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Research Article

SIMULTANEOUS ESTIMATION OF CLOTRIMAZOLE AND TINIDAZOLE IN PHARMACEUTICAL FORMULATION BY HPTLC

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ABSTRACT

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed for simultaneous determination of Clotrimazole and Tinidazole in pharmaceutical formulation. The stationary phase used was precoated silica gel 60F254 TLC aluminum plate, (20×10 cm 2). The mobile phase used was a mixture of toluene: methanol: ethyl acetate: tri ethylamine (5:1:1:0.1v/v/v/v). The detection of spot was carried out at 254 nm. The calibration curve was found to be linear between 100 to 700 ng/spot for Clotrimazole and 200 to 1400 ng/spot for Tinidazole. The developed method can be successfully used to determine the drug content from the pharmaceutical formulation.

Keywords: Simultaneous HPTLC method, Clotrimazole, Tinidazole.

INTRODUCTION

Clotrimazole is a broad spectrum antimycotic drug, it is work by inhibiting the fungal cytochrome P450 3A enzyme, lanosine 14α-demethylase, which is responsible for converting lanosterol to ergosterol, the main sterol in the fungal cell membrane. Chemically it is 1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole[1-3].

Tinidazole is nitro imidazole which has broad spectrum cidal activity against Protozoa. Nitro group of drug is reduced by redox proteins present only in anaerobic organisms to reactive nitro radical which exerts cytotoxic action by damaging DNA and other critical biomolecules. Chemically it is1-[2-(ethanesulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole[5-7].

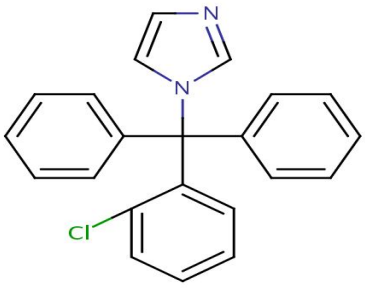

Figure 1: Structure of Clotrimazole[4]

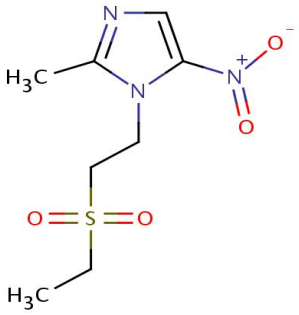

Figure 2 : Structure of Tinidazole[4]

Both drugs are widely used for skin infection and vaginal infection. The indications for the drugs in combination are Tineapedis, Tineacruris, Tineaversicolor, Tineacorporis, Cutaneous candidiasis, Vulvovaginal candidiasis[7].

Various analytical methods have been reported for the estimation of Clotrimazole and Tinidazole in single, in combination with each other and in combination with other drugs that include HPLC[8-14], UV/Vis spectrophotometric[15-19] and HPTLC[20]. Available HPTLC method for Clotrimazole and Tinidazole is performed at g level and we have developed the method at ng level. In available method the limits of detection for clotrimazole and tinidazole were 20 and 60 g mL^{-1}, respectively; the respective limits of quantification were 120 and 200 g mL^{-1}. We have developed method with lower LOD and LOQ level.

MATERIAL AND METHOD

Both API Clotrimazole and Tinidazole were obtained from RK University's drug bank. Toluene, Ethyl acetate and methanol (A. R. Grade) were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Marketed formulation was purchased from local market.

Instrumentation:

Camag TLC system (Muttens, Switzerland) comprising of Camang automatic TLC sampler CAMAG Linomat 5 "Linomat5_171103" S/N 171103 (1.00.12) applicator, syringe capacity 100 l, CAMAG TLC Scanner "Scanner_171010" S/N 171010 (2.01.02), Camag WinCATS software version 1.4.6.2002, Camag twin trough chambers (20×10 cm 2), Camag TLC visualizer, ultrasonicator was used during the study.

TLC plates used were precoated silica gel aluminium plate 60 F_{254}, 20×10 cm^2 with 0.2 mm thickness (E. MERCK KGaA, Mumbai, India).

The chromatographic separation were carried out using precoated silica gel on aluminum plate 60 F_{254}, (20×10 cm^2 and 10×10 cm^2) as stationary phase and toluene: methanol: ethyl acetate: tri ethylamine in the proportion of 5.5:1:1:0.1 v/v/v/v as mobile phase for Clotrimazole and Tinidazole. Ten millilitre volume of mobile phase was placed in twin trough chamber and chamber was saturated for 5 min at 60°C. Detection wavelength for both drug was kept at 254 nm.

For HPTLC analysis, initially various mobile phases were tried in attempts to obtain the best separation and resolution between Clotrimazole and Tinidazole.

Preparation of Standard Stock Solution

Accurately 10 mg of Clotrimazole and 10mg of Tinidazole were weighed separately and transferred into two different 10 ml volumetric flask. Each drug was dissolved in few ml of methanol. The volume was made up to the mark with methanol to give final solutions containing 1000 g/ml of Clotrimazole and 1000 g/ml of Tinidazole respectively.

Preparation of Standard Solution for Binary Mixtures of Clotrimazole and Tinidazole

Transfer 0.5 ml solution of Clotrimazole from standard stock solution (1000 g/ml) and 1.0 ml solution of Tinidazole from standard stock solution (1000 g/ml) in to a 10 ml volumetric flask. The volume was made up to the mark with methanol to obtain a binary mixture containing 50 g/ml of Clotrimazole and 100 g/ml of Tinidazole.

Preparation of Test Solution-1 Tablet Mixtures of Clotrimazole and Tinidazole

Twenty tablets of Clotrimazole and Tinidazole were weighed separately and their average weight was determined and the tablets were crushed to powder sample. From the triturate, a tablet powder equivalent to 5 mg of Clotrimazole and 10 mg of Tinidazole was transferred into 10 ml volumetric flask and dissolved in few ml of methanol. Volume was made up to the mark with methanol. 1 ml of this solution is taken into 10 ml volumetric flask and volume was made up to the mark with methanol to obtain a binary mixture containing 50 g/ml of Clotrimazole and 100 g/ml of Tinidazole.

Preparation of test solution-2 for Binary Mixtures of Clotrimazole and Tinidazole

Twenty tablets of Clotrimazole and Tinidazole in combination contains 200mg Clotrimazole and 500mg Tinidazole were weighed and their average weight was determined and the tablets were crushed to powder sample. From the triturate, a tablet powder equivalent to 10 mg of Tinidazole was transferred into 10 ml volumetric flask and dissolved in few ml of methanol. Volume was made upto the mark with methanol. 1 ml of this solution is taken into 10 ml volumetric flask and volume was made upto the mark with methanol to

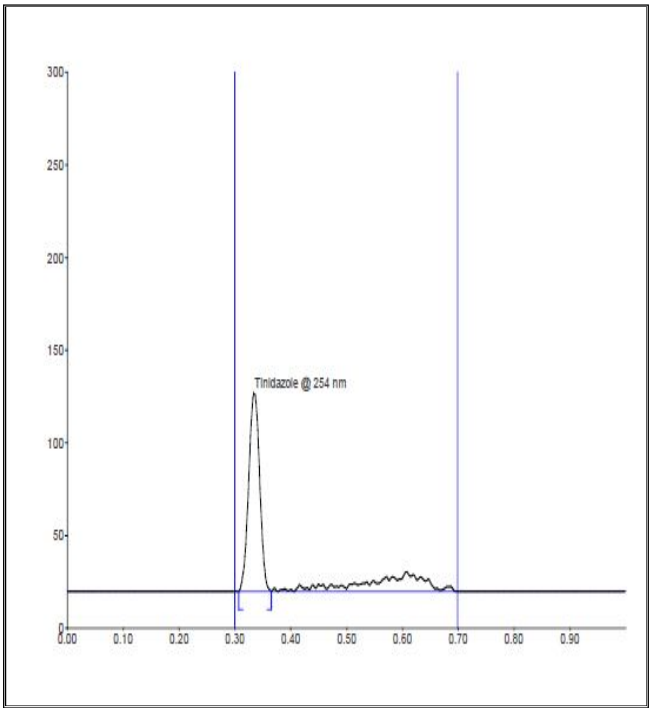

Figure 3: Chromatogram for Tinidazole

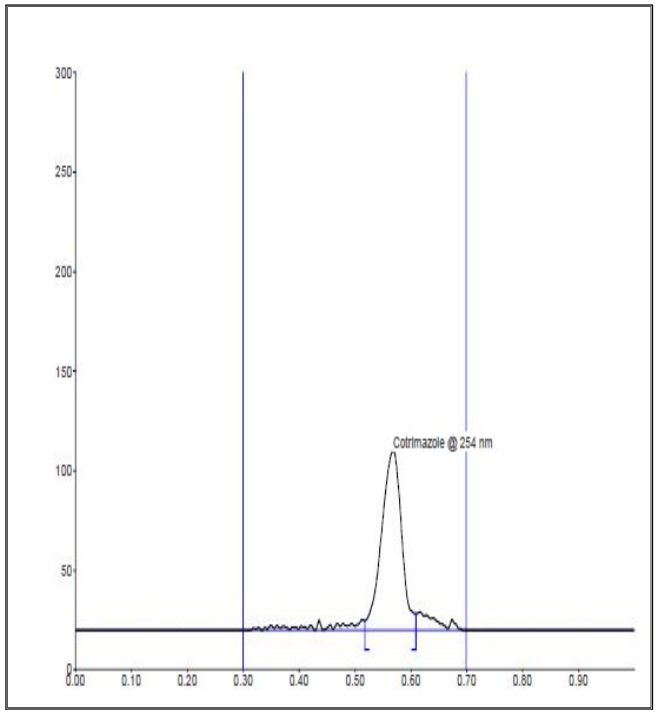

Figure 4: Chromatogram for Clotrimazole

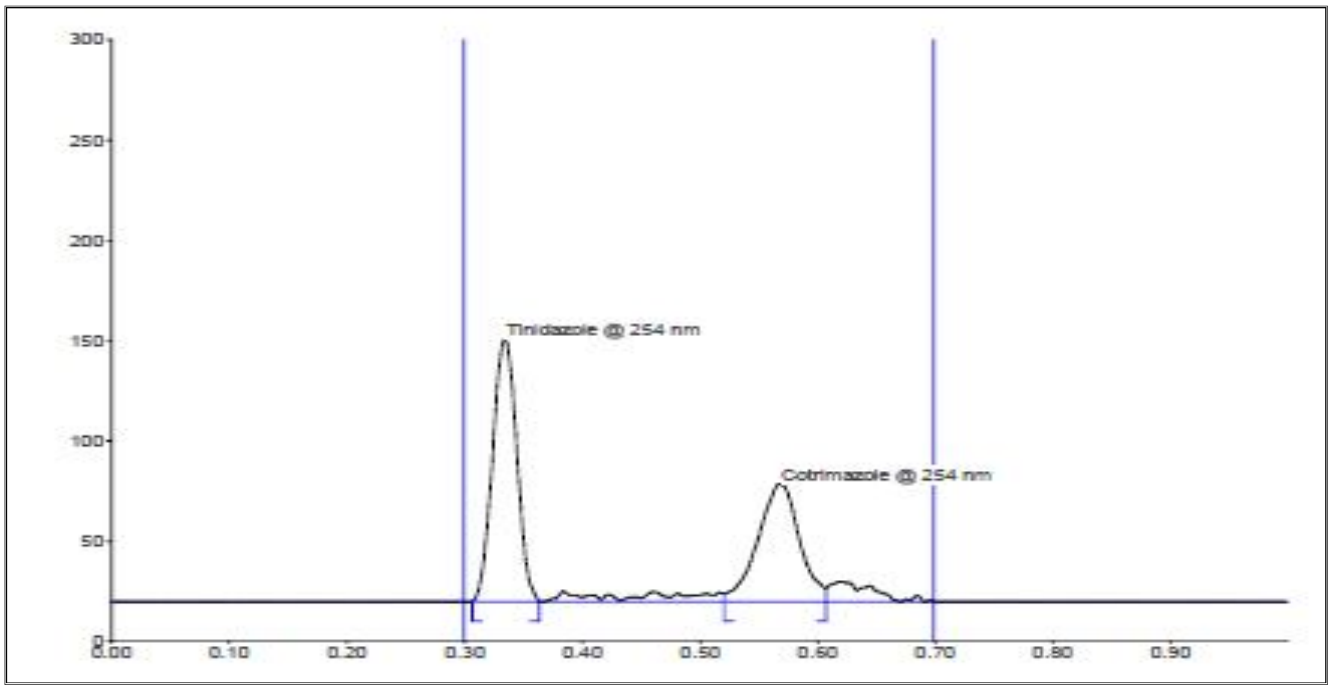

Figure 5: Chromatogram for Tinidazole and Clotrimazole

Table 1: Validation and system suitability parameters

Parameter	CLT	TNZ
Linearity range (ng)	100-700	200-1400
Slope	2.89	2.03
Intercept	258.11	73.23
Regression coefficient (r^2)	0.9984	0.9988
Limit of detection (ng/ml)	22.82	0.87
Limit of quantification (ng/ml)	69.18	2.66
Rf value	0.39	0.67

Table 2: Statistical validation of linear regression of clotrimazole and tinidazole (n=6)

Parameter	Mean		S.D.		R.S.D.		S.E.	
	CLT	TNZ	CLT	TNZ	CLT	TNZ	CLT	TNZ
Slope	2.8982	2.030	0.055	0.031	1.9	1.5	0.022	0.013
Intercept	258.11	73.23	5.13	0.746	1.9	1.0	2.097	0.304
Regression coefficient (r^2)	0.9984	0.9988	0.0005	0.0009	0.05	0.09	0.002	0.0003

S.D. – Standard Deviation R.S.D. – Relative Standard Deviation S.E. – Standard Error

Table 3: Calibration data of clotrimazole and tinidazole (n=6)

Spot No.	Conc. (ng/ml)		Area	
	CLT	TNZ	CLT	TNZ
1	100	200	522.4	490.0
2	200	400	842.7	864.3
3	300	600	1118.7	1315.2
4	400	800	1406.4	1681.0
5	500	1000	1725.3	2083.0
6	600	1200	1990.4	2542.7
7	700	1400	2332.3	2904.6

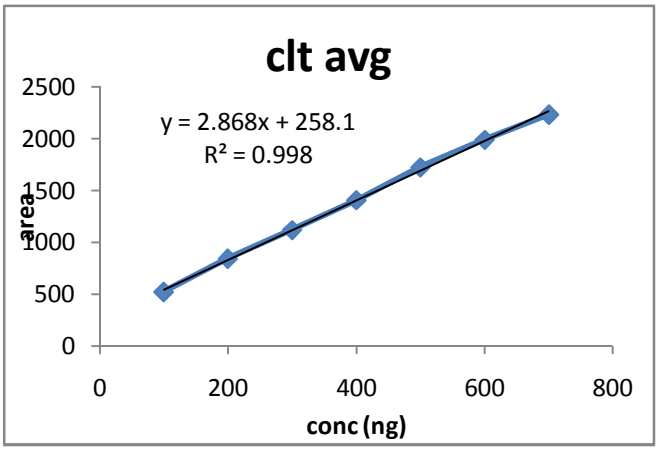

Figure 6: Standard Calibration Curve for Clotrimazole

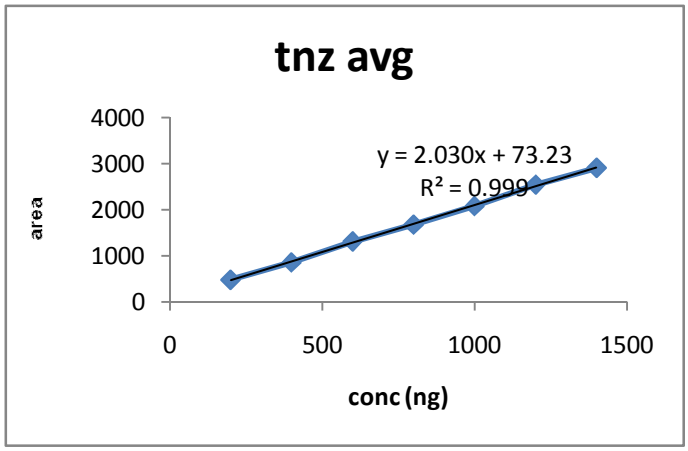

Figure 7: Standard Calibration Curve for Tinidazole

Table 4: Analysis of tablet formulation-1

Sr. No.	Label Claim (mg/tab)		Amount Found (mg/tab)		% of Label Claim	
	CLT	TNZ	CLT	TNZ	CLT	TNZ
1.	250	500	248.72	493.85	99.49	98.77
2.	250	500	246.05	497.95	98.42	99.59
3.	250	500	245.57	496.1	98.23	99.22
4.	250	500	249.87	500.6	99.95	100.12
5.	250	500	247.57	497.05	99.03	99.41
6.	250	500	247.27	498.7	98.91	99.74

Table 5: Analysis of tablet formulation-2

Sr. No.	Label Claim (mg/tab)		Amount Found (mg/tab)		% of Label Claim	
	CLT	TNZ	CLT	TNZ	CLT	TNZ
1.	200	500	200.62	400.08	100.31	100.02
2.	200	500	204.4	396.6	102.27	99.15
3.	200	500	200.88	401.2	100.44	100.3
4.	200	500	205.04	394.72	102.52	98.68
5.	200	500	199.42	395.64	99.71	98.91
6.	200	500	202.64	399.12	101.32	99.78

Table 6: Statistical validation for the tablet formulation-1

Component	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
CLT	99.00	0.64	0.65	0.26
TNZ	99.47	0.46	0.46	0.18

Table 7: Statistical validation for the tablet formulation-2

Component	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
CLT	101.09	1.13	1.11	0.46
TNZ	99.47	0.65	0.65	0.26

* n=6 times

obtain a binary mixture containing 40 g/ml of Clotrimazole and 100 g/ml of Tinidazole.

Chromatographic Development:

From the standard stock solutions appropriate aliquots were applied separately to determine the Rf value of each drug. Now from the binary mixture solution, appropriate aliquots were applied to get the concentration range 100-700 ng of Clotrimazole and 200-1400ng of Tinidazole. Obtained area is plotted against the concentration to get the calibration curve for each drug. From tablet mixture solution appropriate aliquots were applied to determine the concentration of Clotrimazole and Tinidazole present in the tablet formulation.

RESULT AND DISCUSSION

The results obtained by this method are precise and reproducible for the two drugs, Clotrimazole and Tinidazole. Reproducibility of the method was done with six samples of Clotrimazole and Tinidazole. The system suitability parameters were calculated to confirm the specificity of the developed method (Table 1). The statistical parameters confirm the accuracy, precision and reliability of the method. Further this method eliminates complicated extraction of individual drugs for quantitation. Hence the present method is cost effective and faster, can be used for the routine analysis of these drugs from tablets.

REFERENCES

1. Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology. 6th Ed. New York: Churchill Livingstone; 2007: 693
2. Tripathi K.D., Essential of medical pharmacology, New Delhi, Jaypee brother, 6th Ed, pp 761.
3. Indian Pharmacopoeia, Indian Pharmacopoeia Commission Ghaziabad, 2010;Vol 2:1120.
4. http://www.drugbank.ca/druges [Cited 2014 Sep 24].
5. Craig CR, Stitzel RE. Modern Pharmacology with Clinical Application, New Delhi, Wolter Kluwer India Pvt. Ltd. 6th Ed, 2003:598-600.
6. Indian Pharmacopoeia, Indian Pharmacopoeia Commission Ghaziabad, 2010; Vol 3: 2226.
7. Tripathi K.D., Essential of medical pharmacology, New Delhi, Jaypee brother, 6th Ed, pp 798.
8. The British Pharmacopoeia, The department of health, London: Stationary office 2009;2:552
9. Tan SX, Fedorowski J, Moini SH. Development and Validation of a HPLC Method for Clotrimazole Drug Substance Assay and Impurities. United States Pharmacopeia
10. Kumawat P, Jagadish PC, Muddukrishna, Bhat K. Stability indicating hplc method for Clotrimazole lozenges formulation. Inter J Pharm Pharm Sci 2014;6:126-9.
11. The British Pharmacopoeia, The department of health, London: Stationary office 2009;2:2102.
12. Pasha K, Ali A, Bana S, Humair S. Reverse phase – hplc method for the analysis of Tinidazole in pharmaceutical dosage form & bulk drug. Inter J Pharm Pharm Sci 2010;2:46-7.
13. Jansari SK, Patel NB, Patel PR, Patel NN, Desai HT. Development and validation of stability indicating method for simultaneous estimation of Ciprofloxacin HCl and Tinidazole using rp-uplc method. IOSR J Pharm 2012;2:12-9.
14. Vaghela BK, Rao SS. A novel validated stability indicating high performance liquid chromatographic method for estimation of degradation behavior of Ciprofloxacin and Tinidazole in solid oral dosage. J Pharm Bio Sci 2013;5:298-302.
15. Bedair MM, Korany MA, Elsayed MA, Fahmy OT. Derivative spectrophotometric determination of Clotrimazole in single formulations and in combination with other drugs. PubMed 1989:72;432-5.
16. Osama AH, Pakinaz KY. Spectrophotometric determination of Clotrimazole in bulk drug and dosage forms. Sci Direct 1993;40:1289-94.
17. Pandey S, Pandey P, Dubey S, Chaturvedi U, Rai AK. Facile derivative UV spectroscopy method for simultaneous estimation of Tinidazole and Fluconazole. The J. Pharm. Sci 2012; 36:55-62.
18. Nayak K, Khare NK, Sayare A, Ghode P, Lawrence RM. UV spectrophotometric methods for estimation of Norfloxacin and Tinidazole in bulk and tablet dosage forms. Der Pharm Lettre 2012;4:192-8.
19. Bharath SA, Arshad MD, Darak V, Chakravathy KK. Development and validation of simultaneous spectrophotometric estimation of Cotrimazole and Tinidazole in tablet dosage form. IJPI's J Anal Chem 2011;1:12-6.
20. Vaidya VV, Menon SN, Singh GR, Kekare MB, Choukekar MP. An HPTLC method for simultaneous determination of Clotrimazole and Tinidazole in a pharmaceutical formulation. Journal Plan Chromatogr 2007; 20:145-7.

