Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Research Article

Development and Validation of First Order Derivative Method for Metronidazole in Bulk and Tablet Dosage Form by Using UV Spectroscopy.

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Received 17 June 2019; received in revised form 17 August 2019; accepted 25 August 2019

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ABSTRACT
A simple, precise, accurate and less time consuming method was developed for estimation of metronidazole from tablet and bulk formulation by UV Spectrophotometry. This method was successfully applied for the determination of metronidazole in tablet and bulk formulation by using the first order derivative method. It was observed that metronidazole shows zero crossing at 319 nm. Metronidazole showed absorbance maxima (A/dλ) at 300 nm, measurable and minima at 340 nm. Hence wavelength was selected at 319 nm for determination of metronidazole. In this method, metronidazole followed linearity in the concentration range between the 10-16µg/ml with R^2= 0.998. Method was validated for accuracy, precision and linearity.

KEYWORDS
Metronidazole, UV visible spectrophotometer, first order derivative.

1. INTRODUCTION

Metronidazole is chemically a 1-(2-hydroxy-1-ethyl)-2-methyl-5-nitroimidazole, slightly soluble in water, chloroform, alcohol, acetone and in methylene chloride, very slightly soluble in ether. Metronidazole is a prototype of the nitroimidazole class of antimicrobials, it has been evaluated in the treatment of diverse anaerobic and gastrointestinal tract infection.[1,13,15] Metronidazole has often been studied for antibacterial activity against gram negative aerobes and some gram positive bacteria, including *bacteriodes fragilis* that produce β-lactamases.[2,17,19] In healthy human, metronidazole is absorbed rapidly and completely from the gastrointestinal tract and is metabolized in the liver by an oxidative pathway. Liver is the main side of metabolism by side chain oxidation and glucuronide conjugation. A major portion of the dose of the drug is excreted in urine, largely as metabolites. [3, 11, 12, 14, 16, 18] Metronidazole is one of the most widely used antibacterial compounds in the treatment of aggressive periodontitis. According to literature survey it reveals that several methods has been reported for the measurement of metronidazole in bulk and tablet dosage form using mass spectrometry, UV spectrophotometry, HPLC and HPTLC. [4-10] The purpose of the study was to develop simple, precise, accurate, less time consuming and cost effective method for estimation of metronidazole from tablet and bulk formulation by UV Spectrophotometry. In this study, water was used as the solvent which was more economical.

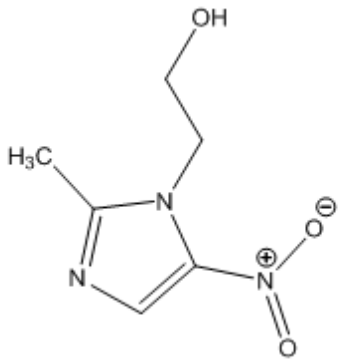

Fig. 1. Structure of Metronidazole.

2. MATERIALS AND METHODS

2.1. Chemical and Reagent

The metronidazole was obtained as a gift sample from Glenmark pharmaceuticals. Double distilled water (Mili Q) was used to prepare working solution for the method. Sample of drug and internal standard were obtained from Jt baker. Analytical grade Methanol was used. Marketed formulation Flagyl, Metrogel and Unimezole was used for the study.

2.2. Instrumentation

A Shimadzu UV/Visible spectrophotometer, model no. UV-1800 was used for the study. The instrument had a spectral band width of 1 nm and wavelength accuracy of ± 0.1 with automatic wavelength correction employing a pair of quartz cells of 1 cm path length.

2.3. Method

2.3.1. Selection of wavelength

In order to ascertain the wavelength of maximum absorption of the drug, (10 µg/ml) solution of the drug in water was scanned using spectrophotometer within the wavelength range of 200-400 nm against water as blank and a sharp peak was obtained at 319 nm (Fig. 2). Calibration curve was plotted by taking absorbance on y-axis and concentration of solution on x-axis (Fig. 3). This method was applied for known sample solution and was found to be satisfactory for analysis of tablet dosage forms.

2.3.2. Preparation of standard stock solution for calibration curve

Standard solution of Metronidazole was prepared by transferring accurately weighed 10 mg of drug into a 100 ml volumetric flask, 20 ml of water added and sonicated till it dissolve completely and the volume was made up to 100 ml using water as a solvent to get the concentration of 100 µg/ml. Then 1 ml of this solution was diluted to 10 ml with water to give 10 µg/ml, similarly concentrations of 11 µg/ml, 12 µg/ml, 13 µg/ml, 14 µg/ml and 15 µg/ml were prepared which were used for the construction of calibration curve (Fig. 3).

2.3.3 Preparation of sample solution

20 tablets were accurately weighed and crushed into fine powder. Tablet powder equivalent to 10 mg of metronidazole was transferred into a 100 ml volumetric flask then added 20 ml of water and sonicated then cooled at room temperature and volume was made up to mark with water and mixed properly. The above solution was filtered through (11µm) whatmann filter paper.

3. METHOD VALIDATION

The proposed method was validated according to ICH guidelines for linearity, accuracy, precision, robustness, LOD and LOQ.

3.1. Linearity

Fresh aliquots were prepared from the stock solution (100 µg/ml) in different concentrations. The plot of absorbance verses concentration of metronidazole was found to be linear in the range of 10-16 µg/ml. Beer's law was obeyed over this concentration range. The regression equation was found to be $y= 0.0505x + 0.0413$ and the correlation coefficient (r^2) of the standard curve was found to be 0.998 (Fig. 2).

3.2. Precision

The precision of an analytical method was expressed as the percent relative standard deviation and standard error of mean of the series of measurements. It was ascertained by the replicate estimation of standard drugs. Intra-day precision was determined by analyzing 12 µg/ml of metronidazole for three times within the day. Inter-day precision was determined by analyzing same concentration daily for three days. The results are reported in Table 1.

3.3. Assay of Metronidazole Tablets

20 tablets were accurately weighed and crushed into fine powder. Tablet powder equivalent to 10 mg of metronidazole was transferred into a 100 ml volumetric flask then added 20 ml of

water and sonicated then cooled at room temperature and volume was made up to mark with water and mixed properly. The above solution was filtered through Whatman filter paper size (11μm) and the absorbance was measured against blank. The amount of Metronidazole was computed by using the equation referring to the calibration curve.

3.4. Recovery study

Accuracy study was carried out by the addition of known amount of the standard drug in the preanalysed tablet formulation in 75, 100, and 125% of the label claim. At each level of amount, three determinations were performed and results are reported in Table 2.

3.5. Robustness

Robustness of the method was determined by carrying out the analysis 12 μg/ml solution under different temperature and wavelength condition. The respective absorbance were noted and the result were indicates as % RSD shown in Table 3 and Table 4.

3.6. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of metronidazole by proposed method were determined using calibration standard. LOD and LOQ were calculated as 3.3σ/s and 10 σ/s respectively, where s is the slope of the calibration curve and σ is the standard deviation of response.

3.7. Development of first order derivative spectra

100 μg/ml Metronidazole standard solutions was separately prepared and scanned in the UV range 200–400 nm, after baseline correction and the spectral data it was then processed to obtain first order derivative spectrum at wavelength interval of 2 nm. The overlain zero-order absorption spectra of Metronidazole were obtained at 319 nm. The absorption spectrum was converted to first-order derivative spectra by using the instrument mode. The calibration curves were prepared in the concentration range of 10-16 μg/ml metronidazole at the wavelength 319 nm.

4. RESULTS AND DISCUSSION

The UV spectroscopy method for the Metronidazole by first order derivative was found to be simple, accurate, economical and reproducible. The drug concentration were found to be linear in the range of 10-16 μg/ml respectively and the correlation coefficient (r^2) value of 0.998 indicates that developed method was linear. The drug showed standard regression equation Y= 0.0505X+0.0413 and molar absorptivity 53.92**.** The intraday precision and interday precision were expressed in terms of relative standard deviation (% RSD), the method was observed as precise with % RSD less than 2. The accuracy of the method was assessed by recovery studies at three different levels i.e 75%, 100%, 125%. The percent recovery was found to be 95.86%, 99.99% and 99.26% (Table No 3). The Limit of Detection and Limit of Quantitation value were found to be 7.12 (μg/ml) and 21.60 (μg/ml) respectively. The assay was done under dissimilar temperature and wavelength condition. The result showed % RSD values within the acceptance criteria i.e. less than 2%. Hence the method was found to be robust.

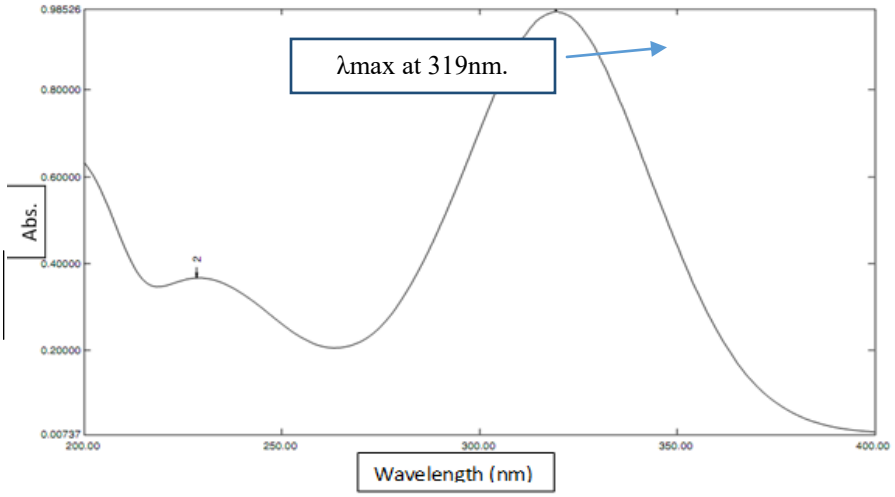

Fig. 2. Spectra of Metronidazole.

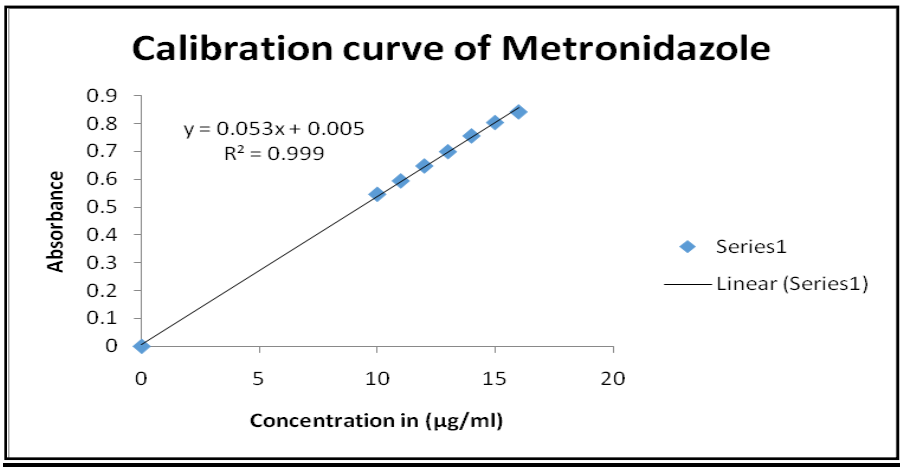

Fig. 3. Calibration curve of Metronidazole.

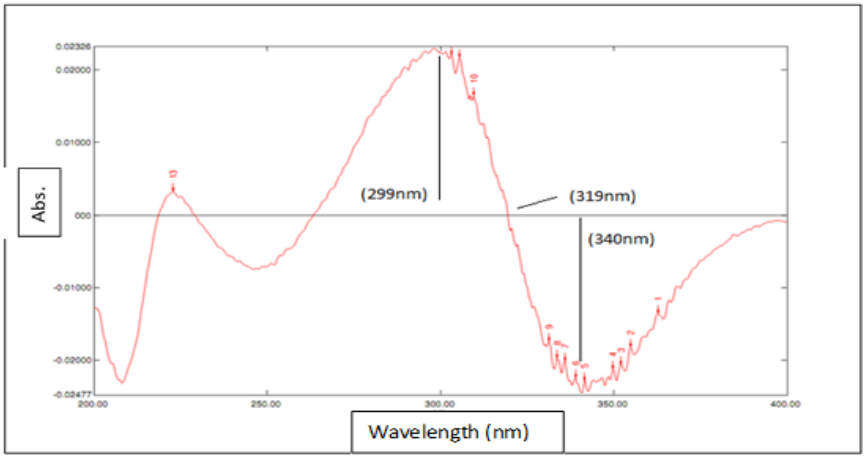

Fig. 4. First order Spectra of Metronidazole.

Table 1. Result of Precision**.**

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

Table 2. Result of Recovery.

Level of Recovery	Drug	Amount of drug added (µg)	Amount of drug std. added (µg)	Method	
				% recovery	%RSD
75%	**Metronidazole**	09	8.611	95.86	0.122
100%		12	11.99	99.99	0.031
125%		15	14.89	99.26	0.128

Table 3. Effect of temperature on a 10 (µg/ml) solution of metronidazole.

Temperature(^{o}C)	Absorbance	Con(µg/ml)	% Recovery	Mean	% RSD
25	0.545	10.04	100.9		
25	0.546	10.01	100.3	100.538	0.331
25	0.545	10.01	100.3		
30	0.544	9.98	100.3		
30	0.545	9.95	99.7	99.766	0.578
30	0.545	9.95	99.1		
35	0.546	9.95	99.1		
35	0.546	9.96	99.1	99.381	0.330
35	0.547	9.95	99.7		

Table 4. Effect of different wavelength of light on a 10 (µg/ml) solution of metronidazole.

Wavelength(nm)	Absorbance	Con(µg/ml)	% Recovery	Mean	% RSD
319	0.545	10.04	99.77		
319	0.544	10.01	99.77	99.7	0.330
319	0.545	10.01	99.2		
320	0.548	9.95	100.07		
320	0.549	9.98	100.090	100.7	0.331
320	0.548	9.99	100.07		
321	0.545	10.01	99.77		
321	0.548	9.98	100.07	99.9	0.333
321	0.547	10.02	99.80		

5. CONCLUSION

The results of developed method for determination of metronidazole indicate that the method is accurate, precise and reproducible. The method was found economical as well. Hence this method can be used for routine analysis of API and commercially available tablet dosage form of metronidazole without interference from commonly used excipients. The solvent used for the proposed method were inexpensive and simple to prepare.

6. ACKNOWLEDGEMENTS

We wish to thank Glenmark pharmaceutical ltd, Taloja MIDC for provide the gift sample of metronidazole and also provide the necessary facility carry out this work.

7. REFERENCES

1. Maliwal D., Jain A., Maheshwari R.K., Patidar V. (2008). Simultaneous spectrophotometric estimation of metronidazole and norfloxacin in combined tablet formulations using hydrotropy. *Research Journal of Pharmacy and Technology*. 1, 4, 357-361.
2. Trivedi K.D. (2013). Development and validation of RP-HPLC method for estimation of metroniadazole and noeflaxocine in suspension form. *International journal in advances in pharmaceutics.* 2, 1, 5-11.
3. James W.T., Leslie T.W. (2007). Drugs used in the chemotherapy of protozoa infections. Goodman and Gilman's Pharmacological Basis of Therapeutics. New York: McGraw-Hill. 9, 1105.

4. Thulasamma P., Venkateswarlu P. (2009). Spectrophotometric method for the determination of metronidazole in pharmaceutical pure and dosage forms. *Rasayan Journal Chem.*2, 4, 865-868.

5. Dingsdag S.A., Hunter N. (2017). Metronidazole: an update on metabolism, structure–cytotoxicity and resistance mechanisms. *Journal of Antimicrobial Chemotherapy.* 73, 2, 265-79.

6. Tiwari G., Tiwari R., Srivastava B., Rai A.K., Pathak K. (2008). Simultaneous estimation of metronidazole and amoxicillin in synthetic mixture by ultraviolet spectroscopy. *Asian Journal of Research in Chemistry.*1, 2, 91-94.

7. Al Shaalan N.H. (2007). Determination of diloxanide furoate and metronidazole in binary mixture using first derivative of the ratio-spectra and high-performance liquid chromatography-UV methods.*American Journal of Applied Sciences.*4, 2, 66-72.

8. Elena G. (2011). Development and validation of a RP-HPLC method for the quantization studies of metronidazole in tablet and powder dosage form. *Journal of medicamental veterinary*.5, 2, 71-73.

9. Kasabe A.J., Shitole V.V., Waghmare V.V., Mohite V. (2009). Simultaneous estimation of metronidazole and ofloxacin in combined dosage form by reverse phase high performance liquid chromatography method. *International Journal of ChemTech Research.*1, 4, 1244-1250.

10. Mishra A.K., Yadava R., Mishra A., Verma A., Chattopadhyay P. (2010). Development and validation of UV spectrophotometric method for the determination of metronidazole in tablet formulation. *International Journal of Pharmaceutical Research and Development.* 2, 6, 001-004.

11. Farhadi K., Bahar S. (2007). Kinetic Spectrophotometric Determination of Metronidazole Benzoate in Surfactant Medium. *Journal of the Chinese Chemical Society*.54, 6, 1521-1527.

12. Hussain S., Ansari M.T. (2011). Quantitation of metronidazole in pharmaceutical suspension using high performance liquid chromatographic method. *Pakistan Journal of Zoology*.43, 5, 909-914.

13. Mishal A., Sober D. (2005). Stability indicating reversed-phase liquid chromatographic determination of metronidazole benzoate and diloxanide furoate as bulk drug and in suspension dosage form. *Journal of pharmaceutical and biomedical analysis.*39, 3, 4, 819-823.

14. Vega E., Dabbene V., Nassetta M., Sola N. (1999). Validation of a reversed-phase LC method for quantitative analysis of intravenous admixtures of ciprofloxacin and metronidazole. *Journal of pharmaceutical and biomedical analysis.* 21, 5, 1003-1009.

15. El-Gizawy S.M. (1995). HPLC analysis of metronidazole and diloxanide furoate in its dosage forms. *Analytical letters*. 28, 1, 83-92.

16. Danao K.R., Hiradere S.M., Moon R.S., Kasture A.V., Yeole P.G. (2010). RP-HPLC simultaneous estimation of metronidazole and diloxanide furoate in combination. *International Journal pharm life Sci.*1, 82-85.

17. El-Ghobashy M.R., Abo-Talib N.F. (2008). Application of derivative ratio and TLC-densitometric methods for determination of a ternary mixture containing metronidazole, diloxanide furoate and mebeverine hydrochloride. *Bull Fac Pharm Cairo Univ.* 46, 75-86.

18. Maher H.M., Youssef R.M., Khalil R.H., El-Bahr S.M. (2008). Simultaneous multiresidue determination of metronidazole and spiramycin in fish muscle using high performance liquid chromatography with UV detection. *Journal of Chromatography B.* 876, 2, 175-181.