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Spectrophotometric determination of metronidazole through Schiff's base system using vanillin and PDAB reagents in pharmaceutical preparations

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Abstract: Two simple sensitive and reproducible spectrophotometric methods have been developed for the determination of metronidazole either in pure form or in their tablets. The proposed methods are based on the reduction of the nitro group to amino group of the drug. The reduction of metronidazole was carried out with zinc powder and 5 N hydrochloric acid at room temperature in methanol. The resulting amine was then subjected to a condensation reaction with aromatic aldehyde namely, vanillin and p-dimethyl amino benzaldehyde (PDAB) to yield yellow colored Schiff's bases. The formed Schiff's bases are quantified spectrophotometrically at their absorption maxima at 422 nm for vanillin and 494 nm for PDAB. Beer's law was obeyed in the concentration ranges 10 to 65 μg mL^{-1} and 5 to 40 μg mL^{-1} with a limit of detection (LOD) of 0.080 μg mL^{-1} and 0.090 μg mL^{-1} for vanillin and PDAB, respectively. The mean percentage recoveries were found to be 100.05 ± 0.37 and 99.01 ± 0.76 for the two methods respectively. The proposed methods were successfully applied to determine the metronidazole in their tablet formulations and the results compared favorably to that of reference methods. The proposed methods are recommended for quality control and routine analysis.

Keywords: spectrophotometry; vanillin; PDAB; metronidazole; pharmaceutical analysis.

Introduction

5-Nitroimidazoles, such as metronidazole, are extensively used as antiamoebic, antiprotozoal and antibacterial drugs. The discovery of the antibacterial and antitrichomonal properties of the antibiotic azomycin led to the investigation of nitroimidazoles as antiparasitic agents [1, 2]. The discovery of the antitrichomonal properties of metronidazole revolutionized the treatment of disease. Although the amoebicidal properties of metronidazole were studied, it was not clinically tested until some years later. In laboratory tests, metronidazole is effective against intestinal amoebiasis in rats and hepatic amoebiasis in hamsters and is also active against *Entamoeba histolytica* in vitro [3, 4]. The initial clinical tests of metronidazole indicated that it was capable of curing invasive amoebic dysentery and amoebic liver abscess [5] Subsequent clinical tests have established metronidazole as the drug of choice in the treatment of all forms of amoebiasis in humans[6, 7].

Metronidazole is officially determined by titrimetry, potentiometry and HPLC methods. Indian Pharmacopoeia [8] describes the non-aqueous titration method using perchloric acid as titrant and malachite green as indicator for the assay of

metronidazole. British Pharmacopoeia [9] describes potentiometric and non-aqueous titration methods using perchloric acid as titrant. United States Pharmacopoeia [10] 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 chromatographic techniques for pharmaceutical analysis. Several methods have been reported for the determination of metronidazole, including spectrophotometry [11, 13], polarography [14]. 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 [15, 22] 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 after converting it to its reduced form by using zinc powder and HCl, as well as the reaction of its reduced product with vanillin and PDAB was studied to establish the optimum reaction conditions, optical characteristics, precision and accuracy of the proposed methods. 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.

Experimental details

Apparatus

An Elico model SL 164 UV-Visible double beam spectrophotometer with 1 cm matched quartz cell was used for recording spectra and absorbance measurements.

Reagents

All reagents used were of analytical grade and were obtained from Qualigens fine chemicals, Mumbai. Metronidazole was kindly supplied by Sarabhai Pharmaceuticals Ltd., Baroda, India. Metronidazole tablets were purchased from a local market. Distilled water was used for the preparation of HCl solution.

Sample preparation

Mehanolic solution of vanillin (4%) and PDAB (3%) was prepared by dissolving 3 and 4 g in a 100 mL volumetric flask containing methanol. A 5 N HCl was prepared by dissolving 43.1 mL of concentrated hydrochloric acid and was diluted to 100 mL with water.

Reduction of the nitro group and preparation of standard drug solution

About 100 mg of metronidazol pure or equivalent tablet powder was accurately weighed and dissolved in 20 mL of methanol. The methanolic solution of metronidazole was treated with 10 mL of 5 N Hydrochloric acid and 0.5 g of zinc powder was added in portions while shaking. After standing for 1hour at room temperature, the solution was filtered using a Whatman filter paper No 41 filter paper to remove the insoluble matter. The residue was washed with 10 mL portions of methanol three times, and the total volume of the filtrate was made up to 100 mL with methanol. The final working standard solution of reduced metronidazole containing 100 μg mL^{-1} was prepared by further dilution.

General procedure

Method A

In method A, fresh aliquots (1.0- 6.5 mL) of standard 100 μg mL^{-1} reduced metronidazole solution were accurately measured and transferred in to a series of 10 mL volumetric flasks by means of a micro burette. To each of the above aliquots, 2.0 mL of 4% (w/v) of vanillin solution in methanol prepared above were added and mixed thoroughly and then the solution was heated in a water bath at 60-70°C for 15 min, and cooled to room temp. After cooling, the volume was brought up to the mark with methanol, mixed well and the absorbance of each yellow colored species was measured after 10 min. at 422 nm against reagent blank. A calibration graph was constructed by plotting the absorbance against the concentration of the drug.

Method B

In method B, fresh aliquots (0.5- 4.0 mL) of standard 100 μg mL^{-1} reduced metronidazole solution were accurately measured and transferred in to a series of 10 mL volumetric flasks by means of a micro burette. To each of the above aliquots, 0.5 mL of 3% (w/v) of PDAB solution in methanol prepared above were added and mixed thoroughly, and then the solution was heated in a water bath at 60-70°C for 15 min, and cooled to room temp. After cooling, the volume was brought up to the mark with methanol, mixed well and the absorbance of each yellow colored species was measured after 10 min. at 494 nm against reagent blank. A calibration graph was constructed by plotting the absorbance against the concentration of the drug.

Assay procedure

Twenty tablets of metronidazole were powdered. An accurate quantity of powder equivalent to 100 mg of metronidazole was weighed. The reduction of metronidazole was carried out by using hydrochloric acid and zinc powder. The resulting filtrate was transferred to a 100 ml volumetric flask and made up to the mark with methanol and an aliquot of this solution was treated as described above for the determination of metronidazole. The amount of metronidazole present in the sample was computed from calibration curve.

Results and discussion

Determination of absorption maximum

Reduced metronidazole when treated with vanillin and PDAB form a yellow product (Schiff's base) in acidic medium. To determine the absorption maxima, 35 μg mL^{-1} (Method A) and 15 μg mL^{-1} (Method B) of the metronidazole was added in a 10 mL volumetric flasks. Then each drug solution was reacted with 2.0 mL vanillin (4%) and 0.5 mL PDAB (3%). The solution was heated in a water bath at 60-70°C for 15 min. After cooling, the volume was brought up to the mark with methanol and mixed well After 10 min., absorption spectra were taken against reagent blank in the range 350-650 nm. The maximum absorption wavelength for metronidazole was found to be 422 nm for method A and 494 nm for method B. The absorption spectrums of the colored product against reagent blank of both the methods are shown in Figure 1. Under the experimental conditions each colorless reagent blank showed a negligible absorbance at the corresponding λ_{max}.

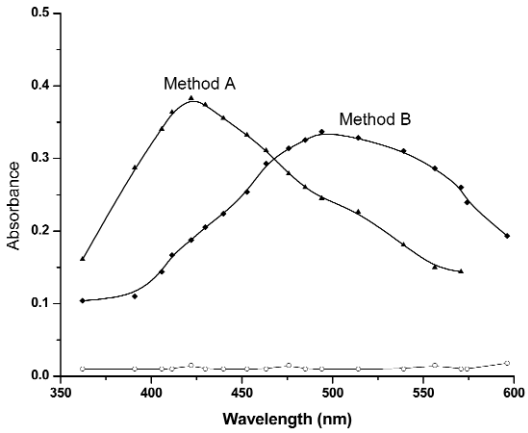
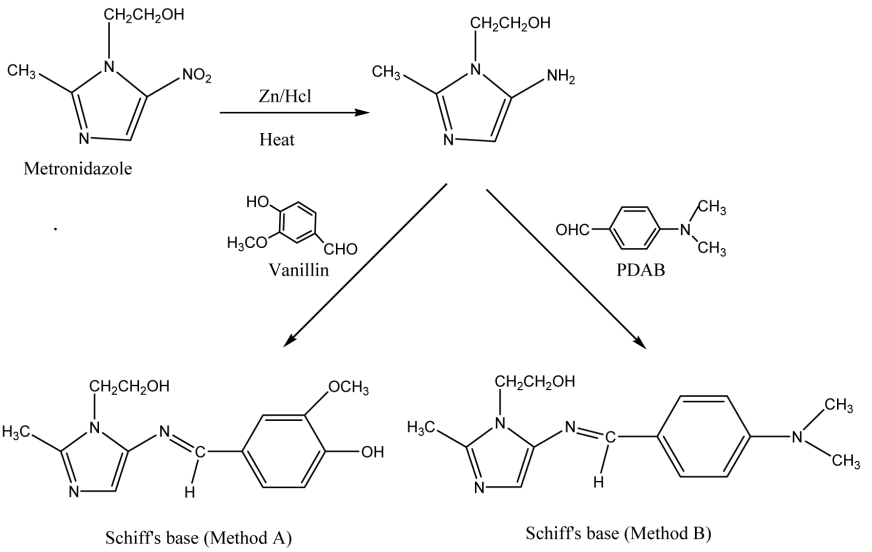


Figure 1. Absorption spectra of Metronidazole (35mg mL^{-1}) with vanillin and Metronidazole (15mg mL^{-1}) with PDAB against reagent blank.

Reaction Sequence

Reduction of the nitro group of the drug was carried out as explained in the experimental section. When the resulting amine undergoes a condensation reaction in acidic medium with vanillin and PDAB, it forms a yellow Schiff's base with maximum absorbance at 422 and 494 nm. The reaction of PDAB and vanillin with pyrroles has been used qualitatively and quantitatively for many years [23, 25]. The reaction with aromatic amines to give Schiff's base is equally well documented [26]. The Wasicky reaction, utilizing aqueous sulphuric acid (94%) solution gives color reaction with alkaloids [27] and purines [28]. In 1994 Werner [29] reinvestigated the reaction with nitrogen compounds and found that in a more dilute aqueous system aromatic compounds react in the presence of mineral acid, provide the $-NH_2$ group is directly attached to the benzene nucleus. The probable reaction mechanism based on the reported method [30, 32] is given in Scheme 1.



Scheme 1. The proposed reactions involved in formation of colored Schiff's bases in acidic medium.

Determination of effective reagents concentration

To study the effect of concentration and volume of vanillin and PDAB on the maximum absorbance, a number of preliminary experiments were carried out. The effect of vanillin and PDAB concentration was studied at 422 and 494 nm. To a series of solutions containing 40 and 20 μg mL^{-1} of metronidazole (100 μg $mL^{?1}$) followed by varying concentration (1% to 6%) of vanillin and PDAB were added for method A and B, then the solution was heated in a water bath for 15 min., at 60-70 ^{0}C and cooled to room temperature. After 10 min absorbance of each solution was measured at 422 and 494 nm. It was observed that the analytical signal increased with an increase in reagent concentration up to 5% (method A and B) and thereafter decreases with an increase in reagent concentration. Therefore the concentration of vanillin and PDAB selected was 4% and 3% for both the methods. Similarly, by fixing the concentration of vanillin and PDAB as 4% and 3% in a series of solutions containing 40 and 20 μg mL^{-1} of metronidazole, different volumes in the range of 1.0-4.0 mL (method A) and 0.1-1.0 mL (method B) were added. Then the solution was heated in a water bath for 15 min., at 60-70 ^{0}C and cooled to room temperature. After 10 min absorbance of each solution was measured at 422 and 494 nm., it was found that 2.0 mL of 4% vanillin solution for method A and 0.5 mL of 3% PDAB for method B was optimal for the formation of color with maximum intensity. Therefore, 2.0 mL of 4% vanillin and 0.5 mL of 3% PDAB were selected for all measurements.

After optimization of chemical variables, the influence of temperature on the colored product was studied at different temperature (50-80 ^{0}C); it was observed that the obtained colored product were stable up to 70^{0}C. However, no considerable improvements were occurred above 80^{0}C therefore 60-70^{0}C was selected as optimum temperature for both the methods.

Optical characteristics and validation of the method

Optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity, for metronidazole, are given in Table 1. Data of the regression analysis using the least squares method made for the calibration curves are also given in the same table. The accuracy and precision of the method were checked by analyzing six replicate samples within the Beer's law range containing the same amount of each drug. The lower values of RSD indicate the good precision and reproducibility of the method. The validity of the proposed pro-

cedure for the determination of metronidazole in their pure state was checked by analyzing this drug using the proposed method. The results obtained for pure drug were reproducible with low relative standard deviations (RSD). The limit of quantification (LOQ) was determined by taking the ratio of the standard deviation (SD) of the blank with respect to water and the slope of the calibration curve multiplied by the factor ten. This means that LOQ is approximately four times greater than LOD. LOD is well below the lower limit of the Beer's law range.

Applicability of the method

The applicability of the proposed spectrophotometric methods for the assay of metronidazole was tested by analyzing various available commercial formulations. The samples were also analyzed using the official method. The results given in Table 2 of the analysis showed that the data are consistent with the label claim of the formulations. The calibration curves showed a linear response over the concentration ranges used in the general procedures as shown in Figures 2 and 3. The RSD values for the reproducibility and recovery studies show that the method is precise and accurate. In addition it is observed that there is no interference (Table 3) from the excipients used in the formulations. Hence, this method can be adopted for the routine quality control of metronidazole in bulk as well as in formulations.

Table 1. Analytical parameters of spectrophotometric methods.

Parameters	Method A	Method B
λmax (nm)	422	494
Beer's Law limits (μg mL^{-1})	10-65	5-40
Molar absorptivity (L mol^{-1} cm^{-1})	1.775 x 10^4	0.3505 x 10^4
Sandell's sensitivity (μg mL^{-1})	0.0096	0.0488
Regression equation** (Y = bx + c)		
Slope(b)	0.1027	0.1866
Intercept(c)	0.0010	0.0439
Correlation coefficient (r)	0.9997	0.9998
% Relative Standard Deviation (R.S.D)*	0.2169	0.2561
% Range of error (Confidence)*		
0.05 level	0.0086	0.0010
0.01 level	0.00125	0.0014
Limit of Detection (μg mL^{-1})	0.0800	0.0900
Limit of Quantification (μg mL^{-1})	0.3160	0.2997
Stability (hr.)	2	2
Color	yellow	yellow

**Y = bX+c, where Y is the absorbance and X is the concentration of drug in μg mL^{-1}.
*Average of six determinations.

Table 3. Determination of Metronidazole[a] in the presence of excipients.

Excipients	Amount taken (μg mL^{-1})	% Recovery + %RSD[b]
Glucose	20	99.5 + 0.54
Sucrose	20	99.47 + 0.25
Lactose	25	99.64 + 0.56
Dextrose	20	100.1 + 0.21
Talc	25	98.96 + 0.82
Starch	20	99.20 + 0.38
Sodium alginate	15	100.25 + 0.64

[a] 30 μg mL^{-1} of Metronidazole Taken.
[b] Average of six determinations.

Table 2. Determination of Metronidazole in Formulations by the Proposed and Reference Method.

Tablet brand name	Labelled Amount (mg/tablets)	% Found ± SD*		
		Reference method[21,22]	Proposed Methods	
			Method A	Method B
Flagyla	400	99.20 ± 0.52	99.23 ± 0.30	99.34 ± 0.48
Metronb	200	98.92 ± 0.66	100.05 ± 0.37	99.01 ± 0.76

*Average of six determinations ± SD
Marketed by Nicholas Piramal[a] and Alkem[b]

Conclusions

The proposed spectrophotometric methods for the determination of metronidazole are simple, accurate, precise and cheap. The statistical analyses show that the data from the proposed method are in good agreement with those of the reported methods. The color reaction does not require stringent conditions nor any specific reagent or buffer. The colored species was stable for more than two hours, which is sufficient time for the analyst to perform the analysis. Moreover they do not require any pretreatment of the drug and tedious extraction procedure. Hence, the data presented in the manuscript by spectrophotometric methods for the determination of metronidazole in its pure and dosage form demonstrate that the proposed methods are accurate, precise and linear. Thus it can be extended for routine analysis of metronidazole in pharmaceutical industries, hospitals and research laboratories.

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