et al*., 1997, Argekar and Shah, 1998, Nadkarni *et al*., 1997, Bhoir *et al*., 1997, Bari *et al*., 1998) and spectrophotometry (Parimoo *et al*., 1996, Amin, 1997, Mohamed *et al*., 1996, Paliwal *et al*., 1998, Paliwal *et al*., 1998, Wrobel *et al*., 1999, Basu *et al*., 1991, Das *et al*., 1992).

Two methods have been reported for simultaneous estimation of MCZ and MTZ. The first one reported spectrophotometric (ratio spectra derivative) and RP-LC (Erk and Levent, 2001) method for simultaneous estimation of the two drugs in ovules. It is very tedious and time consuming and needed the HPLC method for comparison purpose. The HPLC method reported herein does not demonstrate proper resolution of the two drugs, which is required for estimation in presence of the impurities. The second is a RP-HPLC (Akay *et al*., 2002) method with UV detection. This method demonstrates better resolution of the two drugs.

Combination dosages containing these two drugs are available everywhere (e.g., Drez V gel manufactured by Stedman India Ltd., etc.). However there is no method for simultaneous estimation of these two drugs by high-performance thin-layer chromatography (HPTLC). HPTLC is more effective technique for the simultaneous determination in single samples in routine analysis (Agrekar and Powar, 2000). The aim of present study is to develop a simple HPTLC method for simultaneous determination and quantitation of MCZ and MTZ in gels, using Toluene: Chloroform: Methanol (3.0:2.0:0.6 v/v) as mobile phase on silica gel 60 GF_{254} TLC plates (Merck). Quantitative estimation was accomplished by densitometric scanning with UV detector at 240 nm wavelength. The method was successfully applied to the dosage form available.

EXPERIMENTAL

Instrumentation, solvents and chemicals

A Camag Linomat IV sample applicator, a Camag TLC Scanner III controlled by Cats 4.0 version software and a Camag twin trough chamber were used. Merck TLC plate (Art. No.1.05554.0007) coated with silica gel 60 GF_{254} (200 µm thickness) on aluminium sheets were used as the stationary phase.

Pharmaceutical grade of miconazole nitrate was kindly supplied as gift sample by M/s Wokhardt India Ltd., Aurangabad and metronidazole by Nestor Pharmaceuticals Ltd., Faridabad, India. All chemicals and reagents were of HPLC grade and were purchased from Merck Chemicals, India. Drez-V (Gel) (Stedman, India) labeled to contain MTZ 1% and MCZ 2% was purchased locally.

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Chromatographic conditions

Stationary phase – Merck silica gel 60 GF_{254} TLC precoated aluminium plates, 200 μm layer thickness, Mobile phase- Toluene: Chloroform: Methanol (3.0:2.0:0.6v/v), Chamber saturation time - 10 minute, Sample application – 4 mm band, Separation technique - Ascending, Temperature - 25 ± 2ºC, Relative humidity - 60% ± 5, Migration distance – 70 mm, Scanning mode - Absorbance/reflectance, Detection wavelength - 240nm, Band Width- 4 mm, Slit dimension- 3×0.45 mm, Scanning speed- 10 mm/s, Monochromator bandwidth- 20 mm.

Standard solutions

Mixed stock standard solution containing 1.0 mg/ml MTZ and 2.0 mg/ml MCZ was prepared by dissolving in methanol. Working standard solution were prepared by further dilution of stock standard with methanol to give final concentration of MTZ, 100 μg/ml and MCZ 200 μg/ml. This solution was used as working standard for analysis of all the samples.

Sample preparation

An accurately weighed quantity of cream equivalent to about 2 mg of Miconazole nitrate (also equivalent to about 1 mg of metronidazole) was weighed in to 10.0 ml volumetric flask and was dissolved in methanol with shaking for 25 min. The volume was made up to the mark with methanol. The solution was centrifuged and supernatant was used as sample solution.

Calibration procedure

The working standard solution was applied on the TLC plate in the range of 1-10 μl. The plate was developed and scanned as mentioned in chromatographic conditions.

Peak height and peak area were recorded for each drug concentration and the instrument displayed the linear regression curve for concentration vs. peak height/area for both the drugs.

Assay procedure

Two bands of the working standard solution and eight bands of sample solution 5 μl each were applied on TLC plates to give concentration of about 1000 ng/spot for MCZ and 500 ng/spot for MTZ respectively. The plate was developed and scanned under the previously mentioned optimized chromatographic conditions. The amount of MCZ and MTZ present in the applied volume of sample was displayed by the instrument by comparing peak height and area of the sample with that of standard.

Method validation

Precision

Precision of the method was determined by inter-day, intra-day and analyst to analyst variation. System reproducibility was determined by five replicate applications and five times measurement of a laboratory mixture at the analytical concentration. This was performed by preparing laboratory mixtures of miconazole nitrate and metronidazole in the ratio mentioned in marketed formulations. The reproducibility of sample application by measurement of concentration with respect to height and area for active compounds were expressed in terms of SD and %RSD.

Recovery studies

Recovery studies were carried out by applying the method to drug sample to which the known amount of miconazole nitrate and metronidazole has been spiked (standard addition method). Accurately weighed quantities of cream equivalent to about 1.4 mg of MCZ (equivalent to about 70% of the prescribed sample weight as per the proposed method) were transferred to five different 10.0 ml volumetric flasks and 1 to 5 ml of mixed standard solution (conc: MCZ-0.201 mg/ml and MTZ 0.101 mg/ml) were added to them followed by methanol. The flasks were shaken for 30 min and the volumes were made up to the mark with methanol (The drug content in different flasks represents 80-120% of labeled claims with constant amount of excipients). The solutions were then centrifuged and the supernatant was used as sample solution.

Two bands of standard and eight bands of each sample solutions (5 μl each) were applied on TLC plate and the plates were developed and scanned under the optimized chromatographic conditions.

Specificity studies

Sample solutions were prepared by exposing an accurately weighed quantity of cream equivalent to 2 mg of miconazole nitrate and 1 mg of metronidazole to various stress conditions, like at 50°C after addition of 1.0 ml of 0.1 N HCl (acid), at 50°C after addition of 1.0 ml of 0.1 N NaOH (alkali), at 50°C after addition of 1.0 ml of 3.0% H_2O_2 (oxide), at 60°C and in UV-cabinet at 265 nm for 24 hr. The sample solutions were then analyzed by the proposed method.

RESULT AND DISCUSSION

Optimization of procedure

Since both MCZ and MTZ dissolve better in methanol, it was used to prepare standard solutions as well as for extraction from gel.

Various blends of solvent systems in varying proportions were tried as mobile phase. However, mobile phase consisting of toluene: chloroform: methanol (3.0:2.0:0.6 v/v/v), gave good resolution, dense and compact spots along with minimum tailing and Rf values of 0.34 and 0.55 for MTZ and MCZ respectively. The selection of wavelength was based on UV absorbance by both the

drugs with due consideration to their ratio in the formulations. Both peaks were symmetrical in nature and no tailing was observed when scanned at 240 nm (fig. 1). The chamber saturation time with the mobile phase was 10 min at room temperature.

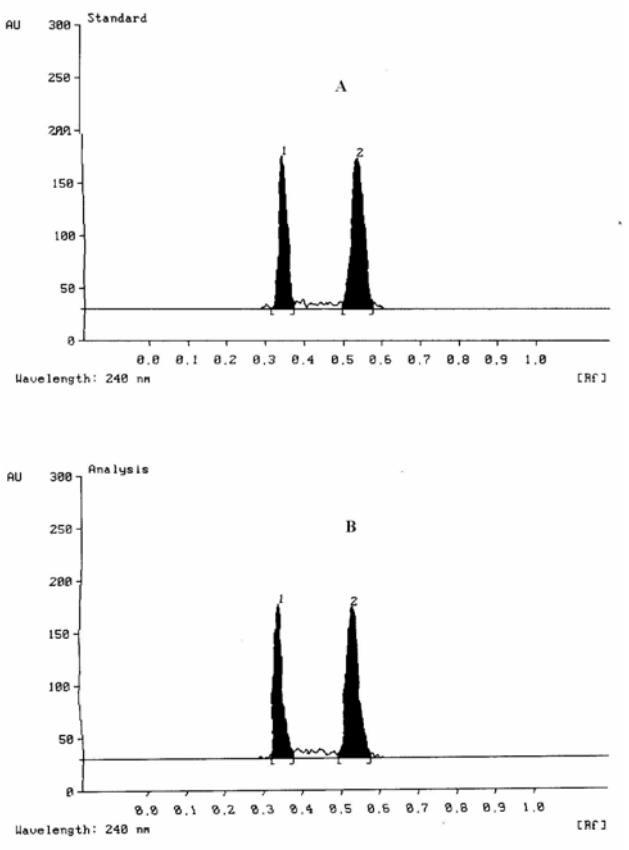

Fig. 1: Densitogram of Metronidazole (500 ng/spot); peak 1 (Rf: 0.34±0.02) and Miconazole nitrate (1000ng/spot); peak 2 (Rf: 0.55±0.02) in standard (A) and sample solution (B).

Linearity

MTZ showed good correlation coefficient in the concentration range of 300-700 ng/spot (r = 0.9971 and 0.9996 by height and area respectively) and MCZ in the range of 600-1400 ng/spot (r=0.9919 and 0.9954 by height and area respectively) (table 1). In case of linear detector response both the drugs in varying concentration show better linear relationship when plotted with concentration *vs* peak height/peak area (fig. 2). Apart from correlation coefficient value the intercept value was not more than 2% of the response observed at 100% concentration in both the cases and hence, single point calibration was used.

Precision

The reproducibility of sample application by measurement of concentration with respect to height and area for active compounds were expressed in terms of SD and %RSD and the results are shown in table 2. The %RSD values depicted in table 3 showed that the proposed method provide acceptable inter-day, intra-day and analyst to analyst variation of MTZ and MCZ.

Recovery studies

The proposed method when used for extraction and subsequent estimation of miconazole nitrate and metronidazole from pharmaceutical dosage form after spiking with additional drug afforded recovery in the range of 98.92-100.13% and mean recovery for miconazole nitrate and metronidazole from marketed formulation are listed in table 4. The results of recovery study indicate that the common excipients present in gel formulation do not interfere with estimation of the two drugs.

Table 1: Linear regression data for calibration curves-HPTLC (n=3)

Parameter	Metronidazole		Miconazole nitrate	
	Height	Area	Height	Area
Linearity (ng/spot)	300-700	300-700	600-1400	600-1400
Correlation coefficient.	0.9971	0.9996	0.9919	0.9954
Slope	263.17	3975.66	91.42	2441.31
Y-Intercept	33.93	329.86	37.87	734.05

Table 2: Results of system reproducibility-HPTLC (n=5)

Parameter	Metronidazole		Miconazole nitrate	
	Height	Area	Height	Area
Mean %estimated* ±S.D.	99.67± 0.87	101.17±0.78	99.81±0.63	100.16±1.15
% R.S.D.	0.873	0.771	0.631	1.148
S.E.	0.39	0.35	0.28	0.51

*Each mean %estimated is a mean of five observations

Specificity

The specificity of the method evaluated on the basis of forced degradation by exposing the sample to various stress conditions has shown reasonable specificity of the proposed method. In case of estimation of metronidazole, the results of the sample treated with a base were much on lower side without additional peaks, indicating that sample has undergone degradation and the degradation product staying at starting point or transformed to non-chromophoric group which does not absorb at wavelength of estimation. In addition to this the results were also low by about 5-7 % in samples treated with hydrogen peroxide and UV radiations compared to the normal samples. In case of miconazole nitrate the sample of UV irradiated and hydrogen peroxide treated sample the results were low by about 5-10%. In case of all other treated samples the results of estimation of metronidazole and miconazole nitrate were close to normal samples indicating that, there is no degradation (table 5).

Analysis of the marketed formulations

The peaks at Rf 0.34 (MTZ) and 0.55 (MCZ) were observed in the densitograms (fig. 1) of the drug samples extracted from gel. There was no interference from the common excipient present in cream. The assay values for both the drugs in formulation suggest that degradation of metronidazole and miconazole nitrate had not occurred in the marketed formulations that were analyzed by this method as shown in table 6. The %RSD value indicated the suitability of this method for routine estimation of metronidazole and miconazole nitrate in pharmaceutical dosage forms.

Table 3: Inter-day, intra-day and analyst to analyst precision studies

Parameter	Metronidazole		Miconazole nitrate	
	Height	Area	Height	Area
Inter- Day				
Mean %estimated *±S.D.	98.86± 0.24	99.42± 0.99	99.97± 0.61	99.26± 0.61
% R.S.D.	0.242	0.996	0.61	0.615
S.E.	0.14	0.57	0.35	0.35
Intra-day				
Mean %estimated* ±S.D.	99.75± 0.63	100.78± 0.42	98.65± 0.47	99.78± 1.27
% R.S.D.	0.632	0.417	0.476	1.273
S.E.	0.36	0.24	0.27	0.73
Analyst to Analyst				
Mean %estimated* ±S.D.	99.41±0.64	99.81±0.16	99.22±0.59	99.66±0.68
% R.S.D.	0.64	0.16	0.59	0.68
S.E.	0.37	0.10	0.34	0.39

*Each mean %estimated is a mean of five observations

Table 4: Result of Recovery Study (n=5)

Parameter	Metronidazole		Miconazole nitrate	
	Height	Area	Height	Area
Cream				
Mean % recovery* ±S.D.	100.13± 1.59	98.92± 0.76	99.49± 1.58	99.63± 1.46
% R.S.D.	1.588	0.768	1.588	1.465
S.E.	0.71	0.34	0.71	0.65

*Each mean %recovery is a mean of five observations

Table 5: Results of specificity study

Condition	Metronidazole (Mean % estimated*)		Miconazole nitrate (Mean % estimated*)	
	Height	Area	Height	Area
Normal	99.85	99.94	99.65	99.78
Alkali	67.16	66.63	99.75	99.10
Acid	97.91	100.67	99.01	97.39
Oxide	95.56	96.28	96.35	95.98
Heat	98.83	100.34	100.20	98.55
UV	93.67	94.05	94.86	90.37

Table 6: Result of estimation of drugs in marketed formulations (n=5)

Parameter	Metronidazole		Miconazole nitrate	
	Height	Area	Height	Area
Cream				
Mean % estimated*±S.D.	98.97±0.43	100.96±0.43	99.08±1.11	98.77±0.88
% R.S.D.	0.434	0.426	1.120	0.891
S.E.	0.19	0.19	0.50	0.39

*Each mean % estimated is a mean of five observations

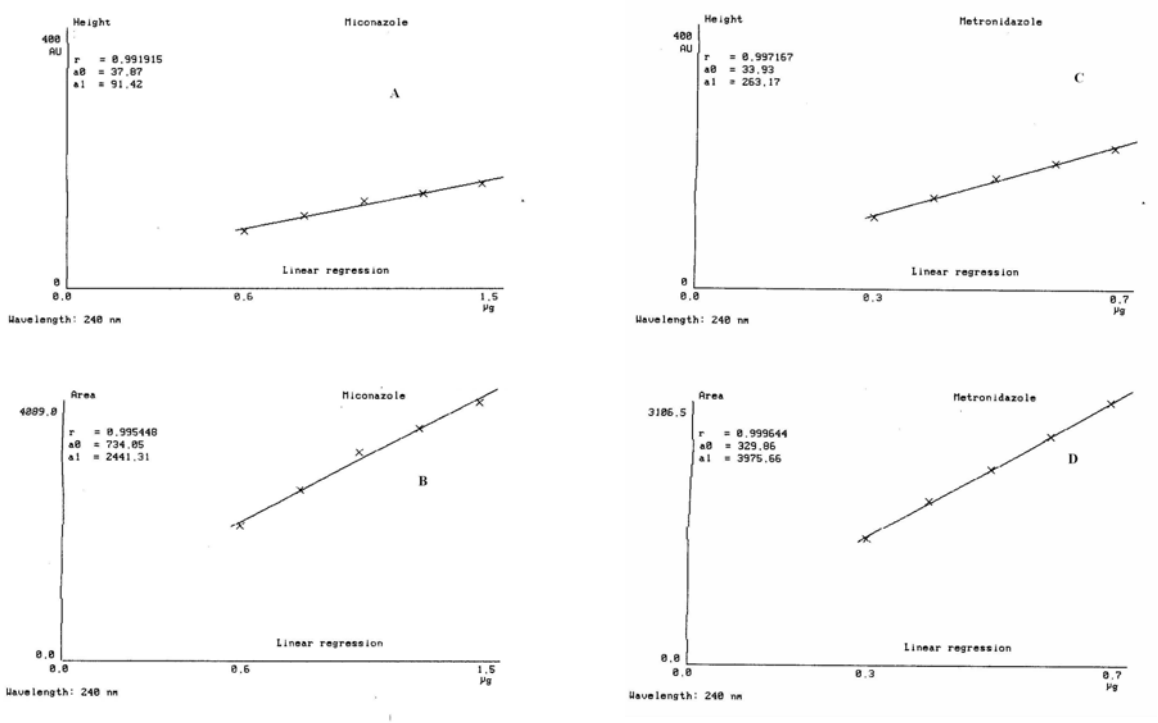

Fig. 2: Linear regression curves of miconazole nitrate by height (A) and area (B) and metronidazole by height (C) and area (D).

CONCLUSION

The method was developed on HPTLC to estimate the two drugs simultaneously in formulation, for the first time in order to analyze more samples in less time. The proposed method is easy to perform, precise, accurate, rapid and reasonably specific and rugged. The whole procedure may be extended to pharmaceutical preparations and other applications on the same drug for routine screening. The method was further validated and the results were found to be concurrent and comparable to the reported method (Akay *et al*., 2002).

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