

## Validated Hptlc Method for Simultaneous Determination of Cefoperazone Sodium and Sulbactam Sodium in Combined Dosage Form

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**Abstract:** A simple, accurate and precise HPTLC method for simultaneous estimation of Cefoperazone sodium and Sulbactam sodium as bulk and dry powder for injection in combined dosages form is described in this paper.

A mobile phase of Chloroform: Ethyl alcohol: Diethyl amine: Water (14: 16:8:1.2 v/v) was used after optimization at different concentrations for the development of densitogram. Aluminum plate coated with the silica Gel 60 F<sub>254</sub> was used as stationary phase. Densitometric evaluation of the separated bands was performed at 274 nm. The R<sub>f</sub> values of Cefoperazone sodium and Sulbactam sodium were 0.41 ± 0.01 and 0.56 ± 0.01 respectively.

The validated method was linear over the concentration range of 200 ng to 900 ng /spot and 400 ng to 1800 ng/spot of Cefoperazone sodium and Sulbactam sodium respectively. Precision of the method was evaluated by interday and intraday RSD. The results were Cefoperazone sodium: Inter day RSD of peak response 1.25 % and Intraday RSD 1.73 % and for Sulbactam sodium Interday RSD of peak response 1.54 % and Intraday RSD 0.98 %. Accuracy was determined in terms of percentage recovery at three concentration levels for Cefoperazone sodium RSD 99.20 %, 99.50 % and 100.32 % and for sulbactam sodium RSD 101.25 %, 100.40 % and 100.60 % respectively. Specificity was determined by spectral analysis of cefoperazone sodium and sulbactam sodium and overlaying the standard spectra and sample spectra respectively. There was no any interference of mobile phase and diluents at the R<sub>f</sub> values of Cefoperazone sodium and sulbactam sodium. Validation was done in accordance with the ICH Guidelines.

**Key words:** High performance thin layer chromatography, microgram, nanogram, Cefoperazone sodium and Sulbactam sodium.

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### I. Introduction

Cefoperazone sodium is chemically Sodium (6R,7R)-7-[[[(2R)-2-[[[4-ethyl-2,3-dioxopiperazin-1-yl]carbonyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]-3-[[[(1-methyl-1H-tetrazol-5-yl)sulphonyl]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. Molecular weight of Cefoperazone is 668 and CAS No. is 62893-20-3

It is Semi-synthetic product derived from a fermentation product. Cefoperazone is a third generation cephalosporin antibiotic. It is one of few cephalosporin antibiotics effective in treating *Pseudomonas* bacterial infections which are otherwise resistant to these antibiotics.

Cefoperazone exerts its bactericidal effect by inhibiting the bacterial cell wall synthesis, and sulbactam acts as a beta-lactamase inhibitor to increase the antibacterial activity of cefoperazone against beta-lactamase producing organisms. It is freely soluble in water, soluble in methanol, slightly soluble in ethanol (96 per cent).

Sulbactam sodium is chemically sodium (2S, 5R)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylate 4, 4-dioxide. It is Semi-synthetic product derived from a fermentation product.

Sulbactam sodium is being used as Beta-lactam antibacterial. Molecular weight is 255.2 and CAS Number is 69388-84-7. It is freely soluble in water, sparingly soluble in ethyl acetate, very slightly soluble in ethanol (96 per cent). It is freely soluble in dilute acids.

Literature survey reveals that the several analytical methods viz. High performance liquid chromatography and UV-VIS spectrophotometric method have been reported for estimation of Cefoperazone sodium and Sulbactam sodium as an individual drug substance and in the combination drug products.

A simple, accurate and precise HPTLC method for simultaneous estimation of Cefoperazone sodium and Sulbactam sodium in the combined dosages form has been developed.

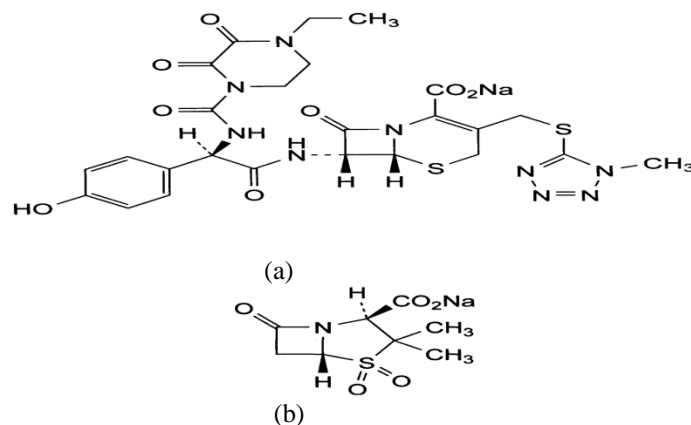


Figure 1. Structure of Cefoperazone Sodium (a) and Sulbactam Sodium (b)

## II. Materials and Methods

### 2.1 Chemicals and Reagents

Cefoperazone sodium and Sulbactam sodium working standards were provided by Aurobindo Pharma Ltd through Mr. Vikrant Tamse as a noble gift samples for validation study. Dry powder injection samples required for method validation were procured from the market. All other reagents used during validation study were of analytical grade.

### 2.2 Instrumentation

The HPTLC method was optimized and validated on the CAMAG HPTLC instrument. CAMAG automatic TLC sampler 4 (ATS4) connected with the win CATS 4 software, CAMAG TLC SCANNER, Integrator controlled by win CATS4 software are the components of the HPTLC instrument. Precoated silica Gel 60 F<sub>254</sub> on aluminium sheets were used as a stationary phase. During development of the plate CAMAG twin trough glass chamber with stainless steel lid was used.

In a 20 x 10 cm twin trough glass chamber (Make: CAMAG), a linear ascending chromatographic development was carried out. During optimization of Method various solvents viz. n-butanol, methanol and water were used. However separation was not achieved. Hence method was optimized with other solvents like Chloroform, ethyl alcohol, diethyl amine and water in the different compositions. A method was optimized with the mobile phase of Chloroform: Ethyl alcohol: diethyl amine: water in the ratio (14: 16: 8:1.2 v/v). The chamber was saturated for 20 minutes. A deuterium lamp was used in the UV range of 190 to 400 nm as a source of radiation. A slit dimension was 6.00 x 0.45 mm, micro, scanning speed was 20 mm s<sup>-1</sup> and data resolution at 100µm/step. Sample was spotted on the silica gel 60 F<sub>254</sub> TLC plate by using CAMAG automatic TLC sampler-4 (ATS). The plates were developed in the CAMAG TLC chamber upto 80 mm. Run time of the analysis was 25 minutes. After development, TLC plate was dried in a current of hot air with the help of hair dryer and dried on a CAMAG hot plate at 120°C for 5 minutes. The contents of Cefoperazone sodium and Sulbactam sodium were evaluated by comparing the peak areas with linear regression.

## III. Standard solution preparation

10 mg of Cefoperazone sodium and 10 mg of Sulbactam sodium standards were accurately weighed and transferred to separate 10 mL volumetric flasks. 2 mL of Methanol was added and sonicated for 5 minutes to dissolve the standards. Then diluted to 10 mL with methanol (Stock solution1 and stock solution 2 for Cefoperazone sodium and Sulbactam sodium respectively) to obtain the concentration of 1 mg /mL and 1.0 mg / mL or 1 µg / µl and 1µg/µl of standard Cefoperazone sodium and Sulbactam sodium respectively.

## IV. Sample solution preparation

Label claim of Cefoperazone sodium and Sulbactam sodium in the combined dry powder injection in one unit was 1000 mg and 1000 mg respectively. To determine the content, 10 vial units were individually weighed. An average weight was recorded. Dry powder from all vials was mixed together to make a pooled sample. A sample weight equivalent to 1000 mg of Cefoperazone and 1000 mg of Sulbactam was weighed in 10 ml volumetric flask. 2 mL of Methanol was added and sonicated for 5 minutes to dissolve. Finally diluted to 10 mL with methanol to obtain the concentration of 1mg /mL and 1mg/mL or 1 µg / µl and 1µg/µl of Cefoperazone sodium and Sulbactam sodium respectively.

## V. Results and discussions

### 5.1 Validation of analytical method

ICH guideline was referred to validate an analytical method. Optimized analytical method was validated for specificity, linearity, accuracy, precision, LOD, LOQ and Robustness.

#### 5.1.1 Specificity

In specificity, Cefoperazone sodium standard, Sulbactam sodium standard, sample solution, diluent and mobile phase were spotted on the TLC plate. TLC plate was developed in a twin trough CAMAG chamber referring the developed method. There was no any interference of mobile phase and diluent at the  $R_f$  value of Cefoperazone sodium and Sulbactam sodium. The separate spectrum of Cefoperazone and Sulbactam sodium were taken. Peak purity of Cefoperazone sodium and Sulbactam sodium was determined by comparing spectrum at three different regions of the spots. i.e Peak start (S) Peak apex(M) and peak end(E) of both the drugs. The bands for Cefoperazone sodium and Sulbactam sodium were confirmed by comparing  $R_f$  values. The  $R_f$  values of Cefoperazone sodium and Sulbactam sodium were 0.41 and 0.58 respectively.

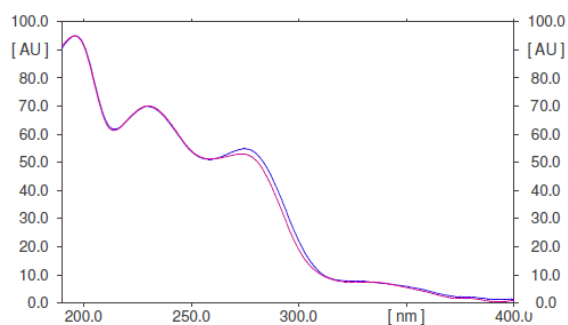


Figure 2. Overlaid spectrum of Cefoperazone sodium standard and Cefoperazone sodium sample

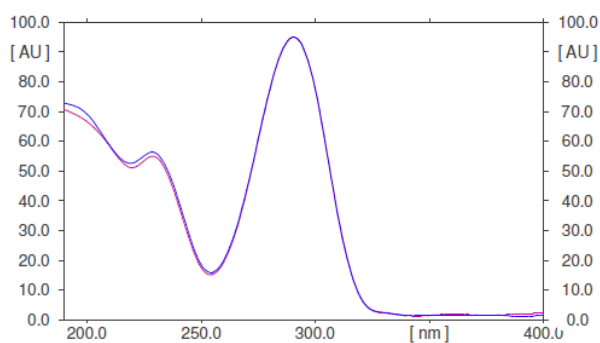


Figure 3. Overlaid spectrum of Sulbactam sodium standard and Sulbactam sodium sample

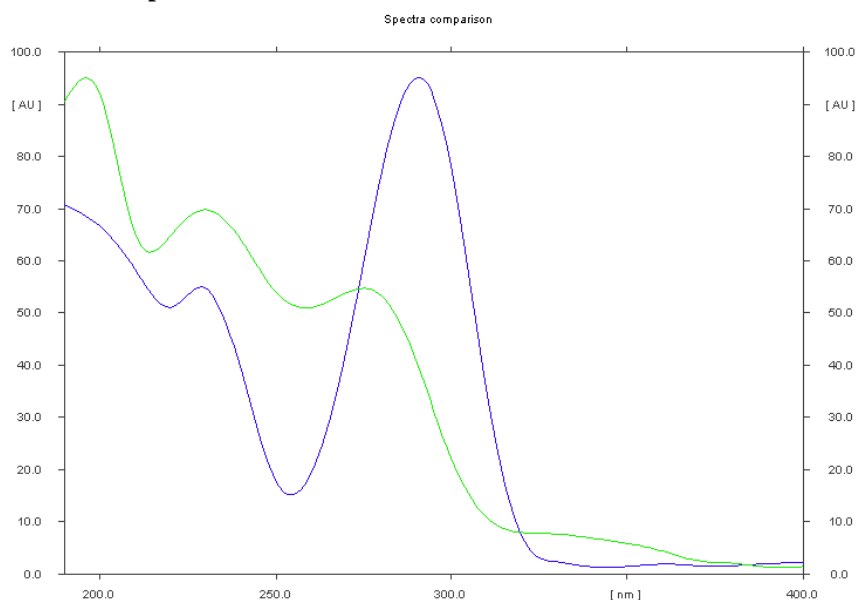


Figure 4. Overlaid spectrum of Cefoperazone Sodium standard and Sulbactam Sodium standard

### 5.1.2 Accuracy

The accuracy of the Cefoperazone sodium and Sulbactam sodium was determined by performing recovery at three different concentration levels. The known concentrations of the samples were spiked with the standard cefoperazone and sulbactam in the concentrations of 400 ng, 500 ng and 600 ng of cefoperazone and 800 ng, 1000 ng and 1200 ng of sulbactam at 80 % 100% and at 120 level with respect to the sample concentration. The spiked samples were analysed by following the proposed analytical method. The percentage recovery was calculated and was in the range of 99.20 % to 100.32 % for Cefoperazone and 100.40 % to 101.60 % for sulbactam respectively. The results are tabulated as under:

**Table 1. : Percentage Recovery of Cefoperazone sodium  
(n=3)**

Sr. No.	Amount of std. Cefoperazone sodium added in ng	Amount of std. Cefoperazone sodium recovered in ng	% Recovery	% Relative Standard Deviation
1	400	396.8	99.20	1.93
2	500	497.5	99.52	1.88
3	600	601.9	100.32	1.83

**Table 2: Percentage Recovery of Sulbactam sodium  
(n=3)**

Sr. No.	Amount of std. Sulbactam sodium added in ng	Amount of std. Sulbactam sodium recovered in ng	% Recovery	% Relative Standard Deviation
1	800	810	101.25	1.59
2	1000	1004	100.40	0.21
3	1200	1207.2	101.60	1.18

### 5.1.3 Precision

The interday and intraday precision of the method were estimated by performing six determinations of Cefoperazone sodium and Sulbactam sodium standard solutions. The analysis was carried by referring the developed method. Analytical results obtained are tabulated as under:

**Table 3: Precision for the Cefoperazone sodium  
(n= 6)**

Conc. of the Cefoperazone sodium (ng/ band)	Inter-day precision		Intra-day precision	
	Mean area (AU)	% RSD	Mean area (AU)	% RSD
500	1583	1.25	1611	1.73

**Table 4: Precision for Sulbactam sodium  
(n=6)**

Conc. of the Sulbactam sodium (ng/ band)	Inter-day precision		Intra-day precision	
	Mean area (AU)	% RSD	Mean area (AU)	% RSD
1000	2788	1.54	2662	0.98

### 5.1.4 Robustness of the method

During Robustness testing, small deliberate changes in the mobile phase composition were done. Effect on the results was examined. Mobile phase having different compositions were tried and chromatograms were run. The small change of  $\pm 0.1$  mL for each component of the mobile phase was done. Also  $\pm 5$  % variation in the mobile during TLC development was used and chromatograph was run.

The robustness of the method was determined at three different concentration levels. The results are tabulated as under:

**Table 5: Robustness testing  
(n=3)**

Parameter	Conc. Level in ng spot <sup>-1</sup> of Cefoperazone	SD of Peak response of Cefoperazone	%RSD	Conc. Level in ng spot <sup>-1</sup> of Sulbactam	SD of Peak response of Sulbactam	%RSD
Mobile phase composition ( $\pm 0.1$ mL)	400	29.14	1.15	800	42.72	1.17
	500	25.0	0.77	1000	42.72	0.92
	600	25.06	0.70	1200	52.55	0.97
$\pm 5$ % variation in mobile phase	400	43.46	1.86	800	57.07	1.61
	500	51.58	1.60	1000	76.16	1.66
	600	23.45	0.65	1200	96.1	1.80

### 5.1.5 Linearity

A series of standard solutions were prepared from the standard stock solutions of Cefoperazone sodium and Sulbactam sodium. Solutions were spotted on the TLC plate in the range of 0.2  $\mu$ l to 0.9  $\mu$ l of Cefoperazone sodium and 0.4  $\mu$ l to 1.8  $\mu$ l of Sulbactam sodium respectively. The corresponding concentrations were in the range of 0.2  $\mu$ g / spot to 0.9  $\mu$ g /spot and 0.4  $\mu$ g/spot to 1.8  $\mu$ g /spot respectively. The linear Correlation coefficient for Cefoperazone sodium was 0.9989 and 0.9978 for Sulbactam sodium respectively.

### 5.1.6 LOD and LOQ

The limits of detection (LOD) and Limit of Quantitation (LOQ) were calculated from slopes of the calibration curve. The Limit of Detection and Limit of Quantitation obtained by this method for Cefoperazone sodium and Sulbactam sodium were LOD= 2.067 mcg, LOQ= 6.266 mcg, LOD= 4.423 mcg and LOQ = 13.403 mcg respectively.

### 5.1.7 Analysis of drug product

Experimental HPTLC results of the amount of Cefoperazone sodium and Sulbactam sodium in the dry powder Injectables expressed as a mg of label claim were in good agreement with the label claim. The drug content was found to be 99.5 % and 100.9 % for Cefoperazone and Sulbactam respectively.

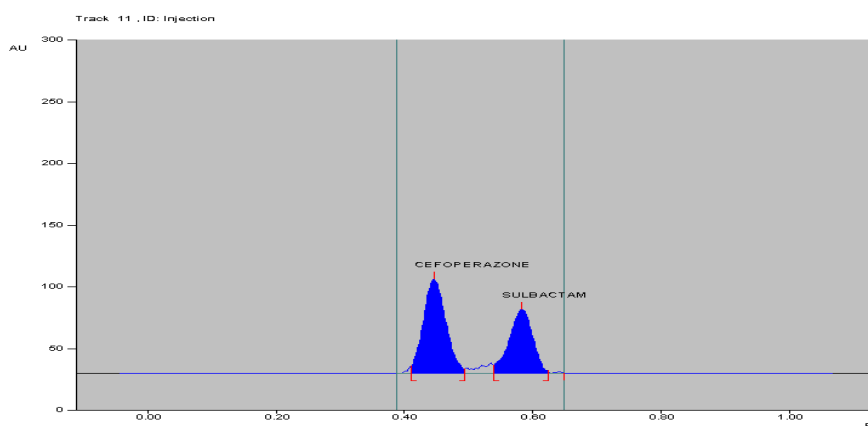


Figure 5. Densitogram of Cefoperazone sodium (  $R_F$  0.41) and Sulbactam sodium (  $R_F$  0.56)

### 5.1.8 Conclusion

HPTLC analysis is rapidly becoming popular in routine analysis. The advantages of these analytical techniques are low operating cost and high sample throughput. This method may be used for simultaneous determination of Cefoperazone sodium and Sulbactam sodium in routine analysis in drug substances as well as drug products. This method may be used for degradation study of the Cefoperazone sodium and Sulbactam sodium. The proposed HPTLC method is simple, accurate, economically chief and reproducible.

### 5.1.9 Acknowledgement

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