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VALIDATED DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF METRONIDAZOLE AND FURAZOLIDONE IN PURE AND IN TABLET DOSAGE FORM

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Abstract

The simple, precise, accurate and economical UV spectrophotometric First Order Derivative method is developed for estimation of metronidazole and furazolidone in pure and tablet dosage form by using distilled water as a solvent. Quantitation was carried out by the proposed method. The spectrum of Metronidazole and Furazolidone was derivatised into first order derivative For Metronidazole. the amplitude of trough at 299.6 nm, crest at 207.8 nm, 246.6 nm and 340.8 nm for D_1 were measured. For Furazolidone the amplitude of trough at 230.8 nm and 332.2 nm and crest at 272.6 nm and 397.0 nm for D_1 were measured. In D_1 method the drug showed linearity in the concentration range of 5-30µg/ml. The method can be used for routine quantitative analysis of metronidazole and furazolidone in pure and tablet dosage form.

Key Words: Metronidazole, Furazolidone, First order derivative method and Validation.

Introduction

Metronidazole chemically is 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol. It is a nitroimidazole antibiotic medication used particularly for anaerobic bacteria and protozoa. Metronidazole is an antibiotic, amebicide, and antiprotozoal. [1] It is the drug of choice for first episodes of mild-to-moderate Clostridium difficile infection.[2]. Furazolidone, chemically 3-{[(5-nitro-2-furyl)methylene]amino}-1,3-oxazolidin-2-one[3], and It is used to treat diarrhoea and enteritis caused by bacteria or protozoan infections[4]. 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[5].

A survey of literature reveals that various methods like GC-FID[6,8], HPLC with UV detection[7], HPLC PDA/MS[9] ,UPLC-MS[10] assay for its quantification in plasma and gastric juice fluids[11] have been reported for assay of metronidazole. United States Pharmacopoeia [12] describes HPLC and non aqueous titration methods for the assay of metronidazole. Several methods have been reported for the determination of metronidazole including Spectrophotometry[13,14,15]. Determination of furazolidone by UV spectrometry in combination was done with other drugs[16]. However there is no spectrophotometric method for the first order Derivative 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.[17]

Materials and Methods

Materials

UV-visible double beam spectrophotometer, JASCO V-630 with spectral bandwidth of 1 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 separately 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 20 µg/ml of metronidazole and 20 µg/ml of furazolidone respectively. The solutions were scanned in the UV region between 400-200 nm and found that metronidazole exhibited λmax at 320.2 nm(fig 1) and furazolidone exhibited λmax at 367.0 nm (fig 2) .

First order Derivative Method

The same spectrum were derivatised into first order derivative. For Metronidazole. the amplitude of trough at 299.6 nm, crest at 207.8 nm, 246.6 nm and 340.8 nm for D_1 were measured. For Furazolidone the amplitude of trough at 230.8 nm and 332.2 nm and crest at 272.6 nm and 397.0 nm for D_1 were measured. The linear regression equations for D1 were calculated for Metronidazole at 207.8 nm y=0.0012x+0.0037 (R^2 = 0.9876), at 246.6 nm y=0.0004x+0.0002 (R^2 = 0.9991), at 299.6 nm y=-0.0013x-0.0002 (R^2 = 0.9994) and at 340.8 nm y=0.0014+0.0002 (R^2 = 0.9986). at fig. no. 1 The linear regression equations for D1 were calculated for Furazolidone at 230.8 nm y=-0.0008x+0.003 (R^2= 0.9893), at 272.6 nm y=0.0009x-0.0031 (R^2 = 0.9925), at 332.2 nm y=-0.0008x-0.0025 (R^2 = 0.9913) and at 397.0 nm y=0.001-0.0032 (R^2 = 0.9928).at fig.no. 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 400-200nm 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 5-30 µg/ml respectively. The regression analysis was carried out for the regression line which estimates the degree of linearity.

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 whatman 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 3.

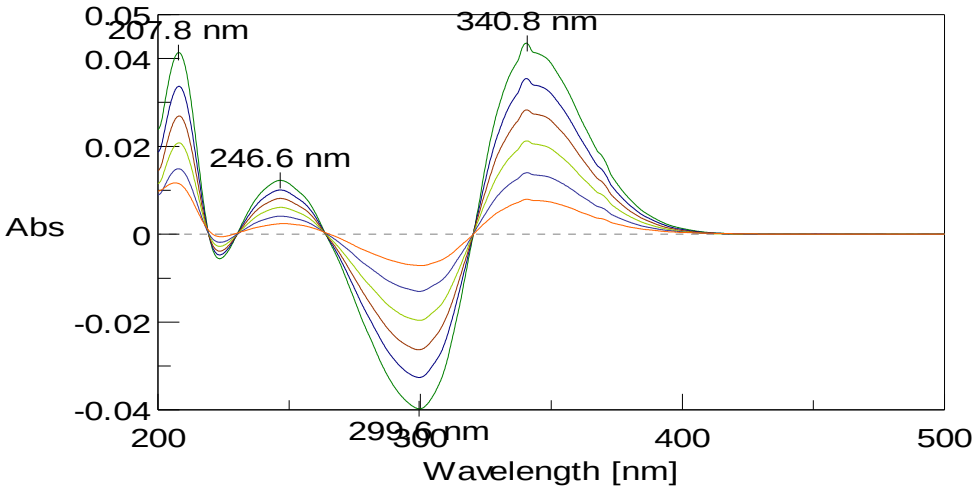

Fig No-1: Overlain spectra of metronidazole (D_1).

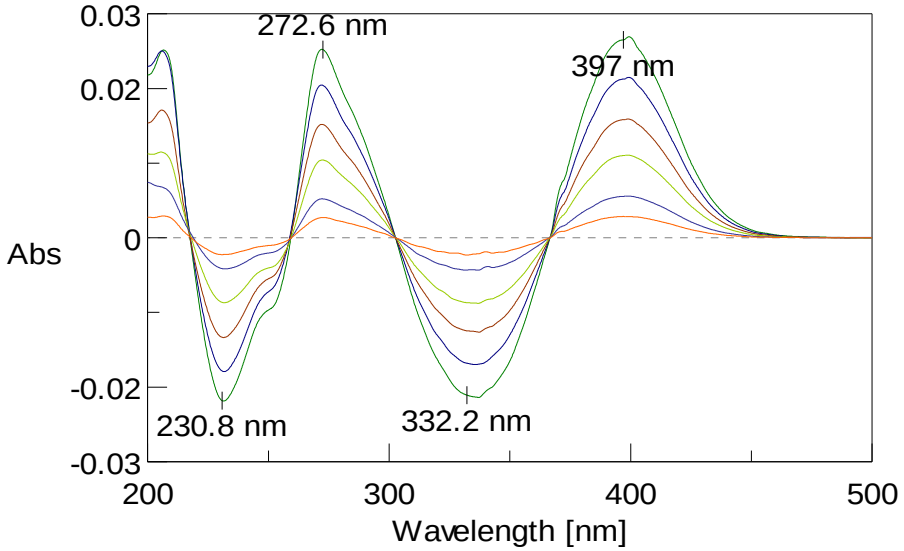

Fig No-2: Overlain spectra of furazolidone (D_1).

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 (fig 3) and 5-50 µg/mL for furazolidone (fig 4). The linearity data for both methods are presented in Table 1 and Table 2.

Accuracy

The accuracy of the methods was determined by performing recovery studies on tablet formulation and for prepared solutions containing known amount of drug by standard addition method in which preanalyzed samples were taken

and standard drug was added at three different levels 80%.100% and 120% as per ICH guidelines. The recovery study performed three times at each level. The results are shown in Table No 3

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 3

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 4

Results and Discussion

First Order Derivative method is developed for estimation of metronidazole and Furazolidone and the linear regression equations for D1 were calculated for Metronidazole at 207.8 nm y=0.0012x+0.0037 (R^2 = 0.9876), at 246.6 nm y=0.0004x+0.0002 (R^2 = 0.9991), at 299.6 nm

y=-0.0013x-0.0002 (R^2 = 0.9994) and at 340.8 nm y=0.0014+0.0002 (R^2 = 0.9986). The linear regression equations for D1 were calculated for Furazolidone at 230.8 nm y=-0.0008x+0.003 (R^2= 0.9893), at 272.6 nm y=0.0009x-0.0031 (R^2 = 0.9925), at 332.2 nm y=-0.0008x-0.0025

(R^2 = 0.9913) and at 397.0 nm y=0.001-0.0032 (R^2 = 0.9928). Results of estimation of marketed tablet formulations were found to be 100.2303±0.2314 with their SD less than 2. Recovery studies were carried out by addition of known amount of standard drug (80,100 and 120% of labeled claim of a tablet) to the preanalysed tablet solution. The % recovery was found to be between 98- 102%, which indicates accuracy and reliability of the validated method as well as noninterference from excipients to the developed method. The intraday and inter day assay was within 2%. The methods were then validated statistically as per the ICH guidelines which yielded good results concerning range, precision, accuracy, specificity and repeatability.

Both the drugs obey Beer's law in the range of 5-30 µg/ml metronidazole and 5-50 µg/ml for furazolidone.

Table-1: Linearity of Metronidazole.

Conc.	207.8 nm	246.6nm	299.6nm	340.8nm
5	0.01154	0.00238	-0.007114	0.00794
10	0.01491	0.0041	-0.01301	0.01399
15	0.02079	0.00611	-0.01961	0.02124
20	0.02689	0.008145	-0.02627	0.02829
25	0.03366	0.01006	-0.03261	0.0354
30	0.04134	0.01225	-0.03973	0.04342

Table-2: Linearity of Furazolidone.

Conc.	230.8 nm	272.6 nm	332.2 nm	397 nm
5	-0.002258	0.002705	-0.002336	0.00285
10	-0.004134	0.005205	-0.00432	0.005544
15	-0.00857	0.0104	-0.008703	0.01101
20	-0.01333	0.01515	-0.01247	0.0158
25	-0.01786	0.0204118	-0.016811	0.02124
30	-0.02182	0.0252	-0.02109	0.02651

Table-3: Analysis Data of Tablet Formulation, Statistical Validation and Recovery studies

Drug	**λmax**	Lable Claim	Lable Claim (%)*	S.D.*	% COV*	S.E.	Amt Added		% Recovery ± S.D #.
							%	mg/mL	
MET	207.8	300	99.8287	0.1227	0.0504	0.0503	80	240	99.576±0.015
							100	300	99.523±0.143
							120	360	99.386±0.141
	246.6	300	99.8532	0.4212	0.1730	0.1727	80	240	99.433±0.195
							100	300	99.633±0.604
							120	360	99.7766±0.369
	299.6	300	100.6035	0.1390	0.0566	0.0570	80	240	100.376±0.140
							100	300	99.9±0.305
							120	360	100.463±0.1504
	340.8	300	100.6359	0.2427	0.0988	0.0995	80	240	99.23±0.141

							100	300	100.456±0.119
							120	360	100.786±0.173
FUR	230.8	100	100.1676	0.03065	0.0125	0.0126	80	80	99.28±0.2116
							100	100	100.6433±0.1106
							120	120	100.566±0.3808
	272.6	100	100.2572	0.2137	0.0874	0.0876	80	80	99.123±0.2122
							100	100	100.543±0.10
							120	120	100.883±0.087
	332.2	100	100.7507	0.3561	0.1460	0.1449	80	80	98.846±0.10
							100	100	100.843±0.126
							120	120	100.34±0.269
	397.0	100	100.4662	0.2059	0.0844	0.0840	80	80	99.486±0.1159
							100	100	100.806±0.155
							120	120	100.8166±0.122

*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-4: 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	0.487	0.517	0.874	0.826	0.943
	FUR	0.158	0.386	0.647	0.980	0.547	0.745
II	MET	0.167	0.299	0.784	0.743	0.971	0.847
	FUR	0.181	0.491	0.987	0.988	0.907	0.828

Table-5: Optical Characteristic for Metronidazole.

Parameter	Values			
	Metronidazole (D1)			
Working λ max in nm	207.8	246.6	299.6	340.8
Correlation Coefficient*	0.9876	0.9991	0.9994	0.9986
Intercept*	0.0037	0.0002	-0.0002	0.0002
Slope*	0.0012	0.0004	-0.0013	0.0014

Table-6: Optical Characteristic for Furazolidone.

Parameter	Values			
	Furazolidone (D1)			
Working λ max in nm	230.8	272.2	332.2	397.0
Correlation Coefficient*	0.9893	0.9925	0.9913	0.9928
Intercept*	0.003	-0.0031	0.0025	-0.0032
Slope*	-0.0008	0.0009	-0.0008	0.001

* Average of six estimation

Conclusion

A new analytical method has been developed to be routinely applied to determine Furazolidone and Metronidazole in pharmaceutical dosage form. In this study, the developed procedure has been evaluated over the specificity, linearity, accuracy, precision and repeatability in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate. Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

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