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Development and validation of new RP-HPLC method for the estimation of ceftriaxone sodium in bulk and pharmaceutical dosage form

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Abstract

A simple, rapid, accurate and precise RP-HPLC method was developed and validated for the determination of ceftrioxone sodium in parentral dosage form. Chromatographic analysis of the drug was achieved on CYBER LAB HPLC comprising of LC- 100 P pump, a variable wavelength programmable LC-UV100 UV detector and SCL system controller. Flowrosil C18 column (250 mm x 4.6 mm, 5 µ) as stationary phase with mobile phase consisting of methanol: water : ortho-phosphoric acid in the ratio of (75 :24.5 :0.5 v/v) . The method showed a good linear response in the concentration range of 10-50 µg/ml with correlation coefficient of 0.9990. The flow rate was maintained at 1.0 ml/min and detection was carried out at 240 nm. The retention time was 3.788 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, solution stability, selectivity and sensitivity. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the determination of ceftrioxone sodium in parentral formulation.

Keywords: Ceftrioxone; RP-HPLC; Parentral dosage form; ICH; Sensitivity

1. Introduction

Ceftriaxone sodium (CFT) is third-generation cephalosporin; the molecular formula is $C_{18}H_{18}N_8O_7S_3$. Chemically (6R, 7R)-7- {[(2Z)-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, scheme I . The CFT is resulting from a fermentation product, this group of unsafe importance because they are able to overcome the blood-brain barrier. However, it has activity broad spectrum next to Gram-negative and Gram-positive bacteria. Ceftriaxone sodium injection is an antibacterial use to treat diseases, for example, lower respiratory tract infections, skin structure infections, urinary tract infections, pelvic inflammatory disease, bone and joint infections also meningitis . More papers were published in articles aimed at expanding and demonstrating HPLC and LC / MS resistors for similarity and evaluation of this active ingredient in pharmaceuticals. The essential examination of the work of organic modifiers on selectivity, determination, and the temperature is an effect on chromatographic behaviour. HPLC has also been developed through the stationary phases that have been improved through the 30-alkyl carbon atoms chains. A number of techniques have been described the determination of ceftriaxone in literature such as HPLC-UV [1-4], HPLC-MS [5,6], UPLC-MS/MS [7,8], spectrophotometry [9- 11] and spectrofluorometric [12] The RP-HPLC [13-20] methods have superiority over the UV spectrophotometric methods [21-24] in terms of selectivity and sensitivity. We here in reported a simple, sensitive, precise, accurate, linear and isocratic RP-HPLC method for the simultaneous quantitative estimation of Ceftriaxone sodium as per ICH guidelines.

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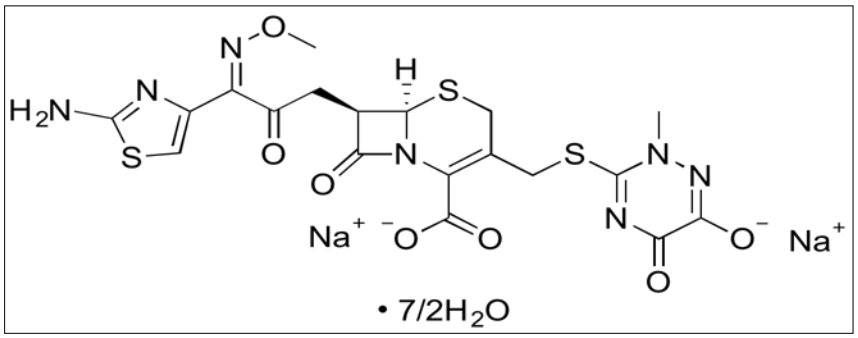

Figure 1 Structure of Ceftriaxone sodium

2. Material and methods

2.1. Instrumentation

Chromatographic separation was performed on a Cyberlab HPLC system equipped with a Flowrosil C18 column (250 × 4.6 mm,with 5 µm particle), single pumps, degasser, variable wave length detector and Rheodyne injector with 20 µl loop volume. 'LC solution' software was used to collect and process the data. Ultra sonicator (Citizen ultra sonicator) was used for sonicating the drug and sample solution.Digital weighing balance (SHIMADZU AUX 220) used for weighing.

2.2. Chemicals and Reagents

All reagents and chemicals used were grade of analytical reagent and H_2O (HPLC grade) was used during the study. Ceftrioxone sodium standard gifted from Sun Pharmaceutical Industries Limited Mumbai, Maharashtra. A commercial injection vial of LEEONE containing 1000 mg of Ceftriaxone sodium was purchased from local medical store.

2.3. Chromatographic conditions

The chromatographic system used for method development and validation includes the LC-P100 pump, variable wavelength programmable LC-UV100 UV detector and SCL20A system controller at CYBERLAB HPLC. A Rheodyne injector 7725i equipped with a 20 µL loop was used and the data was recorded and evaluated using LC solution software version 5.0. Separation was performed at Flowrosil C18 (250 × 4.6 mm i.d., 5µm) at the ambient temperature. A mixture of methanol : water : ortho-phosphoric acid in the ratio of 75 :24.5 :0.5 v/v was found to be the ideal mobile phase for the ideal chromatographic analysis of ceftrioxone sodium. The solvent mixture was filtered through a 0.22 µ membrane filter and sonicated before use. It is pumped through the column at a flow rate of 1.0 mL / min. The injection volume is maintained in the column at 20 µL and room temperature. The column was balanced by pumping the mobile phase through the column for at least 20 min before injecting the drug solution. The detection was monitored at 240 nm. Run time is set to 10 minutes. Optimized chromatographic conditions are shown in Table 1.

Table 1 Optimized chromatographic conditions

Parameters	**Conditions**
Stationary Phase (Column)	C_{18} (250 × 4.6 mm i.d.,5µ)
Mobile Phase	methanol : water : ortho-phosphoric acid (75 :24.5 :0.5 v/v)
Flow rate(ml/min)	1.0 mL/min
Run time(min)	10 min
Column temperature (°C)	Ambient
Volume of injection loop(µL)	20
Detection wavelength(nm)	240 nm
Retention time(min)	3.78

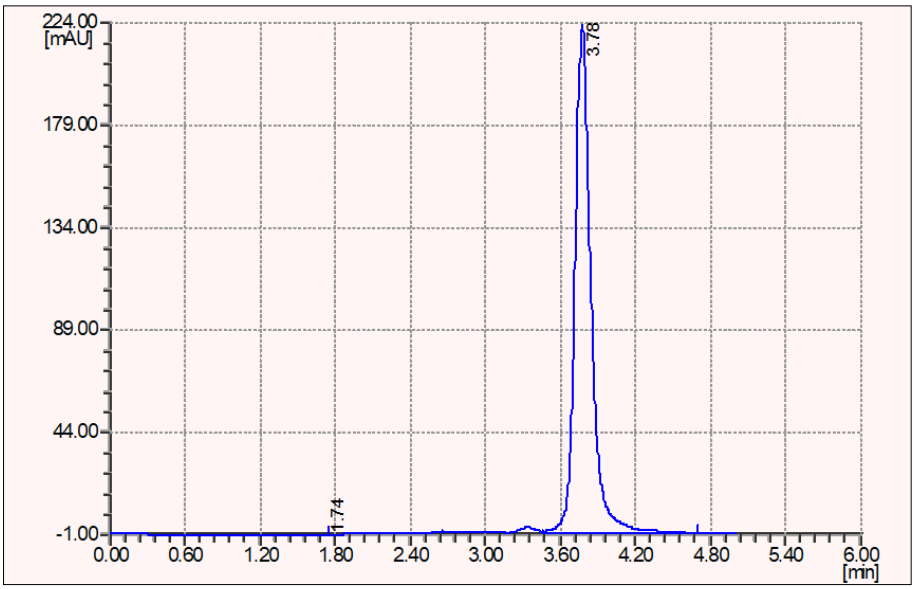

Figure 2 Chromatogram of standard solution of Ceftriaxone sodium

2.4. Preparation of mobile phase

Mobile phase was prepared by mixing 750 mL of HPLC grade methanol with 245 mL water (HPLC grade water) and added 5 ml orthophosphoric acid. The mobile phase was sonicated for 60 min and filtered through the 0.22 µm membrane filter.

2.5. Preparation of standard stock solutions

The standard stock solutions of 1000 µg/mL of the drug were prepared by dissolving 10 mg of ceftrioxone sodium working standard into 10 ml of volumetric flask, Add 10 ml of mobile phase and volume was made up to the mark . Transfer 1 ml of standard stock solution in to 10 ml of volumetric flask and dilute the volume with mobile phase solvent (100 µg/ml). from above solution pipette out 1, 2, 3, 4 & 5 ml into 10 ml volumetric flasks and makeup the volume with mobile phase solvent (10, 20, 30, 40, 50 µg/ml).

2.6. Preparation of sample solution

To determine the content of ceftrioxone sodium in injection dosage form (Label claim: 1000 mg/vial). The powder weight equivalent to 10 mg of ceftrioxone sodium was taken and dissolved in 10 ml of mobile phase. The resulting solution (2 ml) was transferred to a 10 ml volumetric flask and diluted up to the mark with mobile phase. The final solution was filtered through 0.22 µ membrane filter using injection filter. A 20 µL of the filtrate was injected into chromatographic system. The peak area of the ceftrioxone sodium was determined and concentration was found using linear regression equation obtained from calibration curve.

2.7. Method Validation

The developed method was validated as per ICH guidelines [25] by evaluating linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability. Coefficients of variation and relative errors of less than 2 % were considered acceptable.

2.7.1. System Suitability Test

Before performing validation experiments, system suitability test (SST) has to be applied to indicate that HPLC system and method are capable of providing data with admissible quality. SST was performed by investigating capacity factor, tailing factor, theoretical plates number, and also relative standard deviation (RSD) of the peak areas.

2.7.2. Stability

Stability was assessed by analyzing QC standard solutions after keeping them at room temperature for 48 hr. Obtained results were investigated as recovery values and compared to the freshly prepared solutions.

2.7.3. Linearity

A stock solution of ceftrioxone sodium of 1000 µg/mL was prepared with mobile phase. From it, various working standard solutions were prepared in the range of 10 to 100 µg/ml and injected into HPLC. It was shown that the selected drug had linearity in the range of 10–50 µg/mL. The calibration plot (peak area of ceftrioxone sodium versus ceftrioxone sodium concentration) was generated by replicate analysis (n=6) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program.

2.7.4. Accuracy

The accuracy of the method was carried out using one set of different standard addition methods at different concentration levels, 80%, 100% and 120%, and then comparing the difference between the spiked value (theoretical value) and actual found value.

2.7.5. Precision

The precision of the method was ascertained from the peak area obtained by actual determination of six replicates of a fixed amount of the drug (30 µg/mL). The precision of the assay was also determined in terms of intra- and inter-day variation in the peak areas of a set of drug solutions on three different days. The intra- and inter-day variation in the peak area of the drug solution was calculated in terms of relative standard deviation (RSD).

2.7.6. Robustness

Robustness of the proposed method for ceftrioxone sodium was carried out by the slight variation in flow rate, analytical wavelength and mobile phase ratio. The percentage recovery and RSD were noted for ceftrioxone sodium.

2.7.7. Ruggedness

The test solutions were prepared as per test method and injected under variable conditions. Ruggedness of the method was studied by different analysts.

2.7.8. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were established based on the calibration curve parameters, according to the following formulas:

$$LOD=3.3SD/slope$$

$$LOQ=10SD/slope$$

or detection limit=3.3σ/s, quantification limit=10σ/s, where σ is the standard deviation of y-intercept of regression line, and s is the slope of the calibration curve.

2.7.9. Specificity

The specificity of the proposed method was determined against blank and placebo applications. Here mobile phase was used as blank and excipients like starch, lactose, magnesium stearate were used as placebo.

3. Results and Discussion

3.1. Method validation

3.1.1. System Suitability Test

After setting the optimum conditions, system suitability parameters for the developed method were determined and compared with recommended limits. To determine the parameters, the study was performed with standard solution of 30 µg/ml concentration and the results were acquired from six injections. System suitability parameters of the method were demonstrated in Table 2. According to the results, all of the system suitability parameters were within the recommended limits and the method was found to be suitable for the analysis.

Table 2 Results of system suitability test (n = 6)

Parameter	Criteria	Result
Capacity factor(k')	$k' > 2$	3.824
Tailing factor (T)	T < 2	1.3
Theoretical plates (N)	N> 2000	2895
% RSD (peak area)	% RSD ≤ 2	1.21

3.1.2. Stability

The sample solution stability was analyzed by injecting the same solution at 0, 12, 24, and 48 h. Identical change was not observed in the developed method. Also, results were found within acceptable limits (% RSD < 2), which are summarized in Table 3.

Table 3 Stability data of ceftrioxone sodium (standard solutions**)**

Time (hr)	Assay(%)	% Difference
Initial	100.08	----
After 12 hr	100.02	0.05
After 24 hr	99.87	0.21
After 36 hr	99.16	0.92
After 48 hr	98.32	1.76

3.1.3. Linearity and sensitivity

Linearity study was performed with calibration standards with 10, 20, 30, 40, and 50 µg/ml concentrations. The standards were injected in triplicate. Calibration curves were obtained by plotting the peak areas against the given concentrations. The calibration curve was evaluated by the determination coefficient. The determination coefficient (R^2) of the calibration curves was 0.999. Therefore, the calibration curve for ceftrioxone sodium was found to be linear within the range of 10–50 µg/ml concentrations as shown in Fig.3. The regression equations were calculated from the calibration graphs. The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The values of LOD and LOQ are given in Table 4.The low values of LOD and LOQ indicates the sensitivity of method.

Table 4 Spectral and statistical data for determination of ceftrioxone sodium by proposed RP-HPLC method

Parameter	Result
Detection wavelength (nm)	230
Linearity range (µg/ml)	10-50
Coefficient of determination (r^2)	0.9988
Regression equation (Y^a)	Y= 7600.7x – 2060.9
Slope (m)	7600.7
Intercept (c)	-2060.9
Limit of detection, LOD (µg/ml)	0.054
Limit of quantitation, LOQ (µg/ml)	0.16

[a]$Y = mx + c$, where x is the concentration (µg/ml).

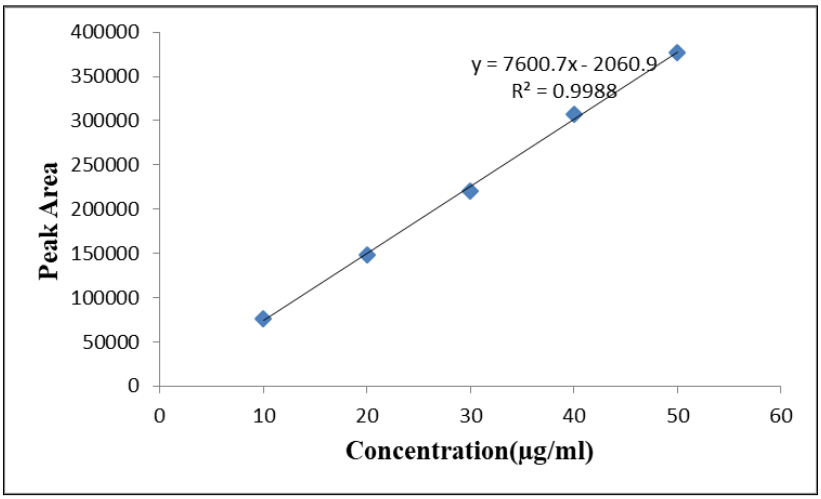

Figure 3 Calibration curve of ceftrioxone sodium

3.1.4. Accuracy

To study the reliability, the suitability, and the accuracy of the method, recovery experiments were carried out. Known quantities of the pure drug were added to the preanalyzed sample to make samples at the levels of 80%, 100%, and 120%, and were assayed by the proposed method. Accuracy was calculated as the percentage of recovery. The recovery and relative standard deviation for each of the analytes are given Table 5. From the recovery studies it is evidence that the method is highly accurate and can give excellent results.

Table 5 Accuracy results

% spike Level	Sample	Amount Added (Std)	Amount Found (µg/ml)	% Recovery	Statistical Parameters
80	30	24	23.76	99.0	Mean = 99.1 SD= 0.458 % RSD=0.462
	30	24	23.90	99.6	
	30	24	23.68	98.7	
100	30	30	29.65	99.5	Mean = 98.9 SD= 0.655 % RSD=0.663
	30	30	29.46	98.2	
	30	30	29.70	99.0	
120	30	36	35.67	99.1	Mean = 98.9 SD= 0.529 % RSD=0.535
	30	36	35.38	98.3	
	30	36	35.74	99.3	

3.1.5. Precision

The precision was demonstrated at three levels: repeatability, intermediate precision, and reproducibility (between laboratories' precision). Each level of precision was investigated by 3 sequential replicate of injections of three concentrations of 20, 30 and 40 µg/mL. The precision was expressed as relative standard deviation (RSD) or coefficient of variation (CV). The results of three levels of precision are shown in Table 6. The developed method was found to be precise as the RSD values for repeatability, intermediate precision and reproducibility studies were < 2 %, respectively as recommended by ICH guidelines (ICH Q2 (R1), 2005).

Table 6 Precision results

Precision	**Results**		
	Concentration(µg/mL)	**% RSD of Peak area**	**% RSD of Retention Time**
Repeatability	20	0.89	0.02
	30	1.21	0.08
	40	1.11	0.12
Intermediate precision	20	1.42	0.08
	30	0.75	0.06
	40	0.67	0.06
Reproducibility	20	1.64	0.11
	30	0.78	0.17
	40	0.85	0.09

3.1.6. Robustness and ruggedness

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (1.0±0.1 mL/min), mobile phase composition (± 5% organic phase) and analytical wavelength (±2 nm). The results are given in Tables 7. The result shown that have the negligible effect on retention time, recoveries and peak area of ceftrioxone sodium indicating the developed method is robustness. Ruggedness of the method was carried out by different analysts. The results are displayed in Table 8.There is no variation in peak areas and retention time of ceftrioxone sodium from studies carried out by two analysts as indicated by % RSD < 2 gives the method ruggedness.

Table 7 Robustness studies

Parameter	**Variation**	**Observed value**			
		% RSD of area	**% RSD of R.T**	**Tailing factor**	**Theroteical plates(N)**
Flow rate (m L/min)	0.9	0.35	0.91	1.5	2541
	1.1	0.59	0.73	1.5	2534
Mobile Phase Composition	80% methanol	0.42	0.14	1.4	2562
	70 % methanol	0.54	0.13	1.5	2551
Wavelength (nm)	242 nm	0.55	0.61	1.5	2545
	238 nm	0.62	0.74	1.5	2552

Table 8 Ruggedness studies

Analyst	**Observed value**			
	% RSD of area	**% RSD of R.T**	**Tailing factor(T)**	**Theroteical plates(N)**
Analyst I	0.45	0.63	1.5	2565
Analyst II	0.52	0.72	1.5	2553

3.1.7. Mobile phase stability

The stability of the mobile phase was evaluated, so the mobile phase was stored at 4–8 °C for 1 week. The aged mobile phase was compared using a freshly prepared one. The mobile phase was stable up to 1 week at 4–8 °C.

3.1.8. Specificity

Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants or matrix. Specificity of an analytical method is its ability to accurately and specifically measure the analyte of interest without interference from blank or placebo. The peak purity of ceftrioxone sodium was assessed by comparing the retention times of standard ceftrioxone sodium and the sample, and good correlation was obtained between the retention time of the standard and sample. Placebo and blank were injected and there were no peaks. There is no interference of blank and placebo on drug peaks hence, the method is specific. The chromatogram for placebo and blank was shown in Fig 4 and 5 respectively.

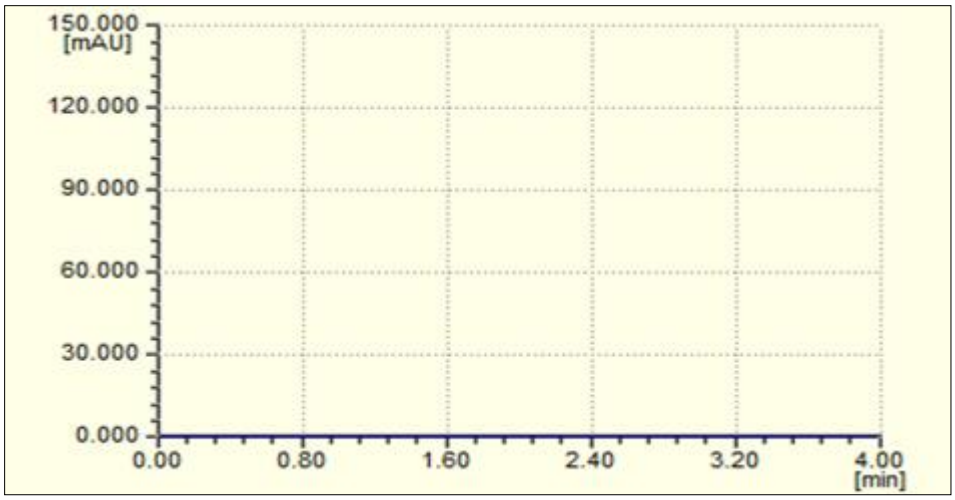

Figure 4 Chromatogram of placebo solution

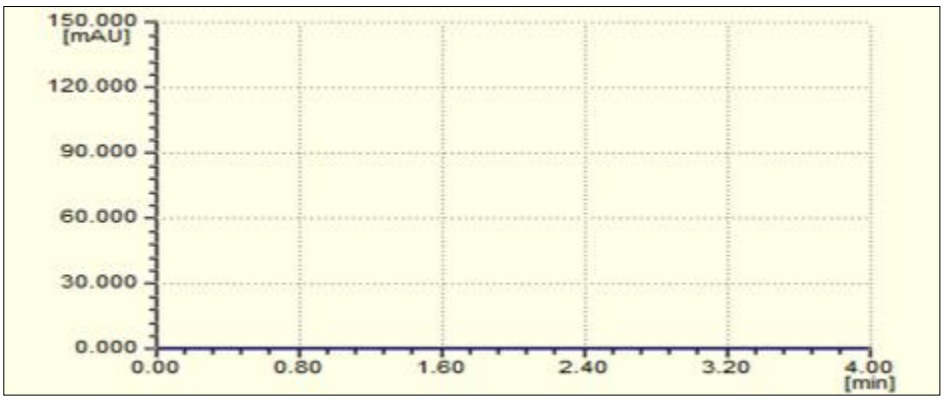

Figure 5 Chromatogram of blank solution

3.1.9. Sample Analysis

The developed and validated method was applied for analysis of tablet formulation contains ceftrioxone sodium. The sample was analyzed in triplicate. Analysis results were evaluated using a calibration curve. The amount of ceftrioxone sodium in the samples was calculated from calibration curve equation and recovery and RSD values were determined. The results of analysis are given in Table 9. The recoveries were in good agreement with the label claims. The chromatogram obtained was clear as shown in Fig. 6. It was concluded that the method can be applied successfully for the analysis of ceftrioxone sodium in injection dosage form.

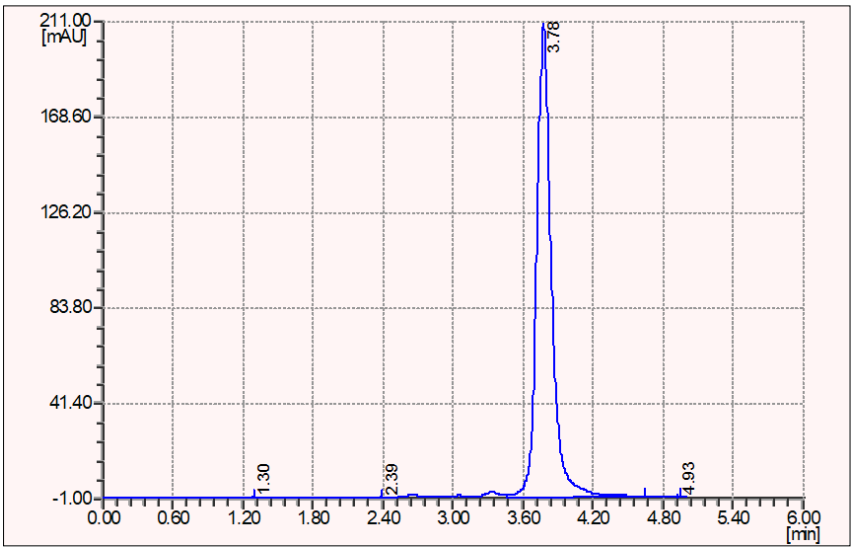

Figure 6 Chromatogram of sample ceftrioxone sodium

Table 9 Assay results from commercial formulation

Tablet	Drug	Labeled Claim (mg)	Amount found (mg)	% Mean Recovery* ± % RSD
LEEONE	Ceftrioxone Sodium	1000 mg	993.2 mg	99.32 ± 0.94

* Average of five determinations

4. Conclusion

The proposed method for the estimation of Ceftrioxone Sodium was validated as per the ICH guidelines and it is simple, specific and economical. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of Ceftrioxone Sodium in pharmaceutical formulations without any interference from the excipient.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed

References

[1] Salman AT . RP- HPLC Estimation of Ceftriaxone Sodium in Pharmaceutical. Egypt. J. Chem. 2021, 64, (9) : 4901 – 4906.

[2] Sun H, Wang H, Ge X. Simultaneous determination of the combined drugs of ceftriaxone sodium, metronidazole and levofloxacin in human urine by high performance liquid chromatography. Journal of Clinical Laboratory Analysis . 2012, 26(6): 486-492.

[3] Sultana N, Arayne M S, Shahzad W. Simultaneous Determination of Ceftriaxone Sodium and Non-Steroidal AntiInflammatory Drugs in Pharmaceutical Formulations and Human Serum by RP- HPLC. Journal Chinese Chemical Society Taipei. 2010, 57(6), 1278-1285.

[4] Shrestha B, Bhuyan NR, Sinha BN. Development and validation of a stability indicating HPLC method for estimation of ceftriaxone in sterile powder for injection without using ion pairing reagent. International Journal of Pharamcy and Pharmaceutical Sciences.2013, 5(3), 948-952.

[5] Kobayashi K, Sato K, Mizuno Y, Katsumata YJ. Capillary high-performance liquid chromatography-fast atom bombardment mass spectrometry of 24 cephem antibiotics. Journal of Chromatography B: Biomedical Sciences and Applications.1996, 677(2): 275-290.

[6] Tange M, Yoshida M, Nakai Y, Uchida T. The role of an impurity in ceftriaxone sodium preparation for injection in determining compatibility with calcium-containing solutions. Chemical & Pharmaceutical Bulletin.2016, 64(3), 207-214.

[7] Page-Sharp M, Nunn T, Salman S, Moore BR, Batty KT, Davis TM, Manning L. Validation and application of a dried blood spot ceftriaxone assay. Antimicrobial Agents and Chemotherapy.2016, 60(1): 14- 23.

[8] Yu X, Tang X, Zuo J, Zhang M, Chen L, Li Z. Distribution and persistence of cephalosporins in cephalosporin producing wastewater using SPE and UPLC–MS/MS method. Science of the Total Environment.2016, 569, 23-30.

[9] Ethiraj R, Thiruvengadam E, Sampath VS, Vahid A,, Raj J. Development and validation of stability indicating spectroscopic method for content analysis of ceftriaxone sodium in pharmaceuticals. International Scholarly Research Notices. 2014, 5.

[10] MJ MH., Marwa S. Cloud Point Extraction Spectrophotometric Method for Determination of Three Types of Cephalosporin via Diazotization Reactions with Different Reagents. Br. J. Anal. Chem. 2017, 4 (16): 24-36.

[11] Rageh AH, El-Shaboury SR, Saleh GA, Mohamed FA. Spectophotometric method for determination of certain cephalosporins using 4-chloro- 7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). Scientific Research.2010, 2(08): 828.

[12] Shah J, Jan MR, Shah S. Development and validation of a spectrofluorimetric method for the quantification of ceftriaxone in pharmaceutical formulations and plasma. Luminescence.2013, 28(4): 516- 522.

[13] Rao MT, Ratna VJ, Rao YS, Kumar TH. Development and Validation of RP-HPLC Method for the Determination of Itraconazole in Bulk and Capsule Dosage Form. Int. J. Pharm. Sci. Rev. Res.2015, 31 : 221-225.

[14] Sindhu SN, Rao YS,Kumar HT, Rao KVP.Method development and validation of RP-HPLC method for estimation of imatinib mesylate in pure and pharmaceutical dosage form. 2015, Der Pharmacia Lettre, 7 (3):33-38.

[15] Kumar TH, Sri, DS, Rao, KVP, Rao YS.Validated RP- HPLC Method For Determination Of Rosuvastatin Calcium In Bulk And Pharmaceutical Formulation.2015, IJPSR, 6(7): 1000-05.

[16] Kumar TH, Prajna CKK, Rao KVP, Rao YS. RP-HPLC Method For Estimation Of Tramadol Hydrochloride And Paracetamol In Pharmaceutical Formulation. GSC Biological and Pharmaceutical Sciences. 2019, 8(1):89-97.

[17] Kumar HT, Asha C, Sankar DG. Estimation of Amlodipine Besylate And Irbesartan In Pharmaceutical Formulation By RP-HPLC With Forced Degradation Studies. International Journal of Applied Pharmaceutics 2019, 11(33):159-167

[18] Kumar TH, Begum A , Sankar DG. RP-HPLC Method For Determination of Azilsartan Medoximil and Chlorthalidone in Tablets exposed to Forced Degradation Condition. Biomedical Research 2019;30(5):775-785

[19] Kumar TH , Asha C. New Validated Stability Indicating RP-HPLC Method For Simultaneous Estimation Of Amlodipine Besylate And Valsartan In Pharmaceutical Formulation. Int. J. Pharm. Sci. Res. 2019; 10(5): 2633-43

[20] Lakshmi GT, Rao YS, Rao KVP, , Kumar HT. RP- HPLC Method for Estimation of Atomoxetine Hydrochloride in Bulk and Pharmaceutical Dosage Form. Research Journal of Pharmaceutical, Biological and Chemical Sciences.2015, 6(2) :1208-1214

[21] Kumar HT, Reddy M., Rishika D, Kumar PR. Estimation of Orlistat by UV Spectrophotometric method. IJPSR. 2011, 2: 2469-2471.

[22] Madhuri PL, Kumar TH, Rao YS, Rao KVP. UV Spectrophotometric Method for Estimation of Sacubitril in Synthetic Mixture. Asian J. Research Chem. 2019, 12: 07-10.

[23] Chaitanya PSV, Kumar TH, Rao YS, Rao KVP. Estimation of vortioxeine in bulk and pharmaceutical formulations by UV spectrophotometry. European Journal of Pharmaceutical and Medical Research. 2019, 6: 389-392.

[24] Sankar DG, Kumar TH, Krishna MV.UV Spectrophotometric determination of Duloxetine Hydrochloride. Asian Journal of chemistry. 2019, 21(6).

[25] ICH Validation of analytical procedures: text and methodology. In International Conference on Harmonization Q2 (R1). Geneva, 2005.