Simultaneous estimation of cefixime trihydrate and ornidazole in combined tablet dosage form by RP-HPLC

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ABSTRACT

A new, simple, rapid, accurate, precise and sensitive method has been developed for the simultaneous estimation of Cefixime trihydrate and ornidazole in their combined tablet dosage form. The method was carried out on a Hiber C_{18} column (250 mm×4.6mm, i.d.5 μm) with a mobile phase consisting of acetonitrile:methanol:water (30:20:50) at a flow rate of 1 ml/min and the detection was carried out at 302 nm. The retention time of Cefixime trihydrate and ornidazole were 2.24 min and 4.31 min. respectively. Linearity for Cefixime trihydrate and ornidazole were found in the range of 10-50 μg/ml and 25-125 μg/ml respectively. The developed method was validated in terms of linearity, accuracy, and precision, limit of detection (LOD) and limit of quantification (LOQ). The proposed method can be used for estimation of both drugs in their combined dosage form.

Key Words: Cefixime trihydrate, ornidazole, RP- HPLC, Validation, Tablet.

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INTRODUCTION

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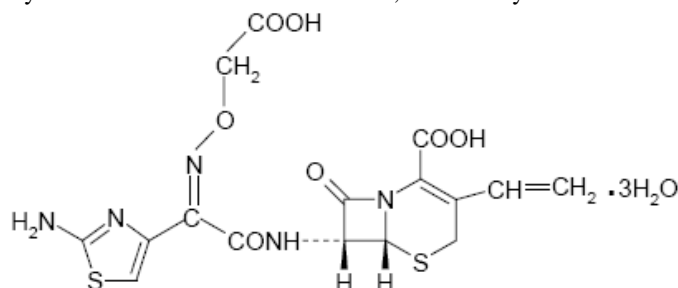

Fig 1. Structure of Cefixime trihydrate

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

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Fig 2. Structure of ornidazole

Both the drugs are marketed as combined dose tablet formulation in the ratio of 200:500 mg CEF: ORD. Literature survey reveals that cefixime can be estimated by spectrophotometrically [3] , HPLC[8] and by HPTLC[9] individually or with other drugs in bulk drugs and in human plasma, while ornidazole can be estimated by spectrophotometrically[10]-[11], HPLC[13] in combination with other drugs. However, there is no analytical method reported for the estimation of CEF and ORD in a combined dosage formulation. Present work describes two methods for simultaneous estimation of CEF and ORD in tablet formulation.

EXPERIMENTAL SECTION

Chemicals and reagents
Standard gift sample of Cefixime trihydrate and ornidazole were obtained from Nucleus formulation Plot No. 3484, 3485, Phase 4, Gidc, Chhatral, District:, Gandhinagar, Gujarat - 382 729, India

Cefixime trihydrate and ornidazole combination tablets (ORNICEF, Cefixime-200 mg Ornidazole-500 mg ; is manufactured by Aristo Pharmaceuticals, India), (CEF-O TAB, Cefixime-200 mg Ornidazole-500 mg ; is manufactured by Piramal healthcare, India), were purchased from the local pharmacy.

Acetonitrile, methanol and water were used of HPLC grade, purchased from RANKEM Ltd.

Instrumentation and chromatographic condition
The LC system (YL-9100) consisted of following components: YL9160 PDA detector, YL9101 vacuum degasser and YL9110 quaternary solvent delivery pump. Chromatographic analysis was carried out on a Hiber C_{18} column (250 mm×4.6mm, i.d.5 μm) using mobile phase of acetonitrile: methanol: water (30:20:50) with flow rate of 1ml/min. Detection of eluent was made at 237 nm by PDA detector. The column was maintained at room temperature and injection volume of 20μl was used. The mobile phase was filtered through 0.45μm Chrom Tech Nylon-66 filter paper.

Preparation of standard solution
Standard stock solution of pure drugs were prepared separately by dissolving 10 mg of each drug with Methanol in 10 ml of volumetric flask and made up to volume to get concentration of 1000 μg/ml.. 0.1 ml stock solution of Cefixime trihydrate And 2.5 ml stock solution of ornidazole were mixed in 10 ml volumetric flask and made up to volume with Methanol to get concentration of 10 μg/ml of Cefixime trihydrate and 25 μg/ml of ornidazole.

Preparation of sample solution
Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 200 mg of Cefixime trihydrate was transferred to 50 ml volumetric flask containing 40 ml of mobile phase, gentle shaking was carried out for 5min and ultra sonicated for 5 min. The volume was made up to the mark with the mobile phase. The tablet sample solution was filtered through Whatman filter paper no.41. 1 ml of filtrate was further diluted to 10 ml of mobile phase to get 400 μg/ml concentrations. From the above solution 1 ml was further diluted to 10 ml of mobile phase and 5 ml was further diluted in 10 ml to get the final concentration 20 μg/ml. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present in tablet was estimated from their respective calibration curve (Table-1).

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System suitability
The system suitability was assessed by six replicate injections of the mixture containing 10 μg/ml of both the drugs. The resolution, peak asymmetry and number of theoretical plates were calculated (Table 2). The obtained values were demonstrated the suitability of the system for the analysis of these drugs in combination.

Method validation
The method was validated for linearity, accuracy, intraday and interday precision, LOD and LOQ, in accordance with ICH guidelines.

Linearity
Aliquots of 0.1,0.2,0.3,0.4 and 0.5 ml from1000 μg/ml standard solution of CEFI and aliquots 2.5, 50, 75, 100 and 125 ml from 1000 μg/ml standard solution of ORNI transferred to series of 10 ml volumetric flasks and made up to volume with mobile phase. Each solution was injected and chromatogram was recorded. Retention time (mean ± s.d.[a]) of CEFI and ORNI were found to be 2.23 ± 0.03 and 4.31 ± 0.03 min respectively. The peak area of CEFI and ORNI in each chromatogram was recorded.
s.d[a]= standard deviation.

Accuracy
To study accuracy of the method, recovery studies were carried out by addition of standard drug sample in a tablet sample at 50%, 100% and 150%. The percentage of recovery was calculated (Table-3).

Precision
It was carried out by preparing 3 replicates of 3 different concentrations within the linearity range and then injecting each solution. The peak area of CEFI and ORNI in each chromatogram was recorded in order to record any intraday variation. To record inter day variation, 3 different concentration solution within the linearity range were analyzed for 3 different days. The peak area of each drug was recorded and % RSD (% relative standard deviation) was calculated for both series of analysis.

Limit of detection (LOD) and limit of quantification (LOQ)
They were calculated as 3.3 σ/S and 10 σ/S respectively. Where σ is the standard deviation of the response (y-intercept) and S is the mean of the slope of calibration plot.

RESULTS AND DISCUSSION

For RP-HPLC method, several different mobile phases were tried and finally mobile phase of acetonitrile : methanol : water (30:20:50) was found to be optimized and well defined. Resolved peaks of CEFI and ORNI with retention time (mean ± s.d.) 2.24 ± 0.03 min and 4.31 ± 0.03 min were obtained respectively. The representative chromatogram of sample solution of CEFI (10 μg/ml) and ORNI (25 μg/ml) is shown in Fig 3 and 3D view of different concentrations of mixed standard solutions of CEFI and ORNI is shown in Fig 4. The calibration curve for each drug was obtained separately by plotting as peak area → concentration over the range of 10-50 μg/ml for CEFI and 25-125 μg/ml for ORNI. From, calibration curve of CEFI (Fig 5), it was found to linear with r^2= 0.9912 and from calibration curve of ORNI (Fig 6) it was found to linear with r^2= 0.9932. The % recoveries for CEFI and ORNI were found to be 99.07%-101.066% and 98.62%-100.011% respectively, which were satisfactory (Table-3). The precision is usually expressed as % RSD. The intraday precision for CEFI and ORNI were found to be 0.17-0.44 and 0.27-0.59 respectively. The inter day precision for CEFI and ORNI were found to be 0.71-0.86 and 0.69-1.01 respectively. The limit of detection (LOD) for CEFI and ORNI were 0.0934 μg/ml and 0.0455 μg/ml respectively. The limit of quantification (LOQ) for CEFI and ORNI were 0.283 μg/ml and 0.138 μg/ml respectively. The system suitability parameters for RP- HPLC are shown in Table-2.
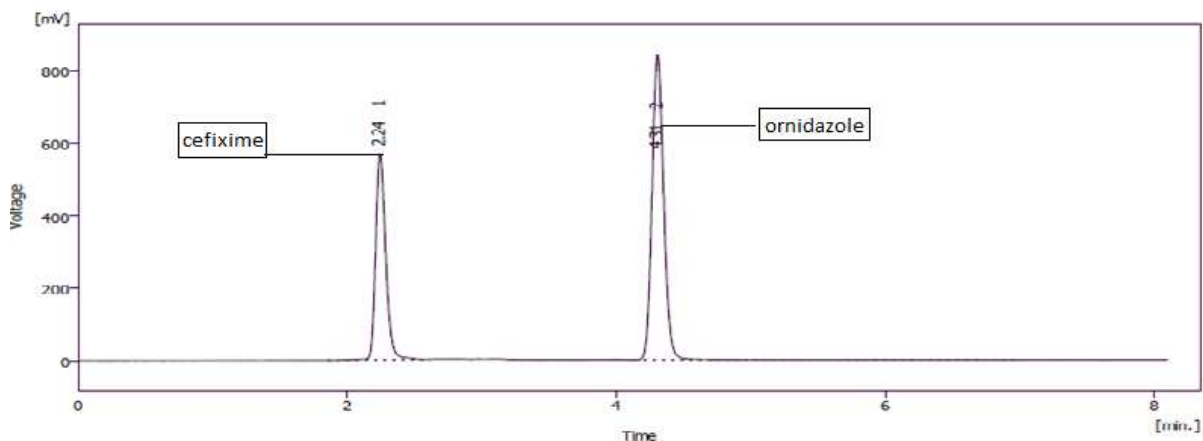
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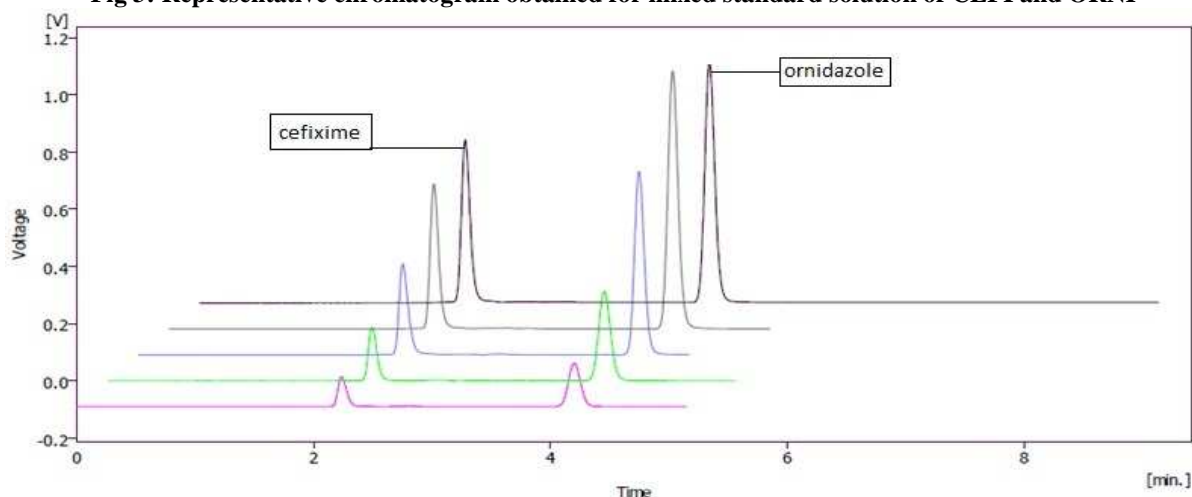**Fig 3: Representative chromatogram obtained for mixed standard solution of CEFI and ORNI**

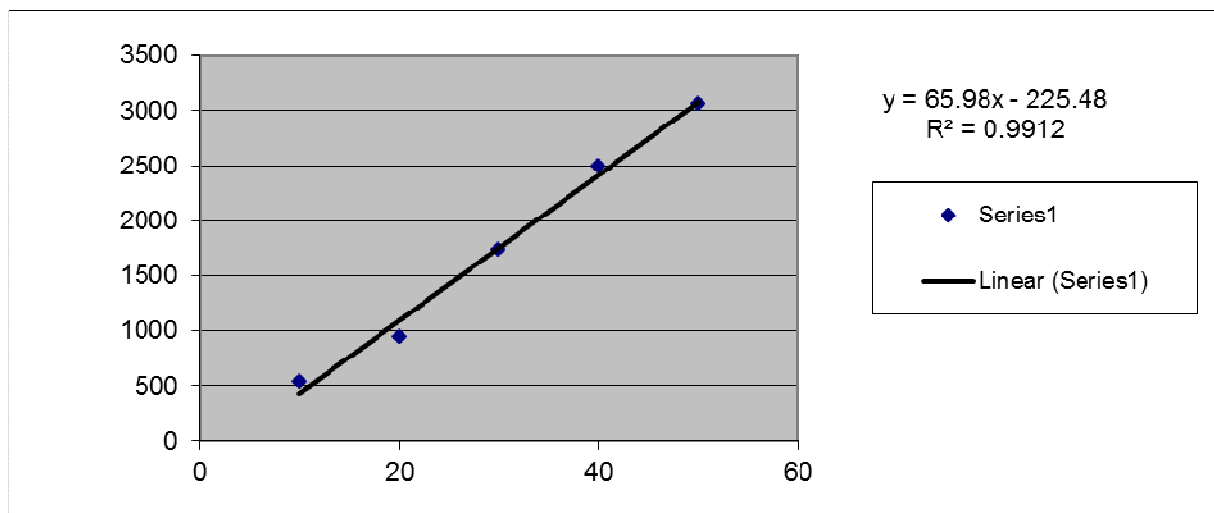**Fig 4: 3D view of different concentrations of mixed standard solutions of CEFI and ORNI**

$y = 65.98x - 225.48$
$R^2 = 0.9912$

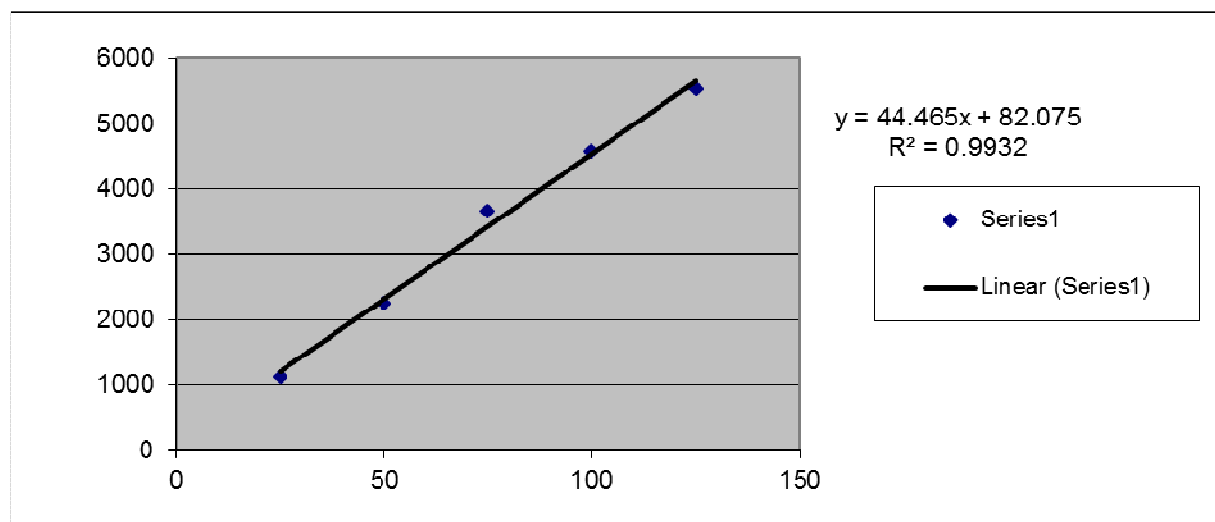**Fig 5: Calibration curve of Cefixime trihydrate**

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Fig 6: Calibration curve of ornidazole
Table 1: Assay results of combined dosage form

Drug	Labeled claim (mg)	Amount found (mg) *	% label claim
CEFI	200	198.14	99.07
ORNI	500	494.8	98.96

*Each value is a mean of six observations.

Table 2: System suitability parameters for RP-HPLC

Sr no.	Parameters	CEFI*	ORNI*
1	No. of theoretical plates	2509	9296
2	Asymmetry factor	0.381	1.04
3	Tailing factor	1.23	0.981
4	Resolution	20.685	-

*Each value is a mean of six observations

Table-3: Recovery studies of CEFI and ORNI

Level of recovery	Amount taken(μg/ml)		Amount added (μg/ml)		Total amount found (μg/ml)*		% recovery	
	Cefi	Orni	Cefi	Orni	Cefi	Orni	Cefi	Orni
0%	20	50	0	0	19.81	49.48	99.07	98.96
50%	20	50	10	25	29.69	76.41	98.97	101.883
100%	20	50	20	50	40.42	100.011	101.066	100.011
150%	20	50	30	75	49.79	123.285	99.58	98.62

Table 4: Summary of validation parameters of proposed RP-HPLC

Parameters	CEFI	ORNI
Linearity (μg/ml)	10-50	25-125
Correlation coefficient	0.9912	0.9932
Slope (m)	65.98	44.465
Intercept (c)	-225.48	82.075
LOD [b] (μg/ml)	0.0934	0.04557
LOQ [c] (μg/ml)	0.2831	0.1381
Accuracy(% recovery)	99.07-101.066	98.62-100.011
Precision (%RSD) [d]		
Intraday (n[e]=9)	0.17-0.44	0.27-0.59
Inter day (n[e]=9)	0.71-0.86	0.69-1.01

LOD[b]= limit of detection; LOQ[c]=limit of quantification; (%RSD)[d]= % relative standard deviation, n[e] = number of observations.

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CONCLUSION

The validated RP-HPLC method employed here is simple, rapid, accurate, precise, sensitive and cost effective which can be used for routine analysis of Cefixime trihydrate and ornidazole in combined pharmaceutical dosage form.

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