INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

VALIDATED SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND FURAZOLIDONE IN PURE AND IN TABLET DOSAGE FORM

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Article Received on: 15/02/12 Revised on: 28/03/12 Approved for publication: 11/04/12

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ABSTRACT
Two methods are developed for simultaneous estimation of metronidazole and furazolidone in pure and in tablet dosage form by using distilled water as a solvent. Quantitation was carried out by the proposed methods namely simultaneous equation (Method I) and absorbance ratio (Method II). The wavelengths selected for Method I were 320.2 nm and 367.0 nm i.e. the respective λmax of both the drugs. In Method II two wavelengths 352.4 nm, the isobestic point and 320.2 nm λmax of metronidazole were selected. Both the drugs obey Beer's law in the range of 5-30 µg/ml for metronidazole and 5-50 µg/ml for furazolidone. The methods are simple, rapid, accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of metronidazole and furazolidone in pure and in tablet dosage form.
KEY WORDS: Metronidazole, Furazolidone, Beer's law, Simultaneous equation method, Absorbance ratio method, Validation.

INTRODUCTION

Metronidazole chemically is 2-methyl-5-nitroimidazol-1-ethanol. It is a nitroimidazole antibiotic medication used particularly for anaerobic bacteria and protozoa. Metronidazole is an antibiotic, amebicide, and antiprotozoal. It is the drug of choice for first episodes of mild-to-moderate Clostridium difficile infection[1]. Furazolidone, chemically 3-{[(5-nitro-2-furyl)methylene]amino}-1,3-oxazolidin-2-one[2], and It is used to treat diarrhoea and enteritis caused by bacteria or protozoan infections[3]. A combination of these drugs is available as tablets for clinical practice. Their combination is used for the treatment of anaerobic infections and mixed infections[4]. United States Pharmacopoeia describes HPLC and non aqueous titration methods for the assay of metronidazole[5] and a UV spectrophotometric assay procedure for furazolidone[6]. A survey of literature reveals that various methods like GC-FID[7], spectrophotometric determination[8], HPLC-PDA[9], assay for its quantification in plasma and gastric juice fluids[10,11] have been reported for assay of metronidazole. HPLC method have been reported for the determination of metronidazole and furazolidone in pharmaceutical preparation[12]. Determination of furazolidone by UV spectrometry in combination was done with other drugs[13]. However there is no UV-spectrophotometric method for the simultaneous determination of the metronidazole and furazolidone in combination. An attempt was made to develop accurate, precise, reproducible and economical methods for the simultaneous estimation of both these drugs in combined dosage form. These methods are validated as per ICH guidelines[14].

MATERIALS AND METHODS

Materials

UV-visible double beam spectrophotometer, JASCO V-630 with spectral bandwidth of 0.5 nm, wavelength accuracy of ± 0.2 nm and a pair of 10 mm matched quartz cells were used. The commercially available tablet, Dependal-M (Label claim: Metronidazole 300 mg, Furazolidone 100 mg) was procured from local market.

Selection of common solvent

After assessing the solubility of drugs in different solvents distilled water has been selected as common solvent for developing spectral characteristics.

Preparation of standard stock solution

The standard stock solution of both metronidazole and furazolidone were prepared separately by dissolving 25mg each of drug in 100ml volumetric flask using distilled water as a solvent to give a concentration of 250 µg/ml.

Absorption maximum (λmax)

The stock solution were suitably diluted with distilled water so as to contain 10µg/ml of metronidazole and 10µg/ml of furazolidone respectively. The solutions were scanned in the UV region between 500-200 nm and found that metronidazole exhibited λmax at 320.2 nm (Figure 1) and furazolidone exhibited λmax at 367.0 nm (figure 2).

Beer's law concentration range

The stock solutions were suitably diluted with distilled water to get concentration range from 5-250µg/ml for metronidazole and furazolidone. The solutions were scanned in the UV region between 500-200nm and their absorbances were measured at respective maxima (λmax) points. Using the absorbance values against concentrations plotted the calibration curve. From the graphs it was found metronidazole and furazolidone obeys Beer's law between 5-30 µg/ml and 5-50 µg/ml respectively. The regression analysis was carried out for the regression line which estimates the degree of linearity.

Stability of absorbance

The stability of the solutions was checked by measuring the absorbance at regular intervals of time. It was observed that the absorbance remained stable for a period of more than 120 minutes which is sufficient for proposed work.

Method I: Simultaneous equation method

Simultaneous equation method was based on the absorption of drugs at the wavelength maximum of each other. Two wavelengths selected for the development of the simultaneous equations were 320.2 nm for metronidazole and 367.0 nm for furazolidone respectively. The absorptivity values determined for metronidazole are 0.0575 (ax1), 0.0092 (ax2) and for furazolidone are 0.0096 (ay1), 0.0244 (ay2) at 320.2 nm and 367.0 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of drugs .

$$C_{MET} = \frac{A_2 \times 0.0096 - A_1 \times 0.0244}{-0.001315} \quad \text{.....Equation 1}$$

$$C_{FUR} = \frac{A_1 \times 0.0092 - A_1 \times 0.0575}{-0.001315} \quad \text{...Equation 2}$$

$$C_{MET} = \frac{Q_M - 0.432}{2.004} \times \frac{A_1}{0.0236} \quad \text{...Equation 3}$$

$$C_{FUR} = \frac{Q_M - 2.436}{-2.004} \times \frac{A_1}{0.0222} \quad \text{....Equation 4}$$

Where, C_{MET} and C_{FUR} are concentration of metronidazole and furazolidone in µg/ml respectively. A_1 and A_2 are absorbance of sample at 320.2 nm and 367.0 nm respectively.

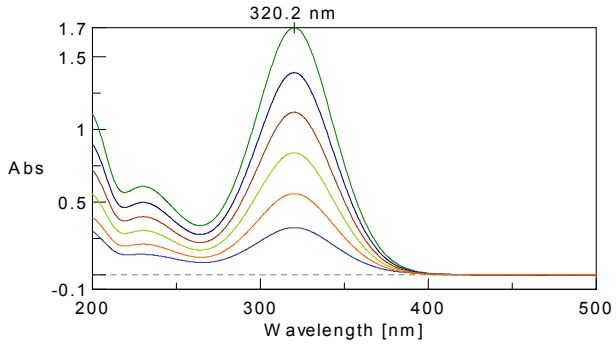

Figure 1: Overlain spectra of metronidazole

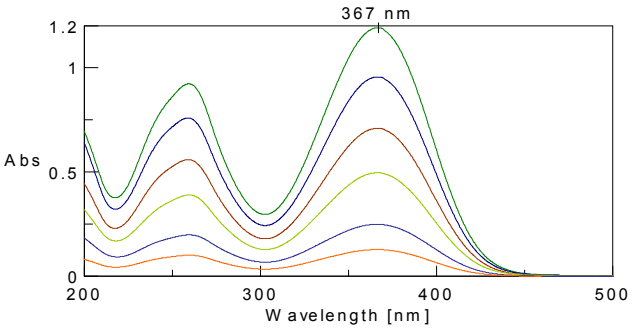

Figure 2: Overlain spectra of furazolidone

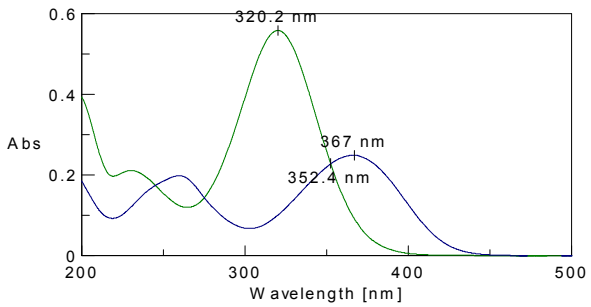

Figure 3: Overlain spectra of metronidazole and furazolidone

Method 2 : Absorbance Ratio / Q Analysis Method

Absorbance ratio uses the ratio of absorbances at two selected wavelengths, one is an isoabsorptive point and other is λmax of one of the two components. From overlain spectra of two drugs, it is evident that metronidazole and furazolidone shows an isoabsorptive point at 352.4 nm, The second wavelength use is 320.2nm, which is λmax of metronidazole (Figure 3) are selected for the formation of Q absorbance equation (Equation 3 and 4). The absorptivity values determined for metronidazole are 0.0236 (ax1), 0.0575 (ax2) and for furazolidone are 0.0222 (ay1), 0.0096 (ay2) at 352.4 nm and 320.2 nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of drugs. The concentration of two drugs in mixture can be calculated from following equation,

Q_M, Q_X, and Q_Y were obtained as bellow:

$$Q_M = \frac{A_2}{A_1}$$

$$Q_x = \frac{ax_2}{ax_1}$$

$$Q_y = \frac{ay_2}{ay_1}$$

Where, C_{MET} and C_{FUR} are concentration of metronidazole and furazolidone in µg/ml respectively.A_1 and A_2 are absorbance of sample at 352.4 nm and 320.2 nm respectively.

Estimation of drugs from tablet dosage form sample solution

Twenty tablets were finely powdered. An accurately weighed quantity of powder equivalent to about 25mg of metronidazole was transferred to a 100mL volumetric flask. The content of the flask was mixed with distilled water and shaken to dissolve the active ingredients and then made up to the volume with the same solvent. The solution was filtered with Whatmann filter paper No:41 and the filtrate was further diluted with distilled water to give a final drug concentration of 20.0 µg/ml and 6.66 µg/ml of metronidazole and furazolidone respectively. Analysis procedure was repeated six times with tablet formulation. The results of tablet analysis are reported in Table 2.

VALIDATION OF THE DEVELOPED METHODS

Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method I and II, the Beer- Lambert's concentration range was found to be 5-30 µg/mL for metronidazole (figure 4) and 5-50 µg/mL for furazolidone (figure 5). The linearity data for both methods are presented in Table 1.

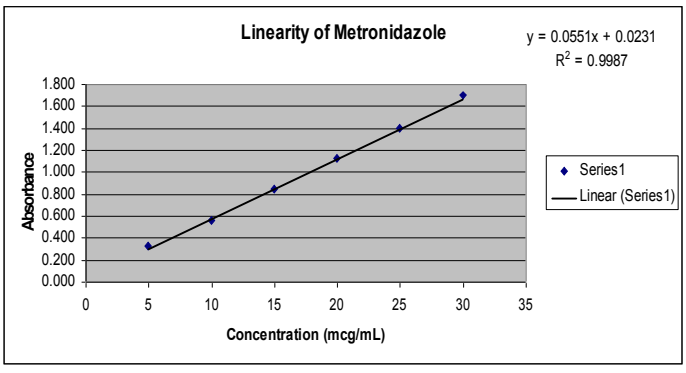

Figure 4: Linearity of metronidazole

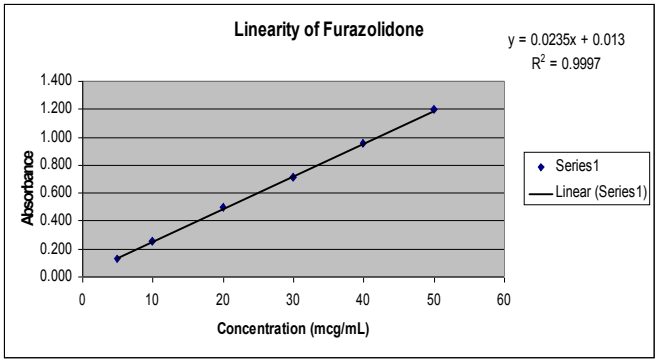

Figure 5: Linearity of furazolidone

Table 1: Optical Characteristics Data of Metronidazole and Furazolidone

Parameters	Values			
	MET	FUR	MET at isobestic point	FUR at isobestic point
Working lmax	320.2 nm	367.0nm	352.4 nm	352.4 nm
Beer's law limit (µg/ml)	5-30	5-50	5-30	5-50
Absorptive Value	0.0575	0.0244	0.0236	0.0222
Correlation coefficient*	0.9987	0.9997	0.9812	0.9971
Intercept*	0.0231	0.013	0.0425	0.0146
Slope*	0.0551	0.0235	0.0616	0.019

Accuracy

To check the accuracy of the proposed methods, recovery studies were carried out at 80,100, and 120 % of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are quoted in Table 2.

Precision

Repeatability

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times with tablets formulation. The standard deviation, coefficient of variation and standard error was calculated. The results of statistical evaluation are given in Table 2.

Intermediate Precision (Interday and Intraday precision)

The interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively. The results of the same are presented in Table 3.

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

The LOD and LOQ of metronidazole and furazolidone by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10 /S, respectively, where S is the slope of the calibration curve and is the standard deviation of response. The results of the same are shown in Table 3.

RESULTS AND DISCUSSION

Linearity range for metronidazole and furazolidone is 5-30 µg/mL and 5-50 µg/mL at respective selected wavelengths. The coefficient of correlation for metronidazole at 320.4 nm and for furazolidone at 367.0 nm is 0.9987 and 0.9997 respectively. Both drugs showed good regression values at their respective wavelengths and the results of recovery study revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed methods. Percentage estimation of metronidazole and furazolidone from tablet dosage form by method I is 99.845 and 99.251 and by method II is 99.844 and 99.358 respectively with standard deviation <2.0 (Table 2). The validity and reliability of proposed methods were assessed by recovery studies. Sample recovery for both the methods is in good agreement with their respective label claims, which suggest non interference of formulation additives in estimation (Table 3). Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval of time and inter assay precision. The standard deviation, coefficient of variance and standard error were calculated for metronidazole and furazolidone. The results were mentioned in Table 2. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter day precision study for both the methods % COV are not more than 2.0% indicates good repeatability and intermediate precision (Table 2). The LOD values are 0.172, 0.348 µg/mL while LOQ values are 0.517, 1.044 µg/mL in method I and the LOD values are 0.249, 0.519 µg/mL while LOQ values are 0.750, 1.560 µg/mL in method II for metronidazole and furazolidone respectively (Table 3). Low values of LOD and LOQ indicates good sensitivity of proposed methods.

Table 2: Analysis Data of Tablet Formulation, Statistical Validation and Recovery studies

Method	Drug	Label Claim mg/tab	Label Claim (%)	S.D.*	% COV	S.E.*	Amount Added		% Recovery # ± S.D
							%	mg/mL	
I	MET	300	99.845	0.066	0.027	0.027	80	240	99.884±0.156
							100	300	99.808±0.397
							120	360	98.748±0.240
	FUR	100	99.251	0.887	0.366	0.364	80	80	99.664±0.575
							100	100	100.185±0.332
							120	120	98.508±0.476
II	MET	300	99.844	0.079	0.032	0.032	80	240	99.817±0.096
							100	300	99.963±0.175
							120	360	99.861±0.331
	FUR	100	99.358	0.587	0.242	0.241	80	80	100.029±.0501
							100	100	99.708±0.325
							120	120	100.184 ±0.542

MET: metronidazole, FUR: furazolidone, S.D.: Standard deviation, COV: Coefficient of variation,S.E.: Standard error, *Average of six estimation of tablet formulation, # Average of three estimation at each level of recovery.

Table 3: Validation Parameters

Method	Drug	LOD* µg/ml	LOQ* µg/ml	Precision(%COV)			
				Intraday n=3	Interday*		
					First day	Second day	Third day
I	MET	0.172	0.517	0.517	0.874	0.826	0.943
	FUR	0.348	1.044	0.647	0.980	0.547	0.745
II	MET	0.249	0.750	0.784	0.743	0.971	0.847
	FUR	0.519	1.560	0.987	0.988	0.907	0.828

MET: metronidazole, FUR: furazolidone, COV: Coefficient of variation, * Average of six determination.

ACKNOWLEDGEMENT

The authors are grateful to P.D.V.V.P.F'S College of Pharmacy, Ahmednagar, India for providing necessary facilities to carry out this work.

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Source of support: Nil, Conflict of interest: None Declared