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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND ORNIDAZOLE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of Cefixime Trihydrate and Ornidazole simultaneously in combined dosage forms. The stationary phase used was precoated silica gel 60F254. The mobile phase used was a mixture of Methanol: Toluene (7:3 v/v) The detection of spots was carried out at 301 nm. The method was validated in terms of linearity, accuracy, precision,LOD andLOQ. The calibration curvewas found to be linear between 60to 160 ng/spot for Cefixime Trihydrate and 150 to 400 ng/spot for Ornidazoleand Itopride is used as internal Standard. The proposed method can be successfully used to determine the drug content of marketed formulation.

KEY WORDS: Simultaneous estimation, HPTLC ,Validation , Cefixime, Trihydrate (CEF), Ornidazole(ORD), Itopride(ITO).

INTRODUCTION

Cefixime trihydrate is Chemically known as (Z)-7-[2-(2-Aminothizol-4-yl)2-(carboxymethoxyamino acetamide]-3-vinyl-3-cephem-4-carboxylic acid trihydrate.OR, (6R,7R)-7-[2-(2 amino-4-thiazolyl) glyoxyl amido]-8-oxo-Vinyl-(carboxymethyl)oxime] with Chemical formulaC_{16} H $_{21}$N $_{5}$O $_{10}$S.Cefixime trihydrate.is White to light white fine crystalline odorless powder slightly soluble in water and alcohol, sparingly soluble in dehydrated alcohol and acetone, freely soluble in methanol, alcohol, glycerol and propylene glycol, practically insoluble in ether, ethyl acetate and used to treatVarious infections including gonorrhea, otitis media, pharyngitis, lower respiratory-tract infections.

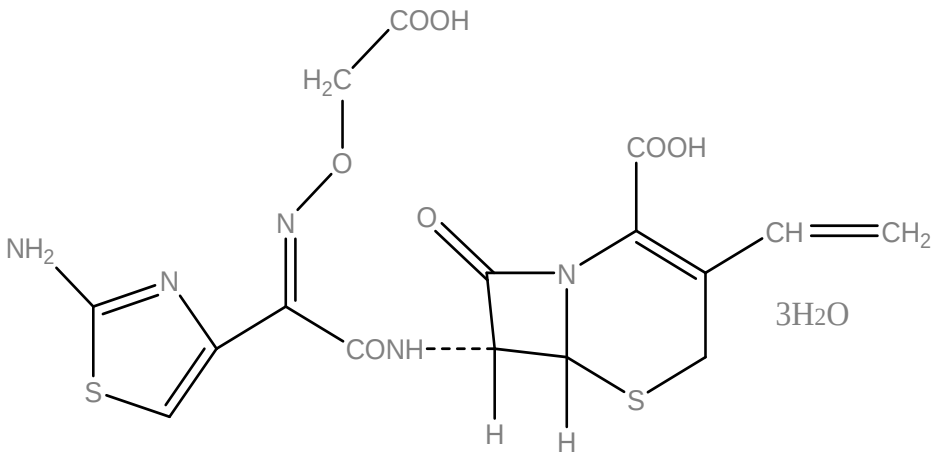


Fig.1: Structure of Cefixime Trihydrate.

Ornidazole isChemically1-chloro -3-(2-methyl-5-nitroimidazole-1yl)propan-2-ol . with chemical formulaC_7 H $_{10}$Cl N $_3$O $_3$White to yellow practically odorless crystalline powder Practically insoluble in non polar solvent solubility is very high in moderately polar solvent like ethanol methanol ,acetonitrile. Therapeutically Used in the treatment of infection due to an aerobe germs such as bactericide,fragiles specifically useful in abdominal and gynecological surgery. Literature surveyreveals that, Cefixime Trihydrate and Ornidazole is official in any of the pharmacopeias like IP, USP. and NF. Hence an attempt has been made to develop a simple, efficient and selective method for the determination of Cefixime Trihydrate and Ornidazole in pharmaceutical dosage forms.

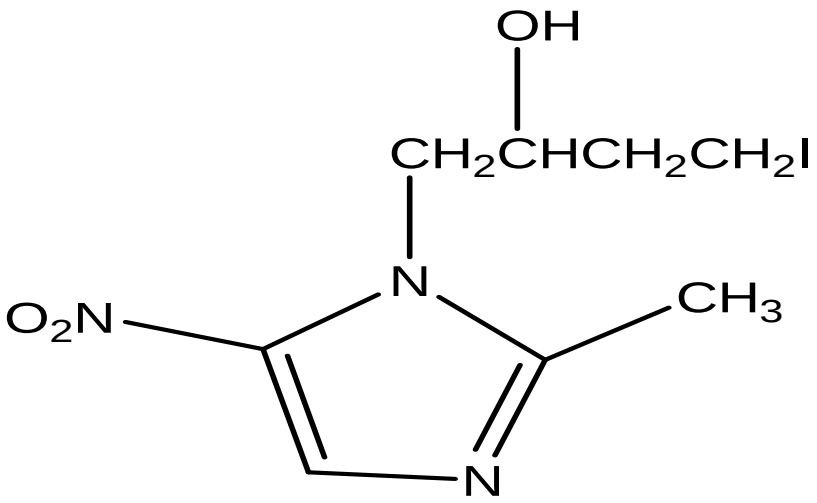


Fig.2: Structure of Ornidazole

MATERIALS AND METHODS

Instrumentation

A CAMAG HPTLC system equipped with Linomat V applicator, TLC Scanner IV, and an integrated software win CATS was used for the analysis. HPTLC was performed on 20 cm × 10 cm aluminium-backed HPTLC plates coated with 0.2 mm layers of silica gel 60 F254 (Merck, India).

Chemicals

All chemicals used for analysis were of analytical or HPLC grade. Gift samples of pure drugs were procured from Biochem industries Mumbai.

HPTLC method and chromatographic conditions

A CAMAG HPTLC system equipped with Linomat V applicator, TLC Scanner IV, and an integrated software win CATS was used for the analysis. HPTLC was performed on 20 cm × 10 cm aluminium-backed HPTLC plates coated with 0.2 mm layers of silica gel 60 F254 (Merck, India). Standard and sample solutions were applied to the plates, as 8 mm bands, distance between tracks 10 mm, under a stream of nitrogen, by means of a Camag Linomat V sample applicator fitted with a 100-µl Hamilton syringe. Plates were then developed, at room temperature, with Methanol and toluene(7:3 v/v) as mobile phase, in a 20 cm × 10 cm Camag twin-trough chamber previously saturated for 10 min. The development distance was 70 mm. After development the plates were removed from the chamber, dried in air, and densitometric scanning was performed at 301nm with a Camag TLC Scanner-4 with winCATS software at a slit width of 6.00 × 0.45 mm, scanning speed of 20 mm/s, and data resolution of 100 µm/step.

Selection of solvent

Methanol was selected as a solvent for preparing drug solutions.

Internal standard

Itopride

Selection of stationary phase

Separation and identification of both the drugs were performed on (20 cm x 10 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) aluminum backed silica gel 60 F254 TLC plates.

Preparation of stock solution and working standard solution

ORD standard stock solution : An accurately weighed quantity of ORD (10 mg) was dissolved in methanol and volume was made up to 10 ml with methanol to prepared. (1000µg/ml) CEF standard stock solution : An accurately weighed quantity of CEF (10 mg) was dissolved in methanol and volume was made in 10ml volumetric flask to prepared (1000 µg/ml). ITO Standered stock solution : An accurately weighed quantity of ito (10 mg) was dissolved in methanol to produce (1000µg/ml) From that solution to prepared a Final concentration solution (100µg/ml). The chromatogram and 3D display is shown in following figure.

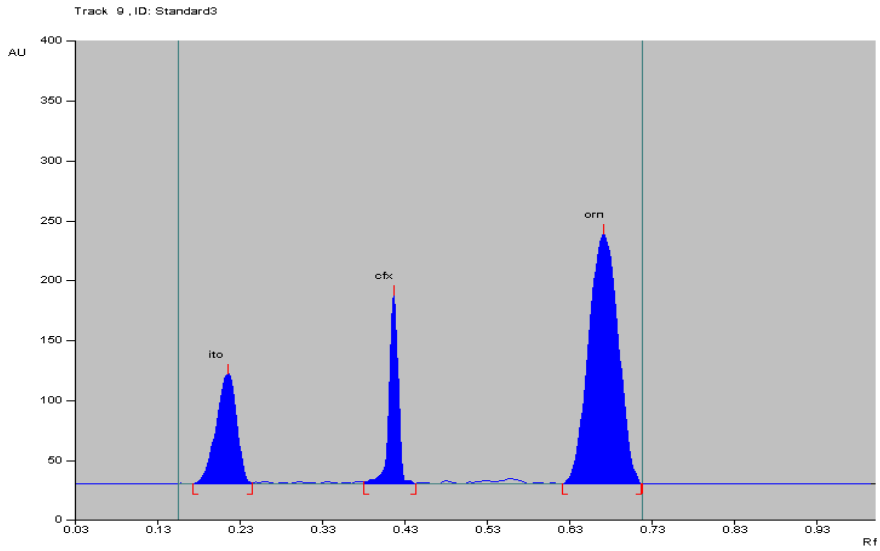


Fig.3: Chromatogram of Standard Cefixime (CFX)and Ornidazole(ORN) with Internal Standered Itopride (ITO).

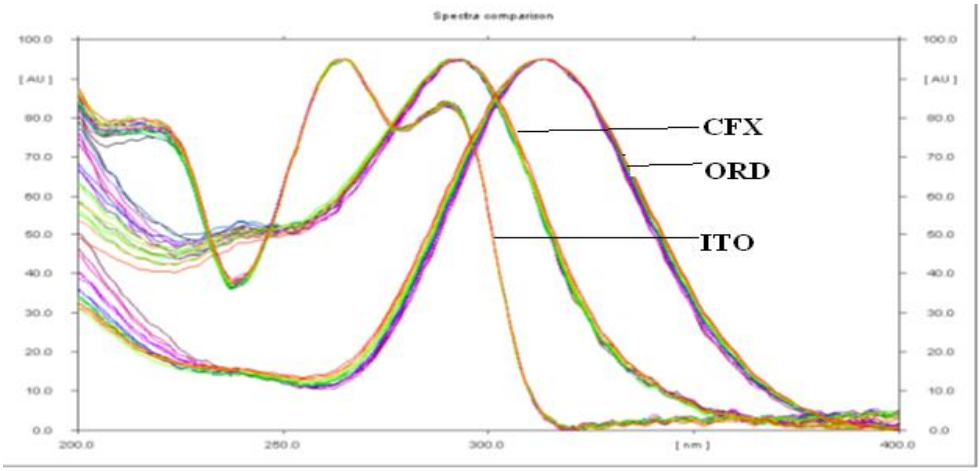


Fig.4: HPTLC Spectro densitogram of standard CFX and ORN With internal std Itopride.

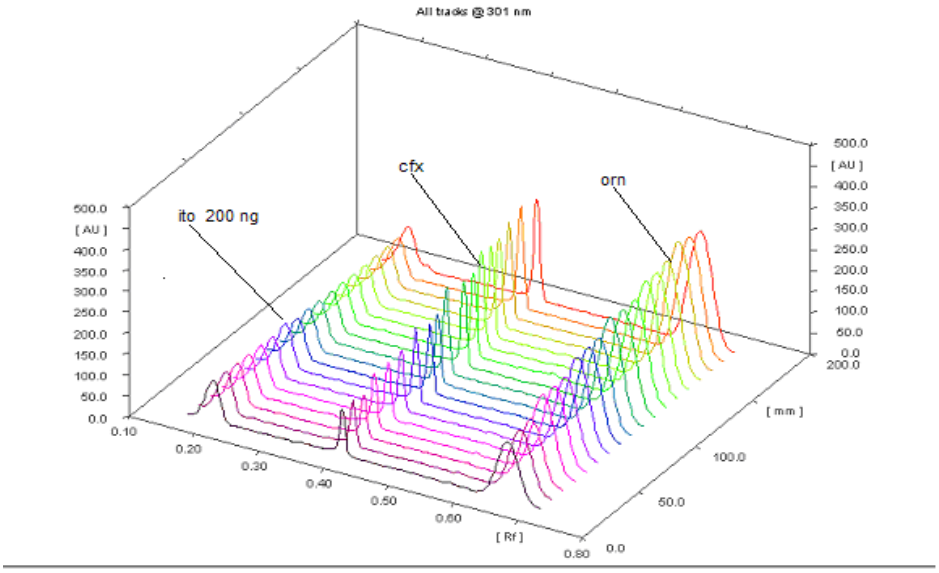


Fig 5 :Standard 3D display of standard CFX and ORN With internal std ITO.

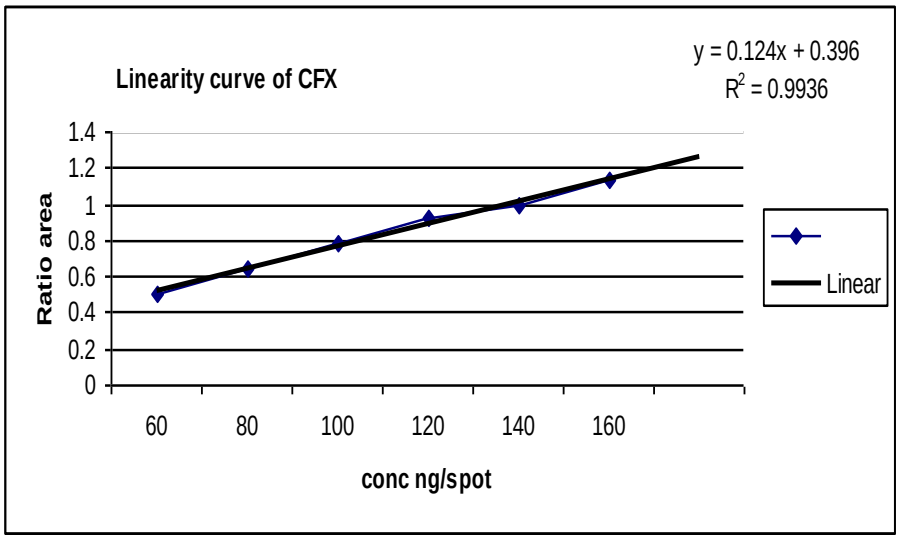
The aliquot portions of standard stock solutions of ORD and CEF were further diluted with mobile phase to get the series of concentration ranging from 150-400ng/spot for ORD and 60- 160 ng/spot for CEF. Each concentration was spotted three times on the plate. Calibration curve was obtained by plotting peak-area ratio on ordinate and corresponding concentration on abscissa, and is given below Table.1

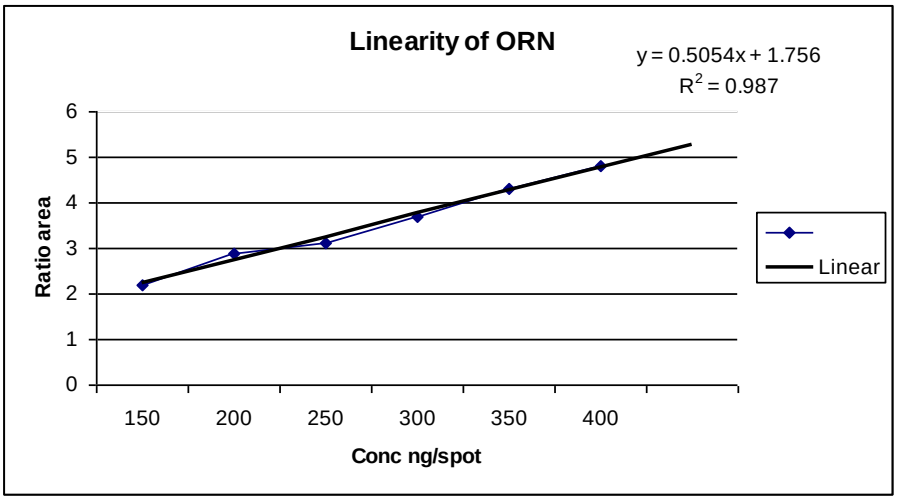
Table No:1Linearity curve

Sr no	Conc ng/spot CFX	Ratio area	Conc ng/spot ORN	Ratio area
1	60	0.5045	150	2.2125
2	80	0.6565	200	2.9527
3	100	0.7599	250	3.1407
4	120	0.9292	300	3.7266
5	140	1.0724	350	4.3297
6	160	1.1346	400	4.8051

Mean of three estimation

Linearity curve of CFX and ORN





Validation of the method

The developed method was validated in terms of linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application.

Analysis of the marketed formulations

Three microliters of sample solutions of the marketed formulation was spotted on to the same plate followed by development scanning. The analysis was repeated in triplicate. The content of the drug was calculated from the peak areas recorded.

Table 2: Analysis of tablet formulation.

Sr No	Conc. ng/spot		Ratio area		Area of Ito 200ng (Y)	Amount Found		% Label claim	
	CFX	ORN	CFX	ORN		CFX	ORN	CFX	ORN
1	120	300	0.92	3.83		123.00	312.24	103.01	104.08
2	120	300	0.88	3.79		117.49	308.34	97.91	102.78
3	120	300	0.91	3.99	1498.74	120.53	319.00	100.44	109.77
4	120	300	0.88	3.85		117.23	314.37	97.69	104.79
5	120	300	0.92	3.85		121.51	314.57	101.26	104.85
6	120	300	0.91	3.66		124.15	294.07	103.46	98.05
Avg								100.62	104.05
±S.D								2.2	1.04
%R.S.D								2.2	1.04

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by performing recovery studies at three different levels of standard additions. Accuracy was checked by adding 80, 100 and 120 % amount of Cefixime Trihydrate and Ornidazole to pre-analyzed sample. Result are shown in Table .3

Table 3: Recovery study

Component	Initial concentration (ng/spot)	Excess drug added (ng)	Drug recovered ng ± SD (n=3)	Drug recovered
CFX	100	80	187.40	104.18%
	100	100	198.70	99.35%
	100	120	217.40	98.90%
ORN	250	200	438.98	97.53%
	250	250	494.95	98.98%
	250	300	546.72	99.40%

Mean of three estimations

Precision study (Intra-day and Inter-day)

The precision of an analytical method is the closeness of agreement between series of measurements obtained from multiple sampling of the same sample. The intra-day precision was determined by analyzing standard solutions in the concentration range of 140ng/ spot to 350 ng/spot for Cefixime Trihydrate and Ornidazole for three times on the same day. While inter-day precision was determined by analyzing corresponding standards daily for 3 day over a period of one week. The results are shown in Table 4& 5.

Table4: Intra day precision

Sr no	Conc ng/spot	Area CFX	Area ORN	Area of ITO ng	Ratio of CFX ORN		Amount found CFX	Amount found ORN	% label claim CFX	% label claim ORN
1	140:350	1543.97	6665.61	1497.75	1.03	4.4	138.93	349.35	99.23	106.59
2	140:350	1560.57	6641.27		1.04	4.41	140.58	376.42	100.42	99.81
3	140:350	1527.74	6511.03		1.02	4.3	137.31	367.41	98.08	104.97
								Mean=	99.24	104.07
								S.D	0.9757	2.1000
								%R.S.D	0.9831	2.0178

Table 5: Inter day precision

Sr no	Conc ng/spot	Area CFX	Area ORN	Area of ITO ng	Ratio of CFX ORN		Amount found CFX	Amount found ORN	% label claim CFX	% label claim ORN
1	140:350	1543.97	6665.61	1497.75	1.03	4.4	138.93	349.35	99.23	106.59
2	140:350	1560.57	6641.27		1.04	4.41	140.58	376.42	100.42	99.81
3	140:350	1527.74	6511.03		1.02	4.3	137.31	367.41	98.08	104.97
								Mean=	99.24	104.07
								S.D	0.9757	2.1000
								%R.S.D	0.9831	2.0178

Mean of three estimation

LOD and LOQ

The limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined and the limit of detection (LOD) of an individual analytical procedure is the lowest amount of an analyte in a sample

which can be detected but not necessarily quantitated as an exact value. LOQ and LOD value represent the sensitivity of the proposed analytical method. LOD and LOQ of the drugs were calculated using the following equations.

LOD = 3.3 x [σ]/S

LOQ = 10 x [σ]/S

Where σ = standard deviation of the response and S = standard deviation and y = intercept of regression lines (Table 1).

Table 6: Limit of detection and limit of quantitation

Drugs	LOD (ng/spot)	LOQ (ng/spot)
CFX	0.425	1.19
ORN	0.736	2.14

Average of five determinations

RESULT AND DISCUSSION

HPTLC method was optimized with a view to develop asimple, accurate method for estimation of drug in pharmaceutical formulation and in bulk drug. UV scanning at 190-450 nm for both Cefixime Trihydrate and Ornidazoleshow that 301 nm is the suitable wavelength for detection of drugs. (Fig.3) The mobile phase methanol and toluene in the ratio of 7:3 v/v. was selected because it gave highest resolution, minimum tailing and Rfvalues of 0.16 and 0.6 for Cefixime Trihydrate and Ornidazolerespectively. (Fig.4). Cefixime Trihydrate and Ornidazole showed linearity in the concentration range of 60ng/spot and 160ng/spot (r2 =0.9937) and 150ng/spot and 400ng/spot (r2 =0.9870) respectively. For HPTLC method the linearity of calibration graphs and adherence of the system to Beer's law was validated by higher value of correlation coefficient. The LOD and LOQ were found to be 0.425 and 1.19µ g/ml for Cefixime Trihydrate 0.736, 2.14µg/ml for Ornidazole respectively for Cefixime Trihydrate and Ornidazole. Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels (80%, 100%, and 120%) by standard addition method. after spiking with additional drug afforded recovery of 98-102% and mean recovery was found to be 100.81% and 98.63% for Cefixime Trihydrate and Ornidazole respectively. The developed HPTLC method was found to be simple, precise, specific and accurate. Therefore this method can be applied for routine analysis of drugs in formulation and in bulk drug.

CONCLUSION

The developed HPTLC method is suitable for simultaneous estimation of Cefixime Trihydrate and Ornidazole in pharmaceutical dosage form is accurate, precise, specific, robust, and rapid. Therefore this method can be applied for routine analysis of drugs in formulation and in bulk drug.

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