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SPECTROPHOTOMETRIC DETERMINATION OF METRONIDAZOLE BY PDAB REAGENT

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ABSTRACT
Metronidazole belongs to the class of medicines known as Antibiotics. It works by killing bacteria or preventing their growth.Therefore it is made up to develop a new simple, accurate, precise, robust method for the estimation of Metronidazole by using PDAB reagent and methanol as solvent. Absorption Spectrum was obtained by scanning from 400-800nm and the maximum absorbance was found at 470nm. Then the method was optimised and validated with various validation parameters as per ICH guidelines. From the linearity studies correlation coefficient was found to be 0.998. From precision and intermediate precision studies %RSD was found to be 0.138 and 0.097 respectively. Assay was conducted by using marketed formulation of Metronidazole and the % purity was found to be 98.5%. Hence the method was suitable for the estimation of Metronidazoleby UV Spectroscopy.

KEYWORDS: Metronidazole, Para-Dimethylaminobenzaldehyde reagent, UV Spectrophotometric determination, Method validation.

INTRODUCTION

Metronidazole is a synthetic antibacterial and antiprotozoal agent that belongs to the nitroimidazole class. It is effective therapy against protozoa such as Trichomonas, Vaginalis, Amoebiasis, and Giardiasis.[1] The initial clinical tests of Metronidazole indicated that it was capable of curing invasive amoebic dysentery and amoebic liver abscess.[2] Metronidazole exerts its action by diffusing into the organism, inhibits protein synthesis by interacting with DNA, and causes a loss of helical DNA structure and strand breakage.[3,4] It may be administered orally, intravenously or topically. It comes in capsules, tablet, topical and intravenous form.[5]

Metronidazole is officially determined by Titrimetry, Potentiometry and HPLC methods described in Indian Pharmacopoeia and British Pharmacopoeia.[6,7] Most of the spectrophotometric methods found in the literature for the determination of Metronidazole in the visible region involve initial reduction by treatment with zinc powder and HCl[8-15] followed by the diazotization and coupling of the resulting amine. In the present work, spectrophotometric method for the estimation of Metronidazole have been developed after converting it to reduced form by using zinc dust and HCl, as well as reaction with PDAB reagent was studied. The structure ofMetronidazole was shown in Fig. no. 1.

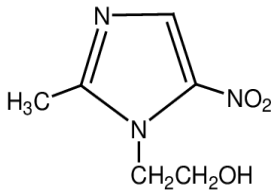

Fig. no. 1: Structure of Metronidazole.

MATERIALS AND METHODS

Instrumentation

PG Instrument (T60 UV- Visible spectrophotometer), UV win software using glass cuvettes of 10 mm path length, weighing balance (Pioneer OHAIUS (Item PA214C).

Materials and Reagents

All chemicals and reagents used were of analytical grade. Para dimethyl amino benzaldehyde, Methanol, Hydrochloric acid were of analytical grade. The Pharmaceutical grade pure drug was kindly provided by CMR College of Pharmacy. Marketed drug obtained from local pharmacy.

Reduction of the nitro group and Preparation of

Standard drug Solution

A Stock Solution of Metronidazole is prepared by taking 100mg of pure Metronidazole drug in a 10ml volumetric flask, then add 500mg of Zinc powder and add 2ml of 5N HCl, boil for 30 mins. Then the solution was filtered using whatman filter paper to remove the insoluble matter. The residue was washed with 10ml portions of methanol three times, and the filtrate was made upto 10ml with Methanol. 5N HCl was prepared by taking 104.2 ml of concentrated acid and is diluted with water to make the volume 250ml.

Determination of λ max of Metronidazole solution

Pipette out 0.1ml from stock solution and add 2ml of PDAB reagent in a 10ml volumetric flask. Make up to 10ml with methanol. Then scan the solution from 400-800nm against reagent blank and maximum absorption was found to be at 470nm.

Preparation of sample solution of Metronidazole

About 10 tablets were weighed and crushed into fine powder. Powder weighed equivalent to 10mg of Metronidazole was transferred into 10ml volumetric flask and made up to 10ml with Methanol and thoroughly agitated for 5 minutes. The content was kept aside for 5 mins, and it is filtered. The filtrate solution was properly diluted with solvent to obtain a required concentration of drug used for the analysis.

Validation Parameters

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, accuracy, precision and robustness of solution.

Linearity

Different aliquots of the working standard of Metronidazole solution ranging from 5-25µg/ml was transferred into a series of volumetric flask. To each flask 2ml of Para-Dimethyl amino benzaldehyde reagent solution was added by means of micro burette and the total volume was made up to 10ml with methanol. The absorbance of each solution was measured at 470nm against blank. The linearity data is shown in table no. 1.

Precision

The reproducibility of the proposed method was determined by evaluating the mid concentration of the standard solution (15µg/ml) at different time intervals. So measure the six replicate absorbance measurements intra and inter-day precision, % RSD was calculated, the data is shown in table no. 3.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out at three different level (50%, 100%,150%). The accuracy was carried out by spiking the sample solution at 3 replicates and data was shown in table no. 6.

Table no. 4: Inter-day Precision of Metronidazole.

Robustness

The robustness of analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of data was shown in table no 7.

RESULT AND DISCUSSION

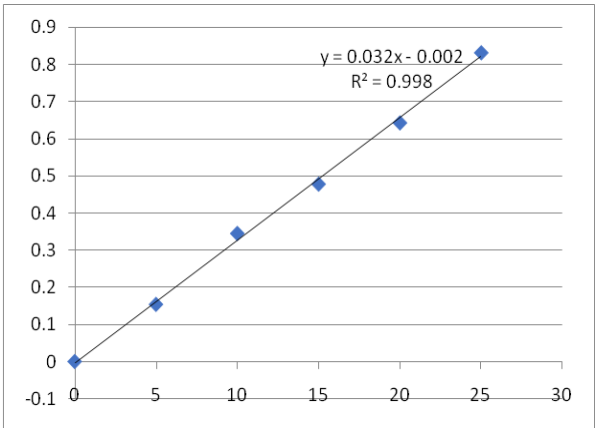

Fig no. 2: Calibration curve of Metronidazole.

Table no. 1: Linearity of Metronidazole.

S. No.	Concentration(µg/ml)	Absorbance
01	5	0.154
02	10	0.345
03	15	0.478
04	20	0.643
05	25	0.832

Table no. 2: System suitability Parameters of Metronidazole.

S. No.	Optical characters	Results
1	λ max	470nm
2	Beers law limit(µg/ml)	5-25(µg/ml)
3	Slope	0.0329
4	Correlation Coefficient	0.9981

Table no. 3: Intraday Precision of Metronidazole.

Concentration (µg/ml)	Absorbance
15	0.476
15	0.475
15	0.477
15	0.476
15	0.478
15	0.474
Mean	0.476
Standard deviation	0.001414
%RSD	0.297104

Concentration (µg/ml)	Absorbance (Day 1)	Absorbance (Day 2)
15	0.480	0.482
15	0.482	0.485
15	0.478	0.481
15	0.476	0.479
15	0.484	0.486
15	0.483	0.484
Mean	0.4805	0.4828
Standard deviation	0.00308	0.00263
%RSD	0.6414	0.5466

Table no. 5: LOD and LOQ of Metronidazole.

Parameters	Metronidazole(µg/ml)
LOD	0.14
LOQ	0.42

Table 6: Accuracy of Metronidazole.

S.no.	Sample level (%)	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	Mean	% Recovery
1	50	15	3	17.46	17.82	99%
	50	15	3	17.83		
	50	15	3	18.19		
2	100	15	6	20.41	20.97	100%
	100	15	6	21.67		
	100	15	6	20.78		
3	150	15	9	23.64	23.97	101%
	150	15	9	24.15		
	150	15	9	24.12		

Table 7: Robustness of Metronidazole.

S.NO.	Wavelength	Absorbance
1	468	0.432
2	470	0.476
3	472	0.497

Table 8: Assay of Metronidazole.

Label claim	Amount found	%Assay
Metronidazole (250mg)	8.5mg	99.78

The result of the method development and validation shows that the Metronidazole assay using PDAB reagent is accurate, precise, and robust. The standard curve is linear over the range of concentrations tested,and the accuracy is within acceptable limits that is determined by %Recovery. The precision of the method is also within acceptable limits as determined by the %RSD that is less than 2. The LOD and LOQ values indicate that the method was sensitive.

CONCLUSION

Based on the experimental results, it can be concluded that the newly proposed spectrophotometric method for the determination of Metronidazole is rapid, accurate, economical. Because of its simplicity, sensitivity the method viable alternative to the HPLC methods. As a results, the proposed method can be used for quality control purposes in the pharmaceutical industry.

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