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RP-HPLC method for the estimation of ceftriaxone and cefdinir third generation cephalosporin in Dosage form

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Abstract: The proposed RP-HPLC methods are developed and subsequently validated which are rapid, sensitive and reproducible for the analysis of ceftriaxone1-8 (A) and cefdinir9-15 (B) in pharmaceutical dosage forms with short analysis time. A mixture of 0.01M Ammonium di hydrogen phosphate(ADP),acetonitrile(ACN), o-phosphoric acid, OPA, (1%) in 5:50:45 (v/v/v) ratio for ceftriaxone and 5% tetra hydro furan (THF), 15% methyl alcohol,40% acetonitrile and 40% (0.1%) ortho phosphoric acid (OPA) for cefdinir were proved to be the most suitable of all the combinations since the chromatographic peaks obtained were well defined, resolved and free from tailing. Flow rates of 1.5 mL/min for ceftriaxone 1.0 mL/min for cefdinir mobile phases were found to be suitable and retension times were 5.3 min. for ceftriaxone 5.1 min. for cefdinir under optimum conditions. Column: A non polar Luna C18 column (250 mm X 4.6 mm,5µ) was chosen as the stationary phase for this study. The spectra of ceftriaxone and cefdinir showed balanced wavelengths at 220 nm and 284 nm respectively.

Keywords-RP-HPLC, Ammonium dihydrogen Phosphate,,acetoninitrile, o-phosphoricacid, Tetra hydro furan and methyl alcohol.

Introduction

Ceftriaxone,(A) chemically known as 6*R*,7*R*)-7-{[(2*Z*)-2-(2-amino-1,3- thiazol-4-yl)- 2-(methoxyimino) acetyl]amino} -3-{[(2-methyl -5,6-dioxo-1,2,5,6-tetrahydro- 1,2,4-triazin -3-yl) thio] methyl}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. .Like other third-generation cephalosporins, it (Ceftriaxone Sodium, CFTS) has broad spectrum activity against gram-positive and gram-negative bacteria. In most cases, it is considered to be equivalent to crfotaxime in terms of safety and efficacy. Ceftriaxone is often used (in combination, but not direct, with macrolide and/or amino glycoside antibiotics) for the treatment of community acquired or mild to moderate health care-associated pneumonia. It is also a choice drug for treatment of bacterial meningitis. In pediatrics, it is commonly used in febrile infants between 4 and 8 weeks of age who are admitted to the hospital to exclude sepsis. It is used for the treatment of gonorrhea, in single intramuscular injection.

Cefdinir, (B) is chemically known as 8-[2-(2-amino-1,3-thiazol-4- yl)-1-hydroxy-2- nitroso-ethenyl] amino-4-ethenyl-7-oxo- 2-thia-6- azabicyclo [4.2.0]oct-4-ene-5-carboxylic acid, a semi-synthetic, broad- spectrum antibiotic in the third generation of the cephalosporin class. It is used to control for common bacterial infections of the ear, sinus, throat, and skin. Therapeutic uses of cefdinir include otitis media, soft tissue infections, and respiratory tract infections,

Including sinusitis, strep throat, community-acquired pneumonia and acute exacerbations of bronchitis.

Experimental

Chemicals

Ammonium di hydrogen phosphate –Aceto nitrile-Tetra hydro Furan-Methanol

A mixture of 0.01M Ammonium di hydrogen phosphate(ADP), acetonitrile(ACN), o-phosphoric acid, OPA, (1%) in 5:50:45 (v/v/v) ratio for A, and a mixture of 5% tetra hydro furan (THF), 15% methyl alcohol,40% acetonitrile and 40% (0.1%) ortho phosphoric acid (OPA) for B were proved to be the most suitable mobile phase of all the combinations since the chromatographic peaks obtained were well defined, resolved and free from tailing. Flow rates of mobile phases will be 1.5 mL/min for A and 1.0 mL/min for B were found suitable in the studied ranges of 1.0—2.0 mL/min.(A), 0.5—1.5 mL/min. (B)

HPLC grade (Merck industries) ortho-phosphoric acid HPLC grade (SD Fine Chemicals, Chennai) were used.

Chromatography

Instrumentation

A Scimadzu HPLC equipped with a Luna C_{18} column (250 mm X 4.6 mm,5µ) an LC 20 AD pump and a SPD 20 AD UV- Visible detector was employed in this study. Chromatographic analysis and data acquision was monitored by using Spinchrome software. A 20 µL Hamilton syringe was used for sample injection. Degassing of the mobile phase was done by using a spectra lab. DGA 20A3 Ultra sonic bath sonicator. A Shimadzu electronic balance was used for weighing the materials.

Wave length: The spectra of dilute solutions of ceftriaxone and cefdinir were recorded on UV

spectrophotometer. The peaks of maximum absorbance wavelengths were observed at 220 nm and 284 nm respectively.

Preparation of solutions

Mobile phase (for A) is a mixture of 0.01M Ammonium di hydrogen phosphate(ADP), aceto nitrile (ACN), o-phosphoric acid, OPA, (1%) 5:50:45 (v/v/v) which was prepared by diluting 5 ml of ADP in a mixture of 50 ml of ACN and 45 ml of OPA in one litre flask. This mixture was used as a diluent for preparing working standard solutions of the drug.

Mobile phase (for B) is a mixture of 5% tetra hydro furan (THF), 15% methyl alcohol,40% acetonitrile and 40% (0.1%) ortho phosphoric acid (OPA) which was prepared by mixing 5 mL

of THF, 15 mL of methyl alcohol,40 mL of acetonitrile and 40 mL of OPA in one Liter flask. This mixture was used as a diluent for preparing working standard solutions of the drug.

About 100 mg of ceftriaxone (or cefdinir) was weighed accurately and transferred into a 100 mL volumetric flask containing 10 mL of mobile phase. The solution was sonicated for 20 min. and then the volume was made up with a further quantity of mobile phase to get 1 mg/mL solution. This solution was suitably diluted with mobile phase to get a working standard solution of 100µg/mL of ceftriaxone (or cefdinir).

Optimization of Chromatographic Conditions: Method development

Column: A non polar C18 column was chosen as the stationary phase for this study.

Retention Time: Under the above optimized conditions retention of 5.3 min. for ceftriaxone 5.1 min. for cefdinir were observed. A typical model chromatograms of ceftriaxone and cefdinir are presented in Fig.1.

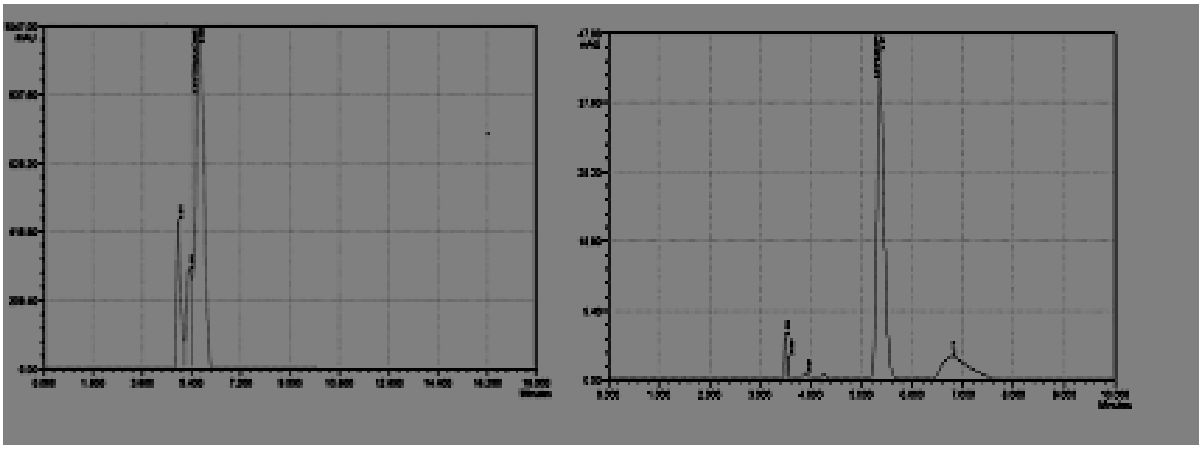

Fig.1.Chromatogram of ceftriaxone sodium and Cefdinir

Validation of the method.

These methods were validated in compliance with ICH guidelines. The following parameters were determined for validation.

Specificity:

The specificity of the methods were assessed by comparing the chromatograms obtained from the drugs with the most commonly used excipients mixture with those obtained from the blank solutions. The blank solution was prepared by mixing the excipients in the mobile phase without the drug. The drug to excipient ratio used was similar to that in the commercial formulations. The commonly used excipients in formulations like lactose, microcrystalline cellulose, ethyl cellulose, hydroxyl propyl methyl cellulose, magnesium stearate and colloidal silicon di oxide were used for the study. The mixtures were filtered through 0.45µ membrane filter before injection.

An observation of chromatograms indicates absence of excipients peaks near the drug peak in the study runtime. This indicates that the method is specific.

Table II. Linearity Data

Concentration(A)	Area (A)	Concentration(B)	Area (B)
0.5 mg/mL	55199	100µg/mL	204562.7
1.0 mg/mL	108561	200µg/mL	352580.9
1.5 mg/mL	158633	300µg/mL	484083.5
2.0 mg/mL	205321.1	400µg/mL	635797.9
2.5 mg/mL	249449	500µg/mL	798418.8

Table III. Regression Characteristics

Parameter	(A) Value	(B)Value
Linearity Range(µg/mL)	50-250	100-500
Slope(a)	97053	1470929.2
Intercept(b)	-0.09922	0.36
Correlation Coefficient	0.999275	0.9993
Regression Equation	Y=97053x-0.09922	Y=1470929.2x+0.36

Linearity:
A plot was constructed between concentrations versus the peak areas noted from the chromatograms. The regression of the plot was computed by least square method. The linearity ranges were found to be 50—250 µg/mL for ceftriaxone, and 100—500 µg/ml for cefdinir. Regression equations were later used to estimate the amount of ceftriaxone and Cefdinir in pharmaceutical dosage forms. The linearity was shown in Fig.2 and the linearity data and statistical parameters for linearity plot are reported in Tables II. and III.

Precision:
The precision of the methods were studied in terms of repeatability in intra-day assay and inter-day assay (intermediate precision). Method repeatability was studied by repeating the assay three times in the same day for intra-day precision, and intermediate precision was studied by repeating the assay on three different days, three times each day (inter day precision).The intra day and inter day variation for determination of ceftriaxone or cefdinir were carried out at four different concentrations. %RSD values are presented in the Table-IV. Shows that the methods provide acceptable (<2) intraday and inter day variation.

Accuracy*:*
Accuracy of the methods were evaluated by standard addition method. An amount of the pure drug at three different concentrations in its solution has been added to the pre analysed working standard solution of the drug. The sample solutions were analysed in triplicate at each level as per the proposed method. The percent recovery and %RSD for recovery at each level are calculated. The results are tabulated (Table-V.). A mean recovery ranged from 99.4 – 99.7 has been obtained by the methods indicate their accuracy.

Table IV. Intra and Inter day precision for A

Concentration of Ceftriaxone µg/mL	Intra-Day Precision			Inter-Day Precision
	Mean Amount found	Amount found	%RSD n=3	Mean amount found
30	29.98	98.9	2.7	30.02
60	60.5	101.25	1.3	59.92
90	89.95	99.91	0.90	89.52
120	120.55	100.68	0.67	119.65

Table IV. Intra and Inter day precision for B

Concentrat ion of Cefdinir µg/mL	Intra-Day Precision			Inter-Day Precision	
	Mean amount found n=3	% Amount found	% RSD	Mean amount found n=3	% Amount found
50	49.78	98.9	1.64	50.02	100.1
100	99.5	101.25	0.82	99.92	99.8
150	149.95	99.91	0.54	149.52	99.2
200	200.55	100.68	0.40	199.65	99.56

Robustness*:*
A study was conducted to determine the effect of deliberate variations in the optimized chromatographic condition of the mobile phase, flow rate, and the pH of the mobile phase. The effect of these changes on the system suitability parameters like tailing factors, the number of theoretical plates, and on assay was studied. A single condition was studied at a time keeping all other parameters constant. The results were found to be within the allowed limits indicate that the methods are robust.Table.VI.

Variation in composition of mobile phase: The effect of variation in percent organic content in mobile phase was evaluated by changing the composition of organic components in the mobile phase. The tailing factor and the number of theoretical plates showed a little change

with change in mobile phase composition. The values are presented in Table-VI.

Table-V. Accuracy Data for A

Amount taken mg	Amount found mg	Percent %Recovery	Mean Recovery	%RSD
0.5	0.494	98.8	99.4	0.49
0.5	0.496	99.2		
0.5	0.502	100.4		
1.0	0.997	99.7	99.5	0.29
1.0	0.993	99.3		
1.0	0.995	99.5		
1.5	1.5002	100.01	99.7	0.26
1.5	1.492	99.46		
1.5	1.498	99.86		

Table.V. Accuracy data for B

Amount taken µg	Amount found µg	Percent Recovery	Mean Recovery
100	99.43	99.53	99.55
100	99.41	99.41	
100	99.73	99.73	
300	299.65	99.88	99.96
300	300.2	100.006	
300	300.05	100.016	
500	499.85	99.97	99.96
500	499.61	99.92	
500	500.02	100.004	

Table – VI. Results of Robustness Study for A

Variation of Mobile Phase			Chromatographic Parameters
$NH_4H_2PO_4$	ACN	OPA	Tailing factor
5	55	40	1.56
10	40	50	1.52
5	45	50	1.52

Table – VI. Results of Robustness Study for B

Variation of Mobile Phase				Chromatographic Parameters	
				Tailing factor	Theoretical plates
THF	ACN	MeOH	OPA		
5	45	10	40	1.58	3490
5	40	20	35	1.47	3502
10	40	15	35	1.6	3570

Table-VII. LOD and LOQ of Ceftriaxone & Cefdinir

Parameter	Value (µg/mL) for A	Value (µg/mL) for B
LOD	10	5
LOQ	40	20

Table- VIII. System Precision for A and B

Injection Number	Peak Area A	Theoretical Plates A	PeakArea B	Theoretical Plates B
1	978285.6	6763	488960.9	3245
2	985840.6	6727	470118.8	3480
3	985647.4	6722	468755.8	3478
4	985361.2	6725	466523.1	3522
5	981549.7	6788	475804.0	3408
6	984542.8	6744	467828.4	3468
Mean	983336.9	–	472998.5	---
SD	3332.2	–	7720.351	----
%RSD	0.338	–	1.7	----

Table-IX. Analysis of formulations & Recovery experiments for A

Sample	Labeled Amount	Amount found	%Recovery
Ciplacef	125 mg	124.86 mg	99.88
Cefast	125 mg	124.55 mg	99.64

Table-IX. Analysis of formulations & Recovery experiments for B

Sample	Labeled Amount	Amount found	%Recovery
Cefdiel	500 mg	499.6 mg	99.92
RTIST	500 mg	495.75 mg	99.15

Variations in flow rates: A study was conducted to determine the effect of variation in flow rate. The system suitability parameters were evaluated at 0.9 mL/min and 1.5 mL/min. The results were within the acceptance crieteria. Hence the allowable variation in flow rate is 1.0 mL/min. to 1.5mL/min.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD = 3.3X S.D of y intercept÷ Slope of Calibration curve

LOQ = 10X S.D of y intercept÷ Slope of Calibration Curve

In this study the analyte response is 10 times greater than the noise response. For this study six replicates of the analyte at lowest concentration in the calibration range were measured and quantified. The LOD and LOQ of ceftriaxone and cefdinir obtained by the proposed methods were 10 and 40 µg/mL and 5 and 20 µg/mL respectively. (Table-VII.)

Results and discussion

In order to effect analysis of the component peaks, different combinations were tested as mobile phase on a C_{18} stationary phase. A mixture 0.01M $NH_4H_2PO_4$ acetonitrile,1% ortho-phosphoric acid in a proportion of 5:50:45(v/v/v) for ceftriaxone and a mixture of 5% tetra hydro furan (THF), 15% methyl alcohol,40% acetonitrile and 40% (0.1%) ortho phosphoric acid (OPA) for cefdinir were proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention times obtained for ceftriaxone and cefdinir were 5.3 min. and 5.1 min. respectively.

Each of the sample was injected six times and the same retention times were obtained in all cases. The peak areas of ceftriaxone and cefdinir were reproducible as indicated by low coefficient of variation. A good linear relationship (r= 0.9992 for A and 0.9993 for B) was observed between the concentration of ceftriaxone (or cefdinir) and the respective peak areas. The regression curves were constructed by linear regression fitting and their mathematical expression were Y=97053X-0.0992 (for A), Y=1470929.2X+0.36 (for B) where Y gives peak area and X is the concentration of the drug. The regression characteristics are given in Table II.. Low % RSD were observed when ceftriaxone solutions containing 30,60,90,120 µg/mL (or 50,100,150,200 µg/mL for cefdinir) were analysed by the proposed methods for finding out intra and inter day variations.

High recovery values obtained from the dosage form by the proposed methods indicate that the methods are accurate(Table IX). The absence of additional peaks indicates non interference of common excipients used in the tablets. The drug content in tablets was quantified using the proposed analytical methods. The tablets were found to contain an average of 99.76 % (A) and 99.53 % (B) of the labeled amount

of the drug. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and percent assay. This indicates that the present methods are robust. The lowest values of LOD and LOQ obtained by the proposed methods indicate the methods are sensitive. The standard solutions of the drugs were stable upto 24 hours as the difference in percent assay is within acceptable limit.

Conclusion

Hence the authors conclude that the proposed RP-HPLC methods are sensitive and reproducible for the analysis of ceftriaxone and cefdinir in pharmaceutical dosage forms with short analysis time.

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