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

Chemate Satyam Z.*, Dongare Umesh S., Jadhav Shweta A., Jadhav Manisha B. 
PDVVPF’s College of Pharmacy, Post - MIDC, Vilad Ghat, Ahmednagar 414 111, (MS), India

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

*Email: satyam_chemat@rediffmail.com

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 $\lambda_{max}$ of both the drugs. In Method II two wavelengths 352.4 nm, the isobestic point and 320.2 nm $\lambda_{max}$ of metronidazole were selected. Both the drugs obey Beer’s law in the range of 5-30 $\mu$g/ml for metronidazole and 5-50 $\mu$g/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. Furazolidone, chemically 3-\{[(5-nitro-2-furyl)methylene]amino\}-1,3-oxazolidin-2-one, and it is used to treat diarrhoea and enteritis caused by bacteria or protozoan infections. 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. United States Pharmacopoeia describes HPLC and non aqueous titration methods for the assay of metronidazole and a UV spectrophotometric assay procedure for furazolidone. A survey of literature reveals that various methods like GC-FID, spectrophotometric determination, HPLC-PDA, assay for its quantification in plasma and gastric juice fluids have been reported for assay of metronidazole. HPLC method has been reported for the determination of metronidazole and furazolidone in pharmaceutical preparation. Determination of furazolidone by UV spectrometry in combination was done with other drugs. 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.

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 nm 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 $\mu$g/ml.

Absorption maximum ($\lambda_{max}$)
The stock solution were suitably diluted with distilled water so as to contain 10$\mu$g/ml of metronidazole and 10$\mu$g/ml of furazolidone respectively. The solutions were scanned in the UV region between 500-200nm and found that metronidazole exhibited $\lambda_{max}$ at 320.2 nm (Figure 1) and furazolidone exhibited $\lambda_{max}$ 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 5-250$\mu$g/ml for metronidazole and furazolidone. The solutions were scanned in the UV region between 500-200nm and their absorbances were measured at respective maxima ($\lambda_{max}$) points. Using the absorbance values against concentrations plotted the calibration curve. From the graphs it was found the calibration plots for metronidazole and furazolidone obeys Beer’s law between 5-30 $\mu$g/ml and 5-50 $\mu$g/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.
Absorbance ratio uses the ratio of absorbances at two selected wavelengths, one is an isoabsorptive point and other is $\lambda_{max}$ of one of the two components. From overlain spectra of two drugs, it is evident that metronidazole and furazolidone shows an isosbestic point at 352.4 nm. The second wavelength use is 320.2nm, which is $\lambda_{max}$ 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, QM, QF, and Qx were obtained as bellow:

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

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

**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 $\lambda_{max}$ of one of the two components. From overlain spectra of two drugs, it is evident that metronidazole and furazolidone shows an isosbestic point at 352.4 nm. The second wavelength use is 320.2nm, which is $\lambda_{max}$ 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, $Q_M$, $Q_F$, and $Q_x$ were obtained as bellow:

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

Where, $C_{MET}$ and $C_{FUR}$ are concentration of metronidazole and furazolidone in $\mu$g/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 $\mu$g/ml and 6.66 $\mu$g/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 found to be 5-30 $\mu$g/mL for metronidazole (figure 4) and 5-50 $\mu$g/mL for furazolidone (figure 5). The linearity data for both methods are presented in Table 1.
The interday and intraday precision was determined by assay precision. Intermediate Precision (Interday and Intraday precision) was performed three times with tablets formulation. The results of statistical evaluation are shown in Table 2. Repeatability was performed for six times with tablets formulation. The results of statistical evaluation are given in Table 2.

**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. Low values of LOD and LOQ indicates good sensitivity of proposed methods.

**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 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

**RESULTS AND DISCUSSION**
Linear 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 range 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% (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.

![Figure 5: Linearity of furazolidone](chart.png)

**Table 1: Optical Characteristics Data of Metronidazole and Furazolidone**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
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<td>Working Imax (nm)</td>
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<td>Beer’s law limit (μg/mL)</td>
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<td>Absorptive Value</td>
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<td>Slope*</td>
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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.

REFERENCES

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

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

<table>
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<tr>
<th>Method</th>
<th>Drug</th>
<th>Label Claim mg/tab</th>
<th>S.D.* % COV</th>
<th>S.E.* %</th>
<th>Amount Added mg/mL</th>
<th>% Recovery ± S.D</th>
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<td>99.70±0.325</td>
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</table>

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

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<tr>
<th>Method</th>
<th>Drug</th>
<th>LOD* µg/ml</th>
<th>LOQ* µg/ml</th>
<th>Intraday % COV</th>
<th>Interday % COV</th>
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<td></td>
<td></td>
<td></td>
<td>First day</td>
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<tr>
<td>I</td>
<td>MET</td>
<td>0.172</td>
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<td>0.517</td>
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