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Research Article

SIMULTANEOUS ESTIMATION OF CLOTRIMAZOLE AND TINIDAZOLE IN A TABLET USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A Simple, efficient and reproducible method for the simultaneous determination of Clotrimazole and Tinidazole from pharmaceutical formulation has been developed using reversed phase high performance liquid chromatography. The separation was carried out using a mobile phase consisting of methanol : acetonitrile (95:5 % V/V). Column used was chromacil C18 (250 X 4 mm internal diameter) 5 µ with flow rate of 1 ml/min. The detection wavelength used was 229nm. The mean retention time of Clotrimazole and Tinidazole were 6.84 min and 5.54 min respectively. Linearity of both drugs are 10-50 µg/ml. Analytical parameters were calculated and a full statistical evaluation included.

Keywords: Clotrimazole, Tinidazole, Reversed phase, High performance liquid chromatography, HPLC, Simultaneous determination.

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 is 1-[2-(ethanesulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole [5-7].

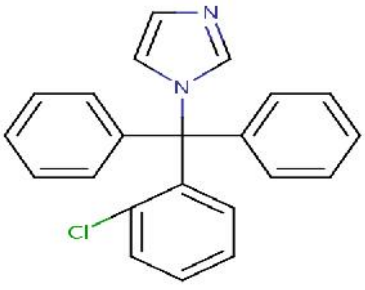

Figure 1: Structure of Clotrimazole[4]

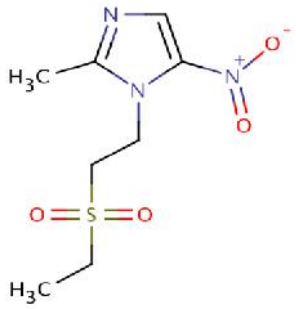

Figure 2: Structure of Tinidazole[4]

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]. Here we have develop a method which gives more reliable parameters like low Rt and simple mobile phase.

MATERIAL AND METHOD

Selection of Wavelength

Using appropriate dilution of standard stock solution of Clotrimazole and Tinidazole both solutions were scanned separately in order to get absorbance. Both the solutions were scanned between 200 – 400 nm using UV-Visible spectrophotometer. Suitable wavelength was selected from the overlay spectra of above solutions. The overlay spectrum for selection of wavelength is given below.

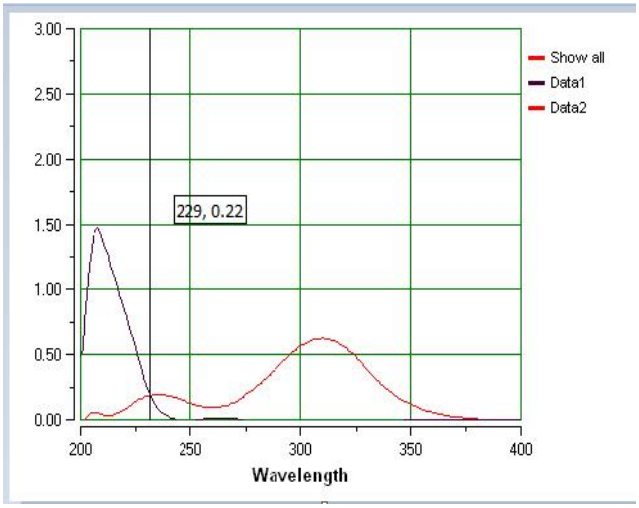

Figure 3: overlay spectrum of CLT and TNZ

Preparation of Standard Stock Solution

Accurately weigh 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 µg/ml of Clotrimazole and 1000 µg/ml of Tinidazole respectively. The solution was subjected to ultrasonication for 20 min and then filtered through 0.2 µm Nylon 6, 6 (N66) membrane filter paper.

Preparation of Standard Solution for Binary Mixtures of Clotrimazole and Tinidazole

Transfer 0.15 ml solution of Clotrimazole from standard stock solution (1000 µg/ml) and 0.30 ml solution of Tinidazole from standard stock solution (1000µg/ml) in to a 10 ml volumetric flask. The volume was made up to the mark with methanol to obtain a binary mixture containing 15µg/ml of Clotrimazole and 30 µg/ml of Tinidazole. The solution was subjected to ultrasonication for 20 min and then filtered through 0.2 µm Nylon 6, 6 (N66) membrane filter paper.

Selection of Mobile Phase

The pure drug of Clotrimazole and Tinidazole were injected into the HPLC system and run in different solvent systems. Different mobile phases like methanol, acetonitrile: methanol, methanol: water, with varying mobile phase ratio were tried in order to find the best conditions for the separation of Clotrimazole and Tinidazole. From the various chromatograms obtained with different mobile phase, methanol: acetonitrile in a ratio of 95:5 is selected for the separation of Clotrimazole and Tinidazole.

Preparation of Mobile Phase

Here, HPLC grade acetonitrile and methanol was filtered through 0.45 µm, 47 mm membrane filter paper and then ultrasonicated for 20 minutes on ultrasonicator. Mobile phase was prepared by mixing 95 ml of methanol and 5 ml of acetonitrile.

Chromatographic Separation

Mixed standard of Clotrimazole and Tinidazole was injected in column with 20 µl micro-syringe. The chromatogram was run for appropriate minutes with mobile phase methanol : acetonitrile (95:5 % v/v) which was previously degassed. The flow rate was set to 1ml/min. and detection was carried out at wavelength 229 nm.

The chromatogram was stopped after complete separation of two drugs. Data related to peak area, height, retention time, resolution etc. were recorded using software.

RESULTS 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 on six samples of Clotrimazole and Tinidazole. The robustness of the method

Figure 3: Chromatogram for Clotrimazole

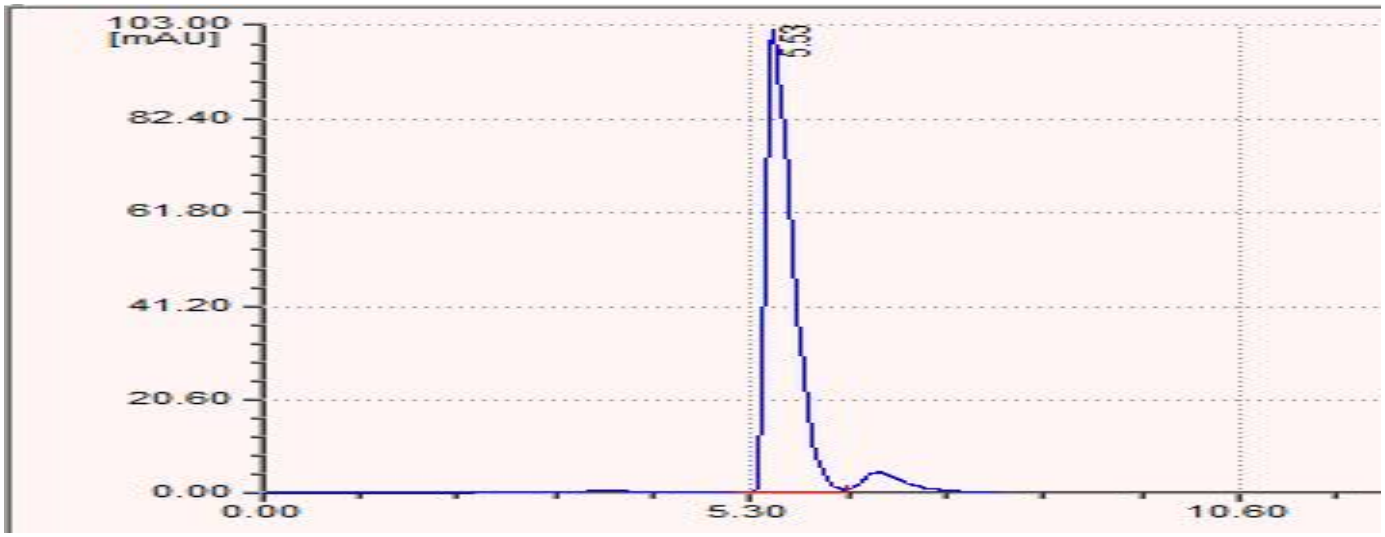

Figure 4: Chromatogram for Tinidazole

Figure 5: Chromatogram for Clotrimazole and Tinidazole

Table 1: Validation and system suitability parameters

Parameter	CLT	TNZ
Linearity range (µg/ml)	10-50	10-15
Slope	10645	4073.1
Intercept	24.01	315.32
Regression coefficient (r^2)	0.9989	0.9997
Retention time (min.)	6.84	5.54
Limit of detection (µg/ml)	0.0001	0.001
Limit of quantification (µg/ml)	0.00029	0.003
Resolution factor	2.51	

CLT- Clotrimazole
TNZ- Tinidazole

Table 2. Calibration table of clotrimazole

Sr. No.	Concentration of Clotrimazole (µg/ml)	Area Under Curve (AUC)
1	10	107331.8
2	20	211526.5
3	30	320184.5
4	40	424973.3
5	50	532860.7

* n=6 times

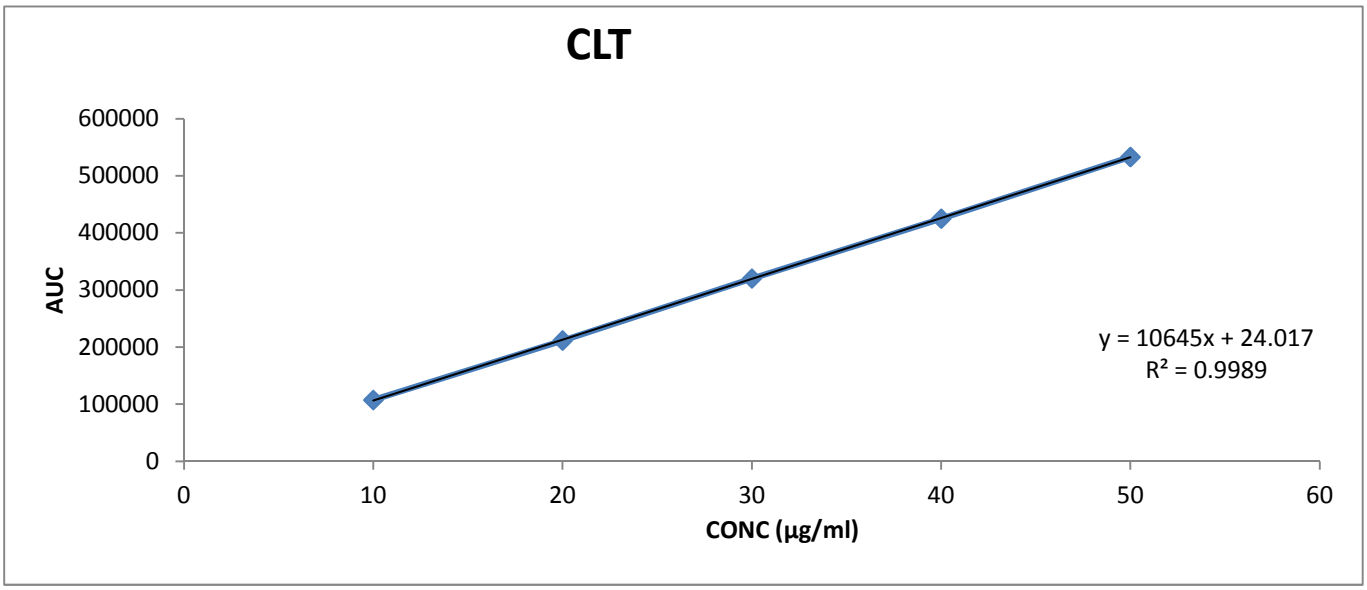

Figure 6. Standard Calibration Curve of Clotrimazole

Table 3. Calibration table of tinidazole

Sr. No.	Concentration of Tinidazole (µg/ml)	Area Under Curve (AUC)
1	10	40819.17
2	20	82326.33
3	30	122520.7
4	40	162467.8
5	50	204401.3

* n=6 times

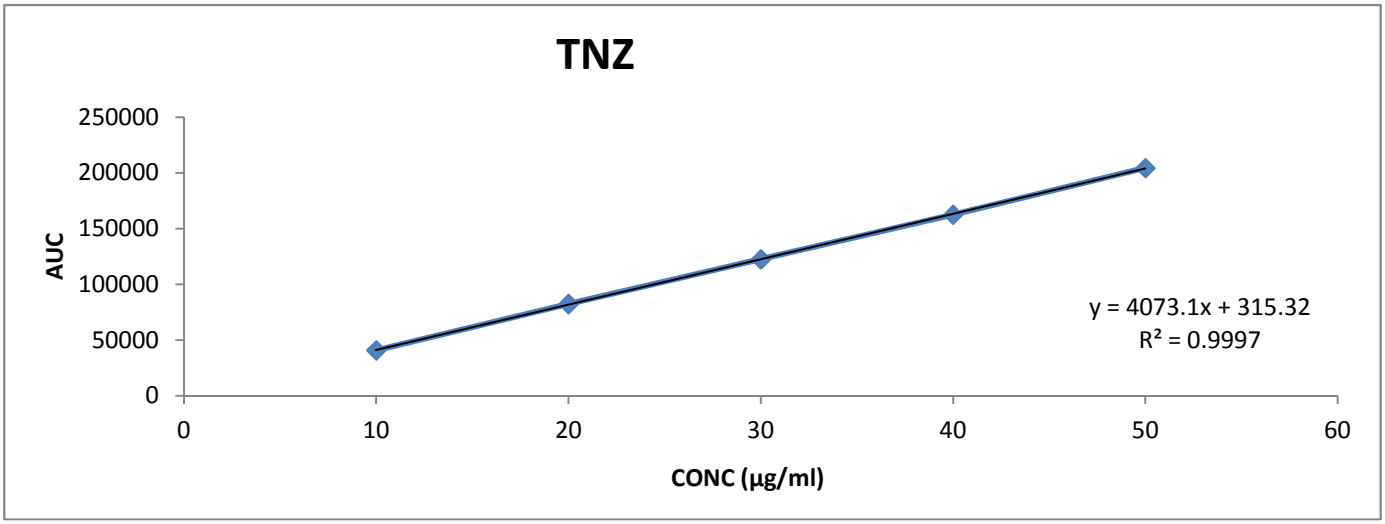

Figure 7. Standard Calibration Curve of Tinidazole

Table 4: Validation of linear regression of clotrimazole

Parameter	Mean	Standard Deviation	% Relative Standard Deviation	Standard Error
Slope	10645.17	179.21	1.680	73.162
Intercept	24.23	0.137	0.565	0.055
Regression coefficient (r^2)	0.9989	0.0004	0.040	0.0001

Table 5: Statistical validation of linear regression of tinidazole

Parameter	Mean	Standard Deviation	% Relative Standard Deviation	Standard Error
Slope	4073.38	31.88	0.782	13.01
Intercept	315.31	1.27	0.402	0.52
Regression coefficient (r^2)	0.9997	0.0003	0.300	0.0001

* n=6 times

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

Table 6: Analysis of marketed formulation for clotrimazole

Label claim (mg/tab)	Amount Present (µg/ml)	AUC	Amount found (µg/ml)	Amount found (mg/tab)	Drug found in formulation (%)
250	15	159534	14.98	249.72	99.89
250	15	159365	14.96	249.47	99.79
250	15	159675	14.99	249.97	99.99
250	15	159354	14.96	249.45	99.78
250	15	159561	14.98	249.77	99.91
250	15	159854	15.01	250.25	100.1

Table 7. Analysis of marketed formulation data for tinidazole

Label claim (mg/tab)	Amount Present (µg/ml)	AUC	Amount found (µg/ml)	Amount found (mg/tab)	Drug found in formulation (%)
500	30	122520	30.03	499.95	99.99
500	30	122786	30.06	500.45	100.09
500	30	122476	29.99	499.45	99.86
500	30	122874	30.09	500.95	100.19
500	30	122385	29.96	498.8	99.76
500	30	122347	30.03	499.95	99.99

Table 8: Statistical validation for the tablet analysis

Brand	Drug	Mean Drug Found in Formulation (%)	Standard Deviation	Relative Standard Deviation (%)	Standard Error
CLODAZ-V6	CLT	99.91	0.121	0.120	0.049
	TNZ	99.96	0.156	0.150	0.064

* n=6 times

Table 9: Recovery data for clotrimazole from formulation

Conc. Level (%)	Amount Present in Formulation (µg /tab.)	Amount Spiked (µg/tab.)	Amount present in powder mixture (µg/tab.)	Amount present in solution (µg/ml)	AUC	Amount Recovered (µg/ml)	Recovery (%)
80	250	200	450	27	28755	27.01	100.03
80	250	200	450	27	28768	27.02	100.08
80	250	200	450	27	28735	26.98	99.96
100	250	250	500	30	31965	30.03	100.1
100	250	250	500	30	31928	29.99	99.98
100	250	250	500	30	31893	29.96	99.87
120	250	300	550	33	35085	32.96	99.89
120	250	300	550	33	35134	33.01	100.03
120	250	300	550	33	35106	32.96	99.88

Table 10: Recovery data for tinidazole from formulation

Conc. Level (%)	Amount Present in Formulation (mg /tab.)	Amount Spiked (mg/tab.)	Amount present in powder mixture (mg/tab.)	Amount present in solution (µg/ml)	AUC	Amount Recovered (µg/ml)	Recovery (%)
80	500	400	900	54	220829	54.01	100.01
80	500	400	900	54	220674	53.96	99.94
80	500	400	900	54	220608	53.95	99.92
100	500	500	1000	60	245040	59.94	99.91
100	500	500	1000	60	245465	60.05	100.08
100	500	500	1000	60	245236	59.98	99.98
120	500	600	1100	66	270011	66.03	100.05
120	500	600	1100	66	269741	65.96	99.95
120	500	600	1100	66	269903	66.01	100.01

Table 11. Statistical validation of recovery studies for clotrimazole

Concentration Level (%)	Mean Recovery (%)	Standard Deviation	Relative Standard Deviation (%)	Standard Error
80	99.95	0.047	0.047	0.027
100	99.99	0.085	0.085	0.049
120	100.00	0.050	0.050	0.029

Table 12. Statistical validation of recovery studies

Concentration Level (%)	Mean Recovery (%)	Standard Deviation	Relative Standard Deviation (%)	Standard Error
80	100.02	0.060	0.059	0.034
100	99.98	0.115	0.115	0.066
200	99.93	0.083	0.083	0.048

Table 13. Robustness data for clotrimazole

Change in Flow Rate					
Flow Rate (ml/min.)	**Amount of drug present (µg/ml)**	**Level**	**Retention Time (min.)**	**Resolution (min.)**	**Amount found (%)**
0.9	15	-1	6.93	1.76	100.04
1	15	0	6.88	1.8	99.94
1.1	15	+1	6.71	1.68	99.67
Change in % of Acetonitrile in Mobile Phase					
% of Acetonitrile in Mobile Phase	**Amount of drug present (µg/ml)**	**Level**	**Retention Time (min.)**	**Resolution (min.)**	**Amount found (%)**
4	15	-1	6.84	1.77	99.98
5	15	0	6.88	1.84	100.1
6	15	+1	6.85	1.79	99.99

Table 14. Robustness data for tinidazole

Change in Flow Rate					
Flow Rate (ml/min.)	**Amount of drug present (µg/ml)**	**Level**	**Retention Time (min.)**	**Resolution (min.)**	**Amount found (%)**
0.9	30	-1	5.76	1.81	99.99
1	30	0	5.54	1.83	99.96
1.1	30	+1	5.68	1.78	100.03
Change in % of Acetonitrile in Mobile Phase					
% of Acetonitrile in Mobile Phase	**Amount of drug present (µg/ml)**	**Level**	**Retention Time (min.)**	**Resolution (min.)**	**Amount found (%)**
4	30	-1	5.62	1.79	99.93
5	30	0	5.54	1.8	99.83
6	30	+1	5.46	1.72	99.89

Table 15. Statistical Validation of Robustness Data

Drug	**Change in Flow Rate**		**Change in % of Acetonitrile in Mobile Phase**	
	Mean Retention Time (min.)	**Standard Deviation**	**Mean Retention Time (min.)**	**Standard Deviation**
CLT	6.84	0.11	6.85	0.02
TNZ	5.66	0.11	5.54	0.08

was confirmed by varying the concentration of organic phase and water, flow rate and temperature. With the increase in the flow rate the retention time and tailing factor decreases and with the decrease in the flow rate the retention time and tailing factor increases. The best result is obtained with the flow rate of 1 ml/min. With the increase in the temperature the retention time is decreases and with the decrease in the temperature the retention time is increases. The best result is obtained with the temperature of 250 C. The results obtained with the variation in the various parameters are within the acceptance limit, so the method was found to be robust in the conditions specified (Table 15). The system suitability parameters were calculated to confirm the specificity of the developed method and shown in Table 1. The high percentage of recovery and low standard deviation data were satisfactory and confirms the accuracy, precision and reliability of the method. Further this method eliminates complicated extraction of individual drugs for quantitation. Both drugs estimated within 8 min, hence the present method is cost effective and faster, can be used for the routine analysis of these drugs from tablets.

CONCLUSIONS:

The method was successfully used to estimate the amount of Clotrimazole and Tinidazole in marketed tablet formulation containing 250 mg of Clotrimazole and 500 mg of Tinidazole. The results obtained were comparable with the corresponding labelled amounts, indicating non-interference of excipients in the estimation.

By observing validation parameters, method was found to be specific, accurate, precise, repeatable and reproducible. This method is simple in calculation, hence can be employed for routine analysis of tablet for assay as well as dissolution testing.

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