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ORIGINAL ARTICLE

Development and Validation of RP HPLC Method for Simultaneous Determination of Cefoperazone sodium and Sulbactam sodium in Dry Injection

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Authors' Contributions
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ABSTRACT

Background: Various analytical techniques are attempted for the qualitative and quantitative study of Cefoperazone sodium and Sulbactam sodium in different pharmaceutical dosage forms. These analytical techniques are not, easy, cost effective, eco-friendly and are so much laborious.

Objective: The main objectives of the study was to develop a simple, green, cost-effective, reproducible and precise isocratic RP-HPLC method for Cefoperazone Sodium and Sulbactam Sodium quantitative and qualitative studies in pure and dry injection powder form in compliance with ICH-Guidelines Q2 (R1).

Methodology*:* The chromatographic separation was carried out on a reversed phase C18 (250mm x 4.6 mm) 5 µm in an isocratic mode using tetrabutylammonium hydroxide solution and acetonitrile (8:3) as mobile phase adjusted pH 4.0 with dilute phosphoric acid and maintained flow rate 1.0 ml/minute at 220 nm. The retention time of Sulbactam sodium and Cefoparazone sodium was 7.4 min and 16.7 min respectively. All HPLC injection volume was 20µl in analysis.

Result: The chromatographic separation was carried out on a reversed phase C18 (250mm x 4.6 mm) 5 µm in an isocratic mode using tetrabutylammonium hydroxide solution and acetonitrile (8:3) as mobile phase adjusted pH 4.0 with dilute phosphoric acid and maintained flow rate 1.0 ml/minute the retention time of Sulbactam sodium and Cefoparazone sodium was 7.4 min and 16.7 min respectively. All HPLC injection volume was 20µl and the retention time for Cefoperazone sodium and Sulbactam sodium was noted about 7.4 min and 16.7 min respectively.

Conclusion: A simple, precise, accurate and cost effective method has been developed by using RP-HPLC. The available method is simple and reliable to determine the Cefoperazone sodium and Sulbactam sodium in different pharmaceutical formulations and in their pure forms.

Keywords: *RP-HPLC, Validation, Method, Development.*

INTRODUCTION

Chemically Cefoperazone (CFN) is (6R,7R)-7-[[(2R)-[[(4-Ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino] (4-hydroxy phenyl) acetyl] amino]-3-[[(1-methyl-1Htetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid [1]. It is a broad spectrum cephalosporin [2] for parenteral purpose used as a bactericidal and mostly used in the treatment of a diversity of bacterial infections [3] spread by Gram-positive and Gram-negative micro organisms [4-6]. Sulbactam is chemically (2S,5R)-3,3-Dimethyl-7-oxo-4-thia-1- azabicyclo [3.2.0] heptane-2-carboxylic acid 4,4- dioxide [7]. It is a penicillanic acid sulphone [8] with glactamase inhibitory actions [9]. It usually has only weak antibacterial function, except for against N.gonorrhoea and N, meningitides but it is an unalterable inhibitor of several p-lactamases 1, 2. (6) [10]. Sulbactam can therefore increase the action of many p-lactam antibiotics against bacteria that are usually resistant as of the production of p-lactamases, such as staphylococci sp., N.gonorrhoea and a number of enterobacteriaceae [11-13]. The in-vitro study of two combinations of Sulbactam and Cefoperazone explains bactericidal activity against various strains of microorganisms [14]. Various analytical techniques were earlier used to determine the estimation in mixtures in pure forms or in pharmaceutical formulations. These methods include spectrophotometric [15-17], HPLC-MS [18,19], HPLC with UV detection [20, 1], HPTLC [4] and UPLC with UV detection [21]. Cefoperazone in USB, BP and not present in IP. The USP and BP explain HPLC method for estimation of Cefoperazone. The literature study exposed that HPTLC, HPLC and Spectrophotmetric methods were mostly used for quantitatively determination of Cefoperazone and Sulbactam [4]. There is no method has been developed for the simultaneous evaluation of Cefoperazone and Sulbactam in combined dosage form. Hence in this study it as attempted to develop an easy, precise, and inexpensive analytical method. This research explains a validated RP-HPLC for simultaneous determination of Cefoperazone sodium and Sulbactam sodium in combination using tetrabutylammmonium hydroxide and acetonitrile (8: 3) adjusted pH 4.0 with ortho phosphoric acid. The column used was Agilent C18, 5µ, 25 cm × 4.6 mm id with flow rate of 1 ml / min using Uv-Vis detection at 220 nm. The chemical structure of Cefoperazone sodium and Sulbactam sodium is given in Figures **1** and **2** respectively.

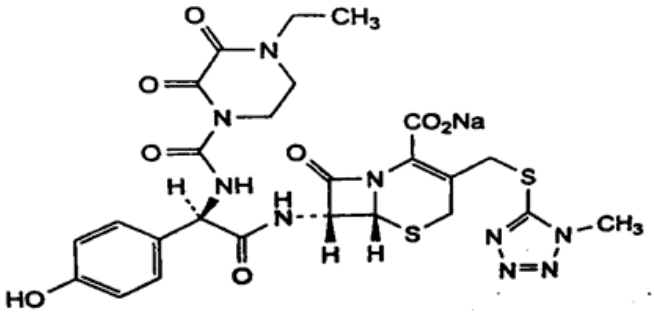

Figure 1. Chemical structure of Cefoperazone sodium.

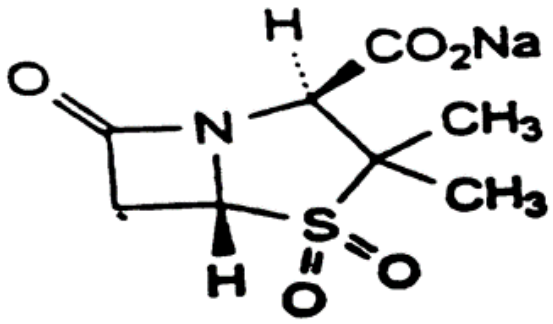

Figure 2. Chemical structure of Sulbactam Sodium.

MATERIALS AND METHODS

Cefoperazone sodium and Sulbactam sodium were donated from Selmore Pharmaceuticals (Pvt) Lahore Pakistan, Tetrabutylammonium (Merck), Acetontrile HPLC grade (Merck) Orthophosphoric Acid (Merck) and purified water was collected from Saturn Pharmaceuticals (Pvt) Lahore Pakistan.

The LC-20 HPLC system (Shimadzu) with isocratic pump was used to develop the new method using C18 (250 x 4.6 column, 5µm, with UV-Vis detector.

Chromatographic Conditions

HPLC System: LC-20 (Shimadzu, Japan)

Column: C18 Agilent (250mmx4.6mm, 5µm

Detection: 220 nm

Flow rate: 1.0/min.

Standard Preparation: Separately, weigh about 50 mg each of the Sulbactam RS and Cefoperazone RS, in a volumetric flask of 50 mL.

Add 30mL of mobile phase to each flask degas it by ultrasonic bath. Build up the volume by mobile process to 50mL after assuring the dissolution. The solution is filtered through a 0.45-micron membrane filter. Weigh 100mg accurately of the content (equivalent to about 50mg of Cefoperazone Sodium and 50mg of Sulbactam Sodium), in 50mL Volumetric Flask. Added approximately 30mL of mobile phase and dissolved sonicate. Upon assuring dissolution, with mobile process make up the volume to 50mL. The solution is filtered through a 0.45 µm membrane filter.

Solubility Assessment: The solubility of Cefoperazone and Sulbactam has been tested in many different solvents; water, acetonitrile, and methanol were tested as solvents for API solubility assessment. One percent solution was prepared separately for each Cefoperazone and Sulbactam and pH was measured by a pH meter that was properly calibrated for each solution.

UV detection: The 20ppm solutions of Cefoperazone and Sulbactam were prepared in a variety of solvents as well as in their mixtures such as CH_3OH, H_2O, ACN (acetonitrile), ACN with water (50:50), CH_3OH with H_2O (50:50) to assess optimum absorption for each API solution. UV / visible scanning were performed at wavelength (200 – 400 nm) and maximum absorbance was perceived in 200-240 nm range for every solution. For the study of the Cefoperazone and Sulbactam at HPLC, intermediate maximum absorbance of 220 nm was chosen.

Mobile Phase Preparation

Used 6.7 ml per liter of tetrabutylammonium hydroxide solution and acetonitrile mixture (3:1). Mobile phase pH 4.0 was adjusted with Orthophosphoric acid.

Standard Preparation: Separately, weigh about 50 mg each of the Sulbactam RS and Cefoperazone RS, in a VF of 50 ml. add 30ml of mobile phase to 50 ml flask and dissolved by sonication. Build up the volume by mobile phase to 50ml after assuring the dissolution. 0.45-micron membrane was used for filtration.

Sample preparation: Precisely measure the material mass of no less than 50 mg Cefoperazone Sodium and Sulbactam Sodium for Injection. Weigh 100mg accurately of the content (equivalent to about 50mg of Cefoperazone Sodium and 50mg of Sulbactam Sodium), in 50ml volumetric flask.

Added approximately 30ml of mobile phase and dissolved by sonication.

Diluents

Mobile phase: Upon assuring dissolution, with mobile phase make up the volume to 50ml. The solution is filtered through a 0.45-micron membrane.

Method Validation: The method was validated with parameters listed below in accordance with ICH guidelines as under:

1. Specificity
2. Precision
3. Accuracy
4. Linearity and Range
5. Detection Limit
6. Quantitation Limit
7. Robustness

1) Specificity

The blank, sample solution, standard solution, and mobile step are prepared and then injected into HPLC according to process development portion. The retention time of any peaks observed was reported in the form of a table in subsequent

2) Precision

System Precision

100% Homogenous concentration sample solution was prepared as provided in the section on method growth. 5 Replicated injections were repeated. The %RSD for APIs assay for repeatability is within acceptance criteria (≤2%)

Repeatability

Six samples were prepared individually at a concentration point of 100 per cent. 3 Replicates were injected from each sample. The standard deviation was measured and tabulated and the relative standard deviation percent. According to the FDA, the percentage of RSD for drug substances should be as much as ≤ 1 percent and for drug product should be as much as ≤ 2 percent.

Intermediate Precision

Composite samples operate on different days for intermediate accuracy, with another separate analyst holding same operating conditions.

Two separate researchers analyzed six sample preparations on day one and collected data (percentage RSD, percentage Assay). The study was replicated using alternative analyst with the same chromatographic conditions and concentration of 100 percent. The findings of the test evaluated on different days by two different researchers do not have %RSD ≤ 2.

3) Accuracy

The accuracy (or trueness) of a method, is describing nearness of the results to accepted or real standard value. Chemical excipients (artificial mixture components) of which well-known amounts of Cefoperazone sodium and Sulbactam sodium to be evaluated were defined at three different levels of concentration as follows:

80% concentration level

Prepare by moving 80 mg sample containing 40 mg of Sulbactam &Cefoperazone into 50ml volumetric flask and filled the flask up to the mark of 50ml with mobile phase.

100% concentration level

Prepare by moving 100 mg sample containing 50 mg of Sulbactam & Cefoperazone into 50mL volumetric flask and filled the flask up to the mark of 50mlwith mobile phase.

120% concentration level

Prepare by transferring 120 mg sample containing 60 mg of Sulbactam &Cefoperazone into 50mL volumetric flask and filled the flask up to the mark of 50mLwith mobile phase.

Three sample solutions will be evaluated in the experimental portion against a simultaneously prepared standard solution. This will measure and record the percentage recovery and the mean recovery. The percentage limit for recovery of spiked placebo should be 98-102% .For each discrete sample, recovery should be within 98-102 per cent.

4) Linearity &Range

The ability of any analytical method, to attain results that are directly proportional to the concentration of analyte in the sample matrix is called linearity. The range of an analytical method gives association to an appropriate level of linearity, accuracy, & precision between for which method has been validated. The quantitative dilutions of concentration stock solution 10mg / ml will prepare five linearity solutions.

Stock Solution Preparation:

In the mobile process, the stock solution will be prepared by separately dissolving 500 mg of Sulbactam Sodium and Cefoperazone Sodium RS in 50ml volumetric flask. Various concentration solutions (600 – 1400 ppm) for obtaining calibration plots will be prepared as follows:

60% concentration level:

Prepare by transferring 3ml in 50ml volumetric flask of growing stock solution via pipette and filled the flask up to the mark of 50ml with mobile phase.

80% concentration level:

Prepare by transferring 4ml in 50ml volumetric flask of stock solution with pipette and filled the flask up to the mark of 50ml with mobile phase.

100% concentration level:

Prepare by transferring 5ml in 50ml volumetric flask of stock solution with pipette and filled the flask up to the mark of 50ml with mobile phase.

120% concentration level:

Prepare by transferring 6ml in 50ml volumetric flask of stock solution via pipette and filled the flask up to the mark of 50ml with mobile phase.

140% concentration level:

Prepare by transferring 7ml in 50ml volumetric flask of stock solution with pipette and filled the flask up to the mark of 50mlwith mobile phase.

Concentrations are plotted in the X axis and peak area in Y axis. 'R^2' (coefficient of correlation) will be ≥0.990.

5) Detection Limit

The minimum level of concentration that can be identified of a specific analytical method but cannot essential quantified with a precise value is LOD. The LOD will be estimated on standard response variance (σ) and slope (S) basis.

$$LOQ = 3.3 \times \left(\frac{\sigma}{S}\right)$$

Where,

σ= Standard reaction variance S= Calibration Curve Slope

6) Quantification Limit

The minimum concentration of a substance that can be measured quantitatively with accurately and precisely. The LOQ will be estimated on standard response variance (σ) and slope (S) basis.

$$LOQ = 10 \times \left(\frac{\sigma}{S}\right)$$

Where,

σ= Response of Standard Deviation S = Calibration Curve Slope

7) Robustness

In robustness small changes are made in the test method to be validated to check consistency and reliability of test method during routine analysis.

7.1) Wavelength Variation ± 2 nm:

Sample solution with the same experimental section method was prepared without any modification, but the wavelength reading modified by ±2 nm (218 & 222 nm for API). Three replication injections were measured as assays at each wavelength.

7.2) Change in Flow Rate ±0.1mL/min

Robustness was checked by change of flow rate. Analysis was conducted at 0.9 &1.1 milliliter per minute and reported results.

7.3) Robustness Regarding Temperature ± 5°C

Mobile step and sample were prepared according to protocol and injected at40°C±5°C for robustness assessment at both 30°C and 40°C temperatures.

RESULTS

The linearity is the ability of any analytical method (given decided range), to attain results that are directly proportional to the concentration of analyte in the sample matrix. The linearity graph for Cefoperazone sodium and Sulbactam sodium has been for illustrated in Figures **3 & 4**. The correlation coefficient (R^2) to be found 1 and average %RSD was attained 0.062% of 60%, 80%, 100%, 120% and 140% solution of Cefoperazone sodium as given in Table **1**.

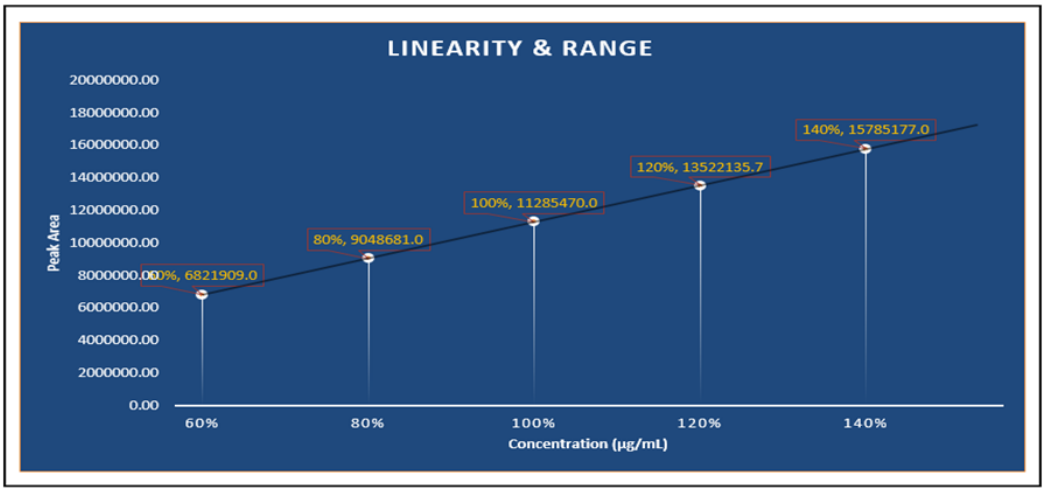

Figure 3. Linearity graph of Cefoperazone sodium.

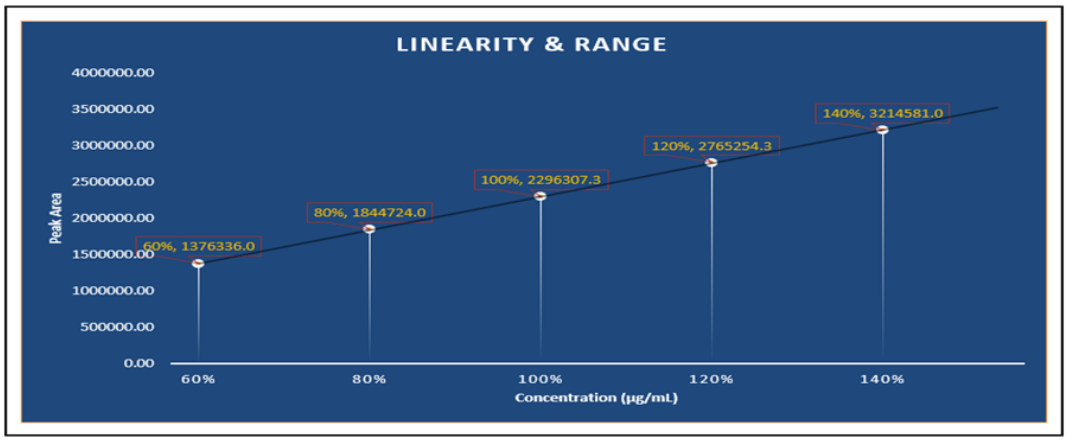

Figure 4. Linearity graph of Sulbactam sodium.

Table 1. Linearity Results of Cefoperazone Sodium.

Cefoperazone Sodium						
Sr. #	**Solution**	**Conc. (µg/mL)**	**Inj. #**	**Area**	**Average**	**% RSD**
1	60 % Solution	600	1	6824372	6821909.00	0.05%
			2	6823177		
			3	6818178		
2	80 % Solution	800	1	9051257	9048681.00	0.05%
			2	9051232		
			3	9043554		
3	100 % Solution	1000	1	11289871	11285470.00	0.05%
			2	11288116		
			3	11278423		
4	120 % Solution	1200	1	13532498	13522135.67	0.09%
			2	13524839		
			3	13509070		
5	140 % Solution	1400	1	15795884	15785177.00	0.07%
			2	15774987		
			3	15784660		
Slope					11199.9953	
Intercept					92679.2000	
Correlation Coefficient					1.0000	
Linear Regression Equation					**y = 1120x + 92679**	

The limit detection was calculated and to be found 5.735 ppm and 13ppm for Cefoperazone sodium and Sulbactam sodium respectively. The quantitation limit for both the actives was calculated so 17.370ppm for Cefoperazone sodium 42.070ppm for Sulbactam sodium was to be found. Repeatability was calculated using a minimum of 9 determinations of 3 concentrations (3 concentrations / 3 replicates each) covering the specified range (800 – 1200 ppm) i.e. 80%, 100%, 120% dilutions were prepared and %RSD was 1.17% as given in Tables **2 & 3** for Cefoperazone Sodium. Accuracy was also calculated using a minimum of 9 resolves of 3 concentrations (3 concentrations / 3 replicates each) of 80%, 100%, 120%. The minimum recovery for Cefoperazone Sodium in dry injection formulation was 98.25% and maximum recovery was 99.33% as given in Table **4**. The robustness of the method was observed with minor changes in method parameters and %RSD was about 0.3% as mentioned in Table **5**.

Table 2. Repeatability data for Cefoperazone Sodium.

Cefoperazone Sodium								
Sr. #	**Name of Solution**	**Weight Taken**	**Conc. (µg/mL)**	**Inj. #**	**Area**	**Average**	**% Assay**	**% RSD**
1	Reference	50	1000	1	11390560	11388536.67	-	0.02%
				2	11389406			
				3	11385644			
2	80 % Solution	40.05	801	1	9044252	9038503.33	99.1%	0.06%
				2	9034811			
				3	9036447			
3	100 % Solution	49.95	0.999	1	11213891	11230537.00	98.71%	0.13%
				2	11240394			
				3	11237326			
4	120 % Solution	60.00	1200	1	13591480	13586418.67	99.42%	0.04%
				2	13586090			
				3	13581686			
Average Assay (%)				100.55%				
Standard Deviation				0.01				
% RSD				1.17%				

Table 3. Intermediate Precision of Cefoperazone Sodium.

Cefoperazone Sodium							
Solution	**Weight Taken (mg)**	**Conc. (ppm)**	**Inj. #**	**Area**	**Average Area**	**Assay (%)**	**% RSD**
Reference	100.0	2000	1	11513208	11506961.33	-	0.05%
			2	11506635			
			3	11501041			
Sample # 1	99.9	1998	1	11554444	11551110.67	45.04%	0.03%
			2	11551512			
			3	11547376			
Sample # 2	100.0	2000	1	11515032	11509820.00	44.93%	0.06%
			2	11512220			
			3	11502208			
Sample # 3	100.0	2000	1	11469299	11471796.67	44.73%	0.04%
			2	11468471			
			3	11477620			
Sample # 4	100.1	2002	1	11517188	11508329.00	44.88%	0.07%
			2	11503483			
			3	11504316			
Sample # 5	100.1	2002	1	11510316	11502964.33	44.81%	0.07%
			2	11504757			
			3	11493820			
Sample # 6	100.0	2000	1	11457633	11462410.67	44.70%	0.04%
			2	11463075			
			3	11466524			
Average Assay (%)			44.85%				
Standard Deviation			0.001				
% RSD			0.29%				

Table 4. Accuracy Results of Cefoperazone Sodium.

Cefoperazone Sodium							
Solution	**Wt. Taken (mg)**	**Conc. (ppm)**	**Inj. #**	**Area**	**Average**	**Recovery %**	**% RSD**
Reference	50	1000	1	11412857	11400493.67	-	0.11%
			2	11401206			
			3	11387418			
80 % Solution	40	800	1	8984491	8979134.33	98.45%	0.05%
			2	8978047			
			3	8974865			
100 % Solution	50	1000	1	11206153	11201224.00	98.25%	0.04%
			2	11197484			
			3	11200035			
120 % Solution	59.95	1199	1	13564867	13577421.33	99.33%	0.08%
			2	13584905			
			3	13582492			
Minimum Recovery (%)			98.25%				
Maximum Recovery (%)			99.33%				
Mean Recovery (%)			98.68%				
Standard Deviation			0.006				
Relative Standard Deviation (% RSD)			0.58%				

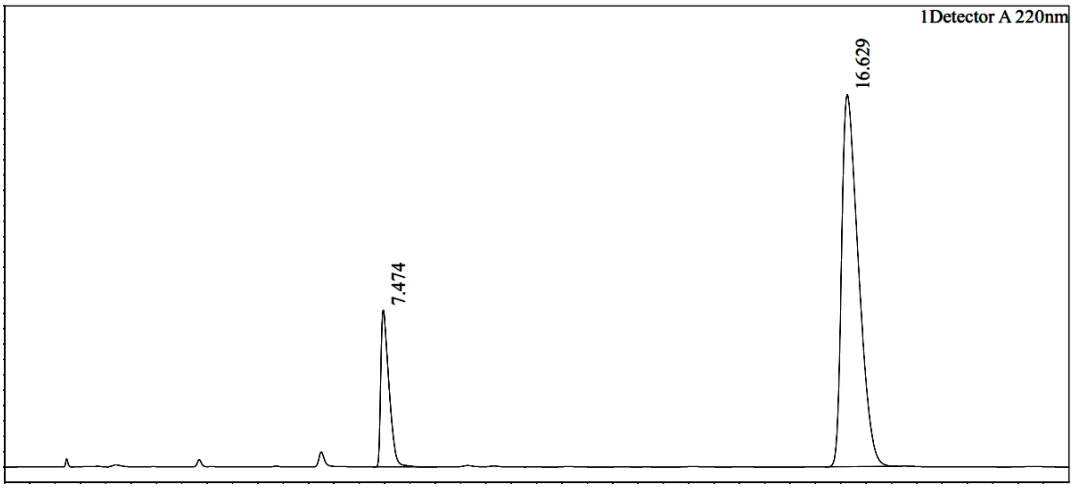

Figure 5. HPLC Chromatogram of Cefoperazone sodium and Sulbactam sodium.

Table 5. Robustness of Method.

APIs	Average Assay (%)	Standard Deviation	%RSD
Cefoperazone	99.90%	0.0032	0.32%
Sulbactum	99.24%	0.0033	0.33%

DISCUSSION

The method was validated as per ICH guidelines parameters of linearity, precision accuracy, LOD, LOQ and robustness [22]. The correlation coefficient (R^2) is about near to 1 as given in Table **1** that indicates that the method is very sensitive and accurate. The method will be suitable if correlation coefficient (R2) of linearity graph is more than 0.995 [23,24]. The LOQ and LOQ for Cefoperazone sodium and Sulbactam sodium was found in ppm these values are in well range hence method is suitable to detect and quantify the both actives (Cefoperazone sodium and Sulbactam sodium) in the dry injection

formulations. The method is very efficient because the recovery of both drugs in the sample is within limits and results are repeatable [25]. All recoveries for the individual should be between 97.0% and 103.0%. The mean recovery should be between 98.0% to 102.0%.

The method was checked by minor variation in flow rate, wavelength and temperature. The absolute difference in the flow rate ±0.1mL / min robustness check, temperature ± 5 ° C, and wavelength ± 2 nm was not more than 2.0 percent, which means that the system is robust for these variables. The results were repeatable as given in Table 5. Hence method is robust and can be used in a routine analysis of Cefoperazone sodium and Sulbactam sodium in pharmaceutical formulation.

CONCLUSION

A simple, precise, cost-effective, robust, and accurate HPLC method has developed for the simultaneous determination of Cefoperazone sodium and Sulbactam sodium from dry injection. The method was validated as per ICH guidelines Q2 (R1) for analytical method validation. Both the actives as sodium Cefoperazone and Sulbactam Sodium were determined in the dosage formulation of dry powder injection. Hence, the developed method can be used in the routine analysis of Cefoperazone sodium and Sulbactam sodium in different dosage forms.

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