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SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND ERDOSTEINE IN PHARMACEUTICAL DOSAGE FORM BY USING REVERSE PHASE - HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT: A simple, precise, accurate and sensitive Reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Cefixime trihydrate and Erdosteine in combined capsule dosage form have been developed and validated. Drugs were resolved on a HiQ Sil C_8 column (25cm X 4.6mm, 5µm), utilizing mobile phase of TetraButyl Ammonium Hydroxide (0.1 N aqueous) pH adjusted to 6.5 with Orthophosporic acid (10% aqueous) : ACN in a ratio of 2:1. Mobile phase was delivered at the flow rate of 1.0 ml/minute. Ultra violet Detection was carried out at 254nm. Separation was completed within 11 minutes. Calibration curves were linear with correlation coefficient 0.998 and 0.997 over a concentration range of 2-22 µg/ml for Cefixime trihydrate and 3- 33 µg/ml for Erdosteine respectively. Recovery was between 99.92-101.12 percent and 100.01-100.34 percent for Cefixime trihydrate and Erdosteine respectively. Method was found to be reproducible with relative standard deviation (R.S.D) for intra and interday precision to be <1.5% over the said concentration range.
Key words: Cefixime Trihydrate, Erdosteine, High Performance Liquid Chromatography, Capsules.

INTRODUCTION

Cefixime trihydrate, is the third generation cephalosporin antibiotic. Cefixime is given orally in the treatment of susceptible infections including respiratory tract infections like acute exacerbations of chronic bronchitis, gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis, and urinary-tract infections(1). It official in USP. Chemially it is 5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-corboxylic acid,7-[[2-amino-4-thiazolyl) [(carboxymethoxy)imino]acetyl] amino]-3-ethenyl-8-oxo-,trihydrate,[6R-[6∝,7β(Z)]]-(6R,7R)-7-[2-(-amino-4-thiazolyl) glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid, 7^2-(Z)-[O-carboxymethyl)oxime](2) Erdosteine [2-Oxo-2-[(tetrahydro-2-oxo-3-thienyl)amino] ethyl] thio]acetic acid is a mucolytic and is official in Martindale(3). It Modulates mucus production, viscosity and increases mucociliary transport, thereby improving expectoration and thus it shows mucolytic and antitussive activity(4-5).

Investigations are done to study effect of mucolytic on antibiotic penetration in sputum and it reveals that mucolytics improve the same(6-8). Hence combination of an antibiotic with a mucolytic is a treatment of choice for acute exacerbations of chronic bronchitis. Also comparative evaluation of cefixime plus erdosteine and amoxicillin plus bromhexine shows that former gives faster and better symptomatic relief and was also better tolerated than later(9). As Combination is not available in the market it was also developed.

There are several investigations concerning the determination of cefixime alone and in combination with other drugs in pharmaceutical preparations and

plasma by UV , HPLC,LC-MS, HPTLC methods(10-14)
One stability indicating HPTLC method is reported for erdosteine(15). Erdosteine and its optical active metabolite have been analyzed by high-performance liquid chromatography using a fluorescent chiral tagging reagent(16). Sensitive determination of erdosteine in human plasma has been achieved by automated 96-well solid-phase extraction and LC–MS–MS(17).
No references have been found for simultaneous quantitative determination of Cefixime trihydrate and Erdosteine in pharmaceutical preparations. Hence attempts were made to develop Simultaneous HPLC method.
In this paper we report simple, accurate, precise and sensitive Reverse phase high performance liquid chromatography method for simultaneous determination of Cefixime trihydrate and Erdosteine in combined capsule dosage form.. The proposed method is optimized and validated according to ICH guidelines(18).

EXPERIMENTAL

Reagents

All chemicals and reagents used were of a HPLC grade or an analytical grade. Cefixime trihydrate was kindly supplied as gift sample by Maxim Pharmaceuticals, Pune and Erdosteine was also obtained as a gift sample. Rest of all chemicals and reagents were obtained as follows.
TetraButyl Ammonium Hydroxide solution (0.1 N aq solution) and Acetonitrile were obtained from Sisco Reasearch Laboratories and Merck Laboratories Pvt Ltd, Mumbai respectively.
Orthophosporic acid (10% aqueous), Dibasic Sodium phosphate and Monobasic Potassium Phosphate solution were obtained from S.d.Fine Chem Ltd. Mumbai.

Equipment

1. HPLC was performed using a Jasco HPLC system 2000 consisting of
a pump PU2080 Plus ,
Rheodyne sample injection port with 20 microlitre loop,
UV detector 2075 plus.
Borwin Software version 1.
Column used was C-8 (4.6 × 250mm, 5µ).
2. Shimadzu Model AY-120 balance
3. Delux 101 pH meter
4. Calibrated glassware were used for the study

Chemicals and materials

Preparation of TetraButyl Ammonium Hydroxide solution

15ml of 0.1 N TetraButyl Ammonium Hydroxide solutions was diluted to 150 ml with HPLC grade water, pH adjusted to 6.5 with Orthophosporic acid (10% aqueous).

Preparation of monobasic potassium phosphate solution

Solution was prepared by weighing 6.8 gm of monobasic potassium phosphate and dissolving it in to 500 ml of water.

Preparation of phosphate Buffer pH 7.0

Buffer Solution was prepared by weighing 7.1 gm of Dibasic Sodium phosphate and dissolving it in to 500mL of water and adjusting its pH to 7 with monobasic potassium phosphate solution

Praparation of Standard Stock Solution

Standard Stock Solution of each drug having concentration of 1mg/ml was prepared by dissolving pure drugs, Cefixime trihydrate and Erdosteine separately in phosphate buffer pH 7.0.

Preparation of solutions for calibration curve

Standard Stock solutions of both drugs were diluted as 1ml to 10ml with phosphate buffer pH 7.0. These solutions were further diluted to get solutions of concentrations 2,6,10,14,18,22 µg/ml and 3,9,15,21,27,33 µg/ml of Cefixime trihydrate and Erdosteine respectively.

Procedure for Sample Preparation /capsule analysis

Sample details:

Composition: Each hard gelatin capsule contains
Cefixime trihydrate equivalent to Cefixime USP 200mg
Erdosteine 300mg.
Gross weight of one capsule: 734 mg
Fill weight of one capsule: 617mg
Contents of 20 capsules were emptied and powdered and capsule powder equivalent to 10 mg of Cefixime trihydrate and 15 mg of Erdosteine was taken in 10mL volumetric flask, and dissolved in sufficient phsophate buffer solution with aid of sonication and volume was made up to 10mL with phosphate buffer solution. Resultant solution was first filtered through whatman filter paper No. 41 and then through 0.45µ filter paper in order to remove the excipients. 1ml of the filtrate was diluted to 10ml with phsophate buffer pH 7.0. Again 1ml of this solution is diluted to 10 ml with phosphate buffer solution to get final concentration of 10 µg/ml and 15 µg/ml for Cefixime trihydrate and Erdosteine respectively.

Dilutions for Precision studies

Precision of the method was checked by 3 replicate readings at 3 concentration levels. Concentration levels

used for Cefixime trihydrate were 10, 14, 18 µg/ml and that for Erdosteine were 15, 21, 27 µg/ml.

Dilutions for Recovery studies

To study accuracy of the method, recovery studies were carried out by addition of standard drug solution to sample at 3 different levels, 80%, 100% and 120% of the test concentration (test concentration is 10 µg/ml for Cefixime trihydrate and 15 µg/ml for Erdosteine)

Robustness studies

Robustness of the method was determined by small, deliberate changes in flow rate, mobile phase ratio, Wavelength of detection and pH of mobile phase. Flow rate was changed to 1 ± 0.5 ml/min. The mobile phase ratio was changed to ± 3% for both components. Wavelength of detection was changed to 254± 2nm; pH was changed to 6.5 ± 0.1

LOD and LOQ Determination

Limit of detection can be calculated by using following formula:

$$LOD = \frac{3.3\ \sigma}{S}$$

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

$$LOQ = \frac{10\ \sigma}{S}$$

Where σ = Standard deviation of the response

S = Slope of the calibration curve

System Suitability Testing

System Suitability Testing is used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. Parameters such as therotical plates, tailing factor, resolution and reproducibility (%RSD for retention time and for area of six replicates) are determined and compared against the specifications. The results of system suitability and system precision were presented in table no-2.

RESULTS AND DISCUSSION

Method Development and Optimisation

Various mobile phases were tried containing methanol, phosphate buffer, acetic acid, but as chemical nature of both drugs is same (acidic) both drugs were eluting out nearly at same retention time. Litreature survey revealed the importance of ion pairing reagents to resolve chemically similar drugs. Ion-exchange chromatography systems have previously been utilized in HPLC analysis of ionic samples. Recently, reversed phase partition chromatography using ion-pair reagents has been developed and utilized. The ionic samples form an ion-pair with ion-pair reagents in the mobile phase to become electrically neutral. The increase in hydrophobic character of the ion-pair results in a greater affinity for the reverse stationary phase and leads to sample resolution. For acidic samples, analysis is performed with pH adjusted to 7.5 with the addition of quaternary ammonium salts to the mobile phase. Acidic samples form an electrically neutral ion-pair with the quaternary ammonium salt eg. Tetrabutylammonium Phosphate, TetrabutylammoniumHydroxide(19).

Use of TetraButyl Ammonium Hydroxide was preferred as it is used as mobile phase component of pharmacopoeial method for Cefixime. Its use with ACN provided good resolution and peak shape. Thus TetraButyl Ammonium Hydroxide pH 6.5: ACN was finalized as mobile phase. By trying various proportions of TetraButyl Ammonium Hydroxide solution and ACN, final proportion selected was 2:1 (TetraButyl Ammonium Hydroxide solution : ACN)

UV absorption spectra of both drugs in the range of 200-400nm showed considerable absorbance for both drugs at 254nm and at the same wavelength, diluents showed no interference. Therefore it was selected as detection wavelength as shown in figure 1.

Flow rate of 1ml/min provided runtime of about 10 minutes and was used as flow rate.

C8 is common stationary phase suitable for many pharmaceuticals and was found to be suitable in this case also.

With above selected method parameters, system suitability testing provided good resolution and reproducibility and was adequate for analysis to be performed. The results of system suitability are shown in table no.2

The method was validated for various parameters as per ICH Guidelines. The results of method validation are shown in table no. 3

Method validation

The linear relationship was observed between the AUC and concentration over the range of 2-22 µg/ml for cefixime trihydrate and 3- 33 µg/ml for Erdosteine. The linearity was expressed as correlation coefficient, which was 0.998 for Cefixime trihydrate and 0.997 for Erdosteine. Correlation coefficient, y- intercept, slope of regression line are shown in table no.3 and Figure -3 and 4

As per ICH guidelines, for assay procedure of active substance or finished product, range should be 80 – 120% of the test concentration. Therefore range of 2-22 µg/ml was selected for Cefixime trihydrate and 3-33 µg/ml was selected for that of Erdosteine.

Precision was carried out as repeatability as per ICH guidelines. It was determined at 3 concentration levels with 3 replicates at each level. For all three

concentration levels % RSD obtained was less than 1.5 % for both Cefixime trihydrate and Erdosteine. The results of precision are given in table no.6

Considering composition of the dosage form, 10 µg/ml of Cefixime trihydrate and 15 µg/ml of Erdosteine was selected as test concentration. The recovery studies were carried out at 80, 100, and 120% of test concentration. The results ranged from 98.88 -100.99 % for Cefixime trihydrate and 99.09 – 101.59 % for Erdosteine. Results of recovery studies are shown in table no.4

Robustness studies were carried out after deliberate alterations of flow rate, mobile phase compositions, wavelength of detection and mobile phase pH. It was observed that the small changes in these operational parameters, did not lead to changes of retention times of peak of interest. Results of robustness studies are shown in table no.5

The proposed method was evaluated in the assay of capsule formulation containing Cefixime trihydrate and Erdosteine. Five replicate determinations were carried out on capsules. Mean % content was 99.38 % for Cefixime trihydrate and that for Erdosteine was 100.33 %. Results of capsule analysis was shown in table no. 7

CONCLUSION

The method described enables the quantification of Cefixime trihydrate and Erdosteine in combined capsule dosage form. The validation data demonstrate good precision and accuracy, which prove the reliability of the proposed method. Hence, this HPLC method can be used routinely for quantitative estimation of both components in capsule dosage form.

Table 1. Conditions Used for Chromatographic Analysis

Sr No	Parameter	Conditions used for Analysis
1	Mobile phase	TetraButyl Ammonium Hydroxide (0.1 N aqueous) pH 6.5 with Orthophosporic acid (10% aqueous): ACN (2:1)
2.	Flow rate	1 ml/min
3.	Detection Wavelength	254 nm
4.	Sample injector	50 µl loop
5.	Column	HiQ Sil C_8 Kya tech (4.6 × 250mm, 5µ)

Table 2. System Suitability Testing

Drug	Retention time (min)	Area	No Of Plates	Resolution	Asymmetry
Cefixime trihydrate	10.00	451091.4	5836.73	0.0	1.1
Erdosteine	5.4	133777.1	4316.31	2.9	1.4

Table 3. Results of validation Parameters

Sr No	Parameters	Results	
		Cefixime trihydrate	**Erdosteine**
1.	Linearity (R^2) Y – intercept Slope of regression line	0.9982 24792 44216	0.9979 1032 9216
2.	%RSD (Indicates precision)	< 2 %	< 2 %
3.	Mean % Recovery	100.52	100.12
4.	Limit of Detection	0.31 µg/ml	0.28µg/ml
5.	Limit of Quantitation	0.94µg/ml	0.84µg/ml
6.	Range	2-22 µg/ml	3-33µg/ml

Table 4. Results for Recovery study

% Added (% of test conc.)	**Amount of drug after std. addition** (µg/ml)		**Mean Area***		**Amount Found** (µg/ml)		**% Recovery**	
	CEF	ERDO	CEF	ERDO	CEF	ERDO	CEF	ERDO
80	18	27	829645.4	250731.7	18.20	27.09	101.12	100.34
100	20	30	908421.2	277569.1	19.98	30.003	99.92	100.01
120	22	33	1002734	305277.2	22.11	33.007	100.53	100.02

*mean area of three replicates

Table 5. Results for Robustness Study

% RSD *Found For Robustness Study								
	Flow Rate **(1mLmin^{-1})**		pH **(6.5)**		Mobile phase ratio (2:1)		Wavelength of detection 254nm	
Drug used	0.9	1.1	6.4	6.6	+3%	-3%	252	256
Cefixime Trihydrate	1.50	1.36	1.21	0.96	1.07	0.81	1.61	1.22
Erdosteine	1.80	1.62	1.28	1.17	1.22	1.59	1.00	1.33

*%RSD for three replicates

Table 6. Results for Method Precision

Sr No	Conc. in µgmL^{-1}	Mean Area	Std Dev	%RSD
Cefixime Trihydrate	10	465939	4195.32	0.90
	14	654518.7	7180.94	1.097
	18	829784.6	3122.34	0.37
Erdosteine	15	139187.3	782.18	0.56
	21	198401.1	3095.89	1.56
	27	248091.3	3565.04	1.43

Table 7. Results for Capsule Assay Study

Sr. No.	Label Claim (µg mL^{-1})		Amount Found (µg mL^{-1})		% of Label Claim	
	Cefixime Trihydrate	Erdosteine	Cefixime Trihydrate	Erdosteine	Cefixime Trihydrate	Erdosteine
1.	10	15	9.87	15.16	98.76	101.10
2.	10	15	10.16	14.83	101.63	98.87
3.	10	15	10.01	15.13	100.11	100.90
4.	10	15	9.81	15.02	98.13	100.13
5.	10	15	9.82	15.09	98.24	100.63

Figure 1. Overlain spectra of Cefixime Trihydrate (CEF) and Erdosteine(ERDO)

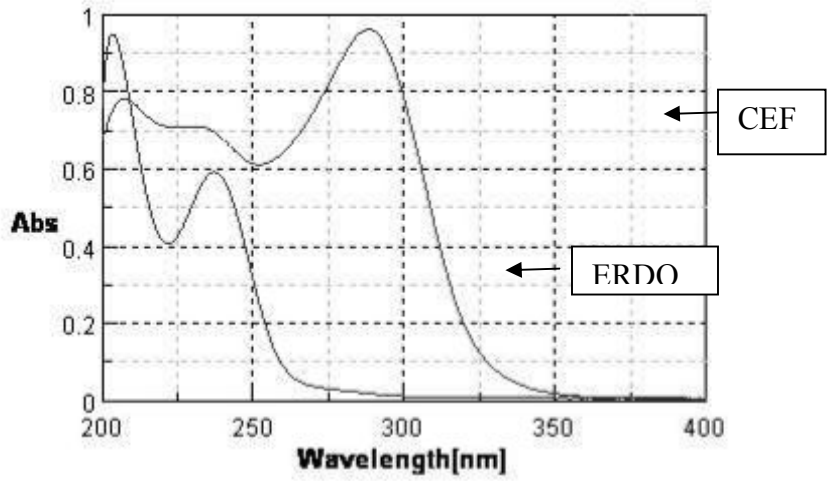


Figure 2. Resolution Study for Cefixime trihydrate and Erdosteine

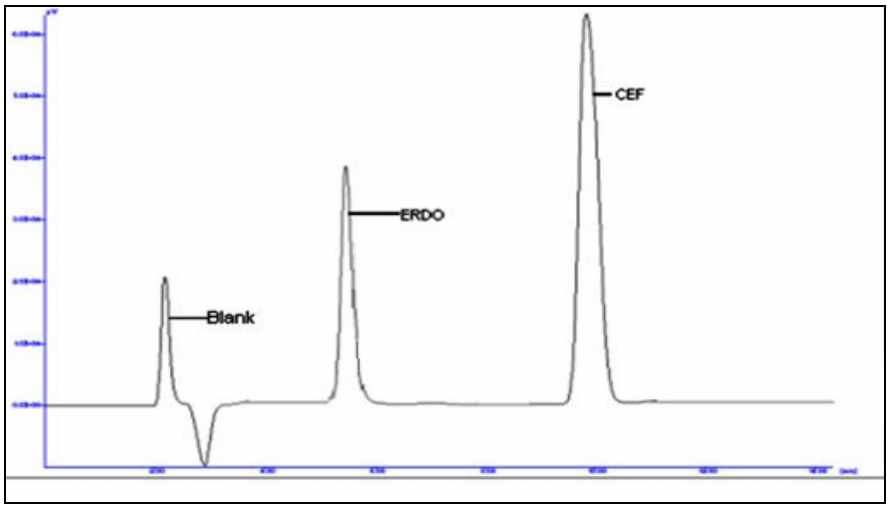


Figure 3. Calibration Curve for Cefixime trihydrate

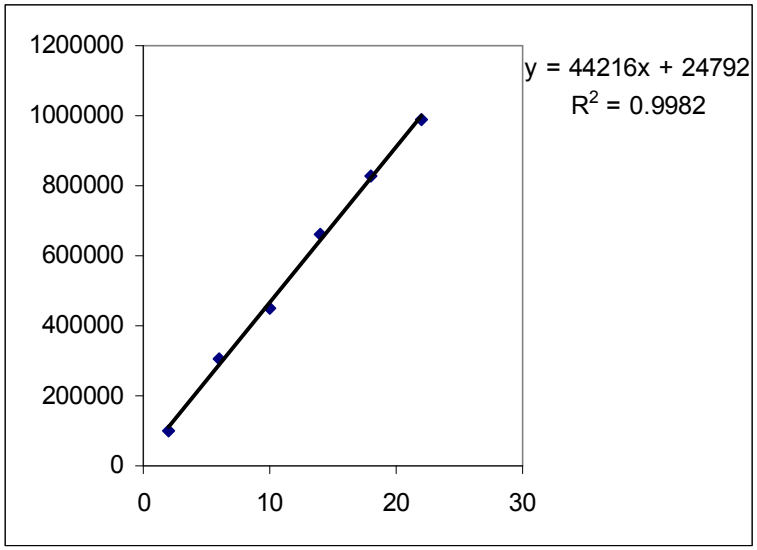
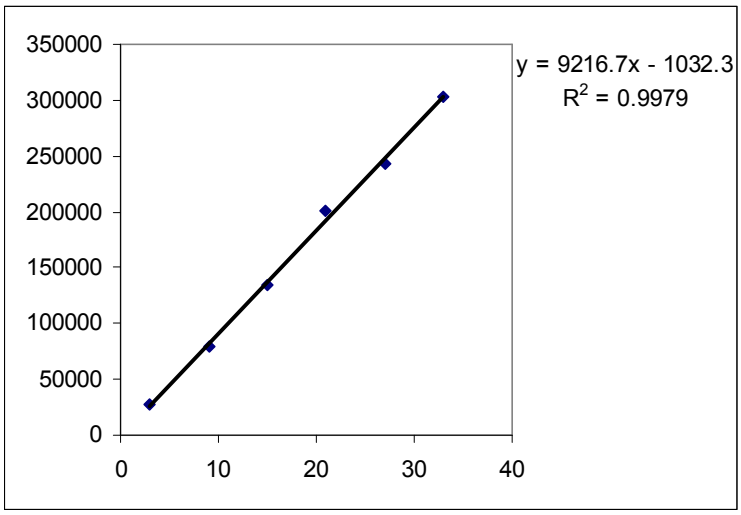


Figure 4. Calibration Curve for Erdosteine



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