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SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF SULBACTAM AND CEFOPERAZONE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
A chromatographic system prominence consisting of quaternary solvent delivery pump, a degasser, an injector, column oven and photodiode array detector, LC 20AT series. C18 (4.6*250) mm, 5 micron column was used. The instrumental settings were a flow of 1ml/min. the injection volume was 20ul. The detection wavelength was 190 nm for all the three analytes
Key words: Sulbactam and Cefoperazone, RP-HPLC, C18 (4.6*250) mm, 5 micron column, Validation.

INTRODUCTION

Cefoperazone (fig-I) is chemically 7-D(-)-(4-ETHYL-2,3-DIOXO-1-PIPERZINE Carboxamido) (4-hydroxyphenyl) acetamido-3-(1-methyl)-1Htetrazol-5-yl)thiomethyl-3-cepham -carboxylic acid. Broad spectrum third generation cephalosporin antibiotic[1, 2] Sulbactam (fig-I) is chemically (2s-cis)-3,3-dimethyl-7-oxo-4-thiol-1-azabicyclo[3.2.0] heptane-2-carboxylic acid4,4-dioxide. In combination with β-lactam antibiotics it acts as an antibacterial[3-6].

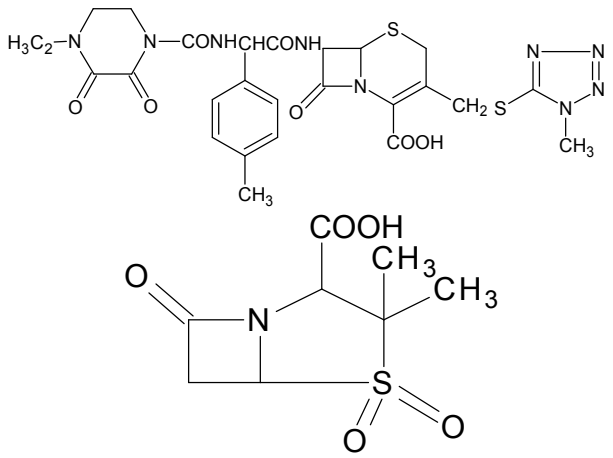

Figure 1. The structure of Cefoperazone and Sulbactam **.**
Cefoperazone

Literature review indicated that HPLC methods have been reported for Cefoperazone and Sulbactam, combination in formulation whereas the reported methods are more time consuming (12 min). Since the selected formulation is a multi-component system, rapid HPLC method was developed for the simultaneous estimation of these drugs in combined dosage forms with internal standard[7-10].

MATERIALS AND METHODS

Cefoperazone,Sulbactam and Ornidazole standards were obtained from Aurobindo Laboratories, Ltd. (Hyderabad, India),methanol, acetonitrile and phosphate buffer(HPLC grade) were obtained from Qualigens Fine Chemicals (Mumbai, India). The (Ceftop) tablets (Ranbaxy laboratories) of the combination of sulbactam and cefoperazone were purchased commercially. Double distilled water was used throughout the experiment. Other chemicals used were have analytical or HPLC grade.

Chromatographic Conditions

A chromatographic system prominence consisting of quaternary solvent delivery pump, a degasser, an column oven and photodiode array detector, LC20-AT series, C18 (4.6*250) mm, 5 micron column was used. The instrumental settings were a flow of 1Ml/min. The injection volume was 20ul.The detection wavelength was 190nm for all three analytes. The peak purity was checked with the photodiode array detector from LC20-AT.

Mobile Phase- The mobile phase consisted of buffer and acetonitrile in the ratio of 35:65(v/v). the pH of the mobile phase was adjusted to 20mM(v/v) of di potassium hydrogen ortho phosphate buffer in the double distilled water. The mobile phase was mixed and filtered through a nylon filter and degassed.

Standard stock solution- Standard stock solutions were prepared by dissolving the drugs in the diluents and diluting them to the desired concentrations. Diluent used for the standards and sample preparations was done as follows. Diluents were composed of water and acetonitrile in the ratio of 50:50(v/v) and diluents b was composed of buffer and acetonitrile in the ratio of 35:65(v/v).

Preparation of Standard Solution:

10 mg of Cefoperazone was taken in a 10 ml standard flask. To this 2 ml of mobile was added for dissolving the drug. Shake it for one min. to get a clear solution and make up the volume to 10 ml with mobile phase (stock solution A).

10 mg of Sulbactam was taken in a 10 ml standard flask and diluted with few ml of mobile phase until the sample dissolves completely and make up the volume to 10 ml with mobile phase (stock solution B).

The internal standard solution was prepared by taking 10 mg of ornidazole in a 10 ml standard flask. It is dissolved by adding 3 ml of mobile phase, shake it for few minutes to get a clear solution and make up the final volume to 10 ml with mobile phase.

The final standard solution was prepared in such a way that each standard flask contains 10, 20, 30, 40 and 50 µg of cefoperazone and sulbactam and 20µg of ornidazole (IS).

Preparation of Formulation Solutions:

500 mg of each cefoperazone and sulbactam was dissolved then extracted with 50 ml of mobile phase (10mg/ml) . This was then filtered and diluted to 10 mg/ml of cefoperazone and sulbactam. From this, 2.5 ml of solution was drawn and mixed with 2 ml of internal standard so that these solutions when diluted to 10 ml with mobile phase contain 25 µg/ml of cefoperazone and sulbactam and 20 µg/ml of internal standard.

Calibration curve solutions- The calibration curve solution containing 5-140ug/ml of cefoperazone and 5-140ug/ml sulbactum and 20ug/ml of ornidazole in each calibration level was prepared.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

Our objective of chromatographic method development was achieve peak tailing factor<2, retention time below 10 minutes, Along with resolution between Cefoperazone, Salbactam and internal standard (Ornidazole)>2.

The chromatographic separation was achieved using C18 (4.6*250) mm, 5 micron column. The chromatographic method was optimized by changing the composition of mobile phase and pH of the mobile phase.

Validation of the method

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out 6 times and the percentage recovery and percentage relative standard deviation of the percentage recovery were calculated and presented in Table1.

Drug	% Recovery		% RSD	
	50% level	100% level	50% level	100% level
Sulbactam	98.8	100.71	0.2015	0.1811
Cefoperazone	97.08	99.60	0.1492	0.2139

Table1: Accuracy (Recovery Studies)

The precision of the method was determined by studying repeatability and reproducibility. The response factor of drug peaks and percentage relative standard deviation were calculated and presented in Table.2&3. The results revealed that the method developed is reproducible.

Repeatability of injection

A standard solution of mixture of drugs was injected 6 times and its % RSD was calculated.

Concentration (µg/ml)	Injection	Peak area		% RSD	
		S	C	S	C
Sulbactam (20 µg/ml) Cefoperazone (20 µg/ml)	1	2120228	9051325	.1146	.0285
	2	2120822	9052672		
	3	2121025	9051821		
	4	2121186	9052461		
	5	2121650	9051923		
	6	2121382	9051627		

Table2: Repeatability of injection

S= Sulbactam, C= Cefoperazone.

The response factor, slope, intercept and correlation coefficient values were calculated. The correlation coefficient of Sulbactam and Cefoperazone were found to be 0.9994 and 0.9998 respectively. The calibration curves were plotted using response factor Vs concentration of the standard solutions. The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the concentration of the analytes. The range demonstrates that the method is linear outside the limits of expected use.

The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the developed methods. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). LOD of Sulbactam, Cefoperazone and Ornidazole were found to be 50, 50 and 100 ng/ml. the LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Sulbactam, Cefoperazone and Ornidazole were found to be 300, 400 and 500 ng/ml.

The resolution, capacity factor, theoretical plates/meter, peak symmetry was calculated for the standard solutions and is presented in Table. 3. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

Drug	R_s	N	K'	α	HETP	LOD ng/ml	LOQng/ml
Sulbactam	5.44	34855	0.334	2.666	28	50	300
Cefoperazone	8.36	39048	0.896		25	50	400

Table3: System suitability studies

The recovery studies in plasma were carried out 3 times and the percentage recovery and percentage relative standard deviations of the percentage recovery were calculated. Among the 4 solvents 1% tri chloro acetic acid (TCA) has showed good recovery. Acetonitrile has given good recovery (90.54%)for Sulbactam but poor recovery(<30%) for Cefoperazone. Where as in the rest 2 solvents 1% H_2So_4 and di ethyl ether drugs recovery was not within limits. The recovery values with 1% TCA were presented in Table4.

S. No.	Name of the Drug	Amount added µg/ml	Amount recovered µg/ml	% Recovery	RSD
1.	Sulbactam	50	42.35	84.7	0.13
2.	Cefoperazone	50	40.28	80.56	0.71

Table 4: Recovery Studies

Hence the developed method for the simultaneous estimation of Cefoperazone and Sulbactam in combined dosage forms is accurate, precise, linear, simple and rapid.

Estimation

Estimation of Cefoperazone and Sulbactam in dosage forms by High Performance Liquid Chromatography was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared and chromatograms were recorded.

The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for 6 times and mean peak area, mean peak area ratio, mean weight of standard drugs, mean weight of sample taken for assay were calculated. The percentages of individual drugs found in formulations, mean, and standard deviation in formulations were calculated and presented in Table 5. The results of

analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

Drug	Label Claim (mg/tablet)	Estimated Amount (mg/tablet)	% Label claim	SD
Sulbactam	500	498.992	99.7984	0.002
Cefoperazone	500	489.578	97.9156	0.024

Table5: Analysis of Formulation

Determination of active ingredients in tablets

The contents of two drugs in tablets were determined by the proposed method using the calibration curve. The results are shown in Table5. The chromatogram of the tablet sample is shown in (FigureII).

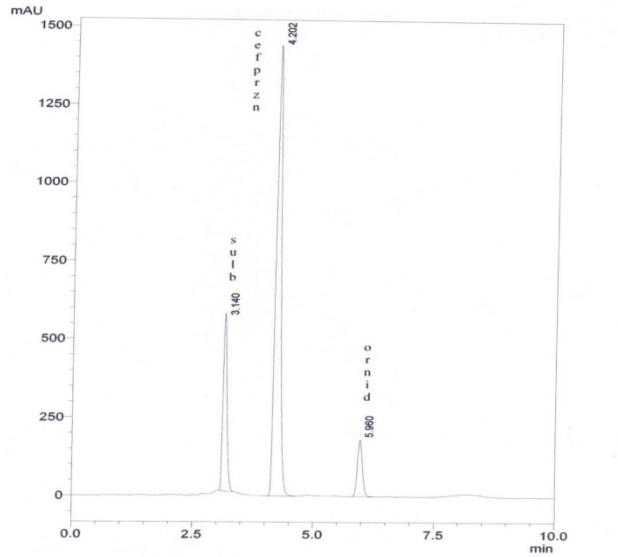

Figure2.A typical chromatogram of the tablet: Sulbactam(3.1), Cefoperazone(4.2),Ornidazole(5.9).

SUMMARY AND CONCLUSION

The scope and objective of the present work is to optimize the chromatographic conditions, to develop HPLC method for the estimation of drugs in selected multi-component dosage form and the same is validated.

Various solvent systems were tried among which sodium phosphate buffer: Acetonitrile with ratio 65:35, pH 3.5 was selected as mobile phase, which gave good resolution and peak shapes. The flow rate was set at 1.0 ml/min. detection was carried out by PDA detector at 190 nm. Quantitation was done by internal calibration method. At the optimum conditions mentioned above, ornidazole was selected as internal standard for the analysis.

The linearity and range was established over the range of 5µg/ml to 140 µg/ml for both Cefoperazone and Sulbactam. The correlation coefficient of Cefoperazone and Sulbactam were found to be 0.9994 and 0.9998. The method was validated for accuracy, precision, and system suitability. The percentage recovery of Cefoperazone and Sulbactam was found to be 98.8 % and 97.04 % for 50%level;100.7%, and 99.6% for 100% level. The low standard deviation value and good percentage recovery indicates the reproducibility and accuracy of the developed method. Similarly the RSD value for precision was also within the acceptable limit.

The chromatographic method developed for Cefoperazone and Sulbactam is said to be rapid, simple, precise, accurate and cost effective that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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