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DEVELOPMENT AND VALIDATION OF A NEW UV SPECTROPHOTOMETRIC SIMULTANEOUS EQUATION METHOD FOR ESTIMATION OF CEFIXIME TRIHYDRATE AND ORNIDAZOLE IN A COMBINED TABLET DOSAGE FORM.

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ABSTRACT

A new UV spectrophotometric simultaneous equation method was developed and validated for the estimation of Cefixime Trihydrate and Ornidazole in pure and pharmaceutical dosage form. Cefixime Trihydrate (Cefix) shows λ_{max} at 288nm and Ornidazole (Orni) shows at λ_{max} 320nm in mixture of methanol and water. The developed method was found to show linearity in concentration (conc.) range of 2-30 µg/ml for Cefixime Trihydrate and Ornidazole with the value of correlation coefficient (R^2) 0.998 and 0.999 respectively. The percent Relative Standard Deviation (% RSD) of inter and intra-day precision studies were found to be within limits of NMT 2% concluding that the present method is precise as per ICH guidelines Q2 (R_1).[1] The developed method can be used for routine estimation of Cefixime Trihydrate and Ornidazole in bulk and pharmaceutical dosage form.

KEYWORDS: UV spectrophotometry, Cefixime Trihydrate, Ornidazole, Absorbance.

INTRODUCTION

Cefixime (CEF) is an oral third generation cephalosporin antibiotic. Chemically, it is (6R,7R)-7- {[2-(2-amino-1,3-thiazol-4-yl)2(carboxymethoxyimino)acetyl]amino-3ethenyl-8-oxo-5-thia-1- azabicyclo-[4.2.0]oct-2-ene-carboxylicacid, clinically used in the treatment of susceptible infections including gonorrhoea, otitismedia, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections.[2]

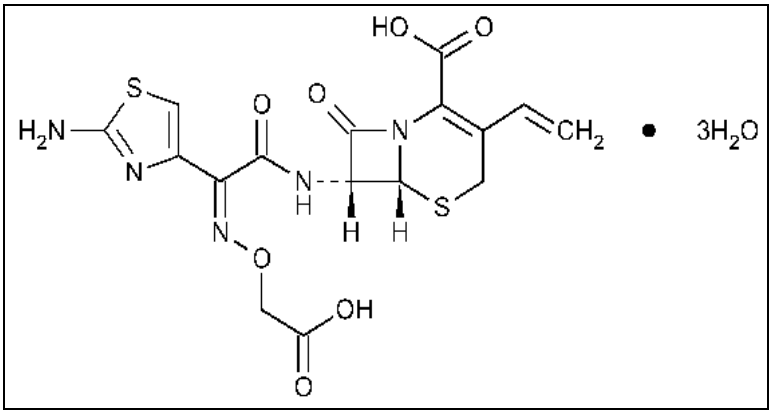

Figure 1: Structure of Cefixime Trihydrate.

Ornidazole7-8 (ORD), chemically 1-chloro-3-(2-methyl-5-nitro-imidazol-1yl)propan-2-ol, is an antimicrobial agent used in treatment of susceptible protozoal infections and anaerobic bacterial infection.[3]

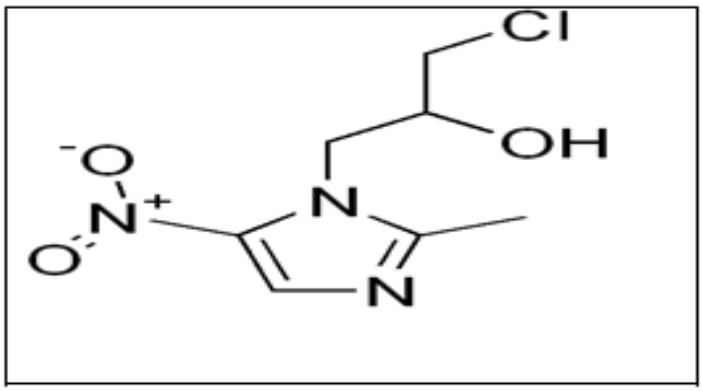

Figure 2: Structure of Ornidazole.

Both the drugs are marketed as combined dose tablet formulation in the ratio of 200:500mg Cefix: Orni. Literature survey revealed that there are few methods reported for the simultaneous estimation of these drugs, individually or with other drugs UV-spectrophotometry,[4-10] are available. Hence present study aim to developing a precise, linear, simple, rapid, validated and cost effective. UV- spectrophotometry method for the simultaneous estimation of these drugs in combined dosage forms.

MATERIALS AND METHODS

INSTRUMENTATION AND APPARATUS

UV analysis was carried out on SHIMADZU UV-2700 UV-VIS Spectrophotometer using UV Probe Ver 2.51 software and all weight measurements were taken on WENSAR

ELECTRONIC BALANCE MAB 220 at room temperature. Methanol (LR) and Deionised water was used for study.

MATERIALS

Cefixime Trihydrate (Cefix) was kindly provided as a gift sample by Maxim Phramaceuticals Pvt Ltd Pune Maharashtra and Ornidazole (Orni) by Cadila Healthcare Ltd, Kundaim, Goa. The pharmaceutical formulation Zifi-OZ (Cefixime Trihydrate-200mg, Ornidazole-500mg) used in this study was procured from local market, which was manufactured by Innova Captab Pvt Ltd.

METHOD DEVELOPMENT

Preparation of standard stock solution

Standard stock solution (1000 µg/mL) of Cefix and Orni was prepared separately by carefully dissolving weighed 25 mg of drug in 2.5ml of methanol in 25 mL volumetric flask and diluting up to the mark with water. 10mL of these solutions were diluted separately up to 100 mL with water to get working stock solution (100 µg/mL). Then, this stock solution was used to prepare further required concentrations.

Determination of Wavelength of Maximum Absorbance (λ_{max})

Solutions of 10 µg/mL of both drugs were prepared from working stock solution and scanned in the range of 200 nm to 400 nm against water as blank. Cefix showed λ_{max} at 288nm and Orni at 320nm.

Preparation of Sample Solutions from Standard Stock Solution

The sample solutions of various concentrations were prepared from the standard stock solution by diluting aliquots of working stock solutions appropriately.

Calibration Curve (Linearity)

A calibration curve was plotted over a concentration range of 2-30 µg/mL for Cefix and Orni. Accurately measured working stock solution of Cefix and Orni (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8 and 3ml) were transferred separately to series of 10 mL volumetric flask and diluted up to the mark with water. The absorbance (Abs.) of both solutions was taken at their respective absorbance maxima. The calibration curves were constructed by plotting concentration against absorbance where each reading was an average of three determinations.

Methodology

Standard drug solutions having concentration 10ug/ml was scanned separately in the range of 200 nm to 400 nm. Maximum absorbance was noticed at 288 nm and 320 nm by Cefixime Trihydrate and Ornidazole respectively. So these two wavelength was preferred as an analytical wavelength. In this method, the absorbance of the solutions were measured at the λmax of both the drugs. The criteria are that the ratios [(A2/A1) / (ax2/ax1)] and [(ay2/ay1) / (A2/A1)] should lie outside the range 0.1-2.0. For this measurement, the standard solutions of Cefix and Orni (10 µg/ml) were scanned separately in the range of 200-400 nm against water as a blank. Data were recorded at an interval of 1 nm. Figure 3 indicates the overlain spectra of the two drugs. Absorbance was measured at selected wavelengths i.e. 288nm and 320nm absorption maxima for Cefix and Orni respectively. The absorbance and Absorptivity values at the particular wavelengths were calculated and substituted in the following equation to get the concentration.

Cx = (A2 Ay1 – A1 Ay2) / (Ay1 Ax2 – Ay2 Ax1) ----- (1)

Cy = (A1 Ax2 – A2 Ax1) / (Ay1 Ax2 – Ay2 Ax1) ------ (2)

Where, A1, A2= Absorbances of mixture at λ1 & λ2 respectively,

ax1 = Absorptivity of Cefix at 288 nm,

ax2 = Absorptivity of Orni at 320 nm.

ay1 = Absorptivity of Cefix at 288 nm.

ay2 = Absorptivity of Orni at 320 nm

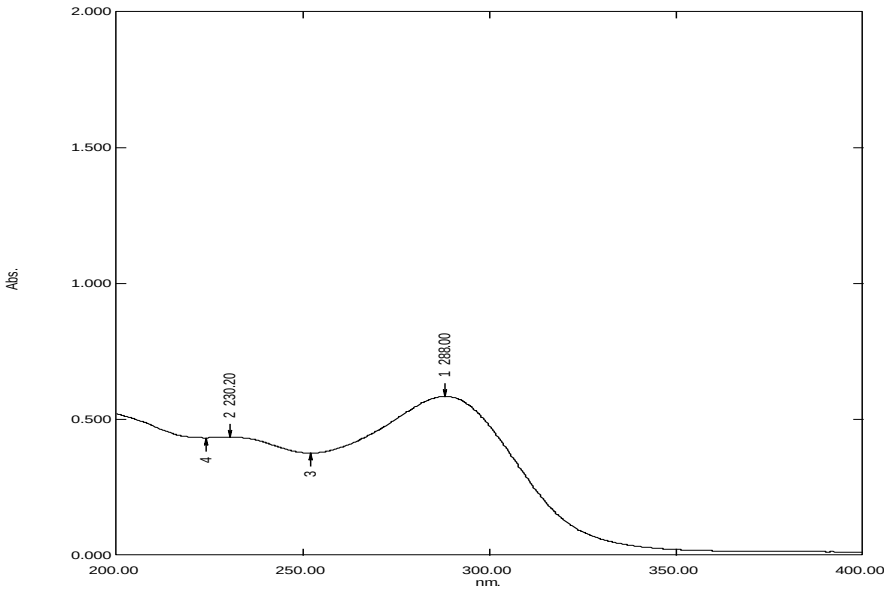

Fig. 3: UV spectra of Cefix in methanol:water(1:9)

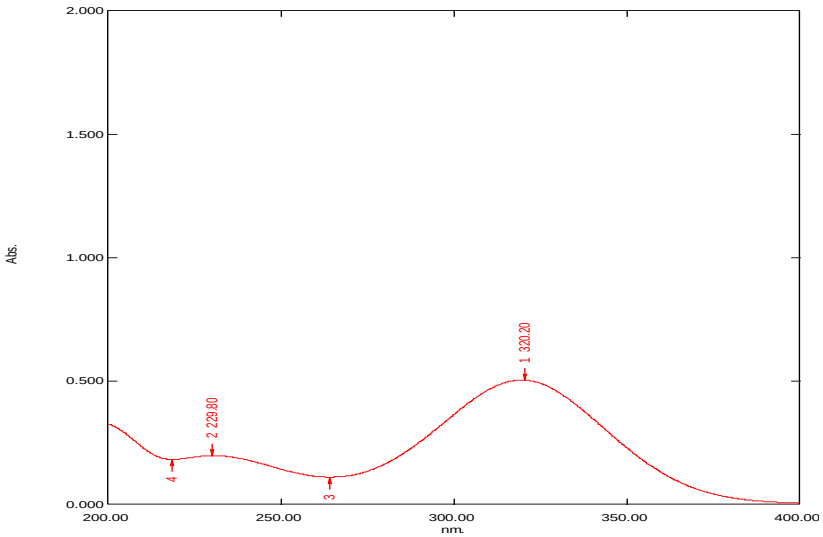

Fig. 4: UV spectra of Orni in methanol:water(1:9)

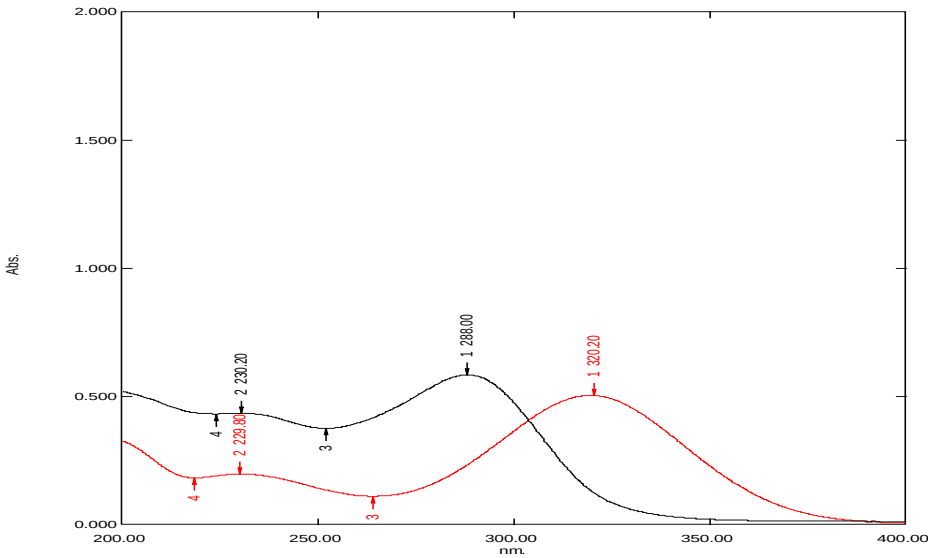

Fig 5: Overlain Spectra of Cefixime Trihydrate and Ornidazole in methnol:water (1:9)

VALIDATION PROCEDURE

The objective of present validation study is to demonstrate whether the developed method is suitable for intended use. The current validation of the analytical procedure has been conducted for parameters like linearity, range, precision and accuracy with respect to ICH guidelines Q2(R1).

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.[11] The linearity was determined by using working standard solutions between 2-

30µg/ml for Cefix and Orni. Calibration curve for linearity range was developed and simple linear regression was performed (Table No. 1 and 2). Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation.

Table 1: Data of Linearity range for Cefix.

Conc. (µg/ml)	Abs. (288nm)	Abs. (320nm)
2	0.087	0.017
4	0.15	0.029
6	0.225	0.044
8	0.314	0.063
10	0.371	0.072
12	0.471	0.094
14	0.526	0.104
16	0.599	0.119
18	0.667	0.132
20	0.763	0.15
22	0.861	0.168
24	0.909	0.178
26	1.003	0.193
28	1.049	0.205
30	1.157	0.225

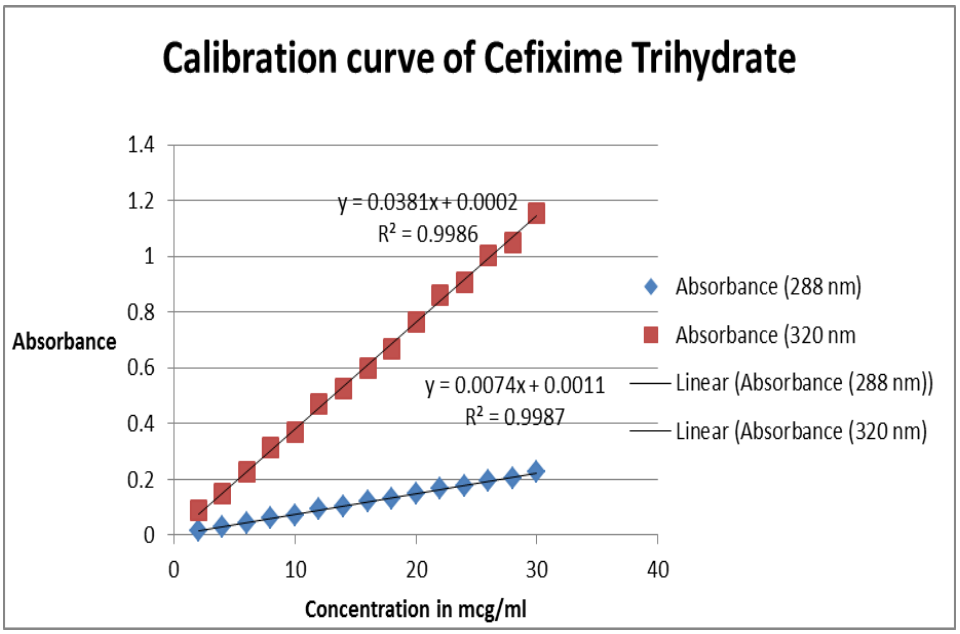

Fig. 5: Calibration Curve of Cefix.

Table 2: Data of Linearity range for Orni

Conc. (µg/ml)	Abs. (320nm)	Abs. (288nm)
2	0.082	0.042
4	0.147	0.072
6	0.215	0.094
8	0.285	0.126
10	0.352	0.156
12	0.425	0.190
14	0.498	0.222
16	0.577	0.260
18	0.627	0.282
20	0.701	0.312
22	0.794	0.357
24	0.864	0.387
26	0.921	0.413
28	1.000	0.448
30	1.094	0.488

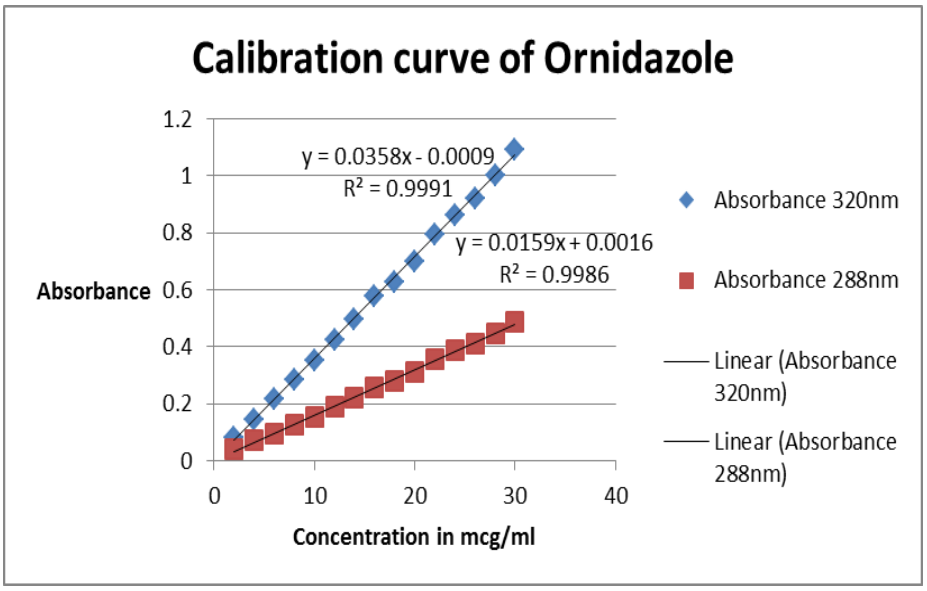

Fig. 6: Calibration Curve of Orni.

Accuracy

The recovery experiments were carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards (80%, 100% & 120%). 10µg/ml and 4 µg/ml of Orni and Cefix were used as nominal 100% sample concentration. The % recovery of the same pure drugs were then determined. Results were expressed in terms of % recoveries. The results are tabulated in Table No. 3 and 4.

Table No. 3: Data of Accuracy studies of Cefix.

Amt in Sample (Tablet) (Mg/ml)	Level of addition (%)	Amt of pure drug spiked (µg/ml)	Total conc. (µg/ml)	Abs (288 nm)	Abs (320nm)	Total content found (µg/ml)	Amt of std drug recovered (µg/ml)	Mean recovery (%)
3.999 of Cefixime Trihydrate	80	3.2	7.2	0.437	0.407	7.278	3.279	102.40
		3.2		0.436	0.406	7.263	3.273	
		3.2		0437	0.407	7.278	3.279	
	100	4	8	0.466	0.411	8.055	4.051	101.55
		4		0.467	0.411	8.084	4.084	
		4		0.466	0.411	8.055	4.051	
	120	4.8	8.8	0.493	0.414	8.788	4.789	99.659
		4.8		0.492	0.413	8.772	4.773	
		4.8		0.493	0.414	8.788	4.789	

Table No. 4: Data of Accuracy studies of Orni

Amt in Sample (Tablet) (Mg/ml)	Level of addition (%)	Amt of pure drug spiked (µg/ml)	Total conc. (µg/ml)	Abs (288 nm)	Abs (320nm)	Total content found (µg/ml)	Amt of std drug recovered (µg/ml)	Mean recovery (%)
10.26 of Ornidazole	80	8	18	0.470	0.688	18.120	7.860	98.479
		8		0.470	0.688	18.120	7.860	
		8		0.471	0.690	18.175	7.915	
	100	10	20	0.495	0.756	20.039	9.779	98.073
		10		0.496	0.759	20.124	9.864	
		10		0.495	0.756	20.039	9.779	
	120	12	22	0.564	0.854	22.603	12.348	102.816
		12		0.564	0.855	22.578	12.318	
		12		0.564	0.854	22.608	12.348	

SAMPLE SOLUTION STABILITY

Stability of the solution was studied by storing the tablet sample solution at room temperature for 3 hours and then analyzed by measuring the absorbance of the solution at 288 nm and 320 nm. % RSD was calculated. The results obtained were compared with the results of the freshly prepared solution.

Table 5: Data of Stability Analysis of the sample solution

	Absorbance of the Sample Solution	
	288nm	**320nm**
Freshly prepared	0.320	0.400
After 1 hour	0.319	0.400
After 2 hours	0.319	0.399
After 3 hours	0.320	0.400
Mean	0.3195	0.39975
SD	0.000577	0.0005
% RSD	0.180704	0.12507

Method Precision

a) Repeatability study

Aliquots of 1 ml each of the working sample solution were transferred to six 10 ml volumetric flask which were then diluted to 10 ml using water (4µg/ml of cefix and 10µg/ml of Orni). The absorbances for each of these solutions were recorded at 288nm and 320nm against reagent blank. The data of repeatability study is shown in Table No. 6.

Table No.6: Data of repeatability study

Concent ration (µg/ml)	**Abs (288 nm)**	**Abs (320 nm)**	**Concentration obtained (µg/ml)**		**Statistical Analysis**		
			Cefix	Orni	Cefix	Orni	
3.999 of Cefixime Trihydarte + 10.26 of Ornidazole	0.320	0.399	4.040	10.225	100.42 %	102.245%	Mean
	0.319	0.399	3.999	10.260			
	0.320	0.399	4.040	10.225	0.529551	0.177511	SD
	0.318	0.398	3.995	10.200			
	0.319	0.399	3.999	10.260	0.527332	0.173613	% RSD
	0.320	0.400	4.027	10.255			

b) Intermediate Precision

The intra-day and inter-day precision of the proposed method was determined by measuring the absorbance of the solutions 6 times on the same day and on 2 different days. The results were reported in terms of percentage relative standard deviation (% RSD). Aliquots of 1 ml each of the working sample solution were transferred to six 10 ml volumetric flask which were then diluted to 10 ml using water (4µg/ml of Cefix and 10µg/ml of Orni). The

absorbance was recorded at 288 nm and 320 nm against the reagent blank. The data of inter-day precision study is shown in Table No.7.

Table No. 7: Data showing intermediate Precision

Conc (ug/ml)	Abs. Day 1		Abs. Day 2		Abs. Day 3		Avg of Abs		% RSD*		Conc found (µg/ml)	
	λ1	λ 2	λ1	λ 2	λ1	λ 2	λ1	λ 2	Cefix	Orni	Cefix	Orni
3.999 of Cefixime Trihydarte + 10.26 of Ornidazole	0.319	0.399	0.319	0.400	0.318	0.399	0.319	0.399	0.1567	0.1104	3.999	10.260
	0.320	0.399	0.319	0.399	0.319	0.400						
	0.319	0.399	0.319	0.399	0.319	0.399						

RESULT TABLE

Table No. 8: Results of validation of parameters of Cefix and Orni.

Parameters	Results	
	Cefixime Trihydrate	Ornidazole
Range (µg/ml)	2-30	2-30
Linearity (µg/ml)	2-30	2-30
Regression Coefficient (R^2 Value)	0.998	0.999
Assay (%)	99.99%	102.26%
Recovery (%)	99.659-102.40 %	98.073-102.81%
Interday Precision (Intermediate)	0.52%	0.17%
Intraday Precision (Repeatability)	0.1567%	0.1104%

RESULTS AND DISCUSSION

The literature survey reveals that there are several validated spectrophotometric, RP-HPLC, HPTLC and other numerous methods available for estimation of Cefixime Trihydrate and Ornidazole in combination as well as individually (as a single component). The attempt was made to develop sensitive, precise & accurate UV spectrophotometric simultaneous equation method for estimation of Cefix and Orni. The method was developed using mixer of methanol and water as the solvent system. It involves formation of λ_{max} 288nm (maximum wavelength of absorption of Cefix) and λ_{max} 320nm (maximum wavelength of absorption of Orni).

The absorbance and Absorptivity values at the particular wavelengths were calculated and substituted in the following equation to get the concentration

Cx = (A2 Ay1 – A1 Ay2) / (Ay1 Ax2 – Ay2 Ax1) ----- (1)

Cy = (A1 Ax2 – A2 Ax1) / (Ay1 Ax2 – Ay2 Ax1) ------ (2)

The linearity range was found to be 2-30µg/ml for Cefix and Orni. The regression coefficient was 0.998 and 0.999 for Cefix and Orni respectively. The method showed mean absolute recovery ranging from 99. 659-102.40% for Cefix and 98.073-102.81% for Orni. Method showed insignificant variation in results, which demonstrated that the method was repeatable with.

a) % RSDs (intraday precision): 0.1567% and 0.1104for cefpo and levo respectively.
b) % RSDs (interday precision): 0.52955% and 0.1736% for cefpo and levo respectively.

CONCLUSIONS

The developed UV Spectrophotometric simultaneous equation method for estimation of Cefixime Trihydrate and Ornidazole is simple, sensitive, precise and accurate. The method could be applied successfully and economically for the simultaneous estimation of Cefixime Trihydrate and Ornidazole in laboratory samples for efficient data generation and for combination formulations of these two drugs in the future.

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