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Development and Validation of First Order Derivative Method for Metronidazole in Bulk and Tablet Using UV Visible Spectroscopy

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Abstract: A simple spectrophotometric method has been developed for estimation of Metronidazole from bulk and tablet dosage form.The calibration curve was observed in the concentration range of 10-16 µg/ml.Inthe First order derivative spectroscopy, the absorbance was measured at λ_{min}=340.00 nm, λ_{max}=300.0 nm & Zero cross=319.0nm. Other studies of assay, accuracy and precision were determined and validated statically. The reproducibility and recovery of all the methods were with % RSD less than 2. The developed methods were found to be precise, accurate,rapid, and specificwhich can be successfully applied for the routine analysis.
Keywords : Assay, Accuracy, Precision, % Recovery, Metronidazole, First order derivative spectroscopy.

Introduction:

Metronidazole (Fig. 1) is chemically a 1-(2-hydroxy-1-ethyl)-2-methyl-5-nitroimidazole.[1] It is a prodrug that acts as antibiotic, antiprotozoal, amoebicidal, bactericidal and trichomonicidal. Metronidazole is converted in anaerobic organisms by theredox enzyme pyruvate-ferredoxin oxidoreductase. Thenitro group of metronidazole is chemically reduced byferredoxin (or a ferredoxin-linked metabolic process) andthe products are responsible for disrupting the DNAhelical structure, thus inhibiting nucleic acid synthesis.[2]

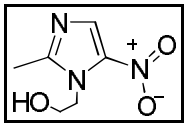

Figure No.1- Chemical Structure of Metronidazole

Literature survey reveals that a few spectrophotometric[3, 4, 5], RP-HPLC[6, 7, 8]methods are reported for the estimation of Metronidazole individually and in combination with other drugs. This study describes new highly sensitive, simple, accurate, precise, rapid, reproducible, economical visible spectrophotometric methods for the determination of Metronidazole in pure and in tablets.

Materials and Methods:

The metronidazole was kindly supplied as a gift sample by Cipla(India) Limited. All agents and chemicals used were of Analytical grade. Metronidazole tablets were purchased from market.

Adouble-beamUV-Visiblespectrophotometer,model UV-1800 (Shimadzu,Japan) having two matched cells with1cm lightpath. A Citizen analytical balance (Sartorius) was used forweighingthe samples.

Preparation of standard stock solutions:

Standard solution of Metronidazole was prepared by transferring accurately weighed 10 mg of drug into a 100ml volumetric flask and the volume was made up to 100ml using water as a solvent to get the concentration of 100µg/ml.

Preparation of calibration curve:

From the standard stock solution fresh aliquots were pipette out and suitably diluted with water to get final concentration in the range of 10-16(µg/ml). The solutions were scanned under 200-400 nm wavelength range and a sharp peak was obtained at 319nm (figure 2). Calibration curve was plotted by taking absorbance on y-axis and concentration of solution on x-axis (figure 3). The method was applied for known sample solution and was found to be satisfactory for analysis of tablet dosage forms.

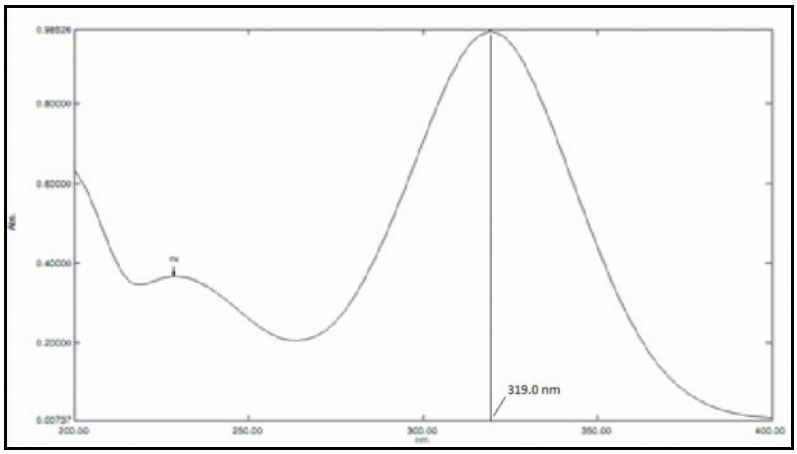

Figure No. 2- Determination of $(_1max$ of Metronidazole std. stock solution

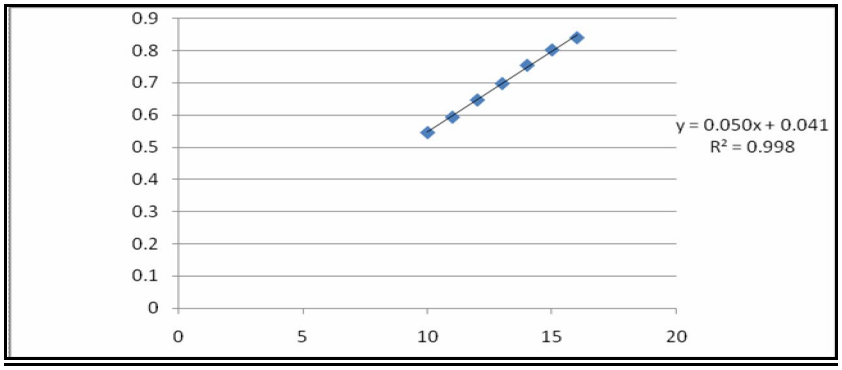

Figure No. 3: Calibration curve of Metronidazole

Development of first order derivative spectra

Working standard solutions of pure single drug was scanned within 400-200 nm after baseline correction and the spectral data was then processed to obtain first order derivative spectrum at wavelength interval of 2 nm. It was observed that metronidazole shows zero crossing at 319.00nm metronidazole showed absorbance maxima at 300.00 nm and minima at 340.00nm. Hence 319.00 nm were selected as analytical wavelengths in this method for determination of Metronidazole. The calibration curves were prepared in the concentration range of 10-16µg/ml for metronidazole at the wavelength 319.00 nm

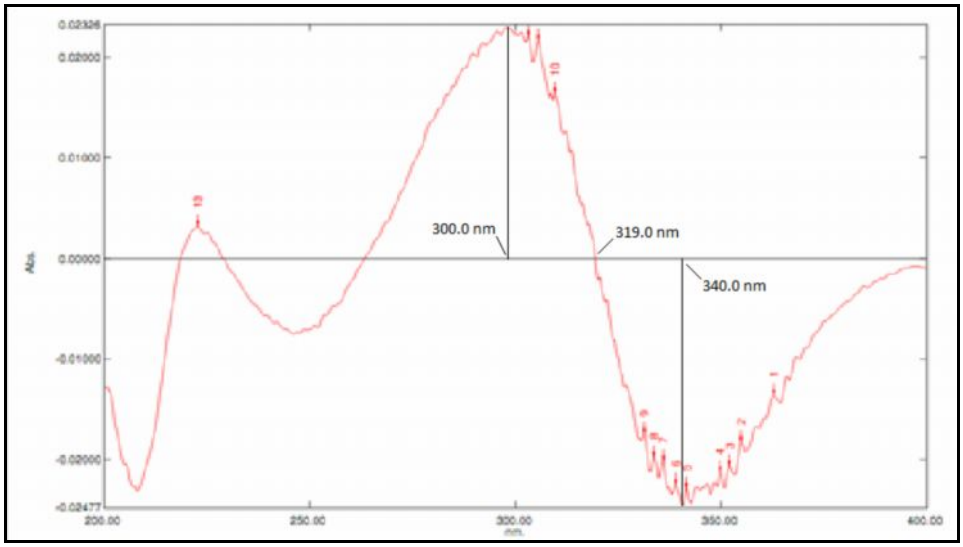

Figure No. 4- First Derivative Spectra of Metronidazole std. stock solution

Assay of Metronidazole Tablets

20 tablets of Metronidazole were weighed, powdered and weight equivalent to 10mg was taken and transferred into a 100ml volumetric flask. Then 20 ml of water was added and kept for 15 min with frequent shaking and the volume was made upto mark with water. The solution was then filtered through whatmann filter paper and the absorbance was measured against blank. The amount of Metronidazole was computed by using the equation referring to the calibration curve (Table 1).

Table No. 1: Results of Analysis of Tablet Formulation

Brand name	Label claim (mg)	Amount of Drug estimated	Percentage label claim	Standard Deviation	Percentage Recovery
Flagyl	400	397	99.25%	1.85	99.37%
Metrogel	200	198	99.00%	1.87	99.25%
Unimezole	200	199	98.50%	0.59	99.14%

Method Validation

The method was validated for different parameters like linearity, accuracy and precision.

Linearity

Fresh aliquots were prepared from the stock solution (100µg/ml) in different concentrations. The samples were scanned in UV –visible spectrophotometer against reagent blank. It was found that the selected drug shows linearity between the 10-16µg/ml (Table 2& 3).

Table No. 2: Linearity results of Metronidazole in water

Concentration (µg/ml)	Absorbance
10	0.5455
11	0.5936
12	0.6471
13	0.6984
14	0.7551
15	0.8031
16	0.8411

Table No. 3: Optical Parameters

Parameter	Result
Absorption maxima	319 nm
Linearity range	10-16(µg/ml)
Standard regression equation	0.0505x+0.0413
Correlation coefficient	0.998
Molar absorptivity	53.92
Standard deviation	0.0098

Accuracy

Accuracy of the method was confirmed by studying recovery at 3 different concentrations 75, 100, and 125% of these expected, in accordance with ICH guidelines. Standard drug solution was added to a pre analyzed sample solution and percentage drug content was measured. The results from study of accuracy were reported in (Table 4).

Table No. 4: Results of Recovery Studies

Level of Recovery	Drug	Amt. of drug added (in µg)	Amt. of drug std. added (in µg)	% Recovery	SD
75%	Metronidazole	9	8.611	95.86	0.00028
100%		12	11.99	99.99	0.00042
125%		15	14.89	99.26	0.00028

Precision

Precision of the method was studied as intra-day and inter day variations. Intraday precision was determined by analyzing 12µg/ml of Metronidazole for three times within the day. Inter-day precision was determined by analyzing same concentration of solutions daily for three days; the results are reported in (Table 5).

Table No. 5: Results of Intermediate Precision

Day	% Label claim estimated* (Mean ± % RSD)
Intra day	11.99± 0.0020
Inter day	11.95± 0.0026

Results and Discussion

Metronidazole in water exhibited λmax at 319nm. The Beer's law was obeyed to the method in the concentration range of 10-16 µg/ml respectively. The optical characteristics such as Beer's law limits (mg/ml),Molar extinction coefficient (L/mol.cm), Regression equation(y), Correlation coefficient calculated from five-six measurements containing 3/4th of the amount of upper Beer's law limits and were calculated for Metronidazole and reported in Table.

Conclusion

The proposed method was found to be simple, sensitive, accurate, precise, and reproducible that can be used for routine quality control analysis of Metronidazole in bulk and in tablet dosage forms.

Table No. 4: Validation parameters for UV-Spectroscopic methods

Parameters	Observations
Linearity range (µg/mL)	10-16µg/mL
Correlation coefficient (R^2)	0.998
Slope	0.0505
Intercept	0.0413

Acknowledgements

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