Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2010, 2(6): 97-104
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X

Validated TLC Densitometric method for the quantification of Cefixime Trihydrate and Ornidazole in bulk drug and in tablet dosage form

Devika G.S[1]*, M. Sudhakar[1]and J. Venkateshwara Rao[2]

[1]*Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Dullapally, Secunderabad, Andrapradesh, India*
[2]*Department of Pharmaceutical Chemistry, Sultan Ul Uloom College of Pharmacy, Secunderabad, Andrapradesh, India*

ABSTRACT

A simple, rapid, accurate and precise densitometric method for determination of cefixime trihydrate and ornidazole in combined tablet dosage forms has been developed and validated. The separation was achieved on Merck TLC aluminium sheets of silica gel 60 F_{254} with n-butanol-methanol-toluene-ammonia 5:2:1:5 (v/v/v/v) as mobile phase. Densitometric quantification was perfomed at 287nm by reflectance scanning. The R_F value of cefixime trihydrate (CEF) and ornidazole (ORZ) were found to be 0.51±0.02, 0.36±0.02 respectively. The method was validated with respect to linearity, precision, accuracy, specificity, robustness and ruggedness, in accordance with ICH guidelines. The calibration curve was linear in the concentration range 360-840ng perband for cefixime trihydrate and 900-2100ng perband for ornidazole. For cefixime trihydrate, the recovery studies results ranged from 99.81-100.25% with RSD values ranged from 0.321-0.721%. For ornidazole, the recovery studies results ranged from 99.21-100.12% with RSD values ranged from 0.260-0.841%. The method proved to be a rapid and cost-effective quality control tool for routine simultaneous analysis of cefixime trihydrate and ornidazole in the bulk drug and in a tablet dosage formulation.

Key words: Cefixime trihydrate, Ornidazole, HPTLC, Densitometric estimation.

INTRODUCTION

Cefixime trihydrate, is the third generation cephalosporin antibiotic. Cefixime (CEF) chemically it is (6R,7R)-7-[[(Z)-2-(2-aminothiazol-4-yl)-2-[(carboxymethoxy) imino] acetyl] amino]-3- ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2- ene-2-carboxylic acid trihydrate[1-4]. Cefixime is given orally in the treatment of susceptible infections including respiratory tract infections, otitis media, pharynsitis ,gonorrhoea, pharyngitis, and urinary-tract

infections[5]. It official in USP[6]. Ornidazole(ORZ) is chemically 1-chloro-3-(2-methyl-5-nitroimidazole-1yl)-propan-2-ol. It used as anti-infective agent [7]. It is not official in any Pharmacopoeia. Literature survey revealed HPLC[7-10],HPTLC and UV[11-20] methods for the analysis of CEF and ORZ as single component system or in combination with other drugs, hence, no official method is reported for simultaneous estimation of CEF and ORZ in formulations. Because of the absence of an official pharmacopoeial method for the simultaneous estimation of CEF and ORZ in tablet dosage form, efforts were made to develop an analytical method for the estimation of CEF and ORZ in tablet dosage form using HPTLC method. The proposed method was optimized and validated as per the ICH guidelines[21].Structure of Cefixime trihydrate and Ornidazole is shown in Figure1.

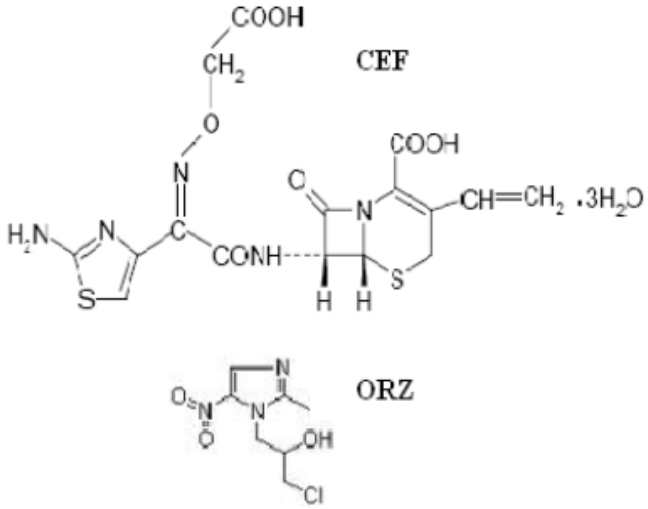


Figure I

Materials:

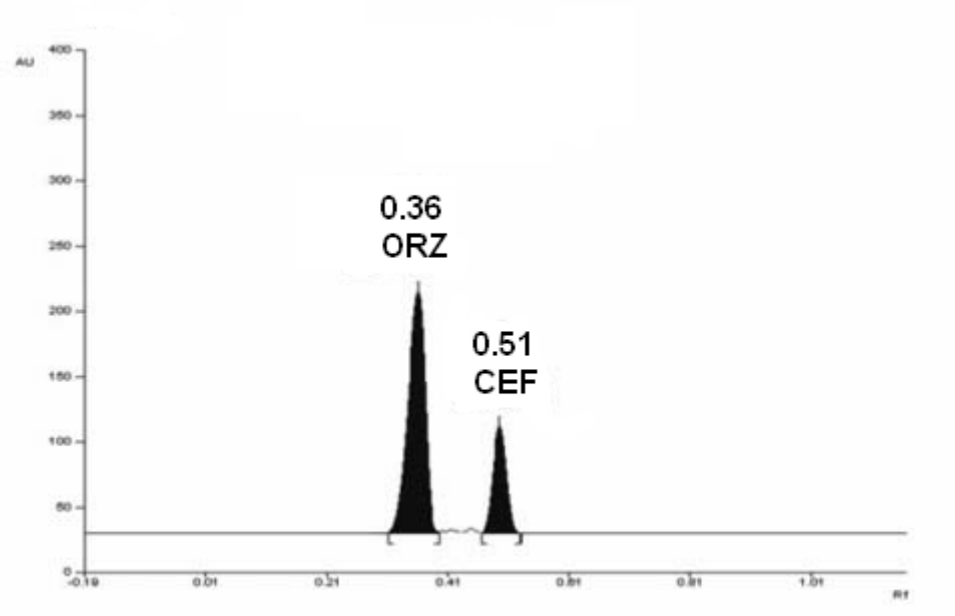
Cefixime trihydrate and Ornidazole were supplied as a gift samples by Orchid pharmaceuticals Chennai) and Emcure Pharmaceutical Ltd, Pune. Fixed dose combination tablets (Cefluv-Oz) containing 200mg of cefixime trihydrate and 500 mg of ornidazole were procured from Wisdom pharma, India. All chemicals and reagents used were of AR grade were purchased from Merck chemicals, Mumbai, India.

Instrumentation and standard chromatographic conditions:

The samples were spotted in the form of bands of width of 6 mm with space between bands of 12 mm, with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel 60 F_{254} aluminum HPTLC plates (20cm×10 cm) with automatic sample applicator LINOMAT V. The plates were prewashed with methanol and activated at110°C for 5 min, prior to chromatography. The linear ascending development was carried out in 20 cm ×10 cm twin trough glass chamber which was already saturated for 10 minutes with the mobile phase consists of using n-butanol-methanol-toluene-ammonia 5:2:1:5 (v/v/v/v). The development distance was 9 cm and the development time 20 min. The plates were dried in a current of air with the help of a hair dryer. Densitometric scanning was performed with a CAMAG TLC Scanner 3 at 287 nm operated by Wincats software version 1.4.2. The source of radiation utilized was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm.

Preparation of Mixed Standard Stock Solution:
0.5 mg /ml of CEF and ORZ prepared by dissolving 25 mg of each drug in 50 ml of methanol. Each solution (2ml) was further diluted to 10ml to furnish stock solution of 100ng μL^{-1}.

Optimization of the HPTLC Method:
Chromatographic separation studies were carried out on the stock solution of CEF and ORZ. Initially on the plates 6µL of stock solution was applied as band 8 mm of width. Plates were developed by linear ascending development using neat solvents like toluene, hexane, methanol, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, etc., without chamber saturation. Based on the results of these initial chromatograms binary and ternary mixtures of solvents were tried to achieve optimum resolution between CEF and ORZ respectively. After several trials, mixture of n-butanol-methanol-toluene-ammonia (5:2:1:5, v/v/v/v) was chosen as the mobile phase for analysis. Other chromatographic conditions like chamber saturation time, run length, sample application rate and volume, sample application positions, distance between tracks, detection wavelength, were optimized to give reproducible R_F values, better resolution, and symmetrical peak shape for the two drugs. Good resolution with R_F value of 0.51 for CEF and 0.36 for ORZ was obtained when densitometric scanning was performed at 287 nm (Fig.II). The spot appeared more compact and peak shape more symmetrical when the TLC plates were pretreated with methanol and activated at 110^{O}C for 5 min. Well- defined spots of standard was obtained when the chamber saturation time was optimized at 20 min at room temperature.



FigureII: Densitogram of Cefixime tri hydrate (R_F 0.51) and Ornidazole (R_F 0.36) of formulation showing no interference of excipients in analysis

Analysis of marketed Formulation:
Quantity of tablet powder equivalent to 25 mg of CEF and 62.5 mg of ORZ was weighed and transferred to a 50 ml volumetric flask containing about 30 mL of methanol, ultra sonicated for 5 min, and diluted to 50 ml methanol. Then it was filtered through Whatmann No 41 filter paper. The 2 ml of sample solution was further diluted to get solutions of 100 ng /µL and 250 ng/µL .Six micro liters of sample solutions were applied as band 8mm at interval under stream of nitrogen. The developed chromatograms were evaluated by scanning in densitometric

mode at 287 nm. The amount of CEF and ORZ present per tablet was calculated by comparing peak area of sample with that of standard. The analytical data are represented in Table 1.

Table 1. Analysis of Tablet Formulation

Drug	Amount present(mg/tab)	Amount found(mg/tab)	% label claim	%RSD*
CEF	200mg	199.98	99.99	0.2458
ORZ	500mg	501.21	100.21	0.2321

** Each value is a mean of six observations*

Validation of the method

Linearity and Range:

For preparation of the calibration plot aliquots of the standard stock solutions of CEF (3.6–8.4µL) and ORZ (9.0–21µL) were applied by over spotting on an HPTLC plate and the plate was developed and scanned as described above. Each standard was analyzed in five replicates and peak areas were recorded. Calibration plots for CEF and ORZ were constructed separately by plotting peak area against respective concentration of CEF and ORZ.

Precision:

Three sets of three different concentrations of standard solution of CEF (400, 600, and800ng per band) and ORZ (1000, 1500, and2000ng perband) were prepared. The intra-day precision of the developed TLC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each on same day. The inter- day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days.

Accuracy:

Accuracy of the method was carried out by applying the method to drug sample(CEF and ORZ combination tablets) to which known amounts of CEF and ORZ standard powder corresponding to 50,100 and 150% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatograms in optimized mobile phase.

Limit of detection and Limit of Quantification:

LOD was calculated from the formula LOD = 3.3σ /S, where σ = Standard deviation of the response calibration curve, S = Slope of the calibration curve and LOQ was calculated from the formula LOQ = 10σ /S, where σ = Standard deviation of the response calibration curve, S = Slope of the calibration curve.

Robustness of the method:

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition like, n-butanol-methanol-toluene-ammonia (5.1:2:1:5, v/v/v/v), (4.9:2:1:5 v/v/v/v), (5:2:1:5.2v/v/v/v), (5:2:1:4.8) were tried and chromatograms were run. The plates were prewashed by methanol and activated at 110^{o}C for 5, 10, 15 min respectively prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40 and 60 minutes. In this also detection wavelength (+/-1nm) is alerted, duration of saturation (+/-5min), development distance (+/-1cm) changes and chromatograms were recorded respectively.

Specficity:
The specificity of the method was determined by analyzing standard drug and test samples. The spot for CEF and ORZ in the samples was confirmed by comparing the RF and spectrum of the spot to that of a standard. The peak purity of CEF and ORZ was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

RESULTS AND DISCUSSION

HPTLC methods are significant methods for Quality assurance of drug molecules. HPTLC has emerged as a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike LC thus reducing the analysis time and cost per analysis. Hence, the method was developed for CEF and ORZ as bulk drug and in pharmaceutical formulation. The method was validated and found to be suitable for routine analysis of the selected drugs. The results of validation studies on simultaneous estimation method developed for CEF and ORZ in the current study involving n-butanol-methanol-toluene-ammonia (5:2:1:5, v/v/v/v) as the mobile phase for TLC are discussed below.

Linearity:
The drug response was linear (r^2 = 0.9994 for CEF and 0.9991 for ORZ) over the concentration range between 360–840ng per band for CEF and 900–2100ng per band for ORZ.and the datas are shown in following tableII.

TableII. Linearity data

Parameter	Cefixime trihydrate	Ornidazole
Linearity range	360-840 ng/band	900-2100ng/band
Slope	1097.313	60.966
Intercept	4.362	23.316
Correaltion Coefficient	0.9994	0.9991
LOD	6 ng/band	20 ng/band
LOQ	30 ng/band	75 ng/band

Precision:
The results of the repeatability and intermediate precision experiments are shown in Table III. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were <2%, respectively as recommended by ICH guidelines.

TableIII. Results of Precision

Drug	Actual conc(ng/band)	Measured conc(ng/band).%R.S.D	
		Repeatabilty	Intermediate
CEF	400	400.07, 0.65	412.21,0.97
	600	611,1.33	615,1.45
	800	825,1.28	814.15,1.37
ORZ	1000	1015.01,0.83	1006.03,1.68
	1500	1501,1.35	1509.12,1.42
	2000	2012.01,1.22	2017.23,1.45

Recovery Studies:
Chromatogram was developed and the peak areas were noted. At each levels of the amount, three determinations were performed. As shown from the data in Table IV good recoveries of the

CEF and ORZ in the range from 99.7 to 100.6% were obtained at various added concentrations. From the data of recovery studies the methods was found to be accurate.

Table IV. Accuracy data

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (nd/band)	Mean Recovery (%)	RSD (%)*
CEF	600	300	901.012	100.112	0.740
	600	600	1200.145	100.01	0.854
	600	900	1499.12	99.94	0.298
ORZ	1500	750	2254.16	100.18	0.487
	1500	1500	2996.21	99.873	0.205
	1500	2250	3755.12	100.13	0.248

**Average from three determinations.*

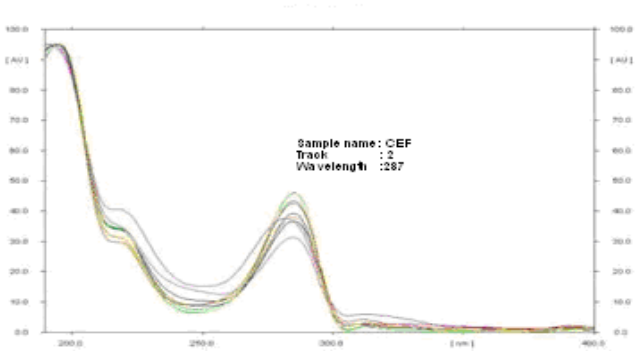
LOD and LOQ

The LOD and LOQ were found to be 6ng/band and 20ngper band respectively for CEF and 30ng/band and 75ngper band respectively for ORZ.

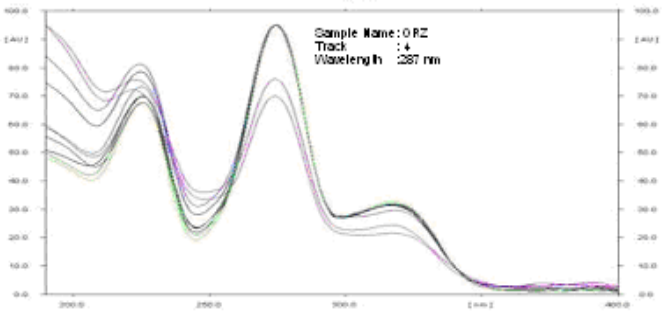
Specficity

The peak purity of both drugs was assessed by comparing the respective spectra of standard drugs and samples at peak start, peak apex and peak end positions of the spot. The peak purity overlay spectra of CEF and ORZ shown in **Fig III & IV** it indicates no interferences of other substances present in the formulation**.** It indicates the specificity of the method.

FigIII: Spectrum of standard Cefixime trihydrate on TLC plate



FigIV: Spectrum of standard Ornidazole on TLC plate



Robustness of the method

The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2%. The low values of % RSD, as shown in Table. V indicated robustness of the method.

Table V. Robustness of the method

S. No	Parameter	Variation	Mean *± RSD	
			CEF	ORZ
1	Mobile phase composition	±2 % (n-butanol)	0.12±0.02	0.76±0.02
2	Chamber saturation period	10%	0.12±0.01	0.77±0.02
3	Development distance	10%	0.13±0.01	0.76±0.03
4	Time from application to development	0,10,20,30	0.12±0.03	0.76±0.01
5	Time from development to scanning	0,10,20,30	0.12±0.00	0.76±0.00

CONCLUSION

The proposed HPTLC method was validated as per ICH guidelines. The %RSD and standard error calculated for the method were low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Hence, it can be concluded that the developed HPTLC method was accurate, precise and selective and can be employed successfully for the estimation of Cefizime trihydrate and ornidazole in tablet dosage form. The method can alsobe used to study the degradation kinetics of CEF and ORZ and also for its estimation in plasma and other biological fluids.

Acknowledgements

The authors are thankful to Orchid pharmaceuticals Chennai and Emcure Pharmaceutical Ltd, Pune for providing gift samples of drugs CEF and ORZ.

REFERCENES

[1] Wison and Gisvold's, Jaime N. Delgado and William A. "Remer's Lippincott – Raven Philadelphia" 10th Eds, pp.287, **1998**.
[2] In: M.J.O.Neil (Ed.), Merck Index, 14th (John Merck research laboratories division of Merck & Co. INC), Whitehouse station, NJ, USA, **1997**,pp1229,1213.
[3]Good mann and Gilmann's The Pharmacological basis of therapeutics,11th Eds,by kaball,**2006**
[4]Roche.G.Cefixime, *Presse Med*,**1989**;18(32):1541-1544.
[5] Wu D.H. A, *ClinTher*,**1993**,15(6):1108-1119.
[6] United state Pharmacopoeia, 27th (pharmacopoeial convention Inc., Twinbrook parkway, Rockville, United state, **2004**) 291-293.
[7] Al-Momani I.F, *J Pharm Biomed Anal*, **2001**; 25(5-6):751-757.
[8] Eric-Jovanovic, S., Agbaba D., Zivanov-Stakic, *J Pharm Biomed Anal*, **1998**; 18(4-5): 893-898.
[9] D. Nagavallai, A.S.K Sankar, K. Anandakumar, K. Karunambigai and M.S.S.N Raju, *Ind. J.of pharm.sci*,**2007**;69:2 page 333-335.
[10] M.Gandhimathi,T.K.Ravi and Nilima shukla, , *Ind. J.of pharm. Sci*,**2006**;68:6 page 838-840.
[11] B. Dhandapani, N. Thirumoorthy, Shaik Harun Rasheed, M.Rama kotaiah and N.Anjaneyalu, *International journal of pharma sciences and research* Vol1 (1):**2010**: page 78-83.

[10] Paramane S, Kothapalli , Thomas A. and Despandey A.D, *Indian journal of pharmaceutical sciences*,**2007**; 69: 525-528.
[12]RanjaneP.N,GandhiS. and Ranher S.S. *Chin.j.chromatogr*.**2008**:26:763-765.
[13] Soma S.M., Vidyasagar V., Narsaiah N., Anandkumar R. and Krishna. *Indian J. Pharm. Sci.* **2005**; 67: 302-306.
[14] Natarajan S and Raman B. *Indian Pharmacist*, **2005**; 4: 79-84.
[15] Nahata CM, *Journal of Liquid Chromatography*, **1991**; 14 (20), 3755-3759.
[16] Shah PB, Pundarikakshudu. K, *Ind. J. Pharm. Sc*., **2006**; 68(1), 90-93.
[17] Jovanovic SE, Agbaba D, Zivanov-Stakie. D and Vladimirov S. *J. Pharm. Biomed. Analysis*,**1998**; 18 (4-5), 893-898.
[18] Liu GU, Sha RG, et al, *Pharm. J*, **1993**; 28 (3), 216-221.
[19] Khaja Pasha, Dr C.S Patil, Dr. K. Vijaykumar, Dr. Sadath Ali and Dr.V.B.Chimkod., *Research J.Pharma.Bio.Chem.Sci*,**2010** vol:(3),226.
[20] Hafiz Muhammad Arshad, Shahnaz Gauhar , Raheela Bano and Iyad Naeem Muhammad, *Jordan Journal of Pharmaceutical* science,. Vol 2,(1):**2009**.
[21] ICH,Q2B,Validation of analytical procedures: Methadology, In proceedings of the International Conference on Hormonisation:Geneva,November,**1996**,1-8.