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Research Article

UV-Spectrophotometric Assay Method Development and Validation of Metronidazole in Bulk and Tablet Formulation

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Abstract

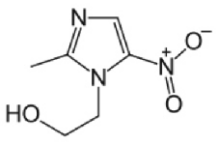
An easy, precise and accurate spectroscopic technique for the estimation of Metronidazole in pure form and tablet dosage form has been developed. The proposed method involves dissolving Metronidazole in 1(M) HCl solution and subjecting the resulting solution to UV Spectroscopic assessment. Absorption maximum was found to lie at about 277nm. Beer's law was obeyed in the concentration range of 2-10 μg/ml. Calibration curve showed linearity between absorbance and concentration as per line equation y=0.0347x-0.0071 with an R^2 value of 0.9974. Validation was performed as ICH guidelines for linearity, accuracy and precision. The assay results were found to be in good agreement with label claim.

Keywords: Assay, Validation, Spectrophotometry, Metronidazole (MND), Tablet dosage form

1.Introduction

Chemically, metronidazole (1) is 2-methyl-5-nitro-1H-imidazole-1-ethanol. It is a 5-nitronimidazole derivative which is bactericidal to anaerobic and microaerophillic microorganisms, including *Bacteroides*, *Clostridium* sp, *Endolimax nana, Entameba histolytica, Fusobacterium vincentii, Gardnerella vaginalis, Giardia lamblia, Peptostreptococcus* and *Trichomonas sp.* These organisms reduce the nitro group and generate metabolites that inhibit DNA synthesis. It has long been the drug of choice for the treatment of trichomoniasis and more recently in combination with iodoquinol for the treatment of symptomatic amebiasis(except in brain). It is also the drug of choice for the treatment of Dracunculus (guinea worm) infestations. It is the alternative drug to treat giardiasis, balantidiasis, blastocystitis and infections by *Entameba polecki*. It is used for treatment and prophylaxis of infections caused by anaerobic bacteria; against GI strains of *Bacteroides fragilis* and vaginal infections by *Gardnerella vaginalis*. Reported to be of value in Crohn's disease.[1]

Review of Literature for MND analysis revealed that several existing methods including different technique such as HPLC with UV detection[2], GC-FID[3], HPLC PDA/MS, UPLC-MS[4] assay for its quantification in plasma and gastric juice fluids have been reported for assay of metronidazole. However there is no simple and accurate method reported for the detection of MND in pharmaceutical formulation by UV spectrophotometry. So there is need to establish a simple, fast, accurate and economic method for determination of Metronidazole in bulk powder and its dosage forms, which can be used in quality control laboratories.



(1)

2.Materials and Methods

2.1 Materials

Metronidazole standard drug was procured as a gift sample from Greenco Biologicals Pvt. Ltd., Kolkata. Metrogyl 200 tablets (J.B Chemicals and Pharmaceuticals Ltd.; 128/1, GIDC, Ankleshwar 393 002) containing Metronidazole IP 200mg, were used as the formulation for analytical study. Analytical grade Hydrochloric acid was procured from Qualigens Fine Chemicals, India. UV-Visible Spectrophotometer (Evolution 201, Thermo Scientific, UK) was used to carry out the assay.

2.2. Standard solution of metronidazole

Ten miligrams of Metronidazole IP was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved in about 50 ml of 0.1(M) HCl and then the volume was made up to the 100 ml mark with 0.1(M) Hcl (solution A) (0.1mg/ml). 5 ml of the stock solution (0.1mg/ml) was transferred to a 100 ml volumetric flask and was diluted to 100 ml with 0.1(M) HCl solution. This 5 μg/ml solution was used as MDZ working standard.

2.3. Determination of λ_{max} and calibration curve

The stock solution (0.1mg/ml) was diluted with 0.1(M) HCl to obtain a solution of concentration 0.01mg/ml. The absorbance of resulting solution was scanned in the UV spectrometer in the range 200-400nm. The absorbance maximum was found at a wavelength at about 277 nm.

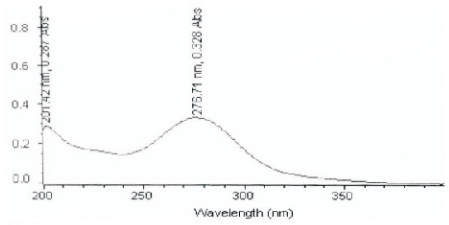


Fig. 1. Scanning curve for Metronidazole in 0.1(M) Hcl in the wavelength range of 200nm-400nm

At this wavelength maximum, calibration curve was drawn by plotting graph between absorbance and concentrations (2-10 μg/ml)

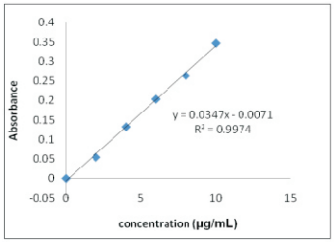


Fig. 2. Calibration curve of metronidazole in 0.1(M) HCl solution

2.4. Determination of active ingredients in tablets

The proposed method was applied to analyze commercially available metronidazole tablets. Each tablet was having content of metronidazole equivalent to 200mg. Ten tablets were weighed and finely powdered. Powder equivalent to 100mg metronidazole was transferred to a 100ml volumetric flask and dissolved in 0.1(M) HCl. By frequent shaking volume was made up to mark with 0.1(M) HCl. The solution (concentration: 1mg/ml) was then filtered through Whatman filter paper (Solution B). This filtrate was diluted suitably with solvent to get the solution of 5µg/ml concentration. The absorbance was measured 0.1(M) HCl solution as blank. The amount of metronidazole was calculated from the calibration curve. The readings were taken in triplicate. Results are shown in Table 1.

Table 1. Data for assay of tablets

Sl. No.	Concentration (µg/ml)	Absorbance
1	2	0.054
2	4	0.132
3	6	0.203
4	8	0.263
5	10	0.346

2.5. Method validation[5]

The following parameter were evaluated for method validation and the test concentration was taken to be 5µg/ml

Table 2. Data for standard curve plotting and linearity determination

Preparation (µg/ml)	Absorbance	Conc. (µg/ml)	Recovery	Mean	%RSD
5	0.170	5.104	102.075	102.459 ± 0.332	0.324
5	0.171	5.132	102.651		
5	0.170	5.132	102.651		

2.5.1. Determination of accuracy

Solutions were prepared in triplicate at levels 80%, 100% and 120% of test concentration using metronidazole working Standard as per the test method and absorbance of each solution was taken in triplicate. The recovery results were recorded. S.D and %RSD were calculated. Results are shown in Table 3.

2.5.2. Tablet formulation

Ten ml of solution a (0.1mg/ml working standard) and 1ml of solution B (1mg/ml tablet powder solution) was transferred to a 100 ml volumetric flask and was diluted to 100 ml with 0.1(M) HCl to obtain a 20µg/ml solution. 50 ml of this solution was transferred to a 100 ml volumetric flask and was diluted to 100 ml with 0.1(M) HCl to obtain a 10µg/ml solution.

Absorbance of this solution was taken in triplicate. The recovery results were recorded; S.D and % RSD were calculated and shown in Table 4.

2.5.3. Determination of precision

Precision of the method was demonstrated by repeatability, intraday

Table 3. Data for determining accuracy for standard

Preparation	Absorbance	Concentration (µg/ml)	Recovery %	Mean	%RSD
80%	0.133	4.038	100.937	100.696 ± 0.416	0.413
80%	0.132	4.009	100.216		
80%	0.133	4.038	100.937		
100%	0.165	4.960	99.193	99.385 ± 0.332	0.335
100%	0.166	4.988	99.769		
100%	0.165	4.960	99.193		
120%	0.203	6.055	100.910	100.910 ± 0.289	0.287
120%	0.202	6.026	100.430		
120%	0.204	6.083	101.390		

Table 4. Data obtained after addition of known amount of pure drug to formulation

Amount of drug added from formulation (µg)	Amount of drug added (µg)	% drug added	Total amount of drug (µg)	Absorbance	Amount recovered (µg)	Recovery %
5	5	100%	10	0.352	10.349	103.487
5	5	100%	10	0.353	10.378	103.775
5	5	100%	10	0.353	10.378	103.775
					Mean ± S.D	103.679±0.188
					% RSD	0.182

and interday variation studies. For repeatability study six samples of same concentration, 5μg/ml, were taken and the absorbances were observed and the %RSD was calculated. The acceptable limit should be within 2%.The results are shown in Table 5.

Table 5. Repeatability

Sl. No.	Concentration (μg/ml)	Absorbance	Mean $\pm$ SD	% RSD
1	5	0.165		
2	5	0.166		
3	5	0.165	0.164$\pm$0.001	0.745
4	5	0.165		
5	5	0.163		
6	5	0.163		

In intraday variation study nine different solutions of same concentration 5μg/ml were analyzed three times in a day *i.e*. from morning, afternoon and evening and the absorbances were noted. From the absorbance result mean, standard deviation and %RSD were calculated. The acceptable limit for intraday variation should be within 1%. Results were shown in Table 6.

In the inter day variation studies, solution of same concentration 5µg/ml were analyzed three times for the three consecutive days and the absorbance result was observed. Mean, standard deviation and %RSD were calculated. The acceptable limit for interday variation should be within 2%. Results are shown in Table 7.

2.5.5. Determination of Robustness

Robustness of the method was determined by carrying out the analysis of a 5μg/ml solution under different temperature and wavelength conditions. The respective absorbances were noted and the results were indicated as %RSD. Results are shown in Table 8 and Table 9.

Table 6. Intraday precision

Concentration (μg/ml)	Time		
	10:30 a.m	2:15 p.m	4:30 p.m
5	0.166	0.165	0.165
5	0.166	0.165	0.166
5	0.165	0.165	0.166
5	0.164	0.163	0.166
5	0.165	0.164	0.163
5	0.165	0.164	0.163
% RSD	0.456	0.496	0.893
Average % RSD		0.615	

Table 7. Interday precision

Concentration (μg/ml)	Day		
	1	2	3
5	0.166	0.167	0.165
5	0.166	0.167	0.165
5	0.165	0.166	0.164
5	0.164	0.166	0.167
5	0.165	0.166	0.167
5	0.165	0.168	0.166
% RSD	0.456	0.490	0.731
Average % RSD		0.560	

Table 8. Effect of temperature on a 5μg/ml solution of metronidazole IP in 0.1(M) HCl

Temperature	Absorbance	Concentration (μg/ml)	Recovery %	Mean	%RSD
25	0.168	5.046	100.922		
25	0.167	5.017	100.346	100.538$\pm$0.332	0.331
25	0.167	5.017	100.346		
30	0.167	5.017	100.346		
30	0.166	4.988	99.760	99.766$\pm$0.577	0.578
30	0.165	4.959	99.192		
35	0.165	4.959	99.192		
35	0.165	4.959	99.192	99.381$\pm$0.328	0.330
35	0.166	4.988	99.760		

Table 9. Effect of different wavelengths of light on a 5μg/ml solution of metronidazole IP in 0.1(M) HCl

Wavelength	Absorbance	Concentration (μg/ml)	Recovery %	Mean	%RSD
276	0.166	4.988	99.770		
276	0.166	4.988	99.770	99.580$\pm$0.329	0.330
276	0.165	4.960	99.200		

Wavelength	Absorbance	Concentration (μg/ml)	Recovery %	Mean	%RSD
277	0.168	5.046	100.922		
277	0.168	5.046	100.922	100.922±0.000	0.000
277	0.168	5.046	100.922		
278	0.167	5.017	100.346		
278	0.166	4.988	99.770	99.962±0.332	0.333
278	0.166	4.988	99.770		

3. Results and Discussion

3.1. Linearity

Linearity was observed within 2μg/ml and 10μg/ml concentration The acceptable limit is, it should be linear in the specified range and the correlation coefficient should not be less than 0.99.The correlation coefficient, $R^2=0.9987$. Hence the relationship between the concentrations and the absorbances of metronidazole showed linearity.

3.2. Determination of active ingredients in Tablets

According to the IP, metronidazole Tablets should contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of metronidazole. Hence the average percentage recovery of 102.459% was found to be within the acceptance limit. %RSD values did not exceed the acceptance limit of 2%.

3.3. Accuracy

According to the IP, metronidazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_9N_3O_3$. The results showed that drug content was within specified limits and the % RSD values did not exceed the accepted limit of 2%. Hence the method can be said to be accurate.

For tablet formulation, the results of percentage recovery (limit 95% to 105%) and %RSD (<2%) were within the specified limits. Hence, the method was found to be accurate for estimation of metronidazole in tablet formulation.

3.4. Precision

The % RSD values for repeatability, intraday and interday precision data were well below the specified limit of 2%, 1% and 2%, respectively. Hence, the method was found to be precise in the specified range.

3.5. Robustness

The assay was done under different temperature and wavelength conditions. The results showed % RSD values to be within the acceptance criteria of 2%. Hence the method was found to be robust in the given conditions.

4. Conclusion

The UV-spectrophotometric method for the estimation of metronidazole in bulk and tablet formulation was found to be accurate, precise and robust. The method was found to be linear over a convinient range, economical and utilised a solvent which can be easily prepared.The above factors make this method suitable for the estimation of metronidazole in bulk drug and in pharmaceutical dosage forms. It can therefore be concluded that this method being accurate, precise, robust, economical, simple and time saving, can be used in laboratories and also for the routine analysis of Metronidazole in bulk preparation and in tablet dosage form.

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Conflicts of interest

The author reports no conflict of interest.

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