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Method development and validation of simultaneous estimation of miconazole and metronidazole in tablet dosage form RP-HPLC method

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ABSTRACT

A new simple, accurate, precise isocratic high performance liquid chromatographic (HPLC) method was developed and validated for the determination of miconazole and metronidazole in tablet formulation. The optimized conditions comprise of column C18,150mmx4.6, 5 µm particle size with a flow rate of 1.0 mL/min, Mobile Phase: 0.1%OPA: Methanol (75:25) mixture was used at a detection wavelength 268 nm. Retention times of miconazole and metronidazole were found to be 2.87min, and 3.75 min respectively. The method was validated in terms of linearity, accuracy, precision, specificity, LOD and LOQ. The LOD values of miconazole and metronidazole were found to be 0.01 and 0.014 µg/mL, LOQ's were found to be 0.03 and 0.04µg/mL respectively. Method is linear in the range of 50 to 150%. This new method was successfully developed and validated as per ICH guidelines, can be utilized for the quantitative estimation of miconazole and metronidazole pharmaceutical dosage forms.

Keywords: Miconazole, Metronidazole, RP-HPLC, Validation, Simultaneous estimation.

INTRODUCTION

Methods are developed for new products when no official methods are available. Alternate methods for existing products are developed to reduce the cost and time for better precision and ruggedness. Trail runs are conducted, method is optimized and validated.

Existing literature reveals that Miconazole and Metronidazole can be analyzed by UV detection, HPTLC, HPLC individually and combination with other drugs in bulk material and pharmaceutical forms. A comprehensive, validated and simple analytical simultaneous method development and validation of Miconazole and Metronidazole is, therefore, crucial. No economic, simple and precise HPLC method was there for simultaneous estimation of Miconazole and Metronidazole in bulk and pharmaceutical dosage forms. Therefore, in proposed project a successful attempt has been made to develop, simple, Accurate, and economic

methods for analysis of combination of Miconazole and Metronidazole tablets validated.

The objective of the present work is to develop and validate a HPLC method for combination dosage of Miconazole and Metronidazole tablets. To be employed in routine analysis. In the method development of Miconazole and Metronidazole we have decided to carry out our project work by incorporating the Reverse phase High performance Liquid chromatography (HPLC).Then the developed method will be validated according to ICH guidelines for its various parameters.

DRUG PROFILE

Miconazole

- Antifungal Agents
- 14-alpha Demethylase Inhibitors
- Molecular formula : C18H14Cl4N2O
- Molecular Weight : 416.129
- Solubility: Freely soluble in methanol, slightly soluble in 95%ethonol, very slightly soluble in water
- Pka: 6.77

Metronidazole

- Antiprotozoal Agents
- Anti-Infective Agents

Molecular formula : C6H9N3O3
Molecular Weight : 171.15
Solubility: Solubility in water at 20□C is 1g/100mL; in ethyl alcohol, 0.5g/100mL; in chloroform, 0.4g/100mL; slightly soluble in ether and soluble in dilute acids.
Pka:15.4.

MATERIALS AND METHODS

Materials

Chemicals/standards and reagents	Equipment's
KH_2PO_4	Electronic Balance
Methanol	Ultra-Sonicator
Water	Heating Mantle
Dipotassium hydrogen phosphate	Thermal oven
Miconazole	pH Meter
Metronidazole	Filter Paper 0.45 microns

METHODS

Preparation of mobile phase

Transfer 1000ml of HPLC water into 1000ml of beaker add 0.2%OPA. Transfer the above solution 750ml of0.1%OPA into 250ml of Methanol, used as mobile phase. After mixing they are sonicated for 20min.

Preparation of the miconazole and metronidazole standard and sample solution

Preparation of Standard Solution

Accurately weigh and transfer 1%w/w of Miconazole and 2%w/w Metronidazole into 100ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with water.
Transfers the above 5ml solution into 25ml volumetric flask dilute to volume with water.

Preparation of sample stock solution

Accurately weigh and transfer equivalent to the 15%w/w of Miconazole and Metronidazole of active ingredients were transfer into a 100ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with water.

Transfers above solution 6ml into 25ml of the volumetric flask dilute the volume with Methanol. And the solution was filtered through 0.45µm filter before injecting into HPLC system.

ASSAY PROCEDURE

Sample preparation

Accurately weighed about 15%w/w(2%Miconazoleand1%Metronidazole) into a 100 ml volumetric flask and 50 ml of mobile phase was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with mobile phase. From the above solution 6 mL is taken and further diluted in 25 ml volumetric flasks with mobile phase.

Standard preparation

Accurately weighed quantity of 1%w/w of Miconazole and 2%w/w Metronidazole was taken in a 100 ml volumetric flask and 50 ml of mobile phase was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with mobile phase. From the above solution 6 ml is taken and further diluted in 25 mL volumetric flasks with mobile phase.

Procedure

Separately injected both the standard (2 injections) and sample preparations (2 injections) into the chromatographic system and recorded the peak area responses.

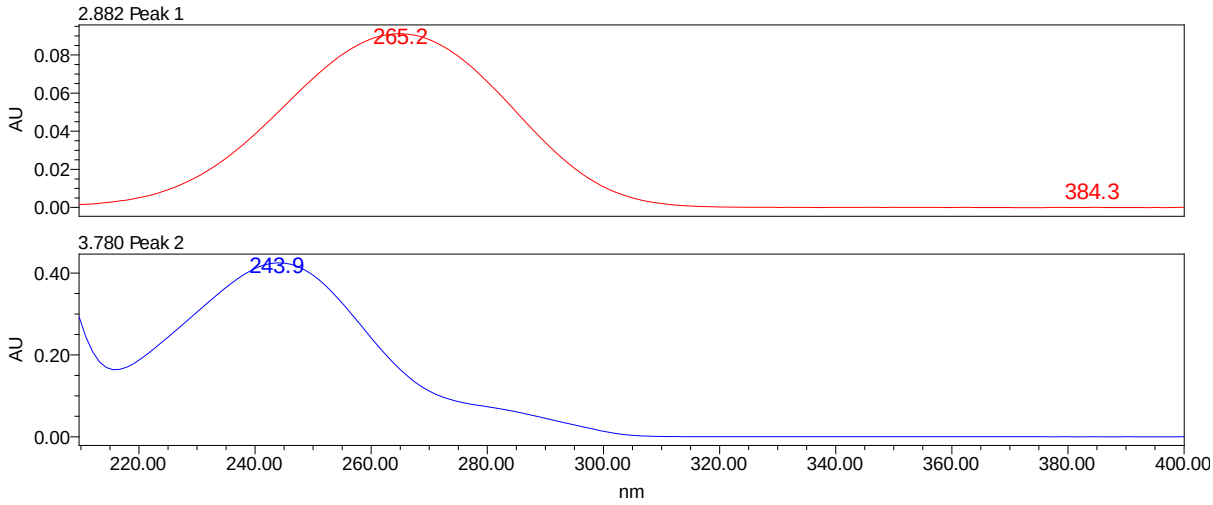
% percentage content =

$$\frac{\text{Sample area} \times \text{Sample dilution} \times \text{Average weight} \times \text{standard weight x purity of working standard} \times 100}{\text{Standard area} \times \text{standard dilution} \times \text{label claim} \times \text{sample weight}}$$

RESULTS AND DISCUSSION

Selection of wavelength

The UV spectrums of Miconazole and Metronidazole under these mobile phase conditions were shown below and from these spectrums, Lambda Max 268 nm were observed**.**



Optimized chromatographic method

Mobile Phase: 0.1%POA: Methanol (75:25)
Column: Phenomenex C18, 150X 4.6, 5µm
Flow Rate: 1.0ml/Min
Temperature: 30˚C
Volume: 10µl
Detector: 268nm

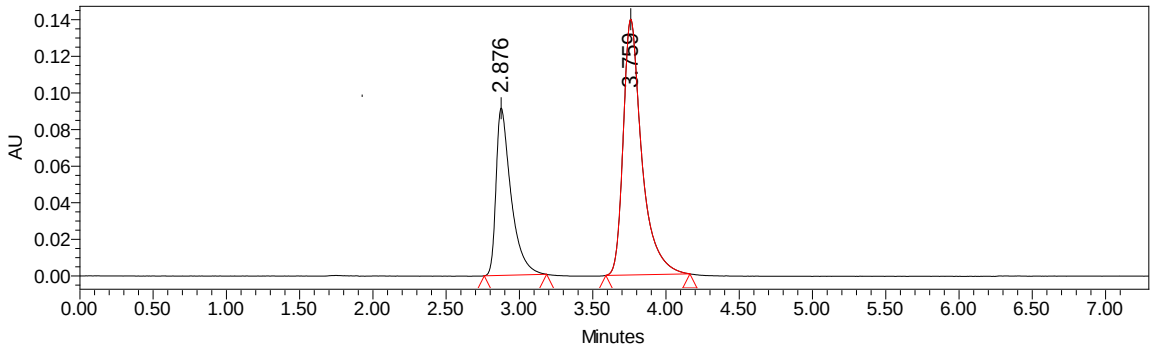


Figure: 3.1 chromatogram of miconazole and metronidazole

	Name	Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
1	Miconazole	2.876	647168		1.77	4048
2	Metronidazole	3.759	1231019	4.25	1.54	4779

Observation

RT was found to be good and the peak symmetry of both drugs were good. And the resolution theoretical plate count and tailing were within the limits and it is used for validation of the method.

Validation of hplc for method development system suitability

Table 3.1: System suitability data of Miconazole and Metronidazole

Parameter	Miconazole	Metronidazole	Acceptance criteria
Retention time	2.880	3.778	+-10
Theoretical plates	3980	4830	>2500
Tailing factor	1.53	1.57	<2.00
% RSD	1.3	0.4	<2.00

Specificity

Table 3.2: Specificity data for Miconazole and Metronidazole

S no	Sample name	Miconazole area	Rt	Metronidazole	Area	Rt
1	Standard	680522	2.880	1145269		3.778
2	Sample	687956	2.882	1144439		3.780
3	Blank	-	-	-		-
4	Placebo	-	-	-		-

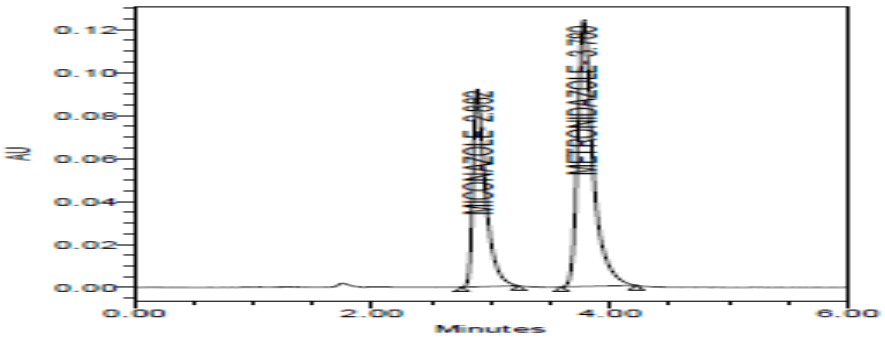


Figure.3.2 chromatogram representing specificity of sample

Precision

Table3.3: Precision data for Miconazole

S.no	RT	Area	%Assay
Injection1	2.882	687956	100
Injection2	2.882	686446	100
Injection3	2.879	683911	100
Injection4	2.885	685156	100
Injection5	2.882	689850	101
Injection6	2.883	688456	100
Mean			100
Std. Dev.			0.32
% RSD			0.32

Table 3.4: Precision data for Metronidazole

S.no	RT	Area	%Assay
Injection1	3.780	1144439	100
Injection 2	3.778	1149354	100
Injection 3	3.775	1146742	100
Injection 4	3.780	1150634	100
Injection 5	3.777	1149534	100
Injection 6	3.780	1151085	100
Mean			100
Std. Dev.			0.22
%RSD			0.22

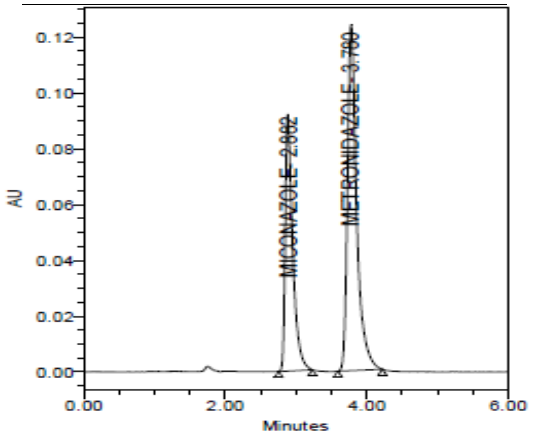


Fig 3.3: Chromatogram for precision injection 1

RESULT

Results of variability were summarized in the above table. % RSD of peak areas was calculated for various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise**.**

Accuracy

Table 3.4 Accuracy (%recovery) results of Miconazole

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	7.50	1.200	1.20	100	100
		2	7.50	1.200	1.20	100	
		3	7.50	1.200	1.21	101	
2	100%	1	15.00	2.400	2.39	100	100
		2	15.00	2.400	2.39	100	
		3	15.00	2.400	2.39	100	
3	150%	1	22.50	3.600	3.59	100	100
		2	22.50	3.600	3.57	99	
		3	22.50	3.600	3.60	100	

Table 3.5 Accuracy (%recovery) results ofMetronidazole

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	7.50	2.400	2.41	100	100
		2	7.50	2.400	2.39	100	
		3	7.50	2.400	2.40	100	
2	100%	1	15.00	4.800	4.81	100	100
		2	15.00	4.800	4.79	100	
		3	15.00	4.800	4.79	100	
3	150%	1	22.50	7.200	7.20	100	100
		2	22.50	7.200	7.21	100	
		3	22.50	7.200	7.18	100	

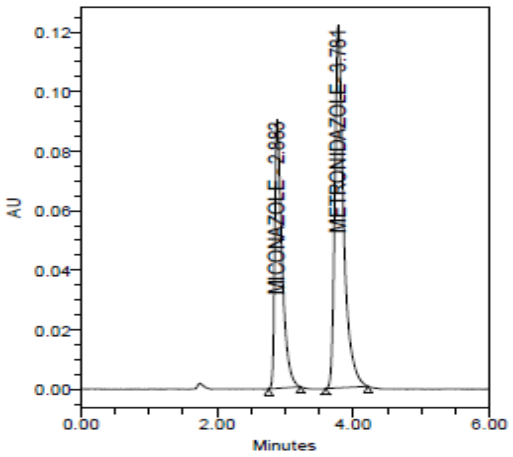


Fig 3.4: Typical chromatogram for Accuracy 100 %

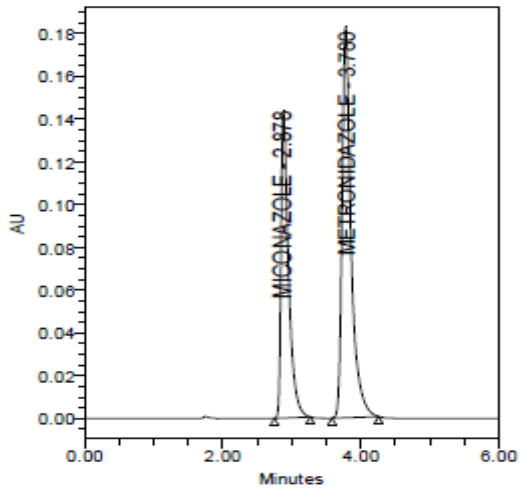


Fig 3.5: Typical chromatogram for Accuracy 150 %

Linearity

Table 3.6: Linearity data for Miconazole

S.no	Conc(µg/ml)	RT	Area
1.	50	2.893	344304
2.	75	2.887	515965
3.	100	2.888	689082
4.	125	2.882	851222
5.	150	2.880	1021771
Correlation coefficient (r^2)			0.999

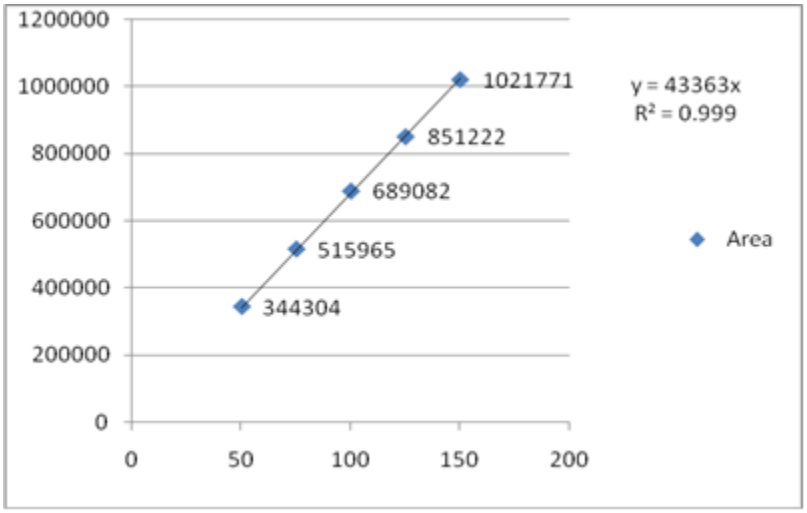


Fig 3.6: Linearity plot of Miconazole

Linearity data for Metronidazole

S.no	Conc(µg/ml)	RT	Area
1.	50	3.785	572341
2.	75	3.781	850562
3.	100	3.785	1141106
4.	125	3.783	1432105
5.	150	3.783	1728967
Correlation coefficient (r^2)			0.999

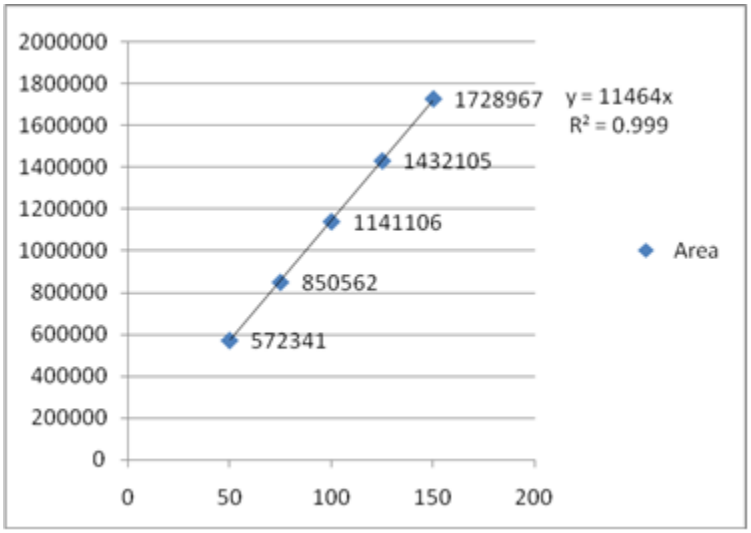
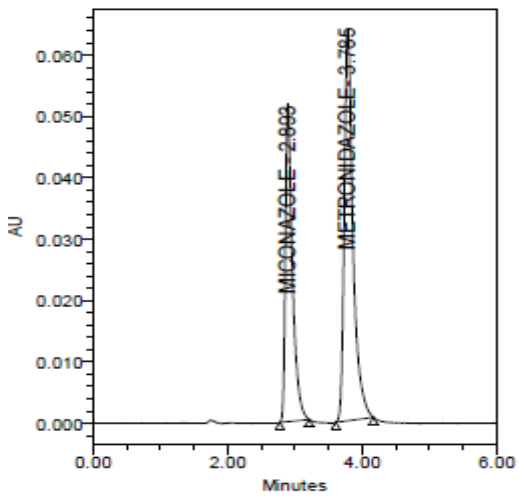


Fig 3.7: Linearity plot of Metronidazole



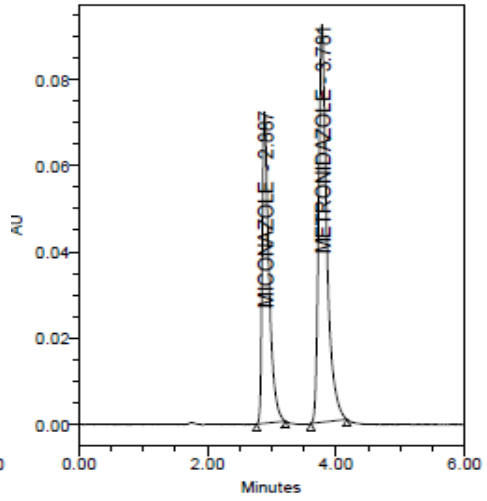


Fig 3.8: Chromatogram representing lineartity1 Fig 3.9:representing linearity 2

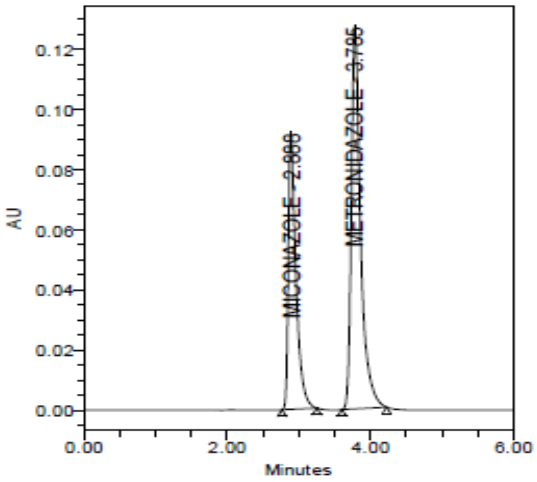
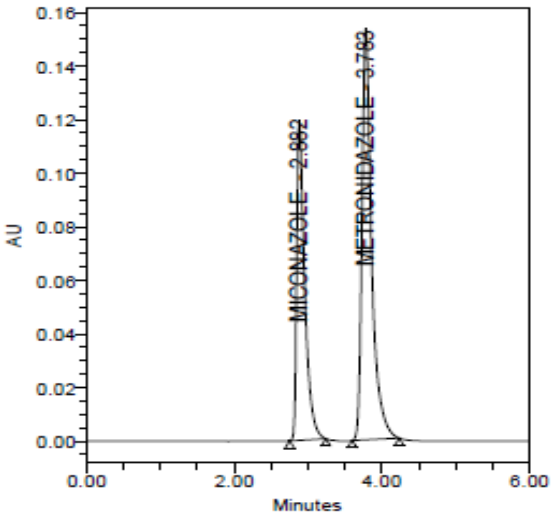


Fig 3.10: Chromatogram representing linearity 3



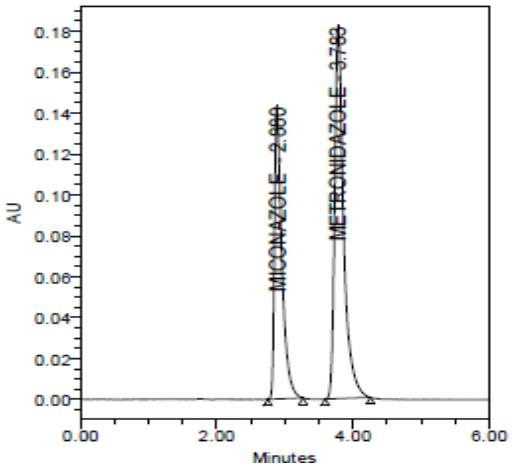


Fig 3.11: Chromatogram representing linearity 4 **Fig 3.12: Representing linearity 5**

RESULT

A linear relationship between peak areas versusconcentrations was observed for Miconazole and Metronidazole in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both Miconazole and Metronidazole which prove that the method is linear in the range of 50% to 150%.

Robustness

Table 3.7: Robustness data for Miconazole

parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate(0.8ml/min)	3.702	3288	1.49
Increased flow rate(1.2ml/min)	2.373	4081	1.37
Decreased temperature(20^0c)	3.704	3187	1.53
Increased temperature(30^0c)	2.376	4044	1.48

Table 3.8: Robustness data for Metronidazole

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate (0.8ml/min)	4.829	4247	1.38
Increased flow rate (1.2ml/min)	3.115	4717	1.54
Decreased temperature(20^0c)	4.838	4253	1.43
Increasedtemperature(30^0c)	3.120	4573	1.53

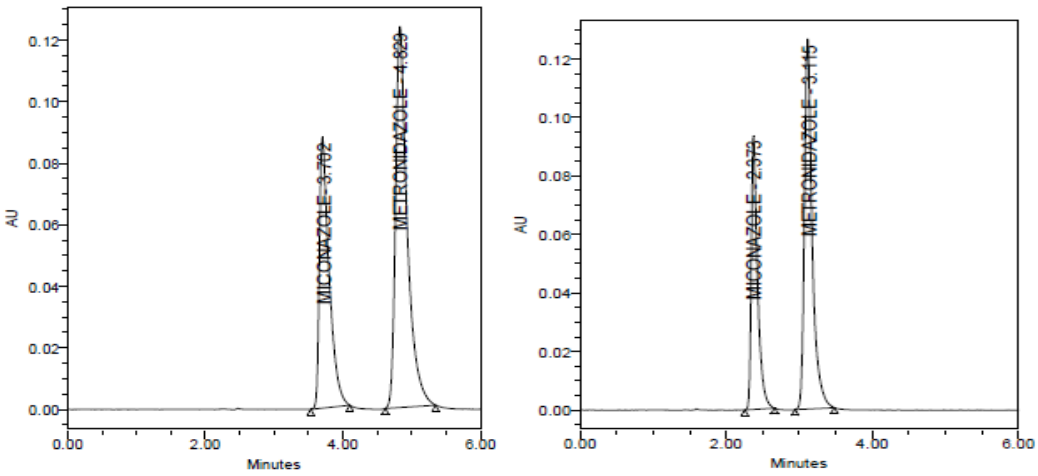


Fig 3.13: Chromatogram for decreased flow rate Fig 3.14: for increased flow rate

Result

The results of Robustness of the present method had shown that changes made in the Flow and Temperature did not produce significant changes in analytical results which were presented in the above table. As the changes are not significant we can say that the method is Robust.

Limit of detection

Minimum concentration of standard component in which the peak of the standard gets merged with noise called the LOD

LOD = 3.3* σ/S

LOD for Miconazole = 0.010, LOD for Metronidazole =0.014

LOD data for Miconazole and Metronidazole

S.no	Sample name	RT	Area
1	Miconazole	2.898	86458
2	Metronidazole	3.782	130012

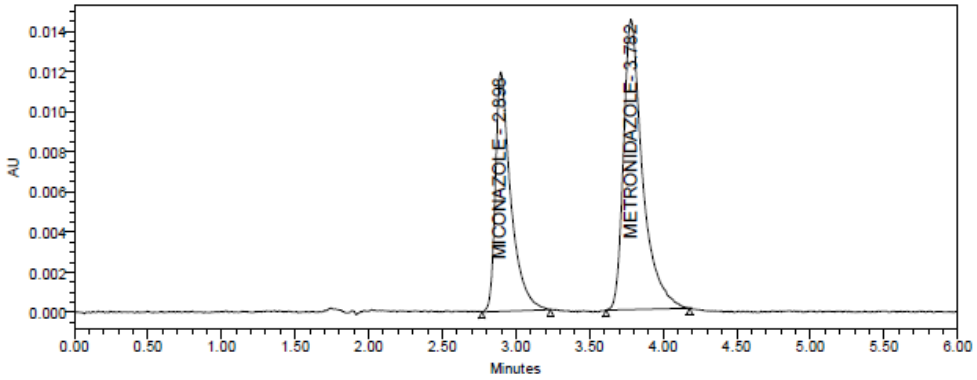


Fig 3.15: Chromatrogram for LOD

Limit of quantification

Minimum concentration of standard component in which the peak of the standard gets detected and quantification

LOQ = 10*σ/S

LOQ for Miconazole =0.034

LOQ for Metronidazole =0.0467

LOQ data for Miconazole and Metronidazole

S.no	Sample name	RT	Area
1	Miconazole	2.898	149834
2	Metronidazole	3.784	231558

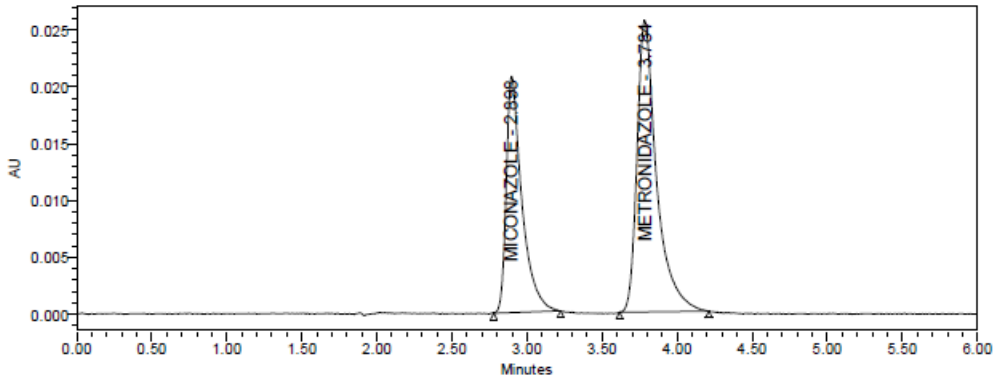


Fig 3.16: Chromatogram for LOQ

CONCLUSION

The study is focused to develop and validate HPLC methods for estimation of Miconazole and Metronidazole in tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analysing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Miconazole and Metronidazole.

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