

## Spectrophotometric Determination of Metronidazole in Pharmaceutical Pure and Dosage Forms using *p*-Benzoquinone

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A simple and accurate spectrophotometric method for the determination of metronidazole in pure and pharmaceutical dosage forms has been developed. The proposed method is based on the reduction of the nitro group to amino group of the drug. This can be achieved by heating a mixture of an alcoholic solution of metronidazole, zinc powder and dilute hydrochloric acid in a water bath at  $90 \pm 5$  °C for 15 min. The cold and clear filtrate reacts with *p*-benzoquinone to develop a purple color, which absorbs maximally at 526 nm. The calibration graph is linear over the concentration range of 15-190  $\mu\text{g ml}^{-1}$  with a molar absorptivity of  $1.09 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ . The proposed method is applied to commercially available pharmaceutical dosage forms and the results are statistically compared with those obtained by the reference method.

**Keywords:** -Metronidazole, *p*-Benzoquinone, Pharmaceutical analysis, Spectrophotometry

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### INTRODUCTION

5-Nitroimidazoles, such as metronidazole, are extensively used as antiamebic, 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. In laboratory tests, metronidazole is found to be 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]. Several

methods have been reported for the determination of metronidazole, including spectrophotometry [8-15,21], polarography [16], titrimetry [17] and HPLC [8]. Most of these are expensive and time consuming. So there is a need to establish a simple, fast and accurate method for the determination of metronidazole in bulk powder and in its dosage forms, which can be used in quality control laboratories. *p*-Benzoquinone was previously reported to be a sensitive reagent for spectrophotometric determination of a considerable number of amine containing medicinal compounds [19,20].

In this work the reduction of metronidazole with zinc dust and HCl, as well as the reaction of its reduced product with *p*-benzoquinone, has been studied to establish the optimum reaction conditions, optical characteristics, precision and accuracy of the proposed method. The standard titrimetric method [17] was also performed for comparing our method statistically and it was found that the results of both methods were not significantly different.

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## EXPERIMENTAL

### Apparatus

A Hitachi spectrophotometer model U 1100 with 1 cm silica cells was used throughout this research work.

### Materials and Reagents

All reagents used were of analytical grade. Metronidazole was kindly supplied by Sami Pharmaceuticals Ltd., Karachi, Pakistan. Metronidazole tablets were purchased from a local market. All water used was double distilled.

### Solutions

Reduced metronidazole standard stock solution was prepared by dissolving 79 mg of metronidazole in 30 ml hot ethanol with 5 ml of 5 N HCl solution and 1 mg zinc dust. The mixture was heated in a water bath at  $90 \pm 5$  °C for 15 min. The residue was then cooled, filtered and washed with ethanol. The volume of the filtrate was made up to 100 ml with ethanol in a volumetric flask to obtain the standard solution with a concentration of  $790 \mu\text{g ml}^{-1}$ .

Eight mg of *p*-benzoquinone was dissolved in a minimum amount of ethanol in a 100 ml volumetric flask and the volume was made up to the mark with ethanol.

### Construction of Calibration Curve

Into a series of twelve 50 ml volumetric flasks containing aliquots of the reduced metronidazole standard stock solution equivalent to  $15\text{-}190 \mu\text{g ml}^{-1}$  (1 to 12 ml), was added 1 ml of *p*-benzoquinone solution and brought the volume up to 50 ml with ethanol. The absorbance was measured at 526 nm against a reagent blank. The calibration curve was constructed by plotting the absorbance against the concentration.

### Procedure for the Assay of Metronidazole in Pharmaceutical Formulations

Twenty tablets of metronidazole were powdered. An accurate quantity of powder equivalent to 100-400 mg of metronidazole was weighed. The reduction of metronidazole was carried out as mentioned above. The resulting filtrate was transferred to a 100 ml volumetric flask and made up to the mark with ethanol. One ml of this reduced metronidazole was further treated with 1 ml of *p*-benzoquinone and the volume

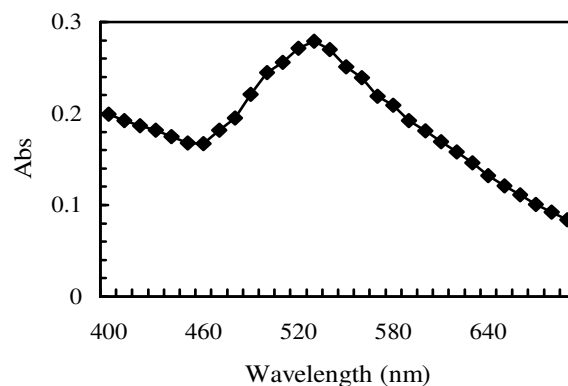


Fig. 1. Absorption spectrum of metronidazole.

was brought up to 50 ml with ethanol in a volumetric flask. The concentration was calculated using the calibration curve.

## RESULTS AND DISCUSSION

### Determination of Absorption Maximum

Reduced drugs when treated with *p*-benzoquinone form a purple color, which absorbs maximally at 526 nm. The absorption spectrum of the product against reagent blank is shown in Fig. 1.

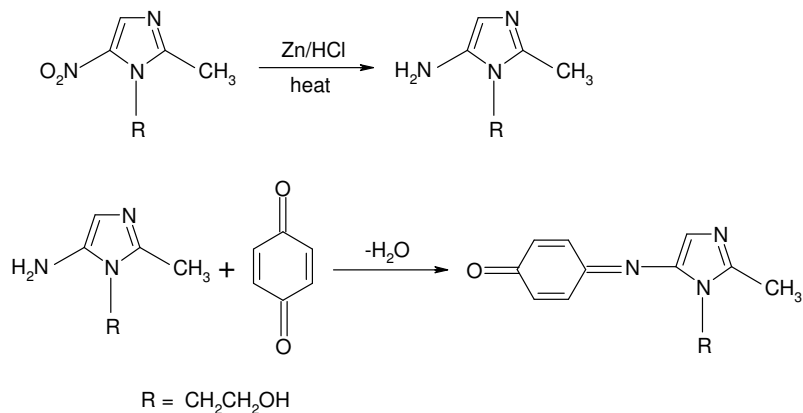
### Reaction Sequence

The reaction between reduced metronidazole and *p*-benzoquinone is shown in Scheme 1.

### Interference Studies

To study the potential interference from the commonly used excipients and other additives such as glucose, lactose, starch, talc, sodium starch glycolate, microcrystalline cellulose, magnesium stearate and ascorbic acid, recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug (metronidazole  $20 \mu\text{g ml}^{-1}$ ), excipients in different concentrations were added and analyzed. Results of the recovery analysis are presented in Table 1. Excipients at the concentrations shown in Table 1 do not interfere with the assay. In addition recoveries in most cases were around 100% and the lower relative standard deviation (RSD) values indicate the good precision of the reference method.

### Spectrophotometric Determination of Metronidazole



*Scheme 1*

**Table 1.** Determination of Metronidazole in the Presence of Excipients

No.	Excipients	Amount taken ( $\mu\text{g ml}^{-1}$ )	Recovery (%) $\pm$ RSD (n = 5)
1	Talc	50	99.7 $\pm$ 0.30
2	Microcrystalline cellulose	300	99.6 $\pm$ 0.25
3	Sodium starch glycolate	100	99.5 $\pm$ 0.25
4	Glucose	50	100.1 $\pm$ 0.30
5	Lactose	300	99.8 $\pm$ 0.40
6	Magnesium stearate	50	99.4 $\pm$ 0.45
7	Starch	200	99.9 $\pm$ 0.25
8	Ascorbic acid	50	99.1 $\pm$ 0.30

**Table 2.** Optical Characteristics and Statistical Data for the Regression Equation of the Proposed Method

Parameters	Values
$\lambda_{\text{max}}$	526
Color	Purple
Stability of color (min)	20
Beer's law limit ( $\mu\text{g ml}^{-1}$ )	15-190
Molar absorptivity ( $\text{l mol}^{-1} \text{cm}^{-1}$ )	$1.09 \times 10^3$
Sandell's sensitivity ( $\mu\text{g ml}^{-1}$ per 0.001 A)	$1.25 \times 10^{-1}$
Regression equation ( $Y^a$ )	
Slope (b)	0.0061
Intercept (a)	0.0016
Correlation coefficient (r)	0.9998
<sup>b</sup> RSD (%)	0.625

<sup>a</sup>  $Y = a + bC$

where C is the concentration of analyte ( $\mu\text{g ml}^{-1}$ ) and Y is absorbance unit.

<sup>b</sup> Calculated from five determinations.

**Table 3.** Determination of Metronidazole Formulations by the Proposed and Reference Method

Formulation	Proposed method		Reference method		t-test	F-test
	<sup>a</sup> Recovery (%)	RSD (%)	<sup>a</sup> Recovery (%)	RSD (%)		
Flagyl	99.20	0.52	99.75	0.30	0.166	2.98
Klint	99.05	0.65	100.05	0.37	0.430	2.97
Metric	98.92	0.66	99.88	0.45	0.411	3.02

<sup>a</sup> Average of 5 independent analyses.

### Optical Characteristics and Validation of the Method

Optical characteristics for metronidazole, such as Beer's law limits, molar absorptivity and Sandell's sensitivity, are given in Table 2. The accuracy and precision of the method was checked by analyzing five replicate samples within the Beer's law range containing the same amount of drug.

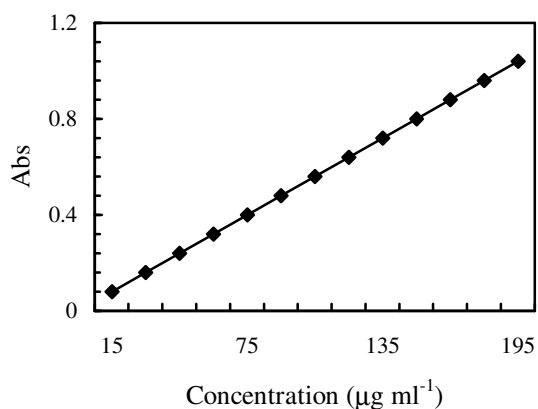
The RSD values are below 0.7%. The lower RSD values indicate the good precision and reproducibility of the method

### Applicability of the Method

The proposed method was successfully applied to the determination of secnidazole in pure and in pharmaceutical dosage forms and the results are compared statistically with the reference method [17] as shown in Table 3. The RSD values are in the range of 0.30 to 0.66% for the reproducibility and recovery studies and show that the proposed method is precise and accurate. The precision and accuracy of the method was further compared statistically using Student's t-test and variance ratio F-test. At a 95% confidence level, the calculated t-values and F-values do not exceed the tabulated values. The calibration curve is linear over the concentration range of 15-190  $\mu\text{g ml}^{-1}$  as shown in Fig. 2.

### CONCLUSIONS

The proposed spectrophotometric method for the determination of secnidazole is simple accurate, precise and cheap. The statistical analyses show that the data from the proposed method are in good agreement with those of the

**Fig. 2.** Calibration curve of metronidazole.

reported method. The color reaction does not require stringent conditions nor any specific reagent or buffer. The color is stable up to 20 min, which is sufficient time for the analyst to perform the analysis. A comparison of the present method with the existing spectrophotometric methods is given in Table 4, which demonstrates the advantages of the proposed method.

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## Spectrophotometric Determination of Metronidazole

**Table 4.** Comparison of Proposed Method with Existing Spectrophotometric Methods

No.	Reagents	$\lambda_{\max}$	Limitations	Reference
1	3-Methyl benzothiazolin-2-one hydrazone (MBTH)	500	MBTH is a costly reagent.	15
2	N-1-naphthyl-ethylenediamine dihydrochloride (NEDA)	520	Involves an additional step of diazotization.	15
3	<i>p</i> -Dimethylaminocinnamaldehyde	510	Condensation reaction. Time consuming.	10
4	$\beta$ -Naphthol	480	Three step processes involves Zn dust reduction, diazotization and coupling.	11
5	Metal and $K_2Cr_2O_7$	502	Involves reduction with Zn-HCl and the use of buffer of pH 2.9 and color formation, and its stability is pH dependent.	12
6	NN-dimethyl- <i>p</i> -phenylenediamine and chloramine-T	540	Involves reduction with Zn-HCl and the use of buffer of pH 7 and color formation and its stability is pH dependent.	13
7	Bromocresol green	654	Involves extraction with $CHCl_3$ and use of buffer of pH 9.5.	14
8	Bromocresol purple	618	Involves extraction with $CHCl_3$ and use of buffer of pH 10.	14
9	8-Quinolinol	500	Reagent is costly and not easily available.	21
10	<i>p</i> -Benzoquinone	526	Two step process. Reagents are cheap and easily available.	This work

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