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Simultaneous Determination of Cefepime, Cefotaxime and Ceftriaxone in Pharmaceutical formulation by HPLC Method

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Abstract: *An analytic high performance liquid chromatography (HPLC) procedure for assay of cefepime, cefotaxime and ceftriaxone has been developed and validated. However, a procedure, which is simple and accurate, required to be developed to be easily employed for quality control. The chromatographic separation was conducted on HPLC Shimadzu using column C18, 5 µm, (250 x 4.6 mm) with a mobile phase was isocratic consisted of Acetonitrile and 0.1% Formic acid* 50%, *in the ratio of 25:75 (by volume) and was delivered to the system at a flow rate of 1.5 ml/min. An injection volume of 20 µl was used for cefepime, cefotaxime and Ceftriaxone. The detection wavelength (λ_{max}) was 260 nm for cefepime, cefotaxime and Ceftriaxone. The calibration curve of cefepime in mobile phase was linear with correlation coefficient (r2) = 0.9992; over a concentration range of 25.0 – 100.0 mg/L for; with a retention time of 1.79 minutes, the calibration curve of cefotaxime in mobile phase was linear with correlation coefficient (r2) = 0.9998; over a concentration range of 50.0 – 200.0 mg/L for; with a retention time of 4.19 minutes. While the calibration curve of Ceftriaxone in mobile phase was linear with correlation coefficient (r2) = 0.9991; over a concentration range of 100.0 – 400.0 mg/L for; with a retention time of 2.86 minutes. The method described is quite suitable for routine analysis in pharmaceutical dosage form.*

Keywords: *RP-HPLC, Cefepime, Cefotaxime, Ceftriaxone, Antibiotics.*

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I. Introduction

Cephalosporins, β-lactam antibiotics are widely used as antibiotics. It has been highlighted as an antibactericidal, which its mechanism of action is closely related to penicillin and cephamicin that are also β-lactam antibiotics. Cefepime is fourth-generation, semisynthetic, broad spectrum, cephalosporin antibiotic for parenteral administration. Chemically, it is 7-[α-(2-aminothiazol-4-yl)-α-(z)methoxyimino acetamido]-3-(1-methylpyrrolidino)-methyl-3-cephem-4 carboxylate, it is characterized by the presence of a positively charged quaternerized N-methyl-pyrrolidine substitution at the 3 position of the cephem moiety, making cefepime a zwitter ion as shown in Fig. 1. It is official in The United States Pharmacopeia and the British Pharmacopeia. It is used clinically for the treatment of lower respiratory tract, intra-abdominal, urinary tract, skin and soft tissue infections and also used for prophylaxis in biliary tract and prostate surgery. Cefepime/TAZ is one of the dosage form combination already licensed and used in Indian hospitals [1, 2].

A literature survey revealed that several liquid chromatography methods had been reported for the determination of cefepime alone [3–6].

Chemically, cefotaxime (Fig. 2) is (7b-(2- (2-aminothiazol-4-yl)-(Z)-2-methoxyimino acetamido)-3-acetoxymethyl-3-cephem-4-carboxylic acid or its sodium salt).

Chemically, ceftriaxone (Fig. 3) is (6R,7R) - 3 [(acetyl - oxy) methyl] - 7 - [[(2Z)-(2-amino-4 thiazolyl)(methoxyamino)- acetyl] amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene- 2-carboxylic acid.

Cefotaxime and ceftriaxone are third generation broad spectrum cephalosporins for parenteral administration and are bactericidal and mainly used in the treatment of various bacterial infections caused by gram positive and Gram-negative micro-organisms [7,8]. The analytical methods focused on separation and determination of a mixture of these compounds by methods used capillary electrophoresis [9], TLC [10,11], HPLC with ammeter [12], voltmeter [13], HPLC with PDA detector [14], and UV detector [15,16].

Therefore, the aim of the present study was to determine main antibiotics, Cefepime, Cefotaxime and Ceftriaxone present in pharmaceutical formulations by reversed phase HPLC.

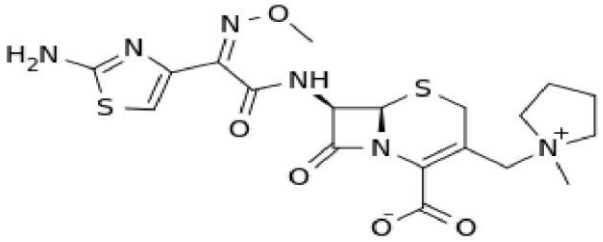

Fig. 1: The Chemical structure of cefepime.

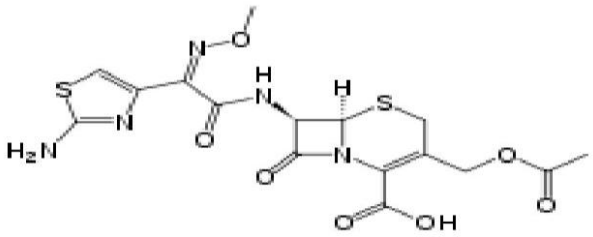

Fig. 2: The Chemical structure of Cefotaxime.

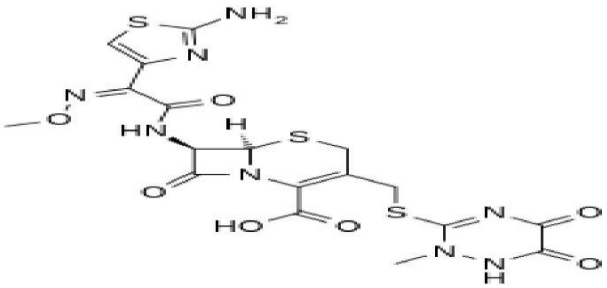

Fig. 3: The Chemical structure of Ceftriaxone.

II. Materials and Methods

All chemicals and reagents used were HPLC grade. Pure standards of Cefepime, Cefotaxime and Ceftriaxone were obtained from Chinese. Acetonitrile was HPLC grade from Romil. Formic acid 50% was HPLC from Sigma Aldrich. Purified HPLC grade water was obtained by reverse osmosis and filtration through a 0.45-µm membrane filter; all used solutions were prepared using applied purification method.

HPLC Instrumentation and Chromatographic Conditions:

Chromatographic separation was performed on C_{18}, 5 µm column (250 mm × 4.6 mm) that was purchased from USA. The optimum mobile phase was prepared by mixing Acetonitrile and 0.1% Formic acid 50%, in the ratio of 25:75 (by volume). The mobile phase was filtered by using a 0.45 µm nylon membrane filter. A wavelength of 260 nm was chosen since it was found to be the most appropriate for the determination of the three active ingredients because both the drugs have sufficient absorption at this wavelength. The flow rate used was 1.5 ml/minute. The injection volume was 20 µl. The total run time of the system was about 5.0 minutes.

Standard and Sample Solution Preparation

A 50 mg of Cefepime, 50 mg Cefotaxime and A 50 mg of Ceftriaxone working standard were weighed and transferred into a 100 ml volumetric flask. 85 ml of the distilled water was added and shake on vortex for 2 min; then was sonicated for 5.0 minutes. Working standard solutions were prepared and further diluted in distilled water to contain a mixture of Cefepime, Cefotaxime and Ceftriaxone in over the linearity range from 25.0 – 100.0 mg/L, 50.0 – 200.0 mg/L and 100.0 – 400.0 mg/L respectively. Mixed well and filtered through 0.45µm filter. Similar procedure was also carried out for sample preparation.

Linearity

Linear calibration plots of the proposed method were obtained over concentration ranges of 25.0-100.0 mg/L (25.0, 40.0, 50.0, 80.0 & 100.0 mg/L) for Cefepime, 50.0-200.0 mg/L for Cefotaxime (50.0, 80.0, 100.0,

160.0 & 200.0 mg/L) and 100.0-400.0 mg/L (100.0, 160.0, 200.0, 320.0 & 400.0 mg/L) for Ceftriaxone. Triplicate injections were made for each standard solution.

Accuracy

Accuracy was evaluated by standard addition method of cefepime, cefotaxime and ceftriaxone. This method known amounts of cefepime, cefotaxime and ceftriaxone were added to the previously analysed sample solution and then experimental and true values were compared. Three levels were made corresponding to 80%, 100% and 160% of the nominal analytical concentration.

Precision

Repeatability was studied by determination of intra-day and inter-day precision. Intra-day precision was determined by injecting five replicates of three different concentrations on the same day and inter-day precision was determined by injecting the same solutions for three consecutive days. Relative standard deviation of the peak area was then calculated to represent precision.

Robustness

Premeditate variations were performed in the experimental conditions of the proposed method to assess the method robustness. For this intention, minor changes were made in mobile phase composition, flow rate and pH of buffer solution. The effect of these changes on chromatographic parameters such as retention time, tailing factor and number of theoretical plates was then measured.

Limit of detection (LOD) and limit of quantitation (LOQ)

Limits of detection (LOD) were calculated according to the expression 3.3σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. Limits of quantification (LOQ) were established by using the expression 10σ/S. LOD and LOQ were experimentally verified by injections of pure standard at the LOD and LOQ concentrations.

III. Results and Discussion

The proposed HPLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of Cefepime, Cefotaxime and Ceftriaxone were shown in Fig. 4. There was clear resolution between Cefepime, Cefotaxime and Ceftriaxone with retention time of 1.79, 4.19 and 2.86 minutes; respectively.

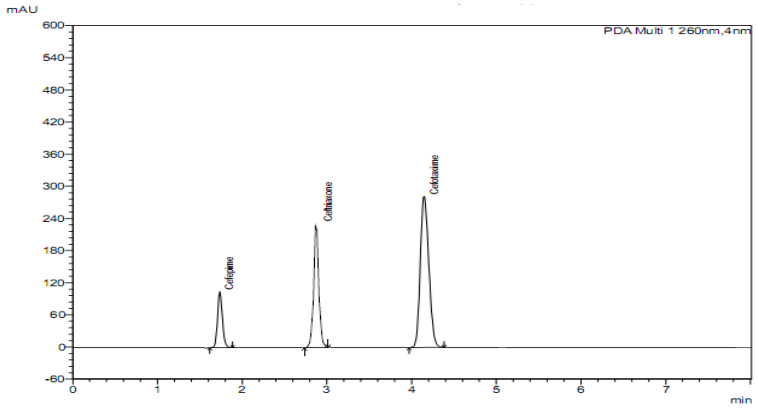

Fig. 4: HPLC Chromatogram for cefepime, cefotaxime and ceftriaxone.

Specificity

Specificity of a method is its suitability for the analysis of a compound in the presence of potential impurities. Placebo, standards, and sample test solutions were all injected at the same wavelength of 260 nm to demonstrate the specificity of the optimized method. A comparison of the retention times of Cefepime, Cefotaxime and Ceftriaxone in sample solutions and in the standard solutions were exactly the same. Fig. 5 showed that there were no interferences at the retention times for Cefepime, Cefotaxime and Ceftriaxone due to the placebo. Therefore, the proposed method is suitable for the quantification of the active ingredients in dosage form.

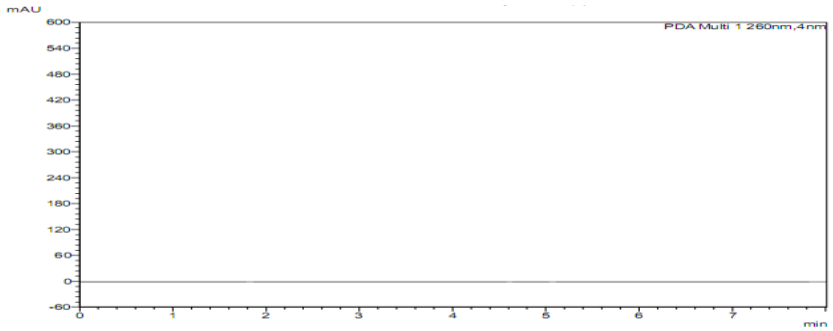

Fig. 5: HPLC chromatogram of Placebo

Linearity

Standard stock solution was diluted to prepare solutions containing of 25.0-100.0 mg/L for Cefepime, 50.0-200.0 mg/L for Cefotaxime and 100.0-400.0 mg/L for Ceftriaxone. Accurately measured working standard solutions of Cefepime (25.0, 40.0, 50.0, 80.0 & 100.0 ml) as shown in Fig. 6 and data are shown in Table 1, Cefotaxime (50.0, 80.0, 100.0, 160.0 & 200.0 ml) as shown in Fig. 7 and data are shown in Table 2, Ceftriaxone (100.0, 160.0, 200.0, 320.0 & 400.0 ml) as shown in Fig. 8 and data are shown in Table 3. Each of the concentrations was injected in triplicate to get reproducible response. Calibration curves were constructed by plotting peak area versus concentration. Each reading was average of three determinations. They were represented by the linear regression equation.

$Y_{Cefepime} = 7856.7237x + 1603.7657$, $r^2 = 0.9992$
$Y_{Cefotaxime} = 17718.6382x + 15571.3571$, $r^2 = 0.9998$
$Y_{Ceftriaxone} = 4149.6321x - 20775.7706$, $r^2 = 0.9997$

Slopes and intercepts were obtained by using regression equation (Y = mx + c) and least square treatment of the results used to confirm linearity of the method developed.

Table 1: HPLC analytical parameters for linearity of cefepime

% of Working Concentration	cefepime	
	Concentration (mg/L)	Observed peak area (mean)
50%	25	191289
80%	40	325746
100%	50	390951
160%	80	634491
200%	100	783275
Slope:	7,856.7237x	
Intercept:	1,603.7657	
r^2	0.9992	

Table 2: HPLC analytical parameters for linearity of cefotaxime

% of Working Concentration	Cefotaxime	
	Concentration (mg/L)	Observed peak area (mean)
50%	50	893312
80%	80	1418769
100%	100	1814787
160%	160	2855772
200%	200	3549214
Slope:	17,718.6382x	
Intercept:	15,571.3571	
r^2	0.9998	

Table 3: HPLC analytical parameters for linearity of ceftriaxone

% of Working Concentration	Ceftriaxone	
	Concentration (mg/L)	Observed peak area (mean)
50%	100	394942
80%	160	642427
100%	200	803223
160%	320	1321309

200%	400	1630785
Slope:	**4,149.6321x**	
Intercept:	**- 20,775.7706**	
r^2	**0.9997**	

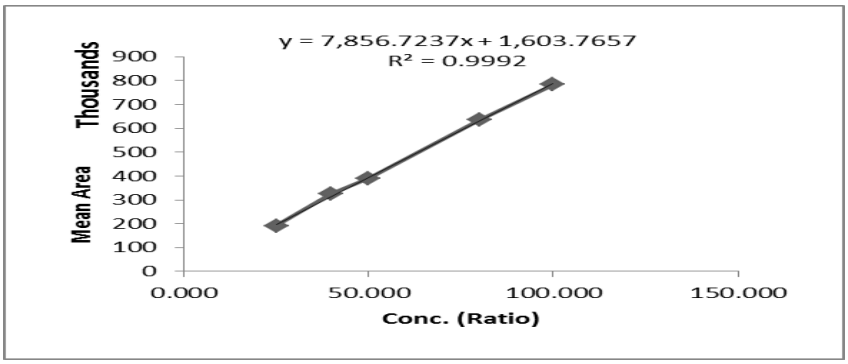

Fig. 6: Calibration curve for cefepime.

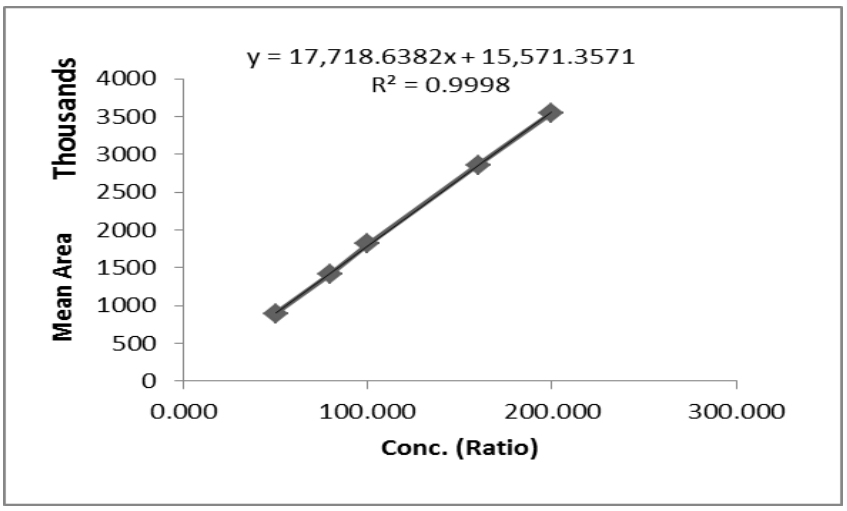

Fig. 7: Calibration curve for cefotaxime.

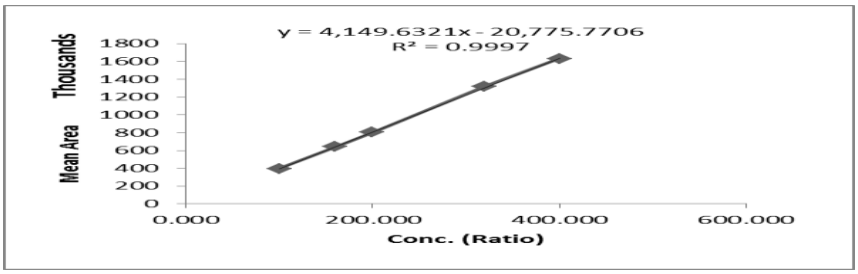

Fig. 8: Calibration curve for Ceftriaxone.

Quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed methods. The LOD is the lowest concentration of the analyte that can be detected with signal to noise ratio (3:1) and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy with signal to noise ratio (10:1). The LOD of Cefepime, Cefotaxime and Ceftriaxone found to be 6.67 mg/L. The LOQ of Cefepime, Cefotaxime and Ceftriaxone found to be 20.0 mg/L.

Accuracy

Accuracy was calculated by addition of standard drugs to preanalyzed sample at 3 different concentration levels and computing percentage recoveries. Standard limit of % recovery study is 98 - 102 % as per ICH guideline. From the studies it was concluded that % recovery study of Cefepime, Cefotaxime and

Ceftriaxone complies with standard limit of ICH guideline. Results of accuracy were proven by the Table 4, 5 and 6.

Table 4: Accuracy and recovery results of cefepime in pharmaceutical market as Pimfast vial.

Working Concentration, (mg/L)	Peak area (Mean*)	Found concentration (mg/L)	% Recovery
40 mg/L	319747	40.49	**101.23**
50 mg/L	390880	49.55	**99.09**
80 mg/L	632494	80.30	**100.37**

Table 5: Accuracy and recovery results of cefotaxime in pharmaceutical market as Rametax vial.

Working Concentration, (mg/L)	Peak area (Mean*)	Found concentration (mg/L)	% Recovery
80 mg/L	1434353	80.07	**100.09**
100 mg/L	1807330	101.12	**101.12**
160 mg/L	2846195	159.75	**99.85**

Table 6: Accuracy and recovery results of ceftriaxone in pharmaceutical market as Rameceftrax vial.

Working Concentration, (mg/L)	Peak area (Mean*)	Found concentration (mg/L)	% Recovery
160 mg/L	643806	160.15	**100.10**
200 mg/L	802370	198.37	**99.18**
320 mg/L	1314886	321.87	**100.59**

Solution Stability

In this study, the mobile phases, the standard solutions, and the sample solution were subjected to long term (3 days) stability studies. The stability of these solutions was studied by performing the experiment and looking for changes in separation, retention, and asymmetry of the peaks which were then compared with the pattern of the chromatogram of freshly prepared solutions

System suitability

The system suitability was determined by injecting six replicates of the standard solutions and analyzing each active ingredient for its peak area, peak tailing factor, resolution, number of theoretical plates, and capacity factor. The values obtained demonstrated the suitability of the system for the analysis of the above drug combinations System suitability parameters might be fall within ± 3% standard deviation range during routine performance of the methods.

IV. Conclusion

The running cost, speed of analysis, high degree of specificity and selectivity plays important role in deciding the suitability of an HPLC method for analysis of pharmaceuticals. The use of isocratic HPLC methods for simultaneous quantification of two or more drugs is getting popularity because such methods not only lower the cost but also increase the speed of analysis. The purposed isocratic reverse phase HPLC method is simple, specific and accurate with a single and low cost solvent system for determination of three different cephalosporins. The simultaneous quantification of the three important cephalosporins with isocratic solvent system in a single run not only saves the solvent but also with a short run time makes it a better choice for the analysis of these drugs in quality control.

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