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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CEFPIROME SULPHATE AND SULBACTAM SODIUM IN BULK AND COMBINED PHARMACEUTICAL DOSAGE FORM

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Abstract: A simple, accurate, precise and sensitive Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed for simultaneous estimation of Cefpirome sulphate (CPS) and Sulbactam sodium (SULS) in bulk and combined pharmaceutical dosage form using BDS Hypersil C_{18} (25cm x 0.46 cm) column and phosphate buffer (pH-5): acetonitrile (40:60 v/v) as mobile phase at flow rate of 1.0 ml/min with detection wavelength 215 nm. Retention time in RP-HPLC were found to be 3.91 min. and 5.78 min. for CPS and SULS. The linearity of CPS and SULS were found in range of 10-30 µg/ml and 5-15 µg/ml, respectively. The method was validated in term of accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per International Conference of harmonization (ICH) guidelines.

Keywords: Cefpirome sulphate (CPS), Sulbactam sodium (SULS), Reverse Phase-High Performance Liquid Chromatography (RP-HPLC)

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INTRODUCTION

Cefpirome sulphate is chemically (6R, 7R)-7-[[2-(2-amino-1, 3-thiazol-4-yl)-2-methoxyiminoacetyl] amino]-3-(6, 7-dihydro-5H-cyclopenta[b]pyridin-1-ium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid; hydrogen sulfate (Figure 1). It is a broad-spectrum semisynthetic β-lactamase resistant fourth generation cephalosporin bearing a quaternary ammonium group at the 3 position of the cephem nucleus. It is used for the treatment of upper and lower urinary tract as well as lower respiratory tract, skin and soft tissue infections. Cefpirome is excreted largely unchanged in the urine with a half-life of 2 hours [1,4].It has an expanded spectrum of activity against Pseudomonas sp., enterococci, and staphylococci, as well as other gram-positive and gram-negative bacteria [2].

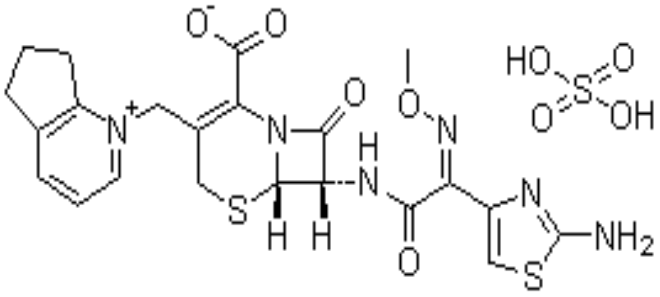

Figure 1. Cefpirome Sulphate

Sulbactam Sodium (SULS) is chemically Sodium (2S, 5R)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylate 4, 4-dioxide (Fig.2) is beta-lactamase inhibitor, enhance the activity of penicillin's and cephalosporin's against many resistant strains of bacteria. It is official in British Pharmacopoeia (BP) and United States Pharmacopoeia (USP), BP describe High Performance Liquid Chromatography (HPLC) [3] and USP also describe HPLC method. Literature survey also reveals Spectrophotometric Methods and RP-HPLC [5, 6] for determination of SULS with other drugs. The combined dosage form of CPS and SULS is also available in the market for systemic system infection. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for simultaneous estimation of CPS and SULS in their combined dosage form. Literature survey does not reveal any simple Spectrophotometric or other method for simultaneous estimation of CPS and SULS in combined dosage form. Hence aim of work is to development and validation of simple, sensitive, specific, accurate, precise and economical Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method for routine analysis of CPS and SULS in their combined dosage form.

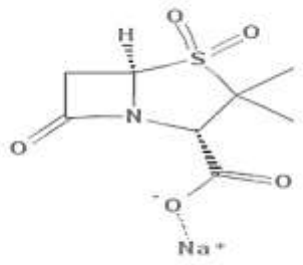

Figure 2. Sulbactam Sodium

In present study Simple, Rapid, Specific and economic RP-HPLC method development and validation for simultaneous estimation of Cefpirome Sulphate and Sulbactam Sodium in their combined pharmaceutical dosage form is reported.

METHOD AND MATERIALS

RP-HPLC Instrumentation and Conditions:

The chromatographic separation achieved by using BDS Hypersil C_{18} (25cm x 0.46 cm) column and phosphate buffer (pH-5): acetonitrile (40:60 v/v) as mobile phase at flow rate of 1.0 ml/min with detection wavelength 215 nm.

Chemicals and Reagents

CPS and SULS were kindly given as a gratis sample by Gitar laboratory, Ahmedabad and Oasis laboratory, Ahmedabad, respectively. The market formulation PIROTUM (CPS 1000 mg and SULS 500 mg) was procured from local market which is manufactured by Venus remedies Ltd, India. Acetonitrile (HPLC Grade) were obtained from Finar Ltd. and Water (HPLC Grade) and O-phosphoric acid were obtained from Merck specialties pvt, Ltd., Mumbai.

Preparation of Stock and Standard Solution

For Cefpirome Sulphate:

Cefpirome Sulphate stock solution (1000 µg/ml): Accurately weighed 10mg of Cefpirome Sulphate was taken in 10ml volumetric flask and diluted with methanol up to the mark.

Cefpirome Sulphate standard solution (200µg/ml): prepared by transferring 2 ml from stock solution, and diluted up to the mark with methanol in 10ml volumetric flask.

For Sulbactam Sodium:

Sulbactam Sodium standard stock solution (1000 µg/ml): Accurately weighed 10 mg of Sulbactam Sodium was taken in 10ml volumetric flask and diluted with methanol up to the mark.

Sulbactam Sodium working standard stock solution (100µg/ml): prepared by transferring 1 ml from stock solution, and diluted up to the mark with methanol in 10ml volumetric flask.

Preparation of standard solution of binary mixtures of Cefpirome Sulphate (20 µg/mL) and Sulbactam sodium (10 µg/mL):

Take 1 mL from the Cefpirome Sulphate stock solution and 1mL from Sulbactam stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Preparation Sample solution:

Sample Stock Solution (Cefpirome sulphate 200 µg/mL, and Sulbactam sodium 100 µg/mL):

Take powdered dosage form 30mg (equivalent to 20 mg of Cefpirome sulphate, and 10 mg of Sulbactam sodium) was transferred to a 100 ml volumetric flask containing 60 ml methanol and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Standard Sample Preparation (Cefpirome sulphate 20 µg/mL, and Sulbactam sodium 10 µg/mL):

Take 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase.

HPLC Method Development and Optimization

Selection of detecting wavelength:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detecting wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. In the present study, drug solution of 20 µg/ml Cefpirome sulphate and 10

µg/ml of Sulbactam Sodium were prepared in methanol. The standard solutions were than scanned in UV region of 200-400 nm and the overlain spectrum were taken.

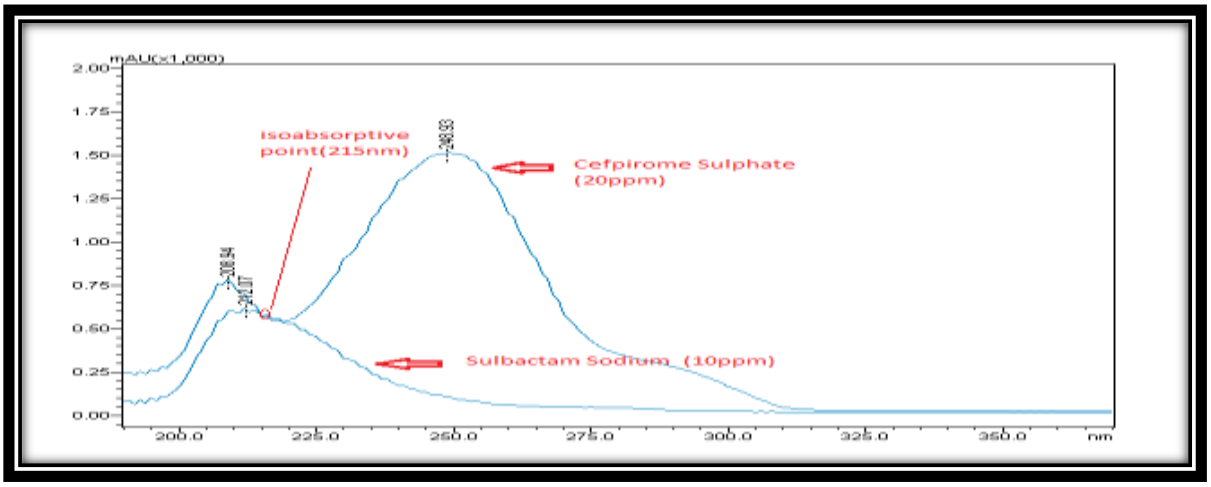

Figure: 1 UV-Spectra of Cefpirome sulphate (20 ppm) and sulbactam sodium (10 ppm) and in Methanol.

Optimization of RP-HPLC method:

The pure drug solution of Cefpirome sulphate (20µg/ml) and Sulbactam sodium (10µg/ml) were injected individually in to HPLC system and allow to run in different mobile phase like Methanol, Water: Methanol, Water: Acetonitrile, Phosphate Buffer: Methanol, Phosphate Buffer: Acetonitrile were tried in order to find the optimum condition for the separation of Cefpirome sulphate and Sulbactam sodium. It was found that mobile phase containing Phosphate Buffer (pH=5): Acetonitrile(40:60 v/v) at a flow rate of 1 ml/min with detecting wavelength 215nm gave satisfactory result with sharp, well defined and resolving peak with minimum tailing as compared to other mobile phases. Under these condition the retention time were typically 3.87 min. for Cefpirome sulphate and 5.683 min. for Sulbactam sodium and optimized chromatographic condition.

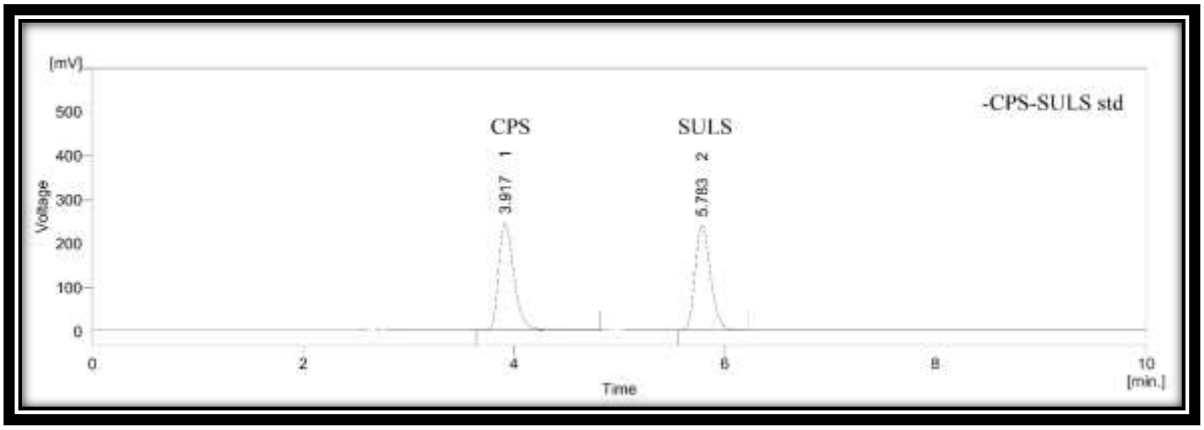

Fig. 2 Chromatogram of standard solution containing 20µg/ml of Cefpirome sulphate and 10µg/ml of Sulbactam sodium using phosphate buffer (pH-5.0): Acetonitrile (40-60 v/v) as mobile phase.

Table: 1 Optimized chromatographic conditions for simultaneous estimation of CPS and SULS.

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Buffer(pH 5.0) : Acetonitrile (40:60)
column	C18 (25cm x 0.46 cm) Hypersil BDS
Flow rate	1ml/min
Runtime	8 min
Injection volume	20 µL
Detection wavelength	215 nm

Validation of RP-HPLC Method [7]:

Linearity

Linearity response for CPS and SULS were assessed in the concentration rang 10-30µm/ml and 5-15µm/ml of standard solution, respectively.

Sensitivity

The sensitivity measurement of CPS and SULS bye the use of proposed method was estimated in term of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated using following equations.

LOD= 3.3*σ/S

LOQ=10* σ/S

Where,

σ= the standard deviation of the response

s= slop of the calibration curve

Precision:

Method precision (Repeatability): Repeatability of sample application was accessed by injecting 20µm/ml of CPS and 10µm/ml of SULS of drug solution six time.

Intermediate precision (System precision): Intermediate precision of method was determined in the term of intra-day and inter-day variation (%RSD).

A) Intra-day precision: Intra-day precision (%RSD) was assessed by analyzing standard drug solution within the calibration range, three time on the same day.

B) Inter-day precision: Inter-day precision (%RSD) was assessed by analyzing drug solution within the range on three different days.

Accuracy

To the pre-analyzed sample, a known amount of standard solution of pure drug (CPS and SULS) were spiked at three different level. This study was carried out at 80%, 100% and 120% level.

5.4.6 Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The important parameter to be studied was the resolution factor between two peaks. The robustness was checked by changing following parameter one by one.

A) Change in the ratio of mobile phase by ±2 ml [Buffer (phosphate buffer, pH 5.0): Acetonitrile (42:58)] and [Buffer (phosphate buffer, pH 5.0): Acetonitrile (38:62)].

B) Change in flow rate by ±0.2 ml/minute (0.8ml/min and 1.2ml/min), after each change, sample solution was injected and% assay with system suitability parameter were checked.

C) Change in pH of mobile phase was ±0.2 pH [Buffer (phosphate buffer, pH 5.2): Acetonitrile (40:60)] and [Buffer (phosphate buffer, pH 4.8): Acetonitrile (40:60)].

System Suitability

To check system suitability Number of theoretical plate, Resolution, Retention time And Tailing factor were determined.

RESULTS AND DISCUSSION

The result of method development and validation study on simultaneous estimation of Cefpirome sulphate and sulbactam sodium in current study involving phosphate buffer (pH-5.0): Acetonitrile (40:60 v/v) as mobile phase for RP-HPLC are given below.

Method development:

CPS and SULS were completely separated on C_{18} column by RP-HPLC using isocratic elution of phosphate buffer and acetonitrile as mobile phase. When the increase the percentage of acetonitrile as compare to phosphate buffer at pH-5.0, a sharp pointed and well separated peak was observed. Eventually proper resolution was achieved at a flow rate of 1 ml/min and using phosphate buffer (pH-5.0): Acetonitrile (40:60 v/v) as the mobile phase for RP-HPLC.

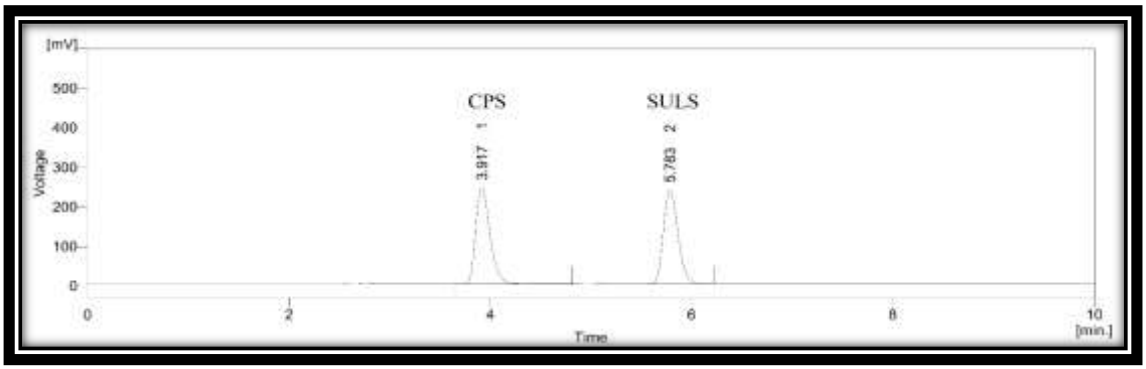

Fig.3 Optimized chromatographic condition

Method validation:

Linearity:

The linearity of analytical procedure is it ability (within given range) to obtain test result which are directly proportional to concentration of analyte in sample. The drug response was linear

(R^2 = 0.9995 for Cefpirome sulphate and 0.9996 for Sulbactam sodium) over the concentration range between 10-30 µg/ml for Cefpirome sulphate and 5-15 µm/ml for Sulbactam sodium. The linear equation for the calibration plots were y=121.32x-25.822 for Cefpirome sulphate and y=239.34x-28.363 for Sulbactam sodium.

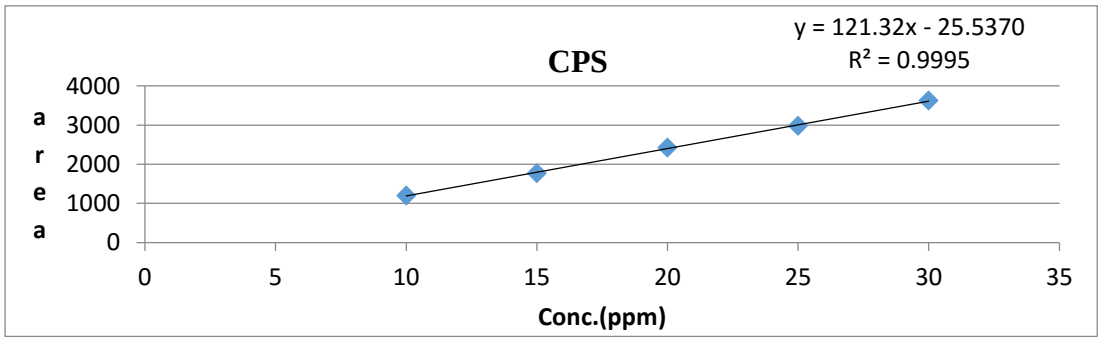

Fig.4 calibration curve of Cefpirome sulphate

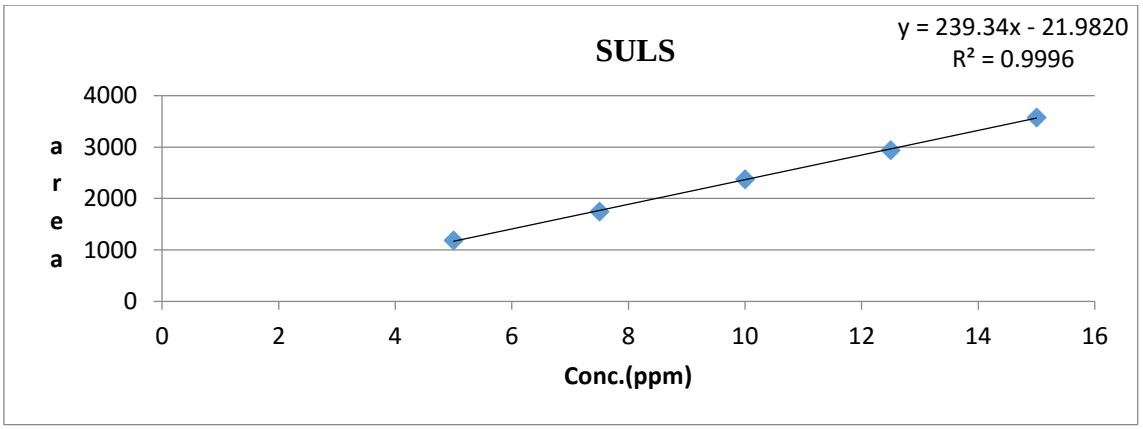

Fig.5 calibration curve of Sulbactam sodium

Table: 2 Calibration curve data for Cefpirome sulphate and Sulbactam sodium.

CPS		SULS	
Concentration(µm/ml)	**Mean Peak Area(n=3)**	**Concentration (µg/ml)**	**Mean Peak Area (n=3)**
10	1199.97	5	1183.831
15	1771.384	7.5	1747.64
20	2421.719	10	2373.567
25	2981.746	12.5	2941.34
30	3627.695	15	3578.706

Correlation coefficient	0.999734244	**Correlation coefficient**	0.999797609
Intercept	25.5370	**Intercept**	21.9820
Slope	121.3	**Slope**	239.3
Regression equation	Y=121.3x-25.5370	**Regression equation**	Y=239.3x-21.9820
LOD (µg/ml)	0.69474	**LOD (µg/ml)**	0.30313
LOQ (µg/ml)	2.10528	**LOQ (µg/ml)**	0.91859

Sensitivity:

The detection limit of an individual analytical procedure is the lowest amount of analyte in sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analyte procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated by respective equations. The LOD value were found to be 0.694 µg/ml and 0.3031µg/ml for Cefpirome sulphate and Sulbactam sodium respectively. The LOQ value were found to be 2.1052 µg/ml and 0.9185 µg/ml for Cefpirome sulphate and Sulbactam sodium respectively.

Precision:

The precision of analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurement obtained from multiple sampling of same homogenous sample under the reproducibility. Precision may be considered at three level: repeatability, intermediate precision and reproducibility [7]. The results of the repeatability, intra-day precision and inter-day precision experiments are shown respectively as given in (Table: 3) and (Table: 4).the developed method was found to be precise as the RSD values for repeatability of intra-day and inter-day precision study were < 2%.

Table: 3 Repeatability study of Cefpirome sulphate and Sulbactam sodium.

Concentration	CPS (20µg/ml)	SULS (10µg/ml)
Area	2412.022	2379.345
	2329.939	2384.207
	2421.761	2328.047
	2426.55	2393.783
	2414.442	2381.731
	2419.234	2386.596

Mean	2403.991333	2375.618
±SD	36.64546219	23.82651
%RSD	1.524359164	1.002961

Table: 4 Intra-day and inter-day precision of CPS and SULS.

Drug	Intra-day precision			Inter-day precision		
	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
CPS	10	1185.55±14.617	1.2329	10	1183.554±14.880	1.2572
	20	2389.965±40.372	1.6892	20	2389.953±38.162	1.5967
	30	3583.186±55.302	1.5433	30	3588.262±43.177	1.2033
SULS	5	1175.902±5.196	0.4418	10	1183.554±14.880	1.2572
	10	2369.065±16.923	0.7143	20	2389.953±38.162	1.5967
	15	3552.438±19.793	0.5571	30	3588.262±43.177	1.2033

Accuracy:

The accuracy of an analytical procedure express the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. As show in (table: 5) and (table: 6), good recovery of the Cefpirome sulphate and sulbactam sodium in the range from 99.4 to 99.8 were obtained at various added concentrations.

For Cefpirome sulphate

10 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 215 nm. The amount of Cefpirome sulphate was calculated at each level and % recoveries were computed.

Table: 5 Recovery data for Cefpirome sulphate.

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	10	8	7.8958	98.697	99.412 ± 0.722
2		10	8	7.9521	99.401	
3		10	8	8.0114	100.142	
4	100 %	10	10	9.8901	98.901	99.436± 0.475
5		10	10	9.9812	99.812	
6		10	10	9.9593	99.593	
7	120 %	10	12	11.9334	99.445	99.406± 0.258
8		10	12	11.8957	99.130	
9		10	12	11.9572	99.643	

For Sulbactam sodium

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 215 nm. The amount of Sulbactam sodium was calculated at each level and % recoveries were computed.

Table: 6 Recovery data for Sulbactam sodium.

Sr. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	5	4	3.9465	98.663	99.885 ±1.126
2		5	4	4.0353	100.882	
3		5	4	4.0044	100.111	
4	100 %	5	5	4.9483	98.966	99.598± 0.649
5		5	5	5.0131	100.263	
6		5	5	4.9783	99.566	
7	120 %	5	6	6.0049	100.082	99.601± 0.490
8		5	6	5.9461	99.102	
9		5	6	5.9771	99.619	

Robustness:

The standard deviation of the area was calculated for each parameter and the %RSD was found to be less than 2%. Result show low value of %RSD as show in (table: 7) and signify the robustness of the method**.**

Table: 7 Robustness data of Cefpirome sulphate and Sulbactam sodium.

Parameter	Normal condition	Change in condition	Drug	Conc.(µg/ml)	Mean area (n=3)±SD	%RSD
Mobile phase ratio (Phosphate Buffer: Acetonitrile)	40:60 V/V	42:59	**CPS**	20	2343.43±46.08	1.9666
			SULS	10	2322.86±20.25	0.8718
		38:62	**CPS**	20	2467.28±34.99	1.4184
			SULS	10	2443.51±16.21	0.6636
Change in flow rate	1 ml/min	1.2 ml/min	**CPS**	20	2345.75±43.60	1.8588
			SULS	10	2328.12±13.35	0.5737
		0.8 ml/min	**CPS**	20	2494.74±37.64	1.5090
			SULS	10	2470.81±14.94	0.6050
Change in pH	5.0	5.2	**CPS**	20	2301.42±40.78	1.7721
			SULS	10	2276.59±21.72	0.9541
		4.8	**CPS**	20	2473.95±31.68	1.2807
			SULS	10	2447.59±14.04	0.573

System suitability:

Various system suitability parameter were calculated. The parameter were found within acceptance criteria (table: 8).

Table: 8 System suitability parameter.

Parameter	Cefpirome sulphate	Sulbactam sodium	Acceptance criteria
Theoretical plate*	3539	7291	>2000
Retention time(min.)*	3.90	5.69	-
Tailing factor*	1.57	1.386	<1.5
resolution	6.986		>2.0

***Mean (n=3)**

Quantitative determination of pharmaceutical formulation:

When dosage form was analyzed, Cefpirome sulphate and Sulbactam sodium gave sharp and well defined peak at retention time 3.93 min. And 5.80 min. respectively, when scanned at 215nm.

Assay result of marketed formulation is shown in (table: 9).

Table: 9 Quantitative determination of pharmaceutical formulation.

Parameter	PIROTUM powder for inj.	
	Cefpirome sulphate	Sulbactam sodium
Actual concentration(µg/ml)	20	10
Concentration obtained(µg/ml)	20.23	9.87
% Assay	101.1805262	98.73553465
%RSD	1.314998836	0.523546982
Limit	90-110%	90-110%

The assay results were comparable to labelled value (500mg Cefpirome sulphate and 250 mg Sulbactam sodium) of each drug in combined dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION

Development and validation of RP-HPLC method was found to be simple, accurate, precise and economical. These method can be applied for routine quantitative analysis of Cefpirome sulphate and Sulbactam sodium in combined and Bulk pharmaceutical dosage forms.

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