Validated Hptlc Method for Simulteneous Determination of Ceftriaxone Sodium and Sulbactam Sodium in Combined Dosage Form

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Abstract: *This paper describes a new simple, accurate and precise HPTLC method for simultaneous estimation Ceftriaxone sodium and Sulbactam sodium as bulk and dry powder for injection in combined dosages form.*
In this method, the densitograms were developed using mobile phase of Chloroform: Ethyl alcohol: Diethyl amine: Water (12: 7:1:0.4 v/v). Aluminum plate coated with the silica Gel 60 F_{254} as stationary phase was used. Densitometric evaluation of the separated bands was performed at 285 nm. The Rf values of Ceftriaxone sodium and Sulbactam sodium are 0.31± 0.01 and 0.58 ± Respectively.
The method was linear over the concentration range of 400 ng to 1200 ng /spot and 300 ng to 7000 ng/spot of ceftriaxone sodium and Sulbactam sodium respectively. Precision of the method was evaluated by calculating RSD for peak response by inerday and intraday analysis. The results were Ceftriaxone sodium: Inter day RSD of peak response 0.88 % Intraday RSD 1.34 % and for Sulbactam sodium Interday RSD of peak response 1.54% and Intraday RSD 1.22%. Accuracy was determined in terms of percentage recovery at three concentration levels for Ceftriaxone sodium =RSD 1.92 %, 1.84 % and 0.66 % and for sulbactam sodium = 1.69 %, 1.79 % and 0.66 % respectively. Specificity was proved by spectral analysis of cetfriaxone sodium and sulbactam sodium and overlaying the standard spectra and sample spectra respectively .There is no any interference of mobile phase and diluents at the RF values of Ceftriaxone sodium and sulbactam sodium. Validation was done in accordance with the ICH Guidelines.
Key words: *High performance thin layer chromatography, microgram , nano gram, Ceftriaxone sodium and Sulbactam sodium.*

I. Introduction

Ceftriaxone sodium is chemically Disodium (6R,7R)-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. It is Semi-synthetic product derived from a fermentation product. Molecular formula of ceftriaxone sodium is $C_{18}H_{16}N_8Na_2O_7S_3$, $3\frac{1}{2}H_2O$. Molecular weight of Ceftriaxone sodium is 662.0 and CAS number is *104376-79-6*. Ceftriaxone sodium is a third-generation cephalosporin antibiotic. Like other third-generation cephalosporins, it has broad-spectrum activity against Gram-positive and Gram-negative bacteria. In most cases, it is considered to be equivalent to cefotaxime in terms of safety and efficacy.

Ceftriaxone sodium is often used (in combination, but not direct, with macrolide and/ or aminoglycoside antibiotics) for the treatment of community-acquired or mild to moderate health care-associated pneumonia. It is also a choice drug for treatment of bacterial meningitis.In pediatrics; it is commonly used in febrile infants between 4 and 8 weeks of age who are admitted to the hospital to exclude sepsis. It has also been used in the treatment of Lyme disease, typhoid fever, and gonorrhea. Ceftriaxone sodium is freely soluble in water, sparingly soluble in methanol, very slightly soluble in anhydrous ethanol.

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. 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. Sulbactam sodium is Semi-synthetic product derived from a fermentation product. 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 have been reported for estimation of Ceftriaxone sodium and Sulbactam sodium as an individual drug substance and in the combination drug by High performance liquid chromatography and UV-VIS spectrophotometric method.

A simple, accurate and precise HPTLC method for simultaneous estimation Ceftriaxone sodium and Sulbactam sodium in dry powder injection manufactured in the combined dosages form has been developed.
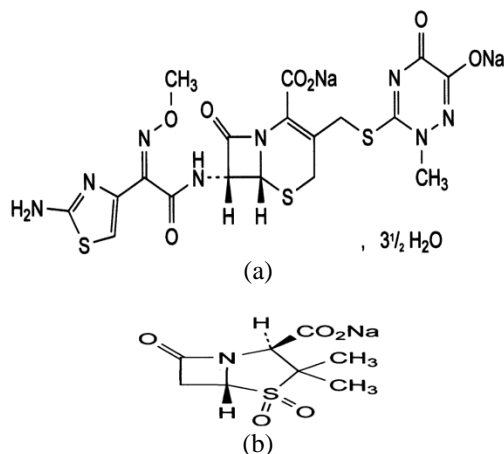
(a)

(b)

Figure 1. Structure of Ceftriaxone sodium (a) and Structure of Sulbactam sodium (b)

II. Materials and Methods

2.1 Chemicals and Reagents

Working Standard of Ceftriaxone sodium and Sulbactam sodium were provided by Aurobindo Pharma Ltd through Mr. Vikrant Tamse-Senior Manager Purchase as Gererous gift samples for study. Dry powder injection samples were procured from the market. All other reagents used were of analytical grade.

2.2 Instrumentation

In this paper, information pertaining to the analytical method validation and evaluation of combination drug has been provided. HPTLC is superior analytical technique in respect of the time and cost of analysis.

HPTLC technique comprises offline stages. These stages are independent of each other. This includes application of samples on the precoated plate, scanning of the developed plates and densitogram etc. The most important features of this technique is to analyse the samples containing multi components in single analysis, application of large number of samples and series of samples by spray techniques. A variety of mobile phases can be developed by using different solvents at different compositions. The mobile phase is being evaporated before the detection step. Application of samples and standard solution is simultaneously being done on the same plate.

CAMAG automatic TLC sampler 4 (ATS4) connected with the win CATS 4 software , CAMAG TLC SCANNER, Integrator controlled by win CATS4 Software, CAMAG twin trough glass chamber with stainless steel lid. Precoated silica Gel 60 F_{254} on aluminium sheets.

In a 20 x 10 cm twin trough glass chamber (Make: CAMAG), a linear ascending chromatographic development was carried out by using mobile phase, Chloroform: Ethyl alcohol: diethyl amine: water in the ratio (12:7:1:0.4 v/v). The chamber was saturated for 20 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. 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 mms^{-1} and data resolution at 100μm/step. Sample was spotted on the silica gel 60 F_{254} TLC plate by using CAMAG automatic TLC sampler-4 (ATS). The plates were developed in the CAMAG TLC chamber upto 80 mm. The contents of ceftriaxone sodium and Sulbactam sodium were evaluated by comparing the peak areas with linear regression.

III. Standard Solution Preparation

10 mg of Ceftriaxone 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. Then diluted to 10 mL with methanol (Stock solution1 and stock solution 2 for ceftriaxone sodium and Sulbactam sodium respectively). 1 ml from stock solution 1 and 5 mL from stock solution 2 were pipetted out in two separate volumetric flasks and diluted to 10 mL with methanol to obtain the concentration of 0.1 mg /mL and 0.5 mg / mL of standard ceftriaxone sodium and Sulbactam sodium respectively.

IV. Sample Solution Preparation

Label claim of Ceftriaxone sodium and Sulbactam sodium in the combined dry powder injection in one unit is 1000 mg and 500 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 ceftriaxone and 500 mg of sulbactam was weighed in 10 ml volumetric flask. 2

mL of Methanol was added and sonicated for 5 minutes to dissolve. Then diluted to 10 mL with methanol to obtain the concentration of 1mg /mL and 0.5 mg/ mL of Ceftriaxone and Sulbactam respectively.

V.   Results and Discussions
5.1 Validation of analytical method
An analytical method developed was validated for the validation parameters Specificity, linearity, accuracy, precision, LOD, LOQ and Robustness.

5.1.1 Specificity
A specificity was determined by analyzing reference standards, samples, diluent and mobile phase being used to verify the interference of mobile phase and diluents during analysis. There was no any interference of Mobile phase and diluent at the *RF* values of Ceftriaxone sodium and Sulbactam sodium. The separated bands of Ceftriaxone sodium and Sulbactam sodium were confirmed by comparing *RF* values. The RF values of Ceftriaxone sodium and Sulbactam sodium were 0.29 and 0.54 respectively. UV spectra recorded at Peak start (S) Peak apex(M) and peak end(E) of both the drugs were also overlapped on each other and were matching.

5.1.2 Accuracy
In method validation, accuracy was being determined by the percentage recovery.
The known concentrations of the samples were spiked with the standard ceftriaxone and sulbactam in the concentrations of 240 ng, 300 ng and 360 ng of ceftriaxone sodium and 1200 ng, 1500 ng and 1800 ng of sulbactam sodium 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 100.78 % to 101.92 % for Ceftriaxone sodium and 100.74 % to 101.82 % for Sulbactam sodium respectively. The detail results are tabulated as under:

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

Sr. No.	Amount of std. Ceftriaxone sodium added in ng	Amount of std. ceftriaxone sodium recovered in ng	% Recovery	% Relative standard Deviation
1	240	244.60	101.92	1.92
2	300	302.43	100.81	1.84
3	360	362.80	100.78	0.60

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	1200	1211.04	100.74	1.69
2	1500	1513.8	100.92	1.79
3	1800	1832.76	101.82	0.66

5.1.3 Precision
The interday and intraday precision of the method were estimated by performing six determinations of Ceftriaxone 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 Ceftriaxone sodium
(n= 6)

Conc. of the Ceftriaxone sodium (ng/ band)	Inter-day precision		Intra-day precision	
	Mean area (AU)	% RSD	Mean area (AU)	% RSD
300	2695	0.88	2713	1.34

Table 4: Precision for the 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
1500	3395	1.54	3400	1.22

5.1.4 Robustness of the method

Following the introduction of small deliberate changes in the mobile phase composition were done and effect on the results were 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.

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^{-1} of Ceftriaxone sodium	SD of Peak response of Ceftriaxone sodium	%RSD	Conc. Level in ng spot^{-1} of Sulbactam sodium	Mean Peak response Sulbactam sodium	%RSD
Mobile phase composition ($\pm$ 0.1 mL)	240	160	1.89	1200	84.4	1.69
	300	170	1.92	1500	110	1.94
	360	57.8	0.60	1800	42.6	0.66
$\pm$ 5 % variation in mobile phase	240	150	1.71	1200	75	1.59
	300	174	1.96	1500	105	1.84
	360	65	0.68	1800	50	0.88

5.1.5 Linearity

A series of standard solutions were prepared from the standard stock solutions of Ceftriaxone sodium and Sulbactam sodium. Solutions were spotted on the TLC plate in the range of 2 µl to 14µl of ceftriaxone sodium and sulbactam sodium respectively. The corresponding concentrations were in the range of 0.2 µg / spot to 1.4µg /spot and 1.0 µg/spot to 7.0µg /spot respectively. The linear Correlation coefficient for ceftriaxone sodium is 0.9998 and correlation coefficient for sulbactam sodium is 0.9953

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 Ceftriaxone sodium and Sulbactam sodium are LOD= 0.28 mcg, LOQ= 0. 859 mcg, LOD= 5.226 mcg and LOQ = 15.838 mcg respectively.

5.1.7 Analysis of formulation

Experimental HPTLC results of the amount of Ceftriaxone 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 in the unit was found to be 100.5 % and 101 % for Ceftriaxone sodium and Sulbactam respectively.
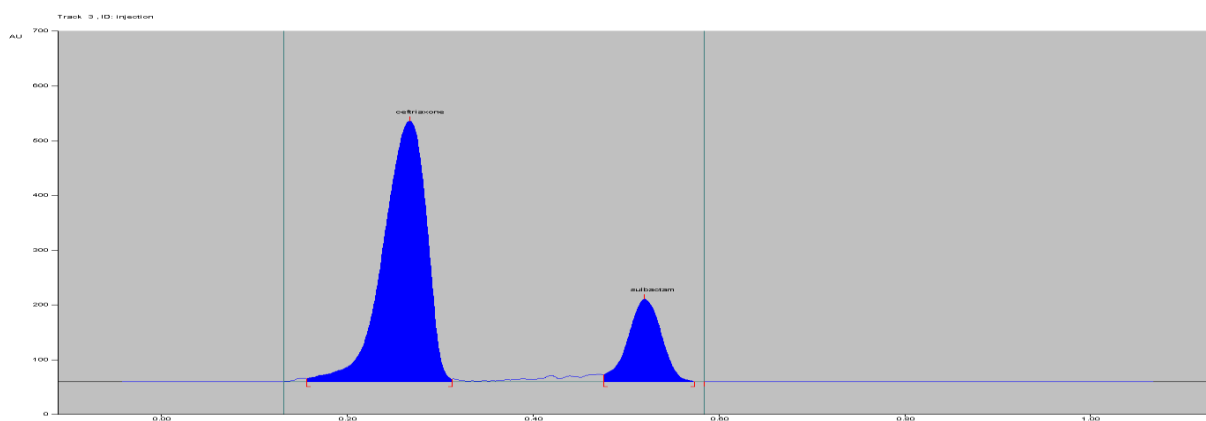

Figure 1. Densitogram of Ceftriaxone sodium (R$_F$ 0.31) and Sulbactam sodium (R$_F$ 0.58)

5.1.8 Conclusion

HPTLC analysis is rapidly becoming popular in routine analysis due to its advantage of low operating costs, high sample throughput. This method can be used for simultaneous determination of Ceftriaxone sodium and Sulbactam sodium in routine analysis.

It may be extended to the degradation study of the Ceftriaxone sodium and Sulbactam sodium and also for its estimation in plasma and other biological fluids.

The proposed HPTLC method is simple, accurate, economically chief and reproducible.

Acknowledgements

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