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Research Article

VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SULBACTAM AND CEFOPERAZONE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

A simple, rapid, accurate and precise isocratic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for simultaneous estimation of sulbactam and cefoperazone in combined dosage form by using phenomenex C-18 column (150mm X 4.6mm, i.d., 5µm,). Mobile phase containing a mixture of buffer (3.3ml of 40% Tetra butyl ammonium hydroxide in methanol was diluted to 1000 ml with distilled water and pH adjusted to 6.6±0.05 with 1% ortho phosphoric acid) and acetonitrile in the ratio of 70:30v/v was used to separation of drugs and flow rate was maintained at 0.8 ml/min. Detection was carried out at 230 nm and the retention times of sulbactam and cefoperazone were found to be 5.33 and 9.32 min respectively. The developed method was validated as per ICH guideline for specificity, linearity, accuracy, and precision and system suitability. The new RP-HPLC method was successfully applied to marketed formulation without any interference from excipients.

Keywords: RP-HPLC, Sulbactam, Cefoperazone and Validation.

Introduction

Sulbactam (SUL) is a β-lactamase inhibitor. This drug is given in combination with β-lactam antibiotics to inhibit β-lactamase, an enzyme produced by bacteria that destroys the antibiotics. IUPAC name of sulbactam is (2*S*, 5R)-3, 3-Dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid 4,4-dioxide.[1] (Fig.1) Its chemical formula is $C_8H_{11}NO_5S$ with a molecular mass of 233 g/mol. In combination with β lactum antibiotics it acts as an anti bacterial.

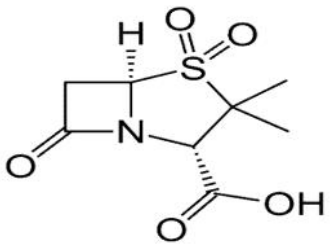

Fig 1: Structure of sulbactam

Cefoperazone (CPZR) 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. IUPAC name of Cefoperazone is (6R,7R)-7-[(2R)-2-{[(4-ethyl-2,3-dioxopiperazin-1-yl)carbonyl]amino}-2-(4-hydroxyphenyl) acetamido]-3-{[(1-methyl- 1H -1, 2, 3, 4- tetrazol-5-yl) sulfanyl]methyl}-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid.[2] (Fig.2) Its chemical formula is $C_{25}H_{27}N_9O_8S_2$ with a molecular mass of 645.67g/mol. The structural formula is given below:

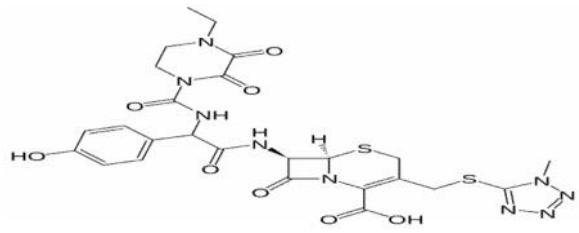

Fig 2: Structure of cefoperazone

In literature survey some analytical methods were found for the quantitative determination of sulbactam alone or in combination with other drugs by RP- HPLC method[3,4] UV-spectro photometric method[5,] and few methods were also reported for the quantitative determination of cefoperazone alone or in combination with other drugs by RP- HPLC method[6,7], UV-spectrophotometric method[8,] voltammetry method[9], HPTLC method[10]. Few analytical methods are also reported in the literature for the combination of these two drugs.[11,12] The objective of study is to develop a new, simple, rapid, accurate and precise isocratic reverse phase high performance liquid chromatography (RP-HPLC) for simultaneous estimation of sulbactam and cefoperazone in bulk and pharmaceutical dosage form.

Materials and Methods

Instrumentation

Chromatographic separation was performed on SHIMADZU liquid chromatographic system LC-2010CHT equipped with quaternary pump, UV/Vis detector and auto Injector. LC solution software was employed for data collecting and processing. Chromatographic Separation was achieved on phenomenex C-18 (150mm × 4.6 mm, i.d., 5µ.) column. Over laid spectrum was recorded by using UV-3000^{+} LABINDIA double beam UV-Visible spectrophotometer (model no.UV-2371) with 1cm matched quartz cells. Weighing was done on Shimadzu electronic balance (AY-120). Global digital pH meter was used to adjust pH of the mobile phase. The mobile phase was degassed and sonicated by using PCI Mumbai 3.5 liter capacity Sonicator.

Chemicals and Reagents:

Sulbactam and cefoperazone gift samples obtained from Jodas Expoim Pvt. Ltd. India, Hyderabad. Acetonitrile was of HPLC grade purchased from E.Merck Specialties (India) Ltd., Mumbai. Ortho phosphoric acid, 40% TBAH in methanol were of AR grade purchased from E.Merck Specialties (India) Ltd., Mumbai. The formulation containing cefoperazone and sulbactam dry powder injection 1gm and 1gm antibiotic was procured from local market.

Preparation of Mobile phase

Preparation of buffer

3.3 ml of 40% tetra butyl ammonium hydroxide in methanol is taken in 1000 ml of volumetric flask and diluted to the mark with Milli-Q water and pH is adjusted to 6.6±0.05 with 1% ortho phosphoric acid. Filtered and degassed the mixture through 0.45 µm membrane filter paper. Mobile phase is prepared by mixing 70 volumes of buffer with 30 volumes of acetonitrile, finally filtered and degassed to remove the dissolved gases.

HPLC Conditions

The mobile phase consisting of buffer: acetonitrile in the ratio of 70:30 v/v was pumped into the column at a flow rate of 0.8ml/min. The detection is monitored at 230 nm with a run time of 20 min. The volume of injection was 20 µl and prior to injection of the drug solution the column is equilibrated for at least 30 min with the mobile phase flowing through the system.

Preparation of Standard Stock Solution

Weighed and transferred 10 mg of sulbactam and 10 mg of cefoperazone into a 20 ml volumetric flask, and added about 10 ml of diluent and sonicated to dissolve. Volume is made up to the mark with diluents.

Preparation of Sample Solution

Take two vials and transfer the powder and mix. From that the powder equivalent to 23.78 mg is taken into 20 ml of volumetric flask. Added 10 ml of diluent, sonicated for 5 min with occasionally shaking and volume is made up to the mark with diluent.

Selection of detection wavelength

Analytical wavelength is selected by scanning sample solutions containing 50µg/ml of sulbactam and 10µg/ml of cefoperazone separately in the range of 200-400 nm. The over laid spectrum of both the drug is given in Fig.3.

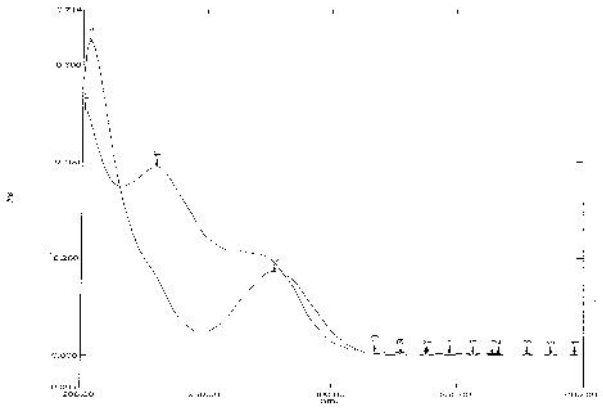

Fig 3: Over laid spectrum of sulbactam and cefoperazone

Optimized chromatographic conditions

Mobile phase : Buffer (pH-6.6±0.05) and ACN in the ratio 70:30 v/v
Stationary phase : Phenomenex C18-Column (150mm X 4.6mm, i.d., 5µm,).
Flow rate : 0.8ml/min
Temperature : 40^0c
Detection wavelength : At 230nm
Diluent : Buffer: ACN (70:30v/v)
Injection volume : 20 µl
Run time : 20 min

Procedure

Suitable aliquots of sulbactam and cefoperazone were taken accurately in 10 ml volumetric flask and diluted it with mobile phase up to the mark to get final concentration in the range 250 µg/ml to 750 µg/ml for the both the drugs. Twenty µl of each standard was injected into the stabilized chromatographic system (n=3). Chromatograms were recorded and peak areas were measured. Calibration curves were constructed by plotting peak area of chromatogram Vs concentration. Regression equations were generated for both drugs and correlation coefficient was found to be 0.998 for both the drugs. A typical chromatogram is presented Fig.4.

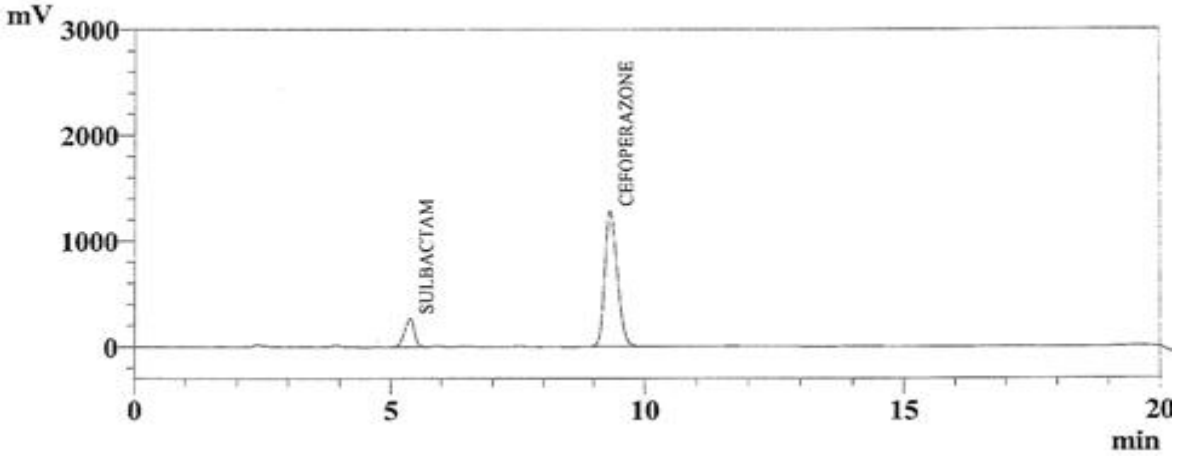

Fig 4: A typical chromatogram of sulbactam and cefoperazone

Method validation

The method was validated according to ICH guidelines with respect to linearity, specificity, precision, accuracy, system suitability.

System suitability studies:

For system suitability, six replicates of standard solutions of Sulbactam and Cefoperazone were injected and studied the suitability parameters like Plate number (N), Resolution (R), Relative time (α), Tailing, and %RSD were studied with the help of standard chromatograms. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method. System suitability parameters were presented in table 1.

Table 1: System suitability parameters of the proposed method

S.No	Parameters	Sulbactam	Cefoperazone
1	Retention time*	5.382	9.317
2	Tailing Factor*	0.886	1.279
3	Resolution*	……	9.553
4	Theoretical plates*	3446	6651
5	%RSD of area under peak*	0.8	0.3

*n=6

Linearity

The linearity of the method was determined at five concentration levels. From each solution 20 µl is injected into the optimized chromatographic system. The calibration curves are constructed by plotting response factor against concentration of drugs (Fig.5 and 6). The slope and intercept value of calibration curves were y = 39001x + 474.2 for sulbactam and y = 20736x + 44367 for cefoperazone. Correlation coefficient was 0.998 for the both the drugs. The linearity data for both the drugs is presented in table 2.

Table 2: Linearity data of the proposed method

S.No	Linearity level	Concentration level µg/ml		Area of the peak	
		SUL	CPZR	SUL	CPZR
1.	I	250	250	1958311	10428354
2.	II	375	375	2897220	15617496
3.	III	500	500	3870287	20955696
4.	IV	625	625	4989750	25491700
5.	V	750	750	5787127	31412140
	Correlation coefficient			0.998	0.998

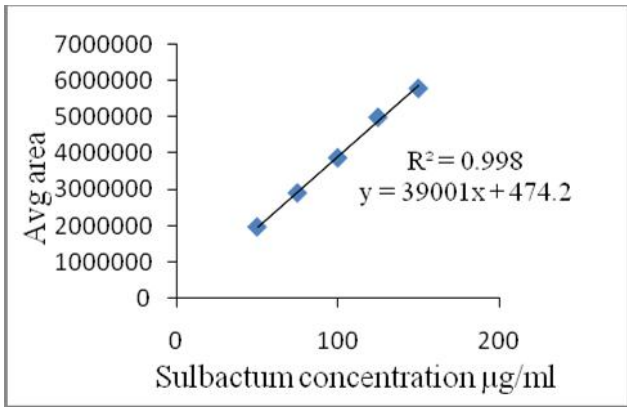

Fig. 5: Linearity curve for sulbactam

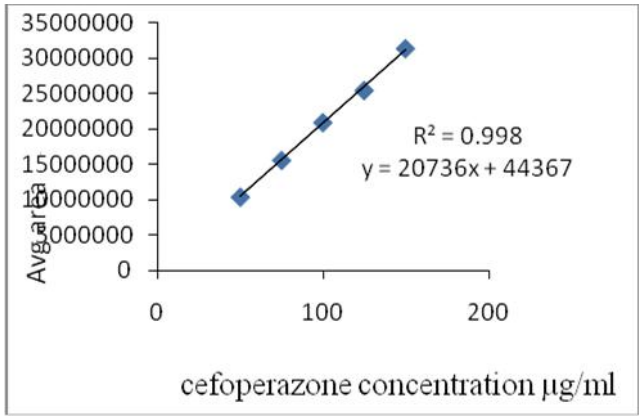

Fig. 6: Linearity curve for cefoperazone

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out by standard addition method at three different levels (50, 100, and 150%). The percentage recoveries were calculated from the data obtained and found to be accurate and the results are given shown in table 3.

Table 3: Results of accuracy of the proposed Method

S.No	Sample	Accuracy	Standard drug µg/ml	Formulation µg/ml	%Recovery
1	Sulbactam	50%	250	500	100.02%
2		100%	500	500	98.94%
3		150%	750	500	100.03%
4	Cefoperazone	50%	250	500	98.98%
5		100%	500	500	100.35%
6		150%	750	500	99.93%

Precision

For precision the sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits and results are tabulated in table 4.

Table 4: Results of precision of the proposed method

S.No.	Injection	Area of salbactam	Area of cefoperazone
1	Injection-1	3588357	21916961
2	Injection-2	3607255	21901270
3	Injection-3	3623665	21756953
4	Injection-4	3629664	21793718
5	Injection-5	3633458	21780433
6	Injection-6	3674354	21833617
	Average	3626126	21830492
	Standard Deviation	28916.72	65973.32
	%RSD	0.80	0.30

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It is observed that there are no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed is robust and results are tabulated in table 5.

Table 5. Results of robustness of the proposed method

S.NO	Condition	Variation	Average peak area		%RSD	
			SUL	CPZR	SUL	CPZR
1	Flow rate	Less flow (0.7ml/min)	4304723	24398390	0.27	0.07
2		Actual flow (0.8ml/min)	3819929	22066321	0.99	0.27
3		More flow (0.9ml/ min)	3414393	19104424	0.17	0.01
4	Temperature	Less temp (38^0C)	3940195	21760337	0.95	0.52
5		Actual temp (40^0C)	3819929	22066321	0.99	0.27
6		More temp(42^0C)	3921278	21410909	0.18	0.61

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. The chromatogram was taken by appropriate dilutions and the amount of each drug present in the sample mixture was determined and it was found that there is no interference with the analyte peak Fig.7 and 8.

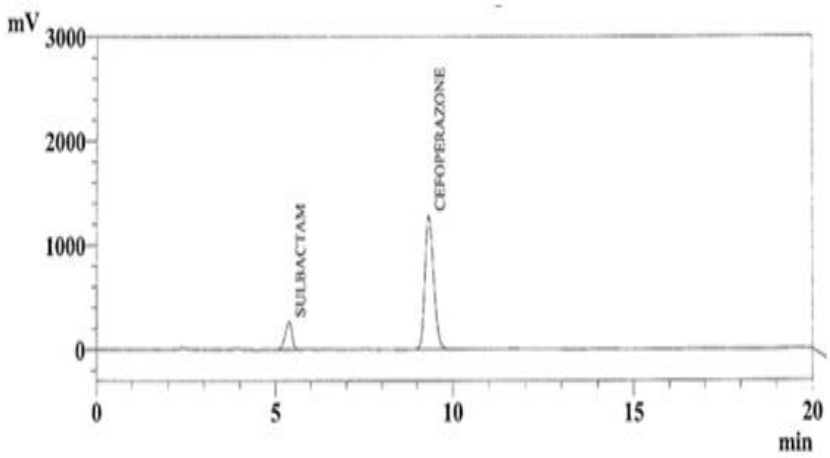

Fig 7: Standard chromatogram for sulbactam and cefoperazone

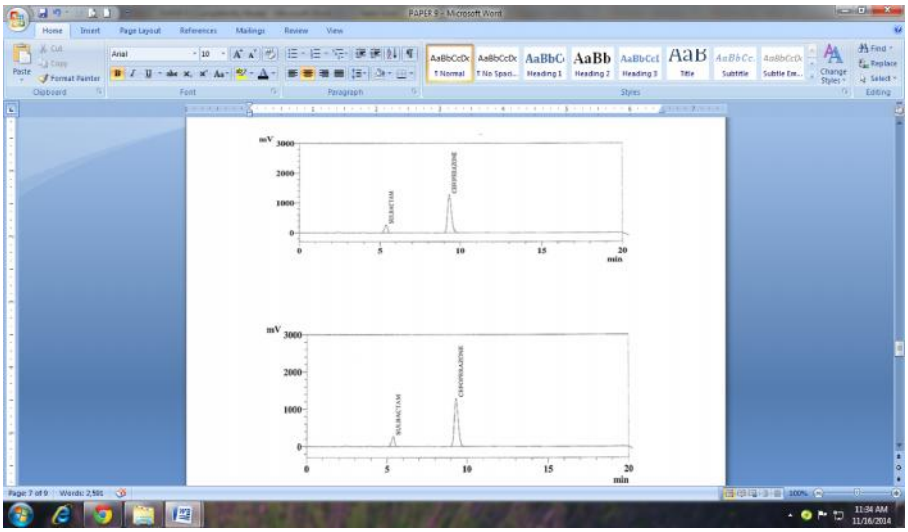

Fig. 8: Sample chromatogram for sulbactam and cefoperazone.

Assay procedure

Two vials are taken and the powder equivalent to 23.78 mg is transferred into 20 ml of dry volumetric flask. 10 ml of diluent, is added and sonicated for 5 min with occasionally shaking. The volume is made up to the mark with diluents ((500µg/ml). In to the optimized chromatographic system 20 µl of sample is injected and chromatograms are recorded and areas are noted and the amount of sulbactam and cefoperazone are determined by substituting in the formula. The %recoveries are determined and presented in the table 6.

Table 6. Assaay of the proposed method

S.No	Label claim	Obtained	% Recovery
1 Sulbactam	1000mg	1002mg	100.26
2 Cefoperazone	1000mg	1003mg	100.30

Results and Discussion

The method was developed by trial and error method. Several columns and mobile phases were tried but finally phenomenex C-18 column (150mm X 4.6mm, i.d., 5µm,) and mobile phase containing a mixture of buffer and acetonitrile in the ratio of 70:30 v/v was found to be most suitable for separation of these two drugs. System suitability parameters like retention time, resolution, tailing and plate count were shown uniformity and %RSD is less than 1.0 and the results are given in table 1 and from the obtained results we can say that the system is suitable for analysis. Linearity plot is obtained by taking concentrations on the X- axis and areas on the Y-axis. Regression equations are found to be y = 39001x + 474.2 for sulbactam and y = 20736x + 44367 for cefopoerazone and calibration curves are shown in fig.5 and fig.6. The method accuracy was evaluated by recovery studies and the percentage recovery of sulbactam and cefoperazone is found to be 100.02% and 98.98 for 50% level; 98.94% and 100.35% for 100% level; 100.03% and 99.93% for 150% level and results is presented in table.no.3.

In method precision %RSD for the area of six replicate injections are found to be within the specified limits and results are tabulated in table 4. Method robustness is carried out by deliberate changes in the optimized conditions and found no marked changes in the Rt and area, which shows that the method is robust and results are given in table 5. Method specificity is concluded by fig.7 and fig.8 which are standard chromatogram and other one is formulation. The placebo and excipients peaks are not observed, hence no interference with standard and analytic peak so it proves method is specific. The assay values obtained are in good agreement with the label claim.

Conclusion

The proposed RP-HPLC method is found to be simple, precise, accurate and sensitive for the simultaneous estimation of sulbactam and cefoperazone in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of sulbactam and cefoperazone in bulk and its pharmaceutical dosage form.

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