

Development and Validation of a High-Performance Liquid Chromatographic Method for Content Assay of Ceftriaxone in Pharmaceutical Dosage

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ABSTRACT

Aim: A sensitive and accurate RP-HPLC method was developed and validated for the quantitative measurement of Ceftriaxone sodium (CEF) in pharmaceutical dosage forms and bulk medicine. **Materials and Methods:** Ceftriaxone sodium was separated on a 245 nm photodiode array detector using a Waters XTerra RP-18 (5 μ m 250x4.6 mm internal diameter) column. **Results:** The method generated an excellent linear response in the concentration range of 0.2-20 μ g/mL with remarkable precision of 0.16-0.7% and accuracy in the percent recovery range of 99.88-99.97%. **Conclusion:** We find the reverse phase HPLC technique to be highly sensitive, accurate, precise and user-friendly and hence recommend it. Therefore, it could be useful in a quality control lab.

Keywords: HPLC, Ceftriaxone, Bulk, Pharmaceutical, Dosage.

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INTRODUCTION

Compared to other cephalosporins, the serum half-life of ceftriaxone sodium (Figure 1) is substantially longer because of its resistance to β -lactamases. Most conditions require no more than one dosage per day. The slow rate of urine elimination and the high plasma protein binding percentage of ceftriaxone contribute to its prolonged half-life. Ceftriaxone is eliminated in the bile and then the urine. Taking probenecid will not cause you to urinate more frequently. It has no non-linear pharmacokinetics and reaches meningitis-effective doses in the cerebrospinal fluid with a relatively low volume of distribution.

Ceftriaxone sodium is a compound with the chemical formula "(Z)-7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetyl-amido]-3-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)thiamethyl]Sodium salt of 3-cephem-4-carboxylic acid".¹ The heterocyclic structure of the 3-thiamethyl group of ceftriaxone is extremely acidic. The distinctive pharmacokinetic features of this drug are thought to originate from its dioxotriazine ring structure. In sonographic studies, ceftriaxone was found to be associated with the development of pseudolithiasis, also known as sludge, in the common bile duct and gall bladder. Certain people, especially those who take ceftriaxone often or at

excessive dosages, may develop cholecystitis. Calcium chelation has been identified as the primary contributor to the problem. A few well-established analytical procedures for determining CEF include high-performance liquid chromatography coupled with detection by Ultraviolet (UV) spectroscopy (HPLC-UV)²⁻⁵ and in a variety of biological samples, such as human plasma⁶⁻¹⁰ and human urine.^{11,12} HPLC is one of many methods that have been reported to be used for the analysis of ceftriaxone sodium in pharmaceuticals.¹³⁻²⁰ These methods exhibit remarkable linearity, accuracy and recoverability. There are, however, drawbacks, such as the fact that their chromatographic duration is rather long (15 min) and that their lack of sensitivity results in a lower limit of quantification. There are a number of issues with these approaches, including their length, breadth, complexity and duration (Table 1). The purpose of the research presented here was to design and validate a quick and easy assay for measuring ceftriaxone sodium concentrations in their pure and pharmaceutical forms.

MATERIALS AND METHODS

Materials

Alkem Laboratories Ltd. in India sent a free sample of ceftriaxone sodium. The solvents we employed were water, methanol and HPLC-grade orthophosphoric acid (Merck Specialities Pvt. Ltd., India). Drug dosages are purchased from a local business.

Stock Solutions

Ceftriaxone sodium was dissolved in water to make a 1000 μ g/mL standard stock solution. In a volumetric flask, the required



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amounts of medication were dissolved in water to produce a stock solution (1000 µg/mL). The stock solution was stored in a dark place at a temperature of 5°C. Calibration curves with concentrations of 0.2, 1, 5, 10, 15, 17 and 20 µg/mL were constructed by serially diluting the stock solution (1000 µg/mL) with the mobile phase. The material was filtered via a 0.2 µm membrane after being diluted with a mobile phase to achieve the desired concentration of pharmaceutical-grade ceftriaxone sodium. For 5 min., it was immersed in an ultrasonic bath. An approximation was made using the known volumes of several medication solutions.

Procedures

Chromatic Conditions

All HPLC analyses were performed on an SPD-prominence diode array detector, LC-20AD solvent delivery pump system, SIL-20A prominence auto sampler and DGU 20A5 prominence degasser from Shimadzu (UFLC) Corporation's Analytical Instruments Divisions in Kyoto, Japan. In addition, Lab Solutions' proprietary data analysis software was employed. Famous Columns uses a CTO-20A type oven to keep the column at an appropriate temperature. The Waters XTerra RP-18 (5 µm, 250×4.6 mm) from Ireland was used for the separation. The mobile phase consisted of a 65:30:5 (v/v) mixtures of methanol, water and orthophosphoric acid solutions and it flowed at a rate of 1.0 mL/min. We used a 20 µL loop for the injections. The chromatographic run lasted for 5 min and the concentration was determined by measuring the absorbance at 245 nm. Before being degassed, the mobile phase was filtered using a 0.2 µm aqua Max-ultra Younglin-Instrument DPL-S filter. The separation procedure was finished at room temperature.

Creating a Calibration Curve

The mobile phase was supplemented with newly prepared, off-site aliquot solutions of ceftriaxone sodium ranging in concentration from 0.2 to 20 µg/mL. Five 20 µL loop injections were conducted with each aliquot solution to verify the repeatability of the detector response at every concentration level. A sample of the resulting chromatogram is displayed in Figure 2. The calibration curve was constructed by relating the average peak area to the concentration of the pharmaceutical solution. Regression analysis was employed to determine the least squares equation and correlation coefficient.

Dosage Forms for Assay

Ceftriaxone sodium was sourced from various local businesses and formulated as follows for the analysis: There are three different types of CEF injections available in India: (A) C Tri (Zuventus Healthcare Ltd., India), which contains 250 mg; (B) Xone (Alkem Laboratories Ltd., India), which contains 500 mg; and (C) Monocef (Aristo Pharmaceutical Pvt. Ltd., India),

which has 1000 mg. A 100 mL calibrated flask was filled with an injectable powder containing 100 mg of ceftriaxone sodium and 60 mL of diluent solution. After that, it was swirled for 20 min. at high speed. After that, everything was mixed together and diluted appropriately that nothing besides water was present. A 0.2 µL filter was employed to filter out impurities from an extract that was around 10 mL in volume. The filtered solution, as was previously mentioned, was diluted with the analytical diluent solution.

Recovery Experiment

There were three separate dosages administered, each comprising a specific quantity of ceftriaxone sodium powder contained in a vial. The total was computed following the suggested procedures and the surplus of pure drug was recovered.

Selectivity Analysis

Standard procedures were followed to assess the concentration of ceftriaxone sodium in synthetic mixes of sodium metabisulphate, sodium chloride and sodium benzoate. Ceftriaxone sodium was extracted using three 20 mL portions of diluent in a mass ratio of 0.5:0.3:0.4:0.2 and the resultant mixture was filtered. After rinsing the filter with diluents, we collected the filtered liquid and the washings in a 100 mL calibrated flask, diluted them to volume and gave the whole thing a good stir. A separate selectivity analysis was run for this purpose. An appropriate sample of the extract was analysed, as was previously mentioned.

RESULTS

The development of a separation technique

The Waters XTerra 5 µm, 250×0.46 mm i.d., RP-18 was able to generate sharp, symmetrical peaks with good sensitivity using a mobile phase of methanol, water and orthophosphoric acid. Several columns were tested before settling on the best one to use in chromatography and many different mobile phases were considered. The XTerra 5 µm RP-18 column proved to be the most successful in attaining the sought-after chromatography and separation under the specified conditions, beating out a low-pressure gradient mode and other columns. Different mobile phase compositions may result in increased sensitivity, sharper peaks and greater separation between peaks. Methanoic acid, trifluoroacetic acid and ethanoic acid were all tried as alternatives to the original methanol in the mobile phase, but none of them produced separations as well as the original. We explored a variety of settings for the pH of the aqueous phase to see if it had any effect on the elution and sensitivity of the peaks. Sodium acetate, ammonium acetate and ammonium formate all had similar effects on pH from 2.3 to 6.5. The effect of the low rate was investigated by changing the mobile phase flow rate from 0.2 to 2.0 mL/min. Even though the injection volume was halved from 30 to 10 µL, the analysis time for ceftriaxone sodium was 3.459

Table 1: Comparison of the proposed HPLC method with existing HPLC and other methods.

Method	Experimental details	Detection	Linear range	QL	Remarks	References
RP-HPLC	Waters XBridgeC18 BEH 2.5 µm (50 mm×3 mm). A mixture of 0.01 mole of o-phosphoric acid with sodium hydroxide buffer and acetonitrile (v/v) 100:12, pH 2.55 as mobile phase.	UV-260 nm	1-20 mg/L	NA	Less accurate and precise, temperature dependent.	12
HPLC	Waters X-bridge C18 column (30 mm×4.6 mm, 2.5 µm silica). A mixture of acetonitrile and 50 mM phosphate buffer (v/v) 8:92, pH 2.4 as mobile phase.	UV-260 nm	5-500 µg/mL	4.6 µg/mL	Less accurate and precise, between- and within-day replicate analysis of quality controls concentration varies 1.8% to 3.6%.	13
HPLC	Waters, located in Saint Quentin, France; Atlantis T3 analytical column (150 mm×4.6 mm×5 m). The mobile phase was a 10 mM phosphoric acid solution, pH 2 adjusted with hydrochloric acid and acetonitrile that had been filtered and degassed. With a flow rate of 2 mL/min, a linear gradient was utilised to increase the acetonitrile concentration from 7% to 19% in 6 min and then from 19% to 49% in 6 to 16 min.	UV -230 nm	5-250 µg/mL	5 µg/mL	Less precise intra-day and inter-day accuracy values is 88%.	14
HPLC	A Kromasil 100-5 C18 analytical column (250×4.6 mm id, 5 µm, AKZO NOBEL, Bohus, Sweden). A mobile phase composed of 1.5 mM potassium dihydrogen phosphate (adjust the pH to 4.5 with phosphoric acid) with 0.0125% triethylamine-methanol (70:30, v/v).	PDA -247 nm	0.24-250 µg/mL	0.17 µg/mL	Least recovery, RSD>1.9%	3
HPLC	C18 column (5 µm, 4.6 mm Ø × 250 mm, Teknokroma S. Coop. C. Ltd. Barcelona, Spain). A mixture of acetonitrile, methanol and triethyl amine buffer (1:1:2 v/v) as mobile phase.	UV -260 nm	0.5-250 µg/mL	0.5 µg/mL	Less precise intra-day and inter-day and percentage of recovery is <99.2	15
UV-vis	A Systronics 2201, UV-visible double-beam spectrophotometer with matched 1 cm path-length quartz cells.	241 nm	5-50 µg/mL	0.1008 µg/mL	Least sensitive and less precise.	16
SPF	RF-5301 PC spectrofluorophotometer (Shimadzu, Japan).	386 nm	0.4-20 µg/mL	0.039 µg/mL	Least accuracy and interference of excipients.	17
RP-HPLC	A Flowrosil C18 column (250 × 4.6 mm, with 5 µm particle). A methanol: water: ortho-phosphoric acid in the ratio of (75:24.5:0.5 v/v) as mobile phase.	240 nm	10-50 µg/mL	0.16 µg/mL	Least stability of mobile phase in room temperature.	18

Method	Experimental details	Detection	Linear range	QL	Remarks	References
HPLC	A Zorbax SB C18 (150×4.6 mm, 5 μm) column. A mixture of 0.2% orthophosphoric acid and ethanol (87:13, v/v).	260 nm	20-120 μg/mL	1.39 μg/mL	Less accuracy, RSD>0.52.	19
HPLC	A Xterra RP-18 (250 cmx4.6 mm, 5 μm). A mixture of 0.2 M tetrabutylammoniumhydroxide buffer and acetonitrile (85:15 v/v) as mobile phase.	UV-254 nm	0.5-150 μg/mL	0.08159 μg/mL	Retention time is high 30.51 min.	20
HPLC	Waters XTerra RP-18 (5 mm, 250×4.6 mm i.d., made in Ireland), A combination of methanol, water and ortho-phosphoric acid (65:30:5 v/v) was used as the mobile phase at a flow rate 1.0 mL/min.	UV-245	0.2 to 20 μg/mL	0.0215 μg/mL	Highly sensitive, simple, precise, accurate, robust and rugged (inter-day and intra-day RSD<0.2%) and accurate (er<0.2%).	This paper

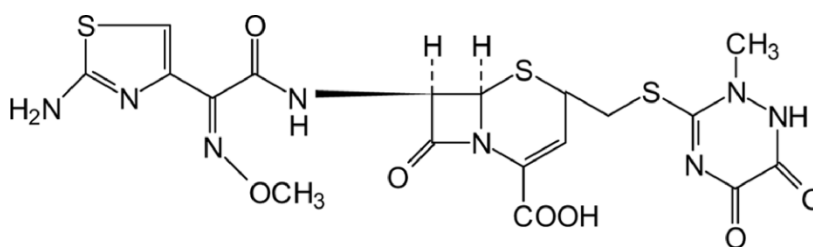


Figure 1: Structure of ceftriaxone.

min. C18 reverse-phase columns were used. Since 1.0 mL/min flow rates offered the highest signal-to-noise ratio and enough time for separation, they were approved. Ceftriaxone sodium is absorbed most strongly at 245 nm; hence, that is the wavelength that will be employed in the experiment.

DISCUSSION

Linearity of Detector Response

How closely the calibration plot of response vs. concentration follows a straight line can be used to evaluate a procedure's linearity. Using the stated chromatographic settings, seven solutions were analysed (concentration range: 0.2-20.0 μg/mL ceftriaxone sodium) to evaluate the linearity and dynamic range of the method. The chromatogram was obtained by injecting 10 μL of solutions into the column. Calibration was performed by plotting the peak area versus concentration of ceftriaxone sodium. The RSD, correlation coefficient and regression parameters are shown in Table 2. Within the $r=0.9999$ region, linearity proved very robust.

Table 2: Spectral and statistical data.

Parameter	Result
Detection wavelength (nm)	245
Linear dynamic range (μg/mL)	0.2-20
Regression equation (Y) ^a	
Slope (b)	91489
Intercept (a)	-2315
Correlation coefficient (r)	0.9999
Limit of Detection (LOD) (ng/mL)	0.00259
Limit of Quantification (LOQ) (ng/mL)	0.0085
% RSD	0.0036

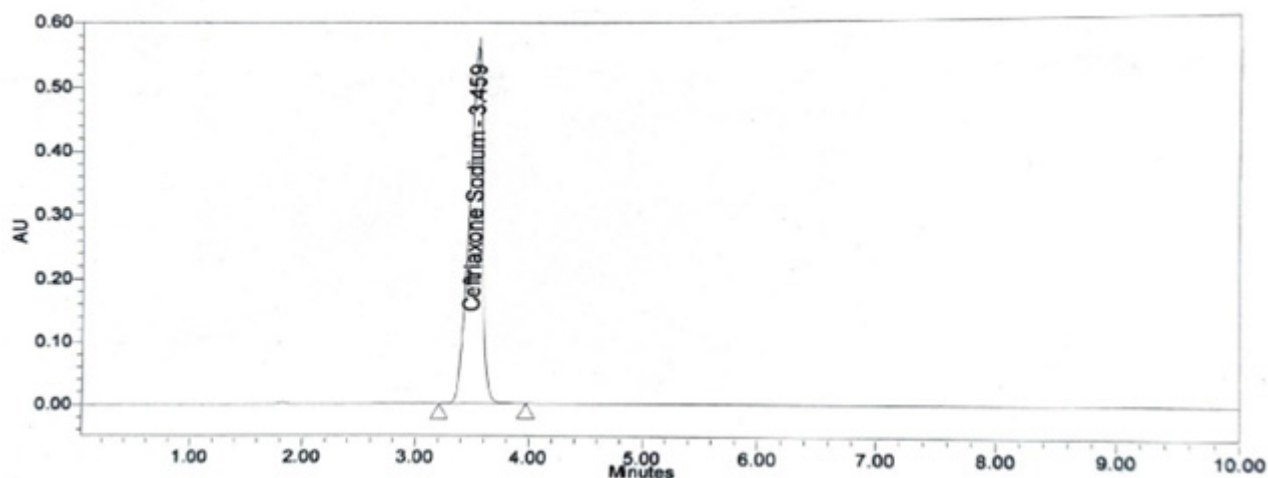
^aY=a+bC, where 'C' is the concentration of drug in μg/mL.

Specificity

Specificity is the capacity of an analytical method to unambiguously identify the analyte in the presence of other components such as contaminants, degradation products and matrix.²¹ We were able to identify the analyte of interest as the only cause of the signal observed by replicating the effects of a placebo in the trial. In accordance with the sample preparation protocol,

Table 3: Relative error and intra-day precision.

Nominal concentration taken, µg/mL	Intra-day accuracy and precision (n=7)		
	CEF found, µg/mL	RE, %	RSD, %
2.0	1.999	0.05	0.065
5.0	4.998	0.046	0.022
7.0	6.995	0.065	0.081
3.0	2.998	0.08	0.0262
6.0	5.996	0.066	0.0373
9.0	8.996	0.046	0.0100

**Figure 2:** Ceftriaxone chromatogram (15 µg/mL).

ceftriaxone sodium-free analytical placebo solutions were made and administered. After the necessary dilution, chromatography was used to examine the drug extract and identify the excipients that were causing interference. The procedure's specificity was confirmed by observing just the ceftriaxone sodium signal in the resulting chromatogram (Figure 2). The slope of the calibration curves for both the drug extract and the standard was evaluated. There was no evidence of a difference in slope across groups, suggesting that excipients did not compromise ceftriaxone sodium's effectiveness.

Precision

The method's dependability was measured by how stable it was from day to day.²¹ Seven replicates of three different ceftriaxone sodium concentrations were assessed both on the same day (intra-day precision) and again five days later (inter-day accuracy). The solutions were injected three times per set. Values for the intraday RSD varied from 0.01% to 0.08% on the mountain peak. Table 3 displays the optimistic tone of the study's findings. Slightly larger RSD values were seen when comparing days. These values varied from 0.05% to 0.19%. Seven replicate tests were performed in standard ceftriaxone sodium solutions by three different

analyzers using two different pieces of equipment and the RSD values were calculated to determine the intermediate accuracy. The RSD ranged from 0.7% to 0.9% between the analysts and it was between 0.5% and 0.8% between the two instruments used.

Accuracy

The precision of an analytical procedure is a measure of how closely the generated value corresponds to the reference value. Results for RE at five different concentrations are shown in Table 3; all of them lie within the linear range of 0.04 to 0.08%. The effectiveness of the synthetic combination was evaluated by mixing ceftriaxone sodium with the placebo as instructed. There was no effect of the co-formulated compounds on the test, as the estimated percent recovery for the active component was 99.92 ± 0.08 (n=7).

Robustness

The method's adaptability was demonstrated by making slight adjustments to two major variables. No changes were made to the flow rate and the mobile phase pH was lowered from 7.0 to 4.5. Minor changes like those indicated above had no appreciable effect on retention time or peak area.

Table 4: Assay results in injection and statistical composition with the reference method.

Pharmaceutical dosage name*	Nominal amount	Percent of label claim \pm SD	
		Reference method	Proposed method
C Tri ^a	250 mg	99.85 \pm 0.06	99.91 \pm 0.04 t=2.09 F=2.25
Xone ^b	500 mg	99.43 \pm 0.44	99.82 \pm 0.38 t=1.89 F=1.34
Monocef ^c	1000 mg	99.31 \pm 0.59	99.75 \pm 0.62 t=1.45 F=1.10

*Marketed by: a. Zuventus Healthcare Ltd., India; b. Alkem Laboratories Ltd., India; c. Aristo Pharmaceutical Pvt. Ltd., India. Tabulated *t*-value at the 95% confidence level is 2.365. Tabulated *F*-value at the 95% confidence level is 3.79.

Table 5: Results of recovery study via standard addition method.

Vial of Injection studied	Method I			
	CEF in Injection μ g/mL	Pure CEF added μ g/mL	Total found μ g/mL	Pure CEF Recovered percent \pm SD*
C Tri 250 mg	9.952	5	14.946	99.88 \pm 0.32
	9.952	7	16.942	99.97 \pm 0.29
	9.952	9	18.951	99.88 \pm 0.18
Xone 500 mg	4.956	5	9.952	99.92 \pm 0.22
	4.956	10	14.952	99.96 \pm 0.16
	4.956	15	19.948	99.95 \pm 0.71
Monocef 1000 mg	14.984	3	17.982	99.93 \pm 0.28
	14.984	6	20.979	99.92 \pm 0.33
	14.984	9	23.980	99.95 \pm 0.16

*Mean value of three determinations.

Ruggedness

We were able to gauge the reliability of the method by injecting the reference working solution into numerous HPLC instruments from various chemical and column manufacturers. The relative strength of the approach was demonstrated by the reproducibility of the detector response and retention times. The RSD (relative standard deviation) values were confirmed to be lower than 0.2% across different days. Consistent detector responses were seen across numerous days, hinting at the method's potential for high-accuracy outcomes over time. The procedure was seen to be too rough and ready as a result.

Application

The tried and proven method was used to determine the concentration of ceftriaxone sodium in three different injection brands having 250, 500 and 1000 mg. This criterion was used for the evaluation since there was little to no difference between

the calibration curve slopes of the injected extracts and the standards. The accuracy and precision of the suggested method were compared to those of the HPLC method described in the literature using the Student's *t*-test and the *F*-test, respectively".¹⁹ Table 4 shows that, at the 95% confidence level and 6 degrees of freedom, there was no statistically significant difference between the computed *t*-values and the tabulated values. The accuracy and precision of the suggested method showed essentially no variance. To test the viability of the proposed methods, recovery experiments were carried out. To arrive at the final tally, the pre-analyzed injectable powder was mixed with three different volumes of pure ceftriaxone sodium. The percentage of pure medication recovered after addition ranged from 99.88 to 99.97%, with Relative Standard Deviations (RSDs) of 0.16 to 0.7%. Traditional injectable additives had no effect on the outcome, as shown in Table 5 of the study. The key characteristics of the method are intra-day and inter-day precision analysis,

as well as a recovery study and a high degree of accuracy and precision. The low LOQ result is further evidence of the method's great sensitivity.

CONCLUSION

It was determined that the reverse-phase HPLC method was sensitive, accurate, precise and simple to implement. In a quality control lab, it could be used to test pharmaceuticals before they reach patients because it doesn't react with routinely used additives. It took around 5 min. to complete the chromatographic procedure.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reversed Phase-High Performance Liquid Chromatography; **CEF:** Ceftriaxone; **UFLC:** Ultra-Fast Liquid Chromatography; **RSD:** Relative Standard Deviation; **SD:** Standard Deviation; **UV:** Ultra Violet; **PDA:** Photo Diode Array; **QL:** Quantification Limit; **LOD:** Limit of Detection; **LOQ:** Limit of quantification; **ICH:** Conference on Harmonization; **NA:** Not available; **SPF:** Spectrofluorometric.

SUMMARY

For the purpose of quantitatively evaluating ceftriaxone sodium in pure medications and dose formulations, a sensitive and precise RP-HPLC technology was developed and authorised. Mobile phase components included orthophosphoric acid, methanol and water and the detector was a 245 nm-active photodiode array. The approach showed an adequate linear response in the concentration range of 0.2-20 µg/mL, with a correlation value of 0.9999. The spike recovery method was shown to be highly accurate, with a range of 99.88% to 99.97% in percent recovery and a precision of 0.16-0.7%.

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