



# **DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CEFOTAXIME SODIUM AND SULBACTAM SODIUM IN BULK AND INJECTION**

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## **ABSTRACT**

A reverse phase high performance liquid chromatographic method was developed for simultaneous determination of cefotaxime sodium and sulbactam sodium in bulk and injection. The separation was made by a Phenomenex Gemini C18 column (250 cm × 4.6 mm, 5 μm) using buffer (40% tetrabutyl ammonium hydrochloride) : acetonitrile : methanol (8 : 1.75 : 0.25, v/v/v) as mobile phase. The validation of the method was performed, and specificity, reproducibility, precision and accuracy were confirmed. The limit of detection was approximately 0.57 μg/mL for cefotaxime sodium and 0.49 μg/mL for sulbactam sodium. Due to simplicity and accuracy, the method is particularly suitable for routine pharmaceutical quality control.

**Key words:** RP-HPLC, Cefotaxime sodium, Sulbactam sodium, Validation.

## **INTRODUCTION**

Cefotaxime sodium (CFT) is chemically a sodium 7-[2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylate (Fig. 1) and it is a third-generation cephalosporin antibiotic<sup>1</sup>. It inhibits bacterial cell wall synthesis by binding to one or more of the penicillin-binding proteins (PBPs), which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls; thus, inhibiting cell wall biosynthesis. Sulbactam sodium (SLB) is chemically sodium (2*S*, 5*R*)-3, 3-dimethyl-

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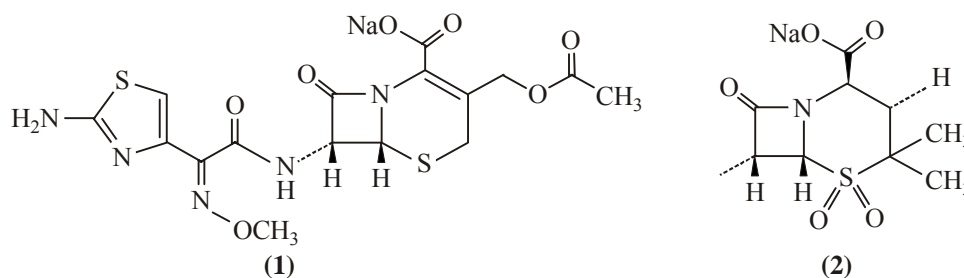
7-oxo-4-thia-1- azabicyclo [3.2.0] heptane-2-carboxylate 4, 4-dioxide (Fig. 1) and is a derivative of the basic penicillin nucleus, which is a beta-lactamase inhibitor<sup>2</sup>. Beta-lactamase inhibitor is a compound that is capable of inhibiting a betalactamase, which in turn is capable of hydrolyzing a beta-lactam antibiotic and protect the actual antibiotic from being inactivated by bacterial beta-lactamase.

In literature, spectrophotometric<sup>3,4</sup>, few HPLC,<sup>5-9</sup> LC/MS/MS<sup>10</sup>, and capillary electrophoresis<sup>11</sup> methods have been reported for determination of CFT alone and combination with other drugs for pharmaceutical formulation and biological fluids. HPLC<sup>12-17</sup>, LC/MS<sup>18</sup> methods have been reported for determination of SLB in combination with other drugs for pharmaceutical formulation and biological fluids. But no method has been developed for combination of CFT and SLB for their simultaneous determination in pharmaceutical formulation. A successful attempt has been made for simultaneous determination of CFT and SLB in combined dosage form. Therefore, it was thought worthwhile to develop a simple, precise, accurate and reliable RP-HPLC method for simultaneous estimation of both the drugs in combined dosage form.

## EXPERIMENTAL

### Standards and reagents

Cefotaxime sodium (CFT) and sulbactam sodium (SLB) were provided by Concept Pharmaceuticals Ltd. (Aurangabad, India) and were used as working standards. The commercially available formulation, Taximax 750<sup>®</sup> injection was used for quantitative determination. Acetonitrile and methanol were HPLC grade and purchased from Merck Chem. Ltd., Mumbai. All solutions were prepared with double distilled R.O. water for HPLC.



**Fig. 1: Chemical structure of cefotaxime sodium (1) and sulbactam sodium (2)**

### Chromatographic system and conditions

The HPLC system used was Shimadzu LC-2010 model composing quaternary pump,

auto sampler, mobile phase degasser, heated column thermostat, and variable UV detector. The mobile phase contained buffer (40% tetrabutyl ammonium hydrochloride) : acetonitrile : methanol (8 : 1.75 : 0.25, v/v/v) and flow rate was maintained at 1.5 mL/min and monitored at 230 nm. Chromatographic separations were performed at ambient temperature on a Phenomenex Gemini C18 column (250 cm × 4.6 mm, 5 μm) and the injection volume was 20 μL.

### Standard stock solution

An accurately weighed 20 mg of CFT and 10 mg of SLB were transferred to 100 mL volumetric flask and volume was adjusted to mark to obtain concentration 200 μg/mL of CFT and 100 μg/mL of SLB.

### Calibration curves

Different aliquots 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 mL of standard stock solution were transferred into 10 mL volumetric flasks and volume was adjusted to mark to obtain concentration in the range 20, 30, 40, 50, 60, 70, 80 and 90 μg/mL of cefotaxime sodium and 10, 15, 20, 25, 30, 35, 40 and 45 μg/mL of sulbactam sodium, respectively. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area vs the drug concentration. The areas exhibited linear responses with  $r^2 = 0.999$  for CFT and SLB. The results are shown in Table 1.

**Table1: Results of linearity studies**

Parameters	CFT	SLB
Linearity range	20 – 90 μg/mL	10 – 45 μg/mL
Coefficient of correlation (r)	0.999	0.999
Regression equation	$Y = 22403 X + 2047$	$Y = 21984 X + 2719$

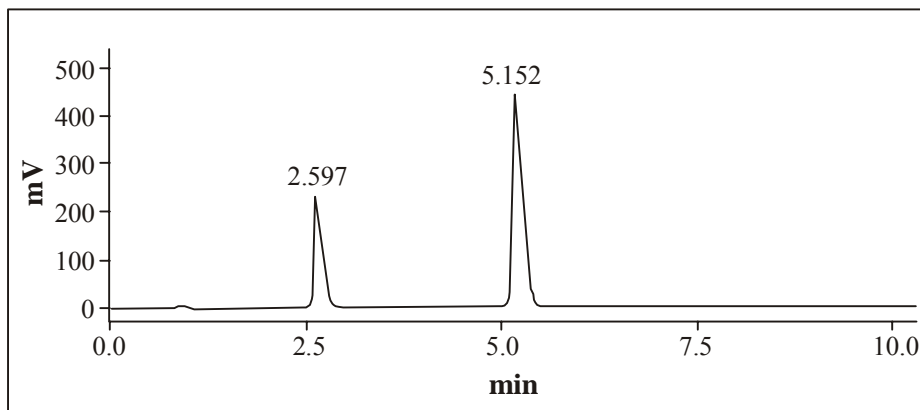
### Analysis of bulk sample

Accurately weighed 20 mg of CFT and 10 mg of SLB were transferred to 100 mL volumetric flask, dissolved in water and volume was adjusted to mark. Appropriate volume, 2.5 mL was transferred into 10 mL volumetric flasks and volume was adjusted to mark to obtain concentration 50 μg/mL of CFT and 25 μg/mL of SLB. The procedure was repeated six times. The typical chromatogram is shown in Fig. 2 and results are given in Table 2.

**Table 2: Assay of bulk sample**

Drug	Amount taken ( $\mu\text{g/mL}$ )	Amount found ( $\mu\text{g/mL}$ ) $\pm$ S.D. (n = 6)	Amount found [%] $\pm$ S.D (n = 6)	% RSD
CFT	50	50.01 $\pm$ 0.08	100.02 $\pm$ 0.16	0.16
SLB	25	24.98 $\pm$ 0.04	99.92 $\pm$ 0.17	0.17

n = Number of repetitions

**Fig. 2: Typical HPLC chromatogram of bulk sample - SLB ( $R_t = 2.5$ ) and CFT ( $R_t = 5.1$ )**

### Analysis of injection

The powder of Taximax 750 injection was weighed and an amount of powder equivalent to 20 mg of CFT was transferred to 100 mL volumetric flask and extracted with water for 20 minutes by shaking mechanically. The solution was diluted to volume with the same solvent and filtered. Appropriate volume, 2.5 mL, was transferred into 10 mL volumetric flasks and volume was adjusted to mark to obtain concentration 50  $\mu\text{g/mL}$  of CFT and 25  $\mu\text{g/mL}$  of SLB. The assay procedure was repeated five times. Chromatogram of injection solution is shown in Fig. 3 and results are presented in Table 3.

**Table 3: Assay of injection**

Drug	Label claim (mg)	Amount found (mg) $\pm$ S.D. (n = 5)	Amount found [%] $\pm$ S. D (n = 5)	% RSD
CFT	500	500.05 $\pm$ 0.84	100.01 $\pm$ 0.16	0.16

Cont...

Drug	Label claim (mg)	Amount found (mg) ± S.D. (n = 5)	Amount found [%] ± S. D (n = 5)	% RSD
SLB	250	249.85 ± 0.39	99.94 ± 0.15	0.15

n = Number of repetitions

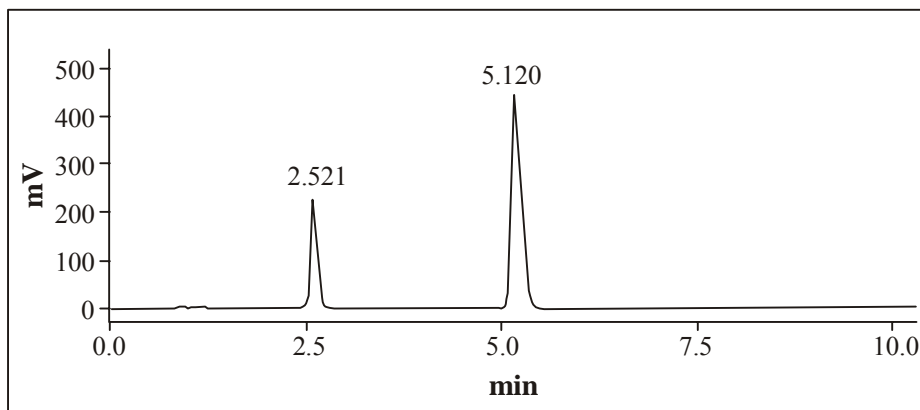


Fig. 4: HPLC Chromatogram of marketed sample SLB ( $R_t$ : 2.5) and CFT ( $R_t$ : 5.1)

### Validation of HPLC method

#### Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day variation was determined by analysing three different concentrations 40, 50 and 60  $\mu\text{g/mL}$  of CFT and of SLB 20, 25 and 30  $\mu\text{g/mL}$ , three times within a day. Inter-day precision was assessed using same concentration of drug (mentioned above) and analysing it for three different days, over a period of week. The results are shown in Table 4.

Table 4: Results from intra-day and inter-day precision

Drugs	Conc. ( $\mu\text{g/mL}$ )	Intra –day Amount found ( $\mu\text{g}$ )		Inter –day Amount found ( $\mu\text{g}$ )	
		(Mean ± SD) (n = 5)	% RSD	(Mean ± SD) (n = 5)	% RSD
CFT	40	39.96 ± 0.25	0.63	40.10 ± 0.44	1.11
	50	50.07 ± 0.04	0.09	50.09 ± 0.14	0.28

Cont...

Drugs	Conc. (µg/mL)	Intra –day Amount found (µg)		Inter –day Amount found (µg)	
		(Mean ± SD) (n = 5)	% RSD	(Mean ± SD) (n = 5)	% RSD
SLB	60	59.83 ± 0.08	0.15	59.90 ± 0.27	0.45
	20	20.01 ± 0.10	0.51	20.03 ± 0.08	0.42
	25	25.03 ± 0.18	0.53	24.98 ± 0.05	0.20
	30	29.94 ± 0.25	0.84	29.99 ± 0.38	1.27

n = Number of repetitions

### Specificity and selectivity

The specificity of the RP-HPLC method was determined by comparison of the chromatogram of mixed standards and sample solutions. The parameters like retention time (*t<sub>R</sub>*), resolution (*RS*) and tailing factor (*T<sub>f</sub>*) were calculated. Good correlation was found between the results of mixed standards and sample solution. The method is quite selective and it showed no interfering peak around the retention time of AMT and FLU and also baseline did not show any significant noise.

### Accuracy

The accuracy of an analytical method is the closeness of the test result obtained by that method to true value. Recovery experiments were performed at three different levels i.e. 80, 100 and 120 %. To the preanalysed sample solutions, known amount of standard drug solutions of CFT and SLB were added to pre-analyzed samples and these were subjected to the proposed HPLC method. The results are shown in Table 5.

### Limit of detection (LOD) and limit of quantitation (LOQ)

The sensitivity of measurement of CFT and SLB by the use of the proposed method was estimated in terms of the LOD and LOQ. The LOD and LOQ were calculated by the use of the equation  $LOD = 3.3 \times N/B$  and  $LOQ = 10 \times N/B$ ; where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. Stock solutions of CFT and SLB were prepared and different volumes in the range 20 - 30 µg/mL of CFT and 10 - 15 µg/mL of SLB were analysed in triplicate. The linearity equation of CFT was found to be  $Y = 21678 X + 15935$  and for SLB  $Y = 24193 X - 16163$ . The LOD and LOQ for CFT were found to be 0.57 µg

and 1.73  $\mu\text{g}$ , respectively (where,  $N = 3754.55$ ,  $B = 21678$ ). For SLB, the LOD and LOQ were found to be 0.49  $\mu\text{g}$  and 1.49  $\mu\text{g}$ , respectively (where,  $N = 3615.47$ ,  $B = 24193$ ).

**Table 5: Results of recovery studies**

Components	Initial Amount ( $\mu\text{g/mL}$ )	Amount added ( $\mu\text{g/mL}$ )	Amount recovered $\pm$ SD ( $\mu\text{g/mL}$ , n = 3)	% Recovered	% RSD
CFT	50	0	50.11 $\pm$ 0.04	100.22	0.08
	50	40	40.09 $\pm$ 0.07	100.23	0.17
	50	50	50.09 $\pm$ 0.13	100.18	0.25
	50	60	60.08 $\pm$ 0.15	100.13	0.25
	25	0	25.03 $\pm$ 0.05	100.12	0.23
	25	20	20.02 $\pm$ 0.12	100.10	0.61
SLB	25	25	25.03 $\pm$ 0.03	100.11	0.13
	25	30	30.01 $\pm$ 0.12	100.04	0.39

### Robustness

The robustness study was done by making small changes in optimized method parameters like change in mobile phase ratio, change in flow rate and change in wavelength. There is no significant impact on retention time and tailing factor.

### Ruggedness

Appropriate concentrations 50  $\mu\text{g/mL}$  of CFT and 25  $\mu\text{g/mL}$  of SLB standard stock solution were prepared and analyzed by two different analysts using same operational and environmental conditions. Peak area was measured for same concentration solutions, six times. The results are shown in Table 6.

### Summary

HPLC method was developed for the simultaneous estimation of CFT and SLB in its injection dosage form. The HPLC analysis was performed on the Phenomenex Gemini C<sub>18</sub> (250 mm  $\times$  4.60 mm), 5  $\mu\text{m}$  particle size in isocratic mode, at 25<sup>0</sup>C temperature using a

mobile phase consisting of buffer : acetonitrile : methanol in the ratio of 8 : 1.75 : 0.25 (v/v/v) at a flow rate of 1.5 mL/min. The detection was carried out at 230 nm. The average retention time for CFT and SLB was found to be 2.59 and 5.15 min, respectively. Linearity was observed for CFT in the concentration range from 20-90  $\mu\text{g/mL}$  ( $r^2 = 0.999$ ) and for SLB 10-45  $\mu\text{g/mL}$  ( $r^2 = 0.999$ ). The sensitivity of the method was assessed by determining LOD and LOQ. For CFT, LOD and LOQ were found to be 0.57  $\mu\text{g}$  and 1.73  $\mu\text{g}$ , respectively, for SLB, LOD and LOQ were found to be 0.49  $\mu\text{g}$  and 1.49  $\mu\text{g}$ , respectively.

**Table 6: Results of ruggedness studies**

Analyst	% Amount found	% RSD	% Amount found	% RSD
	CFT ( $n = 6$ )		SLB ( $n = 6$ )	
I	100.18	0.17	100.01	0.10
II	100.10	0.20	99.96	0.22

The proposed method was applied for injection and % label claim for CFT and SLB, which was found to be 100.01 and 99.94, respectively. The recovery studies were carried out at 80, 100, 120 % level. The % recovery for CFT and SLB was found to be 100.13-100.23 and 100.04 – 100.12, respectively. The % RSD values less than 2 is indicative of accuracy of the method. The method was found to be precise as indicated by the inter-day and intra-day studies. In robustness study, parameters (Change in flow rate and wavelength) were studied and the effects on the results were examined. Low values of % RSD proved that method is robust.

## CONCLUSION

On the basis of results of assay and validation parameters, it was concludes that proposed method was simple, fast, accurate, and precise for simultaneous estimation of CFT and SLB in combined dosage form and can be applied for the routine estimation of CFT and SLB injection.

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