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DEVELOPMENT AND VALIDATION OF A HPLC-UV METHOD FOR SIMULTANEOUS DETERMINATION OF CEFTRIAXONE AND SULBACTAM IN POWDER FOR INJECTION FORMULATION

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ABSTRACT: A simple, rapid, and sensitive high-performance liquid chromatographic method with UV detection has been developed and validated according to the ICH guidelines for the quantization of Ceftriaxone (CEF) and Sulbactam (SUL) in parenteral preparation. Chromatographic separation was carried out in a Hypersil Gold C8 column (250 mm × 4.6 mm; 5 µm particle size) of Thermo Scientific with simple mobile phase composition of 10 ml of 40% Tetra Butyl Ammonium Hydroxide(TBAH) in 1000 ml of water (pH 5.5, maintained by dil Phosphoric acid) and acetonitrile (70:30, v/v) at a flow rate of 2.0 ml min^{-1} with injection Volume of 20 µl where detector was set at 227 nm with a total run time of 10 min. The method was linear over the concentration range of 40-100, µg ml^{-1} for SUL and 80-200 µg ml^{-1} with a correlation coefficient of 0.999 and 0.999 respectively. Limit of quantifications (LOQ) of 8.5, 14.4 and limit of detections (LOD) 2.8, 4.7µg ml^{-1} for CEF and SUL respectively. Accuracy and precision values of both within-run and between-run obtained from six different sets of three quality control (QC) samples analyzed in separate occasions for both the analytes ranged from 98.15% to 99.75% and 0.91% to 1.58%, respectively. Extraction recovery of analytes from 97.57% to 99.03%. The developed and validated method was successfully applied to the quantitative determination of CEF and SUL in pharmaceutical formulation.

INTRODUCTION: Ceftriaxone (CEF) is a broad-spectrum third-generation cephalosporin which is parenterally indicated in several infectious diseases [1]. It has excellent penetration into extra vascular spaces and increased resistance to degradation by β-lactamases.

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Ceftriaxone Sodium is chemically known as, Disodium (6*R*, 7*R*)- 7-[[(2Z)-(2-aminothiazol- 4-yl) (methoxyimino)acetyl]amino]-3-[[(2- methyl – 6 – oxido – 5 – oxo - 2, 5 - dihydro-1,2,4-triazin-3-yl)sulphanyl] methyl] – 8 - oxo - 5 - thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate 3.5 hydrate [2].

CEF contains a highly acidic, heterocyclic system on the 3-thiamethyl group. This unusual dioxotriazine ring system is believed to confer the unique pharmacokinetic properties of this agent. The chemical structure of CEF is shown in **Fig. 1**. CEF is listed in the British Pharmacopoeia [2], United

States Pharmacopoeia [3] and Indian Pharmacopoeia [4]. Sulbactam Sodium is chemically known as Sodium (2*S*, 5*R*) - 3, 3 - dimethyl - 7 - oxo - 4 - thia - 1 -azabicyclo [3.2.0] heptanes - 2 - carboxylate 4, 4-dioxide., belongs to a class of penicillanic acid sulfones [5].

Sulbactam (SUL) is an irreversible inhibitor of many bacterial β-lactamases, *i.e.*, it binds to β-lactamases more readily than CEF. SUL does not have antibacterial activity when used alone; it synergistically expands ceftriaxone's spectrum of activity against many strains of β-lactamase-producing bacteria. The chemical structure of SUL is shown in **Fig. 2**

Literature survey reveals that there are only a few HPLC [5, 6] and Spectroctrophotometric [7] methods available for the determination of both drugs, simultaneously. Reported UV method has used a specific mode that is only available in the sophisticated instruments.

It was found that there are few analytical methods reported for Ceftriaxone and Sulbactam either in individually or in combination with other drugs by spectrophotometry [8-11], HPLC [12-15] HPTLC [16], capillary electrophoresis [17] and differential pulse adsorptive stripping voltammetry [18] Polarographic [19].

The aim of the present study was to develop a simple, sensitive, accurate, versatile, and fast HPLC method for the simultaneous estimation of Ceftriaxone and Sulbactam in pharmaceutical powder for the injection dosage form. The proposed methods were validated in compliance with the ICH guidelines and were successfully applied for the determination of Ceftriaxone and Sulbactam in their pharmaceutical formulations.

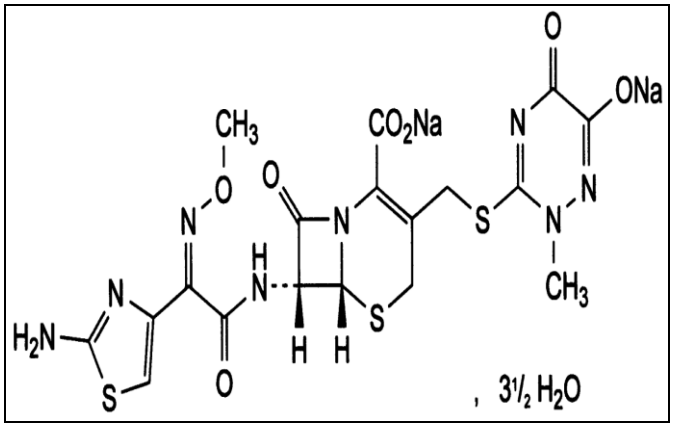

FIG. 1: CEFTRIAXONE SODIUM

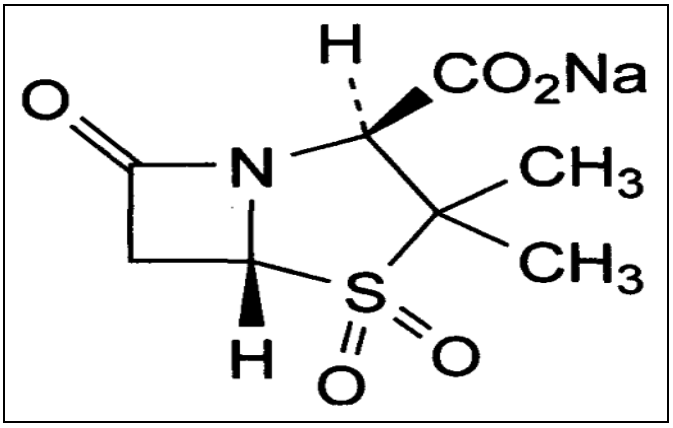

FIG. 2: SULBACTAM SODIUM

MATERIALS AND METHODS:

Chemicals and Reagents: CEF, SUL were procured from the pharmaceuticals industry. Tetra Butyl Ammonium Hydroxide 40% w/w in water analytical grade from Ranchem, Acetonitrile HPLC Grade from Spectrochem., Phosphoric acid analytical grade from Merck (Mumbai, India), HPLC-grade water (resistivity 18.2 MΩ) cm was generated from a Milli-Q water purification system, was used throughout the analysis. Samples are procured from the pharmaceutical industry, and they are considered as Sample I and Sample II respectively, and the samples are powder for injections.

Instrumentation and Chromatographic Conditions: HPLC apparatus consisted of Agilent Technology (USA) Model, G1311A Quaternary pump, G1365D variable wavelength UV detector, Auto-sampler (G1329A), Column oven (G13368) and EZ CHROM ELITE Version 331SOP software. Chromatographic separation was performed isocratically at room temperature using a Hypersil Gold C8 column (250 mm × 4.6 mm, 5 µm particle size) of Thermo Scientific mobile phase composition of 10 ml of 40% Tetra Butyl Ammonium Hydroxide (TBAH) in 1000 ml of water (pH 5.5, maintained by dil Phosphoric acid) and acetonitrile (70:30, v/v) at a flow rate of 2.0 ml min^{-1} where detector was set at 227 nm with a total run time of 10 mins, and sample injection of 20 µL was injected at 37 ºC. The eluent was monitored with a UV detector set at 227 nm.

Preparation of Stock and Working Solutions: 25.8 mg of CEF and 27.5 mg of SUL taken in a 25 ml volumetric flask and dissolving in the mobile phase to obtain a concentration of 1032 µg/ml and 1100 µg/ml respectively. The stock solution stored in amber-colored labeled volumetric flask at 8 ºC.

Preparation of Calibration Standards and Quality Control (QC) Samples: Four calibration standards (CC) of CEF at concentration of 80, 120, 160 and 200 µg ml^{-1} and of SUL at concentration of 40, 60, 80, 100 µg ml^{-1} were prepared by spiking 0.8, 1.2, 1.6, 2.0, ml and 0.4, 0.6, 0.8, 1.0 ml respectively to 10 ml by Mobile phase. Three QC sample 80, 120, 160 µg ml^{-1} for CEF and 40, 60, 80 µg ml^{-1} for SUL were used. All standards stored in the amber-colored labeled volumetric flask at 8 ºC.

Sample Preparation: 86.1 mg of sample diluted to 50.0 ml with mobile phase and mixed properly. Samples were further diluted by mobile phase, which has a final concentration of 100.12µg ml^{-1} of CEF and 50.06 µg ml^{-1} of SUL and then injected into the HPLC system.

Method Validation: The proposed methods were validated in compliance with the ICH guidelines and were successfully applied for the determination of Ceftriaxone & Sulbactam in their pharmaceutical formulations.

This method was validated to meet the acceptance criteria with the ICH guidelines of method validation [20].

Selectivity: The selectivity of the method was determined by analyzing blank (mobile phase), to demonstrate the lack of chromatographic interference at the retention time of the analytes.

Limit of Detection (LOD), Limit of Quantitation (LOQ) and Linearity: Limit of detection (LOD), Limit of quantitation (LOQ) was determined by the following equation $3.3 \times \sigma/S$ and $10 \times \sigma/S$, whereas σ = standard deviation of the response and S = slope of the calibration curve. Calibration curves were acquired by plotting the peak area of the analytes against the nominal concentration of calibration standards. Analyte concentration of different CC and QC samples were prepared as mentioned above.

Accuracy and Precision: Accuracy of an analytical procedure is the closeness of agreement between accepted conventional true values (reference values) and the values found. The accuracy of the proposed methods was tested by the determination of CEF and SUL at different concentration levels within the linear range of each compound.

Precision was studied by determination of intra-day and inter-day precision. Intra-day precision was determined by injecting six standard solutions 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 (RSD %) of the peak area was then calculated to represent precision.

Extraction Recovery: Recoveries of CEF and SUL were determined in the addition standard (80, 120, 160 µg ml^{-1} and 40, 60, 80 µg ml^{-1}) by comparing the experimental and true values.

RESULTS AND DISCUSSIONS:

Optimization of Chromatography: Taking into consideration the instability of Ceftriaxone and Sulbactamin strong alkaline and strong acidic condition, the pH value of the mobile phase should be limited within the range of 3-7. Since, mild acidic pH favors the retention and separation of two drugs on C_8 column.

C_8 columns provide similar selectivity to C_{18} columns but with reduced retention. Reversed-Phase Ion Pair Chromatography is a technique where salt is added to the mobile phase to improve the chromatographic properties. The sample is directed in an aqueous polar mobile phase, commonly including lower alcohol, acetonitrile or other water-miscible organic solvent, together with a counter ion, typically tetrabutylammonium ion (TBA), for anion analysis. After some trials, TBAH with pH 5.5 was finally selected.

Acetonitrile is the most commonly used solvent for LC analysis and often is the first choice for many researchers. Therefore, a binary mixture of acetonitrile and TBAH buffer became the initial mobile phase for the determination of the two drugs. 10 ml of 40% w/w TBAH in 1000 ml water buffer was found to be ideal for our work.

Then, the proportion of acetonitrile and TBAH buffer in the mobile phase was determined by varying the proportion of acetonitrile and TBAH buffer from 20:80, 25:75 to 30:70. Finally, Hypersil Gold C_8 (250 × 4.6 mm, 5µm), the 30:70 ratio of acetonitrile and TBAH buffer with pH 5.5 was employed for the simultaneous determination of the two drugs, this system produced symmetric peak shape, good resolution and reasonable retention time for both the drugs. The retention times of Ceftriaxone and Sulbactam was about 3.2 min and 5.9 min respectively. The total run time is 10 min is taken for the analysis. A typical overlay spectro-photometric examination **Fig. 3** of both ingredients in mobile phase shows the maximum absorbance at 227 nm; hence the wavelength fixed at 227 nm.

Selectivity: The method was found to selective as no significant interfering peak is observed at the retention times of CEF and SUL, which were about 3.2 min and 5.9 min respectively. Total chromatographic run time was 10.0 min. **Fig. 4** and **5** shows the representative chromatograms of blank spiked with analytes.

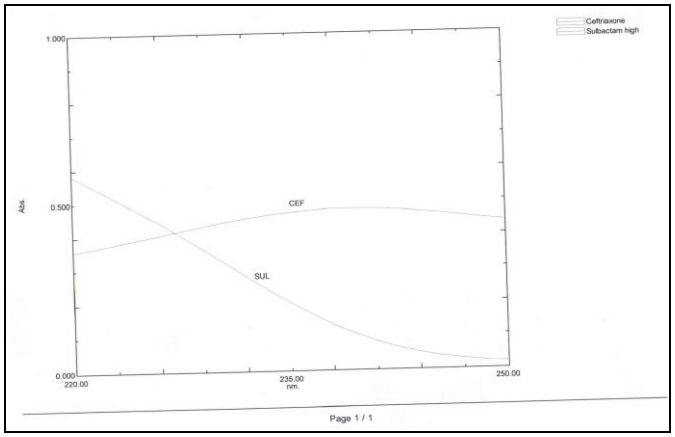

FIG. 3 OVERLAY SPECTRUM

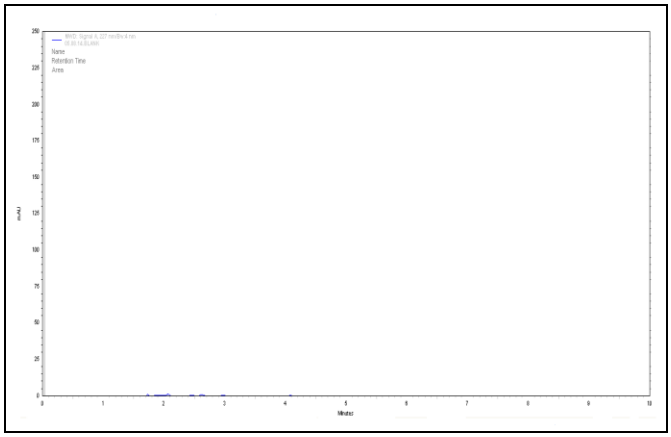

FIG. 4: BLANK CHROMATOGRAM

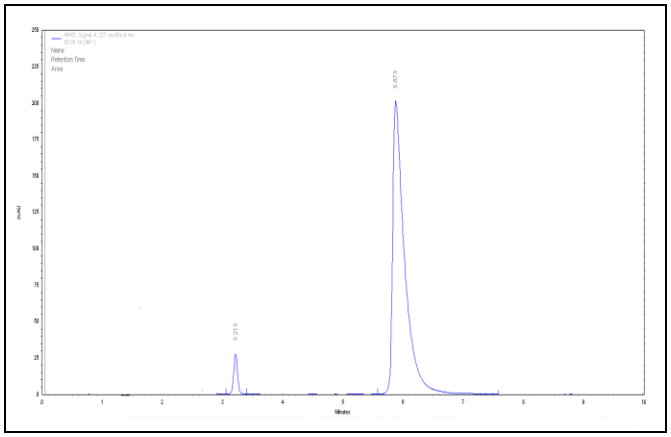

FIG. 5: TYPICAL CHROMATOGRAM OF CEFTRIAXONE AND SULBACTAM

Limit of Detection (LOD), Limit of Quantitation (LOQ) and Linearity: Limit of detection (LOD) was established 2.8 and 4.7 µg ml^{-1} for CEF and SUL, respectively. Limit of quantification (LOQ) was established 8.5 and 14.4 µg ml^{-1} for CEF and SUL respectively. Calibration curves were linear over the concentration range 40–100 µg ml^{-1} and 80-200 µg ml^{-1} for SUL and CEF respectively. Regression coefficient 0.999 and 0.999 for CEF and SUL, respectively **Fig. 6** and **7**. Standard curve had a reliable reproducibility over the standard concentrations across the calibration range. All back-calculated concentrations did not differ from the theoretical value as no single calibration standard point was dropped during the validation.

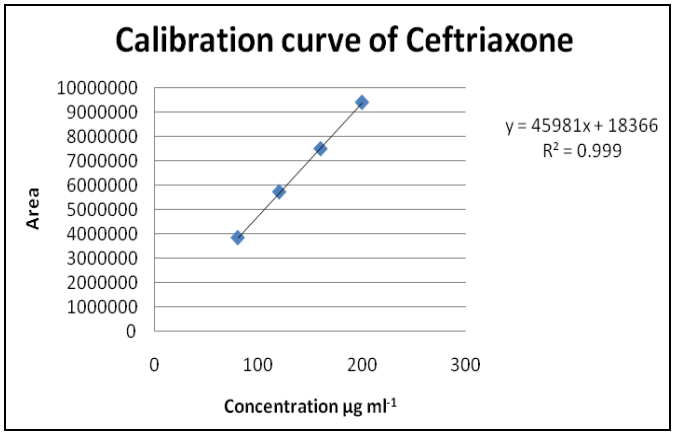

FIG. 6: CALIBRATION CURVE OF CEFTRIAXONE

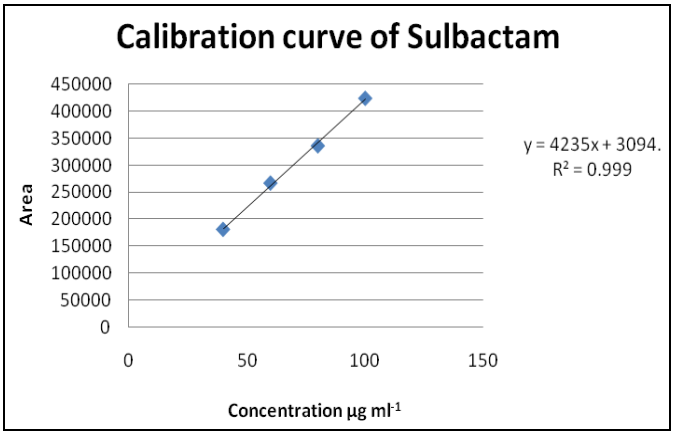

FIG. 7: CALIBRATION CURVE OF SULBACTAM

TABLE 1: ASSESSMENT OF ACCURACY AND PRECISION OF CEFTRIAXONE

QC Sample (µg ml^{-1})	Mean (µg ml^{-1})	S.D.	R.S.D. (%)	Accuracy (%)
Intra Day (n=6)				
80.00	79.05	0.84	1.06	98.82
120.00	118.79	1.19	1.00	98.99
160.00	158.41	1.59	1.00	99.01
Inter Day (n=18)				
80.00	78.97	0.72	0.91	98.71
120.00	118.75	1.09	0.92	98.96
160.00	158.27	1.60	1.01	98.92

S.D. = Standard deviation; R.S.D. (%) (Relative standard deviation) = [(S.D./Mean) × 100], Accuracy (%) = [(Mean / Conc. Added) × 100]; n = number of replicates

TABLE 2: ASSESSMENT OF ACCURACY AND PRECISION OF SULBACTAM SODIUM

QC Sample (µg ml^{-1})	Mean (µg ml^{-1})	S.D.	R.S.D. (%)	Accuracy (%)
Intra Day (n=6)				
40.00	39.90	0.56	1.40	99.75
60.00	58.95	0.72	1.21	98.24
80.00	79.38	0.88	1.11	99.23
Inter Day (n=18)				
40.00	39.50	0.62	1.58	98.74
60.00	58.89	0.67	1.14	98.15
80.00	78.87	0.99	1.25	98.59

S.D. = Standard deviation; R.S.D. (%) (Relative standard deviation) = [(S.D./Mean) × 100]; Accuracy (%) = [(Mean / Conc. Added) × 100]; n = number of replicates

Extraction Recovery: Recovery results were found to be satisfactory as these were consistent, precise, and reproducible are summarized in **Table 3**.

TABLE 3: EXTRACTION RECOVERY OF ANALYTES (n = 6)

Analyte	QC Sample (µg ml^{-1})	Extraction recovery (%)	R.S.D (%)
CEF	80.00	98.03	0.84
	120.00	97.59	0.64
	160.00	98.43	0.52
SUL	40.00	97.57	0.38
	60.00	99.03	0.86
	80.00	98.58	0.70

R.S.D. (%) (Relative standard deviation) = [(Standard deviation /Mean) × 100]; n = number of replicates.

Implementation to Pharmaceutical Formulation: This newly developed method was applied to determine the CEF and SUL in pharmaceutical formulation (powder for injections). Result ware summarized in **Table 4**.

TABLE 4: ESTIMATION OF CEFTRIAXONE AND SULBACTAM IN DIFFERENT FORMULATION

Name	Analyte	Concentration Found mg	%
Sample I	CEF	1016.23	101.62
	SUL	487.06	97.41
Sample II	CEF	995.74	99.57
	SUL	497.85	99.57

CONCLUSION: Here, we have developed and validated an HPLC-UV method that has significant advantages over the previously published method as it provides simple mobile phase composition for chromatographic separation, the shorter run time for analysis, simple sample preparation as well as improved sensitivity. Therefore, this new method leads to a simple, feasible, cost-effective, rapid method with a high degree of accuracy and specificity to quantify simultaneously CEF and SUL in pharmaceutical formulations with HPLC-UV. It will be extremely helpful for successfully analyzing the CEF and SUL in various pharmaceutical formulations.

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CONFLICT OF INTEREST: Nil

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