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[Research article]

Method development and validation for the estimation of metronidazole in tablet dosage form by UV spectroscopy and derivative spectroscopy**K.Narendra reddy and MK.Ranganath**

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ABSTRACT

Two simple, precise, rapid, sensitive and accurate spectrophotometric methods have been developed for the estimation of metronidazole in pure form and in tablet dosage form. Metronidazole has absorbance maxima at 313 nm in methanol: water (80:20) for UV spectroscopy and absorbance minima at 298nm for first order derivative spectroscopy. The linearity was obtained and obeyed Beer's law in the concentration range of 4 - 12 µg/ml and correlation coefficients were 0.9995 and 0.9897 for UV spectroscopy and derivative spectroscopy respectively. The precision values for UV and derivative spectroscopy were found to be 0.35-0.64 and 1.78-1.92 with mean accuracies 98.60-99.70% and 97.55-103.80% respectively. Limit of detection values for UV and derivative spectroscopy were found to be 0.23 and 1.01 respectively. Limit of quantification for both UV and derivative spectroscopy were found 0.69 and 3.06 respectively. The methods were validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated methods were successfully used for the quantitative analysis of API and tablet dosage form.

Keywords: Metronidazole, Beer's law, derivative spectroscopy, ICH.**INTRODUCTION**

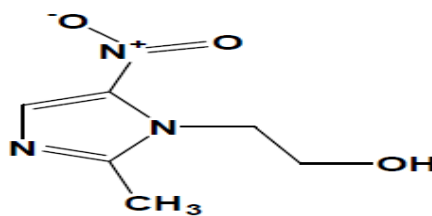
Metronidazole is chemically 2-(2-methyl-5-nitro-1H imidazol-1-yl) ethanol.¹ It is a nitro imidazole anti-infective medication used mainly in the treatment of infections caused by susceptible organisms, particularly anaerobic bacteria and protozoa, it is a drug used for the treatment of trichomonal vaginitis, amoebiasis, giardiasis, and certain anaerobic bacterial infections in humans². Metronidazole is a nitroimidazole derivative which is bactericidal, amoebicidal and trichomonocidal. It was reduced by low-redox-potential electron transfer proteins (e.g. nitroreductases such as ferredoxin) to unidentified polar product(s) which

lack the nitro group. The reduction product(s) appears to be responsible for the cytotoxic and antimicrobial effects of the drug which include disruption of DNA and inhibition of nucleic acid synthesis.³⁻⁷

United States Pharmacopoeia⁸ describes HPLC and non-aqueous titration methods for the assay of metronidazole. Visible spectrophotometry, because of simplicity and cost effectiveness, sensitivity and selectivity, and fair accuracy and precision, has remained competitive in an era of chromatographic techniques for pharmaceutical analysis. Several methods have been reported for the determination of metronidazole, including spectrophotometry,⁹

polarography.¹⁰ 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 followed by the diazotization and coupling of the resulting amine. All these methods are less sensitive, involve tedious procedures such as heating and extraction, utilize costly reagents and involve an additional diazotization step. In the present study, two

spectrophotometric methods for the quantitative estimation of metronidazole have been developed to establish optical characteristics, precision and accuracy of the proposed methods.¹¹ The methods are simple, rapid, sensitive and are successfully applied to determine the metronidazole in their pharmaceutical formulations. Furthermore, they do not need costly instrumentation required for published HPLC methods.



Structure of metronidazole

MATERIALS & METHODS

Instrument

Techcomp UV visible double beam spectrophotometer model 2301 with 1cm matched quartz cells were used for all the spectral measurements.

Chemicals and reagents

Distilled water, methanol, metronidazole raw material and tablets (METROGYL 400). Solvent mixture of methanol and water (80:20) was used as a solvent for development of spectral characteristics. All the chemicals used were of analytical grade.

Preparation of standard solution

An accurately weighed quantity of metronidazole (50 mg) was transferred to a 50ml volumetric flask and dissolved and diluted to the mark with solvent to obtain working standard solution having concentration of 1000µg/ml. From this solution 1ml of solution was pipetted out and transferred to a 50ml volumetric flask and diluted to the mark with the solvent. The concentration of the solution was 20µg/ml.

Absorption maximum

The standard solution was scanned in the spectrum mode over the range of 200-400 nm. Metronidazole showed an absorbance maxima peak at 313nm.

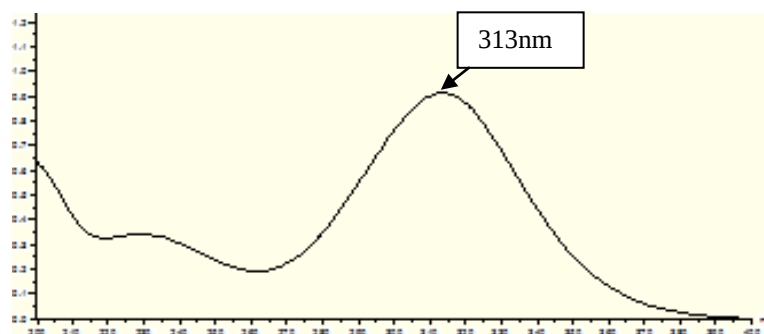


Fig. 1: Spectrum showing absorption maxima at 313nm for metronidazole

Beer's law concentration range

Aliquots of standard solution 2-3ml were transferred into a series of 10ml volumetric flasks and makeup to volume with solvent up to the mark.

The absorbance of these solutions were measured against blank at the wavelengths of 313 nm and 298 nm for UV spectroscopy and derivative spectroscopy respectively. The absorbance values

against the concentrations were plotted in the calibration curve (Fig.2,3). From the calibration curve it was found metronidazole obeys Beer's law in the concentration range of 4-12µg/ml. The regression analysis was carried out for the regression line which estimates degree of linearity.

Preparation of sample solution

Twenty tablets were weighed accurately and ground into a fine powder. An accurately weighed quantity of tablet powder equivalent weight to 50mg of metronidazole was transferred to a 50ml volumetric flask and dissolved and diluted to the mark with solvent to obtain sample stock solution having concentration of 1000 µg/ml. The solution was filtered through a filter paper (whatman. 41) to get the clear solution. From this solution 1ml of solution was pipetted out into a 50ml volumetric flask and diluted to the mark with the solvent. Concentration of the solution was 20µg/ml.

Validation

Both the methods were validated in compliance with ICH guidelines.

Accuracy (recovery study)

The accuracy of an analytical procedure expresses the closeness of agreement between the value

$$LOD = 3.3 \times \sigma/S$$

Where,

σ = the standard deviation of the response and
S= slope of the calibration curve.

Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The limit of quantification (LOQ) of the drug was derived by

$$LOQ = 10 \times \sigma/S$$

Where,

σ = the standard deviation of the response and
S= slope of the calibration curve.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The results were represented in table. 01.

which is accepted either as a conventional true value or an accepted reference value and the value found.

To study the accuracy of the proposed method, recovery studies were carried out at three different levels (50%,100% and 150%). The results were represented in table. 01.

Method precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The results were represented in table. 01.

Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The limit of detection (LOD) of the drug was derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOQ) using the following equation designated by International Conference on Harmonization (ICH) guidelines. The results were represented in table. 01.

calculating the signal-to-noise ratio (S/N, i.e., 10 for LOQ) using the following equation designated by International Conference on Harmonization (ICH) guidelines. The results were represented in table. 01.

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The results were represented in table. 01.

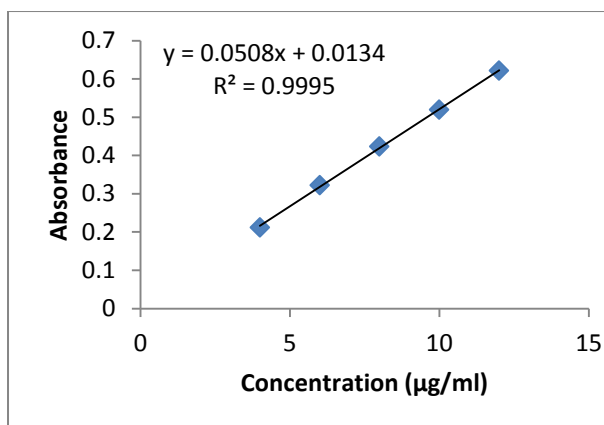


Fig.2: Linearity curve for metronidazole

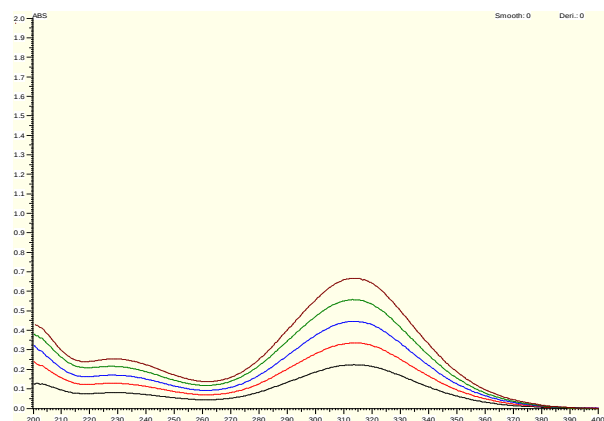


Fig. 4: Overlay spectra of metronidazole

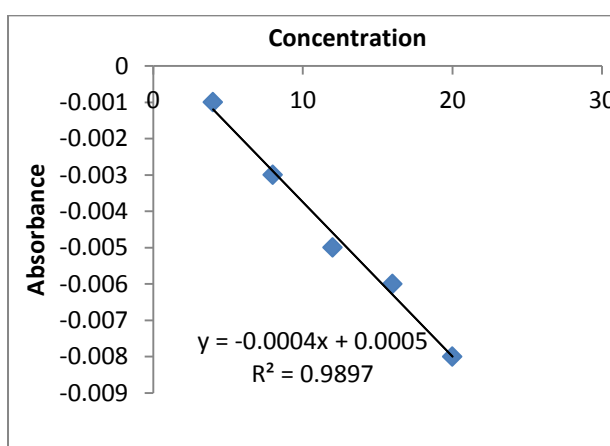


Fig. 3: Linearity curve for metronidazole UV first order derivative spectroscopy

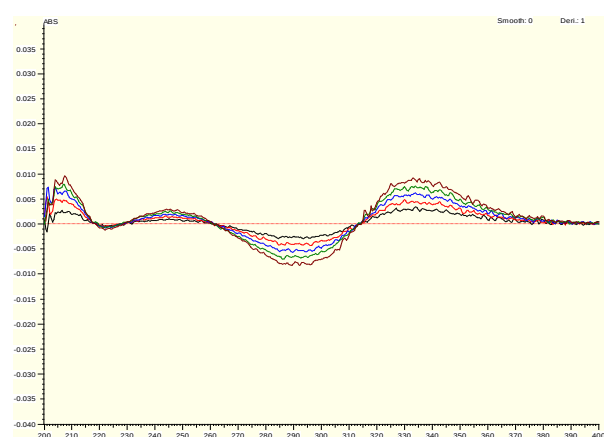


Fig. 5: First order derivative overlay spectra of metronidazole

Table. 01: Regression analysis data and summary of validation parameters for the proposed UV spectroscopic method

Parameters	UV spectroscopy results	First derivative spectrometry results
λ max (nm)	313nm	298nm
Linearity range (µg/ml)	4 -12	4-12
Correlation coefficient (R^2)	0.9995	0.9897
Regression equation ($y = mx + c$)	$Y = 0.0508x + 0.0134$	$y = -0.0004x + 0.0005$
Slope (m)	0.0508	-0.0004
Intercept (c)	0.0134	0.0005
Standard deviation (S.D.)	0.0035	0.00015275
% Relative standard deviation (%RSD)	0.35	1.91739569
Repeatability	0.35-0.64	1.78-1.92
Interday (n=3)	1.24-1.44	1.08-1.59
Intraday (n=3)	0.89-1.48	1.25-1.56
Accuracy (% Recovery) (n=3)	98.50 -99.70	97.55-103.80
Limit of detection (LOD) (µg/ml)	0.23	1.01
Limit of quantification (LOQ) (µg/ml)	0.69	3.06

RESULTS & DISCUSSION

The linearity for metronidazole was found in the concentration range of 4 to 12 µg/ml ml and the correlation coefficient values for UV and derivative spectroscopy were 0.9995 and 0.9897 respectively. The precision values were 0.35-0.64 and 1.78-1.92 with mean accuracies 98.50 -99.70% and 97.55-103.80% respectively for UV and derivative spectroscopy. Limit of detection values for UV and derivative spectroscopy were found to be 0.23 and 1.01. Limit of quantification for both UV and derivative spectroscopy were found 0.69 and 3.06 respectively. The reproducibility, repeatability and precision of both methods are very good as shown by the low values of standard deviation and relative standard deviation (%RSD). The % recovery value in the range of 98.50 -99.70 and 97.55-103.80 for UV and derivative spectroscopy to the pharmaceutical formulation indicates non-interferences from the formulation excipients. Optical characteristics of these methods and summary of validation parameters for metronidazole were given in table. 01.

CONCLUSION

The results of these developed methods for determination of metronidazole indicate that these methods were accurate, precise and reproducible. Both the methods are economical as compared to other reported analytical methods. Hence these methods can be used for routine analysis of API and commercially available tablet dosage form of metronidazole without interference from commonly used excipients. The solvents used for the proposed methods were inexpensive and simple to prepare. These methods adopted to be used in a quality control laboratory for routine drug analysis.

ACKNOWLEDGEMENT

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