INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.7 | Issue 4 | Oct - Dec -2018
Journal Home page: www.ijpar.com

Research article *Open Access*

Estimation cefixime and ornidazole simultaneous in tablet dosage form by RP-HPLC method

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cefixime and Ornidazole in Tablet dosage form. Mobile phase containing Buffer and Degassed Methanol and Buffer in the ratio of 60:40 V/V, has been pumped through column at a flow rate of 1ml/min. Buffer used in this method was of di-sodium hydrogen phosphate and potassium dihydrogen phosphate. Temperature was maintained at 30°C. Optimized wavelength for Cefixime and Ornidazole was 254 nm. Retention time of Cefixime and Ornidazole were found to be 2.1 min and 4.9 min. %RSD of the Cefixime and Ornidazole were and found to be 0.21 and 0.26 respectively. The percentage recovery was obtained as 99.81% and 99.76% for Cefixime and Ornidazole respectively. LOD, LOQ values are obtained from regression equations.

Keywords: Cefixime, Ornidazole, Simultaneous estimation,

INTRODUCTION

The term 'Chromatography' covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase. Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces or basing on the size of the particles (e.g. Size exclusion chromatography).

Reversed Phase Chromatography

The objective was to make less polar or non polar so that polar solvents can be used to separate water-soluble polar compounds. Since the ionic nature of the chemically modified silica is now reversed i.e. it is non-polar or the nature of the phase is reversed [1-7].

Simple compounds are better retained by the reversed phase surface, the less water- soluble (i.e. the more non-polar) they are. The retention decreases in the following order: aliphatics >

induced dipoles (i.e. CCl_4) > permanent dipoles (e.g.$CHCl_3$) > weak Lewis bases (ethers, aldehydes, ketones) > strong Lewis bases (amines) > weak Lewis acids (alcohols, phenols) > strong Lewis acids (carboxylic acids). Also the retention increases as the number of carbon atoms increases.

As a general rule the retention increases with increasing contact area between sample molecule and stationary phase i.e. with increasing number of water molecules, which are released during the adsorption of a compound. Branched chain compounds are eluted more rapidly than their corresponding normal isomers.

MATERIALS AND METHODS

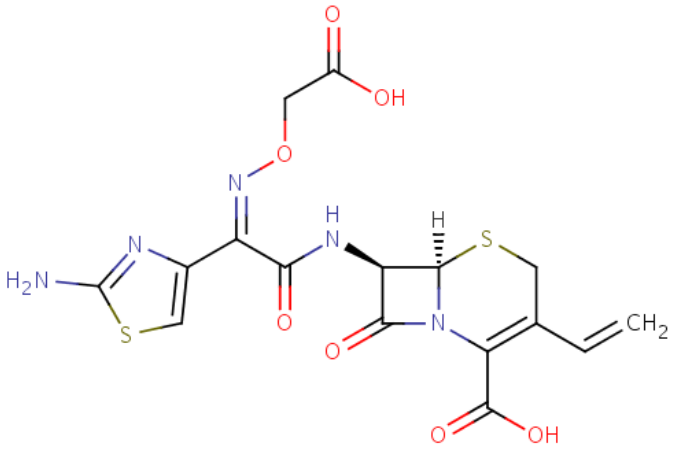

Fig 1: Structure of Cefixime

Chemical Formula: $C_{16}H_{15}N_5O_7S_2$
Molecular Weight: 453.45 g/mole
IUPAC :(6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy)imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.
Category : Anti Bacterial agent

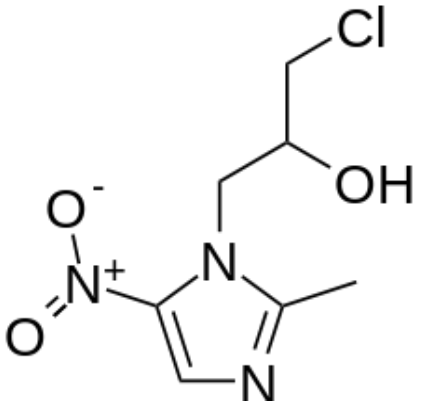

Fig 2: Structure of Ornidazole

Chemical formula: $C_7H_{10}ClN_3O_3$
Molecular Weight: 219.625 g/mole
IUPAC : 1-chloro-3-(2-methyl-5-nitro-1*H*-imidazol-1-yl)propan-2-ol
Category : anti Bacterial agents.

OPTIMIZED METHOD

Mobile Phase: Degassed Methanol and Buffer in the ratio of 60:40 V/V.

Preparation of (KH2PO4 0.1M) buffer

Weight 3.8954g of di-sodium hydrogen phosphate and 3.4023 of potassium dihydrogen phosphate in to a beaker containing 1000ml of distilled water and dissolve completely. Then pH is adjusted with orthophosphoric acid and then filtered through 0.45µm membrane filter.

Preparation of stock solution

Reference solution: The solution was prepared by dissolving 20.0 mg of accurately weighed Cefixime and 25.0 mg Ornidazole in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicate for 20min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min.

Preparation of working standard solution

The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Cefixime and Ornidazole above, sonicated and filtered through 0.45µ membrane [8-15].

Preparation of sample drug solution for pharmaceutical formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg Cefixime and 50 mg Ornidazole was weighed and dissolved in the 70 mL mobile phase with the aid of ultrasonication for 20 min. The content was diluted to 100 mL with mobile phase to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to give a test solution containing 20 µg/mL Cefixime and 50 µg/mL Ornidazole.

Procedure for calibration curve

The contents of the mobile phase were filtered before use through 0.45micron membrane and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug solutions, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The chromatographic separation was achieved using a mobile phase consisting of Methanol : Buffer at 60:40V/V the eluent was monitored using Pda detector at a wavelength of 254nm .The column was maintained at ambient temperature (27^0C) and an injection volume of 20µl of each of standard and sample solutions were injected into the HPLC system to get the chromatograms. The retention time, peak areas of drug was recorded graph was plotted by taking concentration of the drug on x-axis and peak area on y-axis. A typical chromatogram of Cefixime and Ornidazole [16-19].

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -ODS C_{18}(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : Buffer (60:40)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	254nm
Drug RT (min)	2.9min for CEFIXIME and 4.1 for ORNIDAZOLE.

RESULT AND DISCUSSION

Method development

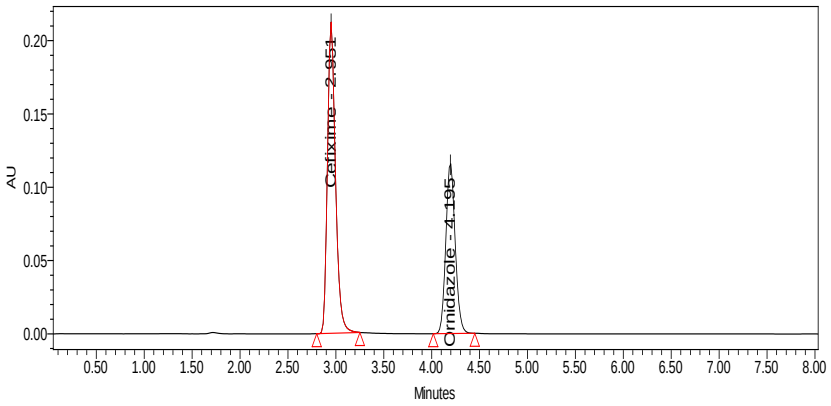

Fig 3: Chromatogram of standard

Table 2: Retention time of Cefixime and Ornidazole

S.NO	Name of the peak	Retention time(min)
1	Cefixime	2.951
2	Ornidazole	4.195

System suitability

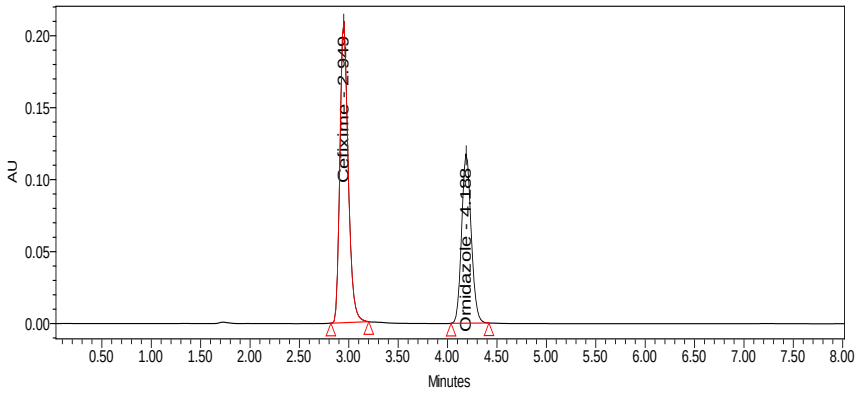

Fig 4: System suitability Chromatogram for standard

Specificity

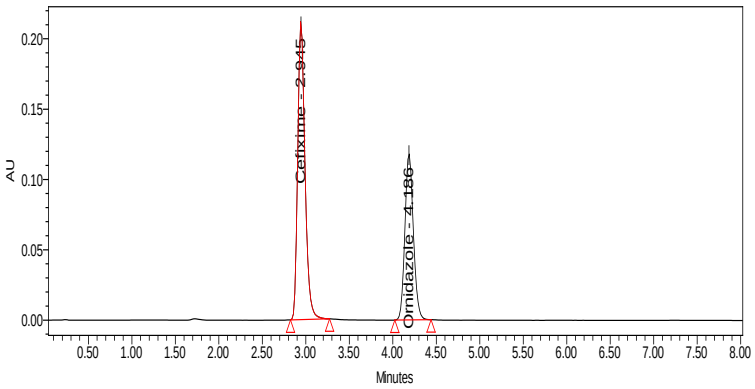

Fig 5: Chromatogram of standard

Precision

Table 3: Data of Repeatability (System precision) for Cefixime

	Injection	Peak Areas of Cefixime	%Assay
Concentration 40ppm	1	1146923	99.65
	2	1143596	99.08
	3	1158293	99.98
	4	1147283	100.04
	5	1152490	100.16
Statistical Analysis	**Mean**	1149717	99.78
	SD	5754.015	0.435569
	% RSD	0.500472	0.43652

Table 4: Data of Repeatability (System precision) for Ornidazole

	Injection	Peak Areas of Ornidazole	%Assay
Concentration 40ppm	1	801690	98.84
	2	797631	99.69
	3	805783	100.05
	4	801496	101.11
	5	806432	100.96
Statistical Analysis	**Mean**	802606.4	100.13
	SD	3590.034	0.937203
	% RSD	0.447297	0.935987

Precision

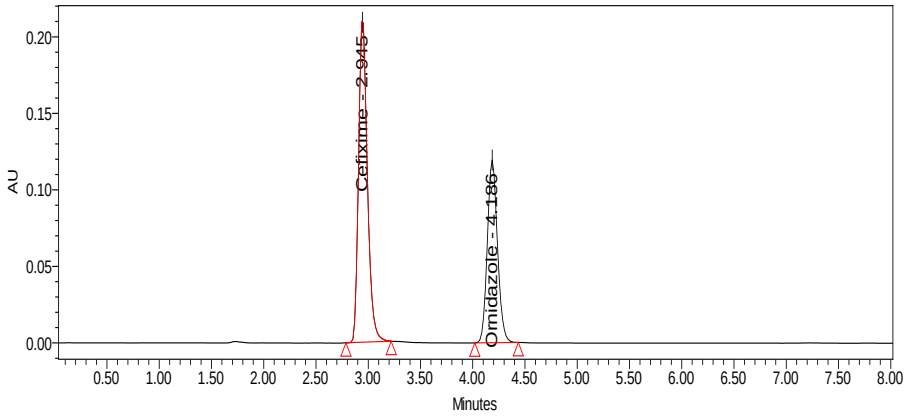

Fig 6: Chromatograms of system suitability

Method precision

Table 5: Data of Repeatability (Method precision) for Cefixime

	Injection	Peak Areas of Cefixime	%Assay
Concentration 40ppm	1	1152293	99.55
	2	1146923	99.88
	3	1147283	99.40
	4	1152490	99.56
	5	1139272	99.85

	6	1147283	99.40
Statistical	**Mean**	1147591	99.67
Analysis	**SD**	4815.615	0.250093
	% RSD	0.419628	0.250913

Table 6: Data of Repeatability (Method precision) for Ornidazole

	Injection	**Peak Areas of Ornidazole**	**%Assay**
Concentration	1	805783	99.85
40ppm	2	801690	99.96
	3	801496	100.53
	4	806432	100.30
	5	797564	100.08
	6	801496	100.53
Statistical	**Mean**	801593	100.20
Analysis	**SD**	3262.714	0.290477
	% RSD	0.406614	0.289873

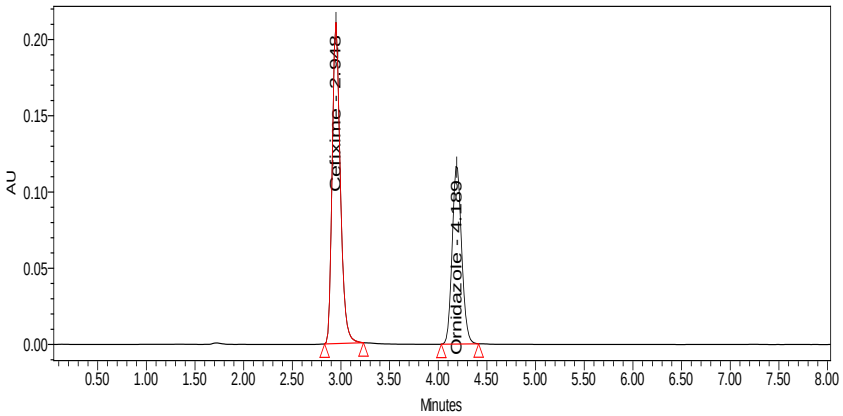

Fig 7: Chromatograms of Repeatability

Intermediate precision

Table 7: Data of Intermediate precision (Analyst 2) for Cefixime

	Injection	**Peak Areas of Cefixime**	**%Assay**
Concentration	1	1139272	98.80
40ppm	2	1140892	99.54
	3	1136601	99.98
	4	1141067	100.02
	5	1136024	101.08
	6	1140892	99.54
Statistical	**Mean**	1139125	99.82
Analysis	**SD**	2281.417	0.755001
	% RSD	0.200278	0.756312

Table 8: Data of Intermediate precision (Analyst 2) for Cefixime

	Injection	Peak Areas of Cefixime	%Assay
Concentration 40ppm	1	797564	99.85
	2	795138	99.68
	3	795685	100.08
	4	800569	100.01
	5	797049	99.52
	6	795685	100.08
Statistical Analysis	**Mean**	796948.3	100.37
	SD	1998.386	0.337086
	% RSD	0.250755	0.337299

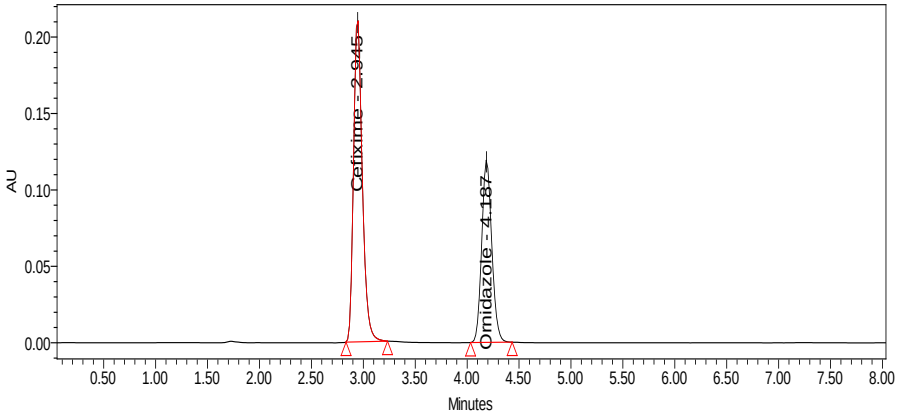

Fig 8: Chromatograms of Intermediate precision

Accuracy (Recovery)

Table 9: Data of Accuracy for Cefixime

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.85	99.25	**MEAN**	99.88
50% Injection 2	20	19.96	99.80		
50% Injection 3	20	20.12	100.6	**%RSD**	0.67
100 % Injection 1	40	39.74	99.35	**MEAN**	99.81
100 % Injection 2	40	40.08	100.2		
100% Injection 3	40	40.24	100.6	**%RSD**	0.399
150% Injection 1	60	59.04	98.40	**MEAN**	99.19
150% Injection 2	60	59.62	99.36		
150% Injection 3	60	59.89	99.81	**%RSD**	0.72

Table 10: Data of Accuracy for Ornidazole

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.86	99.30	**MEAN**	99.46
50% Injection 2	20	19.98	99.90		
50% Injection 3	20	19.84	99.20	**%RSD**	0.38
100 % Injection 1	40	39.54	98.85	**MEAN**	99.76
100 % Injection 2	40	39.82	99.55		
100% Injection 3	40	39.96	99.9	**%RSD**	0.189
150% Injection 1	60	59.92	99.86	**MEAN**	100.0067
150% Injection 2	60	60.08	100.13		
150% Injection 3	60	60.02	100.03	**%RSD**	0.136

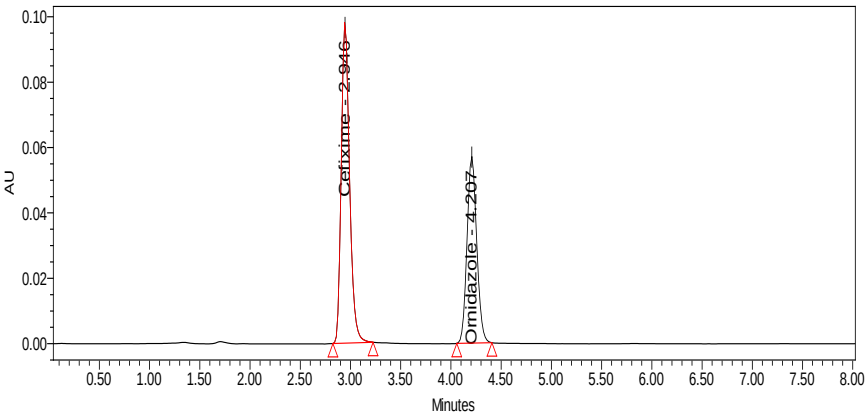

Fig 9: Chromatograms for accuracy (50%)

Linearity

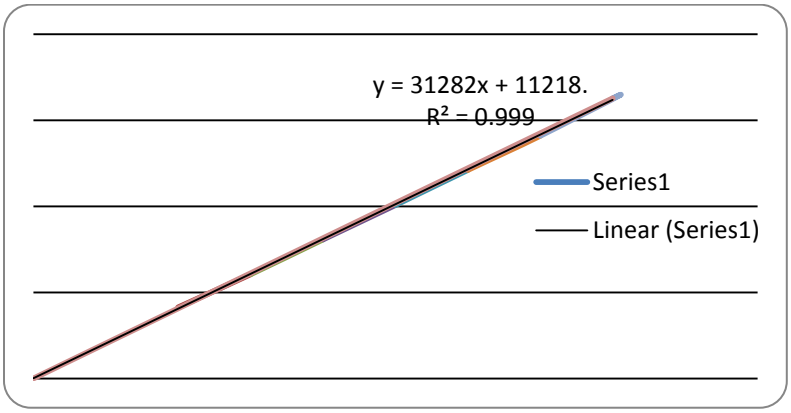

Fig 10: Linearity Plot (Concentration Vs Response) of Cefixime

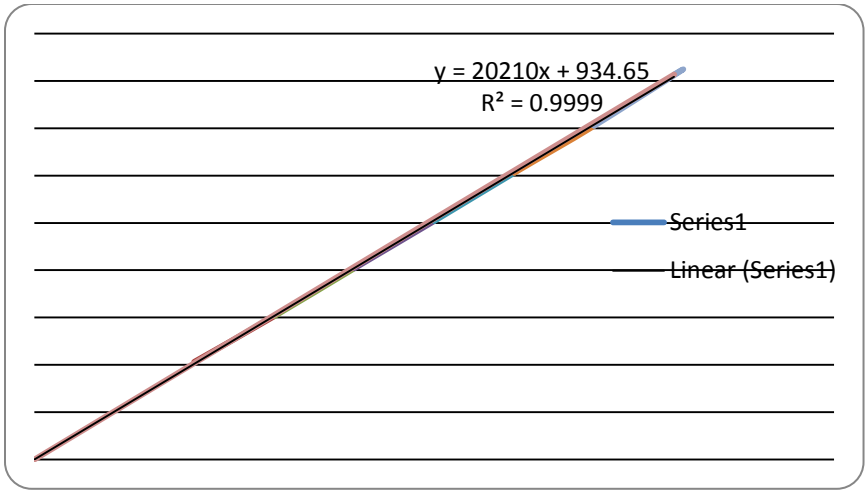

Fig 11: Linearity Plot (Concentration Vs Response) of Ornidazole

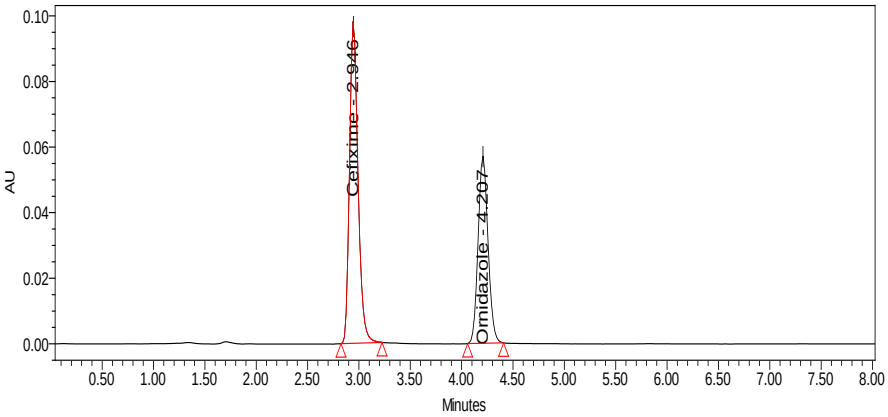

Fig 12: Chromatograms for 20 ppm

Ruggedness

System to System variability

Table 11: Data of system to system variability (Cefixime) System-2

S.NO:	Peak area	Assay % of Cefixime
Mean	1151146	99.78
%RSD	0.540725	0.43652

Table 12: Data of system to system variability (Ornidazole) System-2

S.No.	Peak area	Assay % of Ornidazole
Mean	802266.8	100.11
%RSD	0.413454	0.838768

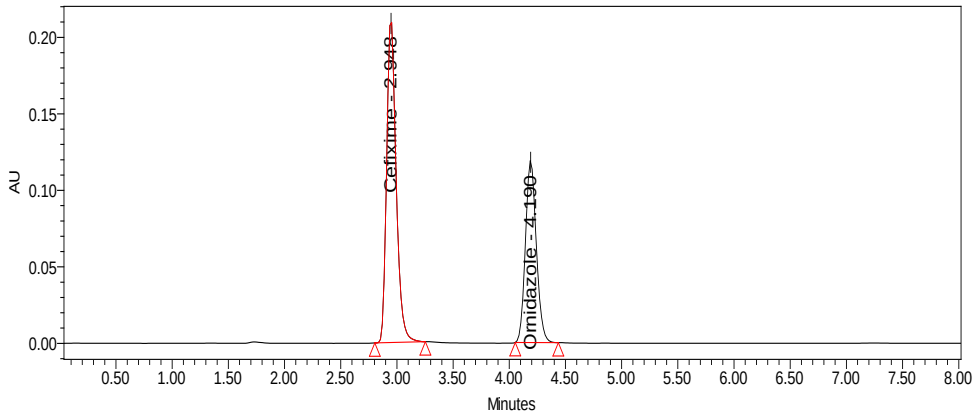

Fig 13: Chromatograms of system to system variability

Robustness

Table 13: Data for Effect of variation in flow rate (Cefixime)

Flow 0.8 ml	**Std Area**	**Tailing factor**		**Std Area**	**Tailing factor**		**Std Area**	**Tailing factor**
	1139272	1.238915		1146923	1.251658		1152293	1.262464
	1140892	1.230637		1143596	1.245435		1146923	1.251658
	1136301	1.240858		1158293	1.262464		1147283	1..237018
	1141067	1.238995		1147283	1.237018		1152490	1.239010
	1136024	1.241073		1152490	1.239010		1139272	1.238915
			Flow 1.0 ml			**Flow 1.2 ml**		
Avg	1138711	1.236496	**Avg**	1149717	1.247117	**Avg**	1148852	1.245813
SD	2431.578	0.005254	**SD**	5754.015	0.010328	**SD**	7076.841	0.010984
%RSD	0.213538	0.424907	**%RSD**	0.500472	0.008282	**%RSD**	0.615992	0.00881712

Table 14: Data for Effect of variation in flow rate (Ornidazole)

Flow 0.8 ml	**Std Area**	**Tailing factor**		**Std Area**	**Tailing factor**		**Std Area**	**Tailing factor**
	797564	1.099100		801690	1.122813		805783	1.121321
	795138	1.103929	**Flow 1.0**	797631	1.112181		801690	1.122813
	795685	1.111477	**ml**	805783	1.121321		801496	1.124805
	800569	1.117660		801496	1.124805	**Flow 1.2**	806432	1.123373
	797049	1.119004		806432	1.123373	**ml**	797564	1.099100
Avg	797201	1.110234	**Avg**	802606.4	1.120899	**Avg**	801593	1.118282
SD	2124.413	0.008622	**SD**	3590.034	0.00503	**SD**	3613.298	0.047969
%RSD	0.500472	0.77655	**%RSD**	0.447297	0.004488	**%RSD**	0.450203	0.965376

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

From the linearity plot the LOD and LOQ are calculated

Cefixime

LOD = _0.25
LOQ = 0.77

Ornidazole

LOD = 0.34
LOQ = 1.05

CONCLUSION

In the current approach, the present recovery was found to be 98.0-101.50 was linear and precise

over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 2.9 cefixime and 4.1 for Ornidazole. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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