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Der Chemica Sinica, 2017, 8(1):133-137

ISSN : 0976-8505
CODEN (USA): CSHIA5

Simultaneous Determination of Fluconazole and Tinidazole in Combined Dose Tablet using High Performance Thin Layer Chromatography

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ABSTRACT

This paper presents an HPTLC method, validated for simultaneous determination of fluconazole (FLZ) and tinidazole (TNZ) in a combined dose tablet. Chromatography was performed on silica gel 60F_{254} plates with 250 µm thickness, using Toluene: Ethyl acetate: Chloroform: Methanol (1.2:3:2:0.8 v/v) as a mobile phase. For the proposed method, linearity (FLZ-r^2 > 0.996; TNZ-r^2 > 0.997), sensitivity (FLZ-3.04 ng/spot; TNZ-11.12 ng/spot), recovery (FLZ-98.66%-99.13%; TNZ- 99.26%-100.54%), and repeatability were found to be satisfactory. The HPTLC method offers a simple, sensitive, rapid and cost effective technique that can be applied successfully in routine quality control of formulations containing FLZ and TNZ.

Keywords: Fluconazole, Tinidazole, HPTLC

INTRODUCTION

Fluconazole (FLZ), 2-(2,4-Difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl) propan-2-ol) (**Figure 1A**), is a triazole antifungal drug which in sensitive fungi, inhibits a cytochrome P450 dependent enzyme resulting in impairment of ergosterol synthesis in fungal cell membranes. It is widely used as a broad-spectrum antifungal agent for the treatment and prophylaxis of fungal infections of a deep organ. Tinidazole (TNZ), 1-[2-(Ethylsulphonyl) ethyl]-2-methyl-5-nitroimidazole (**Figure 1B**) is widely used to treat infectious diseases such as amoebiasis, giardiasis, trichomoniasis and those caused by anaerobic bacteria. To treat systemic fungal infection, a combination of these drugs is used either as two different tablets in a form of a kit or a combination of two drugs as a single tablet. Literature cited various methods to estimate FLZ in biological fluids, similarly, few methods such as I.R. spectroscopy [1], UV spectrophotometry [2-6], microbiological assays [7] and TLC [8] for determination of FLZ in pharmaceutical formulations.

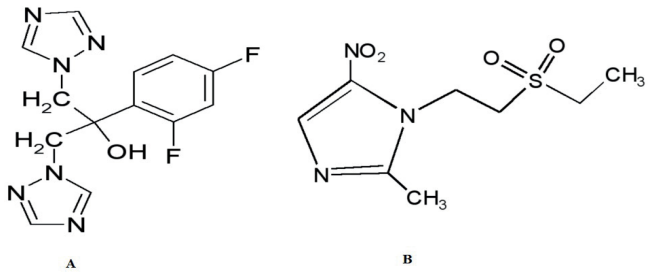

Figure 1: Structure of Fluconazole (A) and Tinidazole (B).

The literature survey revealed HPTLC [9,10] and HPLC [11,12] methods to estimate TNZ from dosage forms, the study of TNZ hydrolysis [13] and its degradation behavior [14] by HPLC along with various methods such as UV [15], Micellar HPLC and UV derivative [16] and RP-HPLC [17-19] methods for simultaneous determination of TNZ and FLZ in dosage forms. However, the literature did not cite any method for simultaneous determination of FLZ and

TNZ by HPTLC. Therefore, we have attempted to develop an HPTLC method for determination of FLZ and TNZ from combined tablet dosage form.

High-performance thin-layer chromatography (HPTLC) is a simple, rapid, and highly efficient method, which allows analysis of several samples simultaneously using a small amount of solvent. This reduces the time, cost of analysis, and decreases the possibility of environmental pollution.

MATERIALS AND METHODS

Experimental

Materials and reagents

Zim Labs Nagpur, kindly gifted FLZ and Suven laboratories, Chennai, kindly supplied TNZ. All other chemicals and solvent were of analytical grade and purchased locally.

Flucoti tablets (labeled claim: tinidazole 1000 mg and fluconazole 75 mg) manufactured by Fourrts (India) Pvt. Ltd., Chennai, were purchased from local pharmacy.

Standard solutions

Stock solutions of individual drug and a mixed standard stock solution (containing FLZ 500 µg/ml and TNZ 6.65 mg/mL) were prepared separately in methanol and stored at 4°C. An accurately measured, 3.0 ml mixed stock standard solution was diluted to 10.0 ml with methanol to give working standard solution (conc: FLZ-150 µg/mL; TNZ-1.995 mg/mL).

Sample solutions

A sample of 20 tablets were accurately weighed and powdered. Tablet powder equivalent to about 12.5 mg of FLZ and 166.63 mg of TNZ was accurately weighed, dissolved using 15 mL of methanol and sonicated for about 15 minutes. The volume was made up to 25.0 ml and then filtered. 3.0 ml of the clear filtrate was diluted to 10.0 ml with methanol.

Procedure

This procedure was finalized based on trial and error. Working standard solution was accurately applied as a band 4 mm wide with a Camag 100 µl syringe (Hamilton, Bonaduz, Switzerland) on a pre-coated silica gel $60F_{254}$, (10 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany) HPTLC plates, using a CAMAG Linomat V (Switzerland) sample applicator. Precoated HPTLC plates were washed with methanol and activated at 110°C for 5 min prior to chromatography. Spots were applied at a constant rate of 5.0 s/µl and space between two bands was 4 mm. The linear ascending development was performed in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase Toluene: Ethyl acetate: Chloroform: Methanol (1.2:3:2:0.8 v/v) for 10 min at room temperature (25°C ± 2) at a relative humidity of 60% ± 5. The length of chromatogram run was 70 mm. Camag TLC scanner III performed densitometric scanning in the reflectance-absorbance mode at 205 nm.

Validation of the developed method

The proposed method was validated, for linearity, accuracy, precision, and specificity.

Linearity

Mixed standard solutions containing FLZ in the range of 0.45 µg/ml-1.2 µg/ml and TNZ in the range of 5.99 µg/ml-15.99 µg/ml were analysed to obtain the calibration curve. Correlation coefficient (r^2) was estimated to determine linearity.

Precision

The system repeatability (intraday precision, percentage RSD) was assessed by six determinations of sample solutions and standard solutions. Intermediate precision was considered by three different analysts on different days (day 1, day 3 and day 5).

Accuracy

Accuracy was estimated by standard addition method by evaluating percentage recoveries of the known quantities of combination of FLZ and TNZ added to the solutions with marketed formulations.

Specificity

Specificity of the method towards the drugs was established by attempting deliberate degradation of the two drugs with exposure to stress conditions like acidic (0.1 M HCl), alkali (0.1 M NaOH), an oxidizing agent (3% H_2O_2), heat (60°C) and UV rays. After 24 hours, samples were diluted with methanol and analysed by proposed method.

Applicability of the developed method to marketed formulation

Accurately, 6 µl of the sample solution (FLZ: 0.75 µg/spot, TNZ: 9.99 µg/spot) was spotted on the HPTLC plate followed by development and scanning as described in the experimental section.

RESULTS AND DISCUSSION

Optimization of HPTLC method

To develop a robust mobile phase, the mixture of Toluene: Ethyl acetate: Chloroform: Methanol (1.2:3:2:0.8 v/v) was optimized resulting in well-resolved and compact spots free of tailing with better sensitivity. The observed Rf values for FLZ and TNZ were 0.27 ± 0.012 and 0.51 ± 0.022 respectively. **Figure 2** depicts the chromatograms obtained from standard and sample drug solutions.

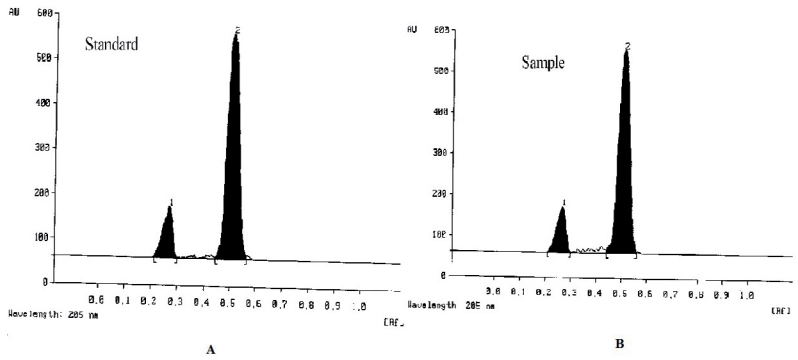

Figure 2: Chromatogram of fluconazole (1) and tinidazole (2) in standard (A) and sample (B), solution.

Method validation

The response was linear in the range, from 450 ng/band to 1050 ng/band for FLZ and 5985 ng/band to 13965 ng/band for TNZ. Correlation coefficients (r) value were >0.99 for both the drugs. The limit of detection for TNZ and FLZ were 11.12 and 3.04 ng/spot respectively, and limit of quantitation values were 37.32 and 14.24 ng/spot. The effect of random events on the precision of the analytical method due to a different day and different analyst variation or intermediate precision, expressed in terms of percentage RSD, shows that the method is precise (**Table 1**). The result obtained (**Table 2**) suggest the accuracy of the method without any interference by additives in tablet formulation. The results of specificity studies have shown that the sample undergoes degradation on exposure to alkaline conditions as depicted in **(Figure 3)**. The chromatogram shows extra peaks at 0.07, 0.12 and 0.37 (Rf). The degradation products are well resolved from the target analyte, thus affording precise determination of FLZ and TNZ in stressed conditions.

Table 1: Results from study of intermediate precision.

Parameter	Tinidazole	Fluconazole
	By Peak Area	By Peak Area
Analyst-1 [percentage estimated]	99.63	99.09
Analyst-2 [percentage estimated]	100.89	99.14
Analyst-3 [percentage estimated]	98.87	100.12
Interday precision [percentage RSD, n=6]	0.69	0.36
Intraday precision [percentage RSD, n=6]	0.42	0.64

Table 2: Recovery studies of tinidazole and fluconazole (n=6).

Drug	Amount of standard drug added (mg)	Amount of standard drug recovered	Recovery (%)
TNZ	16.66	16.54	99.26
	50.02	49.73	99.43
	83.42	83.87	100.54
FLZ	1.25	1.24	99.13
	3.75	3.67	98.66
	6.25	6.19	99.07

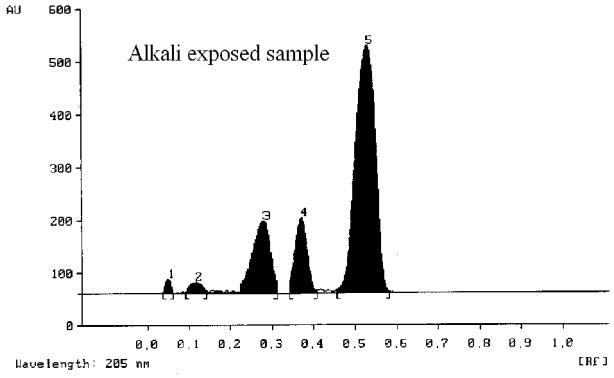

Figure 3: Chromatogram of tinidazole and fluconazole (sample) exposed to alkaline stress for 24 h at 50°C (peak no. 3 Fluconazole, peak no. 5 Tinidazole and degradation products-peak no. 1, 2 and 4).

No degradation was observed in the case of, samples exposed to other stress conditions. Applicability of the method was verified by determination of FLZ and TNZ in a combined dose tablet. The results obtained (**Table 3**) suggest suitability of the method for routine analysis.

Table 3: Summary of results of estimation in tablet formulation (n=6).

Sample	Constituents Estimated	Labeled Claim (mg/ tab)	Mean Amount Estimated (mg/tab)	Mean Percent of labeled Claim ± SD, (%RSD)
				By Peak Area
Tablet Formulation	Fluconazole	75	75.62	100.82 ± 0.92, (0.91)
	Tinidazole	1000	993.4	99.34 ± 0.33, (0.33)

The matrix did not affect the quantitative determination of two drugs. This has been established by the recovery studies where, finely powdered tablet was spiked with standard drug and amount of recovery calculated. The percentage recovery obtained indicates the efficacy of the method to quantify FLZ and TNZ in combined tablet dosage form.

CONCLUSION

The proposed chromatographic method enables quantitative determination of FLZ and TNZ without the interference of degradation products of TNZ. HPTLC combined with densitometry is a technique, which is a simple and low-cost method of drug assay. The proposed method is rapid, sensitive, and suitable for routine control of fluconazole and tinidazole in pharmaceuticals.

The developed method can be applicable to quantify FLZ and TNZ in their single dosage form. Moreover, it is able to resolve TNZ from its degradation products; hence it can be used as a stability indicating analytical method for TNZ.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. N. J. Gaikwad, Professor and Head, Department of Pharmaceutical Sciences, R.T.M. Nagpur University, Nagpur for providing laboratory facilities. Authors are also thankful to Zim Laboratories, Nagpur India, for providing gift sample of fluconazole and Suven laboratories Chennai for providing tinidazole as a gift sample. One of the authors is thankful to University Grant Commission, New Delhi India, for supporting the project financially, under Rajiv Gandhi National Fellowship Scheme.

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