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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF METRONIDAZOLE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new RP-HPLC method for the quantitative determination of Metronidazole was developed and validated as per ICH guidelines. The drugs were injected into YL-instrument 9300 Model with Hypersil C_{18} column (250×4.6, 5 µm) maintained at ambient temperature and effluent monitored at 314 nm. The mobile phase consisted of Methanol: Water (50:50 V/V). The flow rate was maintained at 1.0 ml/min. The calibration curve for Metronidazole was linear from 6-40µg/ml (r^2 for Metronidazole = 0.99). The proposed method was adequate, sensitive, reproducible, accurate and precise for the determination of Metronidazole in bulk and pharmaceutical dosage form.

KEYWORDS: Metronidazole, Linearity, Validation.

INTRODUCTION

Metronidazole, is an antiprotozoal medication. It is used either alone or with other antibiotics to treat pelvic inflammatory disease, endocarditis and bacterial vaginosis. It is effective for dracunculiasis, giardiasis, trichomoniasis and amebiasis. It began to be commercially used in 1960 in France. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. It is available in most areas of the world. It belongs to the class nitroimidazole. It inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. This function only occurs when metronidazole is partially reduced and because this reduction usually happens only in anaerobic cells, it has relatively little effect upon human cells or aerobic bacteria. It is chemically 2-(2-methyl-5-nitroimidazol-1-yl) ethanol with molecular formula $C_6H_9N_3O_3$ and molecular weight of

171.156 g/mol. It is soluble in water, ethanol, methanol, chloroform and DMSO.[1,6] Various analytical methods have been reported for the estimation of Metronidazole, including spectrophotometric methods and HPLC. HPLC is the most widely used technique for the estimation of Metronidazole in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. Different reagents are used for assay of Metronidazole which are quite expensive and need complex and sophisticated instrumentation. The present research work describes a HPLC and UV spectrophotometric method for estimation of Metronidazole in API.[7,11] The present method aims at developing a simple, accurate and precise RP-HPLC method for the estimation of Metronidazole in bulk and Pharmaceutical dosage forms.

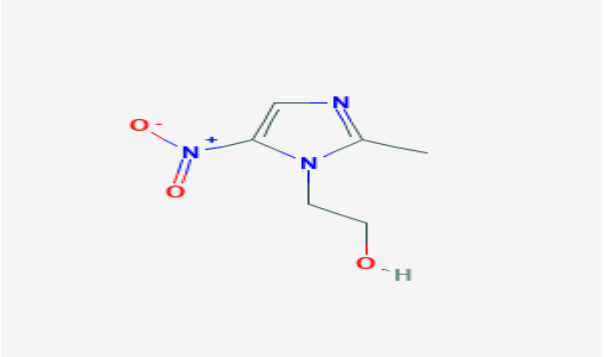

Fig. 1: Chemical structure of Metronidazole.

MATERIALS AND METHODS

Chemicals and solvents: The API of Metronidazole was obtained from Yarrow chemicals, India. HPLC grade water (prepared by using 0.45 Millipore Milli –Q) was procured from Standard Reagents, Hyderabad. HPLC grade Methanol was bought from Merck, Mumbai.

Instrumentation: A YL- instrument 9300 module equipped with a UV spectrophotometer for finding out the λmax values of the drugs was used throughout this study. An Hypersil C_{18} (250×4.6,5mm) column was employed for the method development. The chromatographic system was monitored by Autochrome software. Analytes were monitored by UV detection at 314 nm using an isocratic mode with Methanol: water in the ratio 50:50 as mobile phase. The flow rate was set at 1.0 ml/min and effluent was monitored at 314 nm. The temperature and run time were maintained at 25°C and 5 min. respectively. Solubility of the compounds was enhanced by sonication on an ultrasonicator.

Selection of mobile phase

The objective of this experiment was to optimize the method for estimation of Metronidazole based on the literature survey. Various mobile phases were tested to select the best possible system. The various mobile phases used included water: Acetonitrile (50:50), water: methanol (70:30), Acetonitrile: water (40:60). Better peak resolution and adequate retention time were obtained with the ratio of Methanol: water (50:50).

Preparation of Mobile Phase

The mobile phase was prepared by mixing 500 ml of methanol and 500 ml of water in a 1000 ml clean and dry flask. The mobile phase was then degassed using Ultra-Sonicator to remove dissolved gases and the resultant mobile phase was filtered through a 0.45 µm membrane filter under vacuum.

Preparation of Standard solution

Standard solution was prepared by accurately weighing 100 mg of Metronidazole and transferring them into a 100 ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10 mins and then made upto volume with the mobile phase. The resultant solution was filtered through a 0.45 µm membrane filter under vacuum. From this 1 ml of solution was taken & made upto 10 ml with mobile phase. The solution was sonicated for about 10 mins. From this solution 2ml was taken and made upto 10ml with mobile phase which gives a concentration of 20mcg/ml. This solution was passed through 0.45 µm membrane filter before injecting into HPLC system.

Preparation of Sample solution

Sample solution was prepared by taking 20 tablets and triturating into fine powder and accurately weighing the amount of drug equivalent to 100 mg of Metronidazole and transferring them into a 100 ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10 mins and then made upto volume with the mobile phase. The resultant solution was filtered through a 0.45 µm membrane filter under vacuum. From this 1 ml of solution was taken & made upto 10 ml with mobile phase. The solution was sonicated for about 10 mins. From this solution 2ml was taken and made upto 10ml with mobile phase which gives a concentration of 20mcg/ml. This solution was passed through 0.45 µm membrane filter before injecting into HPLC system.

Prior to validation studies blank solution was injected and chromatogram was noted. System suitability studies were performed using the standard solution of Metronidazole. Optimized conditions maintained where the drug was eluted with good retention time and peak area which was shown in the fig 3.

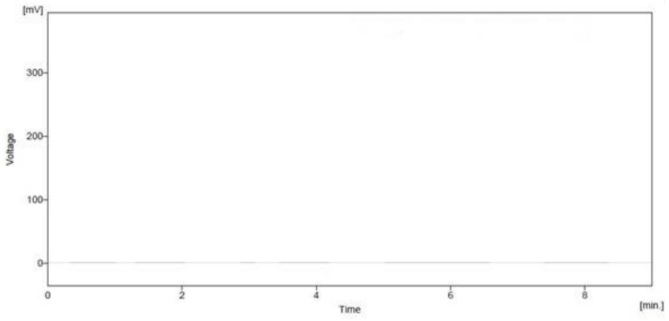

Fig. 2: Blank chromatogram.

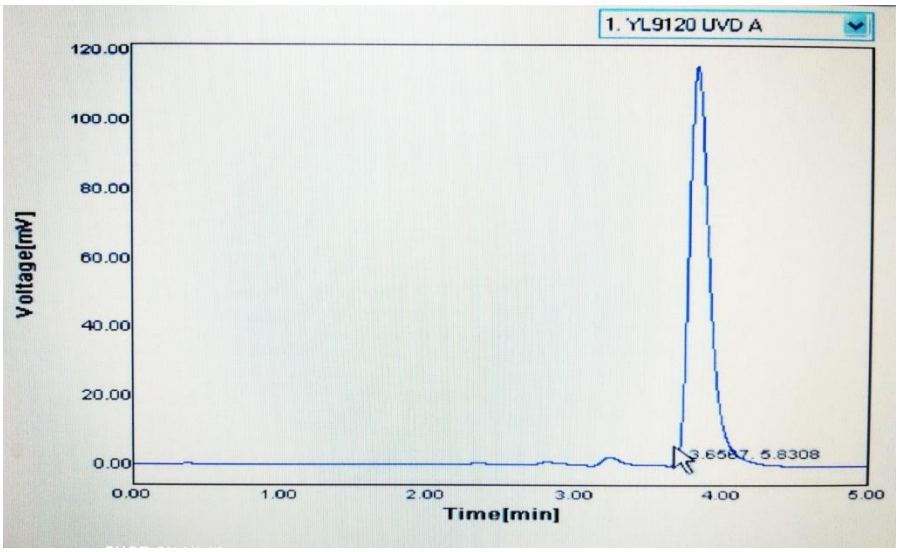

Fig. 3: Optimized Chromatogram.

System suitability

The standard solution of 20mcg/ml was used as system suitability solution which was injected for about 6 times. The peak area, tailing factor and number of theoretical plates were noted and results are tabulated below.

Table 1: System suitability data of Metronidazole.

Sample ID	Concentration(µg/ml)	Retention Time	Theortical plates	Tailing factor
100%	20	3.65	5709	1.37
100%	20	3.64	5836	1.38
100%	20	3.65	5928	1.35
100%	20	3.65	5953	1.34
100%	20	3.65	5938	1.34
100%	20	3.66	5047	1.35
Mean	20	3.6567		
Standard Deviation		0.0028		
%CV		0.076		

Linearity: The linearity of the method was established by determining the absorbance of different concentrations of Metronidazole over a range of 6-40µg/ml respectively.

Table 2: Linearity data of Metronidazole.

S.No	Concentration of Metronidazole (mcg/ml)	Peak area
1	0	0
2	6	550
3	10	710
4	16	1064
5	20	1371
6	24	1593
7	30	2030
8	40	2644

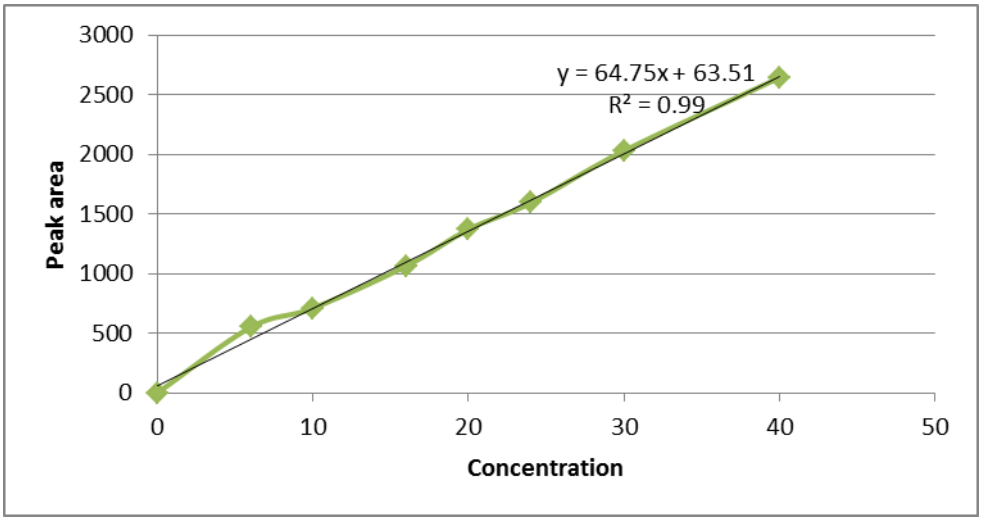

Fig. 4: Calibration curve of Metronidazole.

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by analyzing the samples were carried out by analyzing the measured concentration and

the added concentration of the drug. Each sample was injected thrice. The percent recoveries of the drugs were estimated.

Table 3: Accuracy data of Metronidazole.

S. No	Accuracy level	% Recovery	Avg.% Recovery
1	80%	100.85	100.58
2	80%	100.48	
3	80%	100.42	
4	100%	97.65	97.61
5	100%	97.29	
6	100%	97.90	
7	120%	99.12	99.17
8	120%	99.24	
9	120%	99.16	

Precision: Precision is one of the important factors which determine the reliability of an analytical method. The precision of the developed method was tested and was found to be suitable. Both system and method precision were performed and are given in table 4,5.

Table 4: Method precision data of Metronidazole.

S.No	Injection number	Retention time of Metronidazole	Area of Metronidazole
1	Injection 1	3.62	1395
2	Injection 2	3.66	1387
3	Injection 3	3.65	1371
4	Injection 4	3.65	1369
5	Injection 5	3.66	1350
	Average		1374.4
	Standard deviation		17.458
	% RSD		1.270

Table 5: System precision data of Metronidazole.

S .No	Injection number	Retention Time of Metronidazole	Area of Metronidazole
1	Injection 1	3.66	1395
2	Injection 2	3.65	1367
3	Injection 3	3.65	1380
4	Injection 4	3.33	1369
5	Injection 5	3.68	1375
	Average		1377.2
	Standard deviation		11.189
	% RSD		0.812

Robustness: The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like volume of injection, wavelength which may differ but the responses were still within the limits of the assay.

Table 5: Robustness data of Metronidazole.

Proposed variations		Retention time	Theoritical plates	Assymetric factor
Variation in flow rate	0.9 ml	3.66	5938	1.37
	1.1ml	3.65	5836	1.38
Variation in mobile phase	40:60	3.52	5928	1.35
	55:45	3.66	5953	1.36

Ruggedness: Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It was checked that the results were reproducible under different analysts.

Table 6: Ruggedness data of Metronidazole.

	Retention time of Metronidazole	Area of Metronidazole
Analyst(1)100%	3.65	1351
Analyst(2)100%	3.66	1368

Assay: Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out.

Table 7: Assay data of Metronidazole marketed formulation.

Drug	Labelled claim(mg)	Drug found	% Purity
Sample 1	100mg of Metronidazole	99.7mg of Metronidazole	99.7%
Sample 2	100mg of Metronidazole	98.6mg of Metronidazole	98.6%

DISCUSSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and economical RP-HPLC method. It was successfully applied for the determination of Metronidazole in pharmaceutical dosage forms without the interferences of other constituents in the formulations. Different mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was done based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Methanol:Water (50:50) with 1.0 ml/min flow rate was quite robust.

The optimum wavelength for detection was 314 nm at which better detector response for drug was obtained. The average retention time for Metronidazole was found to be 3.65 mins. The % RSD values in the system suitability were found to be less than 2%. The calibration was linear in concentration range of 6-40 mcg/ml for Metronidazole**.** The low values of % RSD indicate the method is precise and accurate.

Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. These results show the accuracy and reproducibility of the assay. Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions; the % RSD. reported was found to be less than 2%. The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. There was no significant difference in the results achieved by the proposed method.

CONCLUSION

The proposed method for the assay of the Metronidazole in the commercially available tablet formulation was found to be precise, simple, accurate, economical, and rapid. It can be easily adopted for routine quality control for monitoring the assay in the bulk drug samples, in-process samples, and for the finished tablet formulation.

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