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Simple UV Spectrophotometric Assay of Metronidazole

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Abstract

Metronidazole is a derivative of benzimidazole. It is being used widely for the treatment of amoebiasis. An efficient, simple and least time consuming spectrophotometric method for the assay of metronidazole has been developed. Comparison of assay of three different brands of metroni-dazole has also been available in public medical stores of Karachi, Pakistan. The assay is based on the ultraviolet UV absorbance maxima at about 340 nm wavelength of metronidazole using water as solvent. A sample of drug was dissolved in water to produce a solution containing metronida-zole. Similarly, a sample of ground tablets of different brands were dissolved in water and various dilutions were made. The absorbance of sample preparation was measured at 340 nm against the solvent blank and the assay was determined by comparing with the absorbance of available brand. The method can be applied for the routine QC quantitation of metronidazole in tablet formulation actively.

Keywords

Metronidazole, Assay, UV Spectrophotometry

Subject Areas: **Inorganic Chemistry, Medicinal Chemistry**

1. Introduction

Metronidazole (**Figure 1**) has been used for the treatment of infections for more than 45 years and is still in use for the treatment of amoebiasis, giardiasis and trichomoniasis. Anaerobic bacterial infections caused by, *Fuso-bacteria*, *Bacteroides* species and *Clostridia* are best treated by metronidazole therapy [1]. Metronidazole is a nitroimidazole derivative. It is a recommended treatment during pregnancy for infections with bacterial vagino-sis and *Trichomonas vaginalis* [2]. The metronidazole treatment protocols commonly employed are three times per day for three to five days [3]. It is typically administered to adults in doses of 250 mg three times a day for five to seven days and 15 mg/kg three times a day for five to seven days in children. From all around the world,

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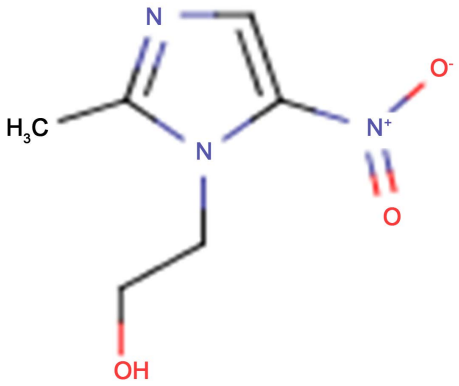

Figure 1. Structure of metronidazole.

therapeutic failure of metronidazole and the drug for giardiasis in humans, have been increasingly reported [4]. It is prescribed commonly for a wide range of non-parasitic infectious diseases. The chances of the development of clinically drug-resistant strains of *Helicobacter pylori HP*, leading cause of gastro intestinal GI cancer may increase by overusing metronidazole for the treatment of parasitic infections. According to previous reports metronidazole toxicity may induce several neurologic side effects, including ataxic gait, peripheral neuropathy, dysarthria, encephalopathy and seizures [5]. We have already performed these types of assay for different drugs for the measurement of active substances [6]-[12].

2. Methodology

UV visible 1601 Shimadzu double beam spectrophotometer was used to spectram measurement Water was used as a solvent for performing assay.

2.1. Wavelength Selection

About 100 ppm of metronidazole solution was accurately prepared in water. This solution was scanned in the 200 - 400 nm UV region. The wavelength maxima (λ_{max}) was observed at 340 nm and this wavelength was adopted for absorbance measurement.

2.2. Standard Stock Solution

Accurately weighed 10 mg of metronidazole standard was transferred to a volumetric flask and add sufficient water to produce 100 ml.

2.3. Sample Preparation

The three different brands (flagyl, gramex, metrozine) were purchased from different medical store located in Karachi, Pakistan. All tablets of each brand have same batch number and were labeled to conatin metonidazole 400 mg per tablet. All the three brands have 5 year shelf life.

The serial number as an identification of purchased brands is given in **Table 1**. 20 tablets of three different brand of metronidazole from the marketed sample were weighed and crushed uniformly with the help of a mortar and pestle. By calculating the average weighed sample powder equivalent to 10 mg of metronidazole was transferred into a volumetric flask containing 10 ml water. The solutions were sonicated for about 5 min and than make up volume up to 100 ml with water.

3. Procedure

After preparation of standard and tablet solutions, strength of solution 100 ppm in 100 ml absorbance of the sample preparation and standard preparation in 1 cm cell at the wavelength of maximum absorbance at about 340 nm, using a spectrophotometer, using the blank solution. Calculate the quantity in mg, of metronidazole per tablet.

4. Results and Discussion

Results are given in Tables 2-4. Pharmaceutical assay of metronidazole was carried out on different brands using spectrophotometer. Table 1 shows brand name, average weight and amount use for preparation of 100 ppm solution, absorbance and % assay of different brands. Table 2 shows the descriptive statistic of drugs with SD and standard error.

Test of hypothesis *i.e.* ANOVA and multiple comparison of different brands of metronidazole are given in Table 3. It shows highly significant difference of p = 0.005 and F value is 8697.899 of all brands with df 2, 12 within and between groups. Table 4 shows multiple comparison of all brands with each other and the results show highly significant p values, p = 0.000 in each case. This proposed method for assay of commercially available metronidazole tablet formulation is very simple, economical, accurate, least time consuming and rapid. It

Table 1. % assay of different brands of Metronidazole.

Brand Name	Serial no.	Average wt of tablet mg	Wt for 100 ppm	Absorbance at 340 nm	% assay
Flagyl	MET-1	511.8	12.7	2.40	100%
Gramex	MET-2	750	18	2.850	118.75%
Metrozine	MET-3	742	18	2.772	115.5%

Table 2. Descriptive statistics of different brands of Metronidazole.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
MET-1	5	2.4060	0.00548	0.00245	2.3992	2.4128	2.40	2.41
MET-2	5	2.8460	0.00548	0.00245	2.8392	2.8528	2.84	2.85
MET-3	5	2.7664	0.00590	0.00264	2.7591	2.7737	2.76	2.77
Total	15	2.6728	0.19822	0.05118	2.5630	2.7826	2.40	2.85

Table 3. ANOVA.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.550	2	0.275	8697.899	0.000
Within Groups	0.000	12	0.000		
Total	0.550	14			

Table 4. Multiple comparisons.

Dependent Variable: absorbance							
	(I) Brands	(J) Brands	Mean Difference (I - J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	MET-1	MET-2	−0.44000*	0.00356	0.000	−0.4495	−0.4305
		MET-3	−0.36040*	0.00356	0.000	−0.3699	−0.3509
	MET-2	MET-1	0.44000*	0.00356	0.000	0.4305	0.4495
		MET-3	0.07960*	0.00356	0.000	0.0701	0.0891
	MET-3	MET-1	0.36040*	0.00356	0.000	0.3509	0.3699
		MET-2	−0.07960*	0.00356	0.000	−0.0891	−0.0701

*The mean difference is significant at the 0.05 level.

can be used for routine QC quality control analysis in the API, and tablet formulation.

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