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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SULBACTAM AND CEFOPERAZONE IN DOSAGE FORM AND IN PLASMA

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ABSTRACT

A simple, precise and accurate RP-HPLC method has been developed for the simultaneous estimation of sulbactam and cefoperazone in dosage form and in plasma using ornidazole as an internal standard. The chromatographic separation was achieved on a phenomenex phenyl hexyl column (250mmX4.6mm,5µm) using a gradient system with mobile phase composed of acetonitrile-phosphate buffer(sodium phosphate-20mM)(65:35v/v)pH 3.5 adjusted with orthophosphoric acid at a flow rate of 1 ml/min and detector wavelength190nm.The retention time of sulbactam and cefoperazone was 3.1 and 4.1.The validation of the proposed method was carried out for specificity, linearity, accuracy, precision, robustness and limit of detection and quantitation.The linear dynamic ranges were from 10-50µg/ml for sulbactam and 10-50µg/ml for cefoperazone.Limit of detection and quantitation for sulbactam were 50ng/ml and 300ng/ml for cefoperazone were 50ng/ml and 400ng/ml respectively. The developed method can be used for routine quality control analysis of titled drugs in combination in formulation and in plasma.

KEYWORDS*:* RP-HPLC, Sulbactam, Cefoperazone

INTRODUCTION

Cefoperazone (Fig.1)7-D (-)-(4-ethyl-2, 3-dioxo-1-piperzinecarboxamido (4-hydroxyphenyl) acetamido-3-(1-methyl)-1Htetrazol-5-yl) thiomethyl-3-cepham-carboxylicacid is a broad spectrum third generation cephalosporin antibiotic[1].It is active against many gram positive and gram negative organisms. Sulbactam (Fig.2) (2S-cis)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid 4,4dioxide is a ß-lactam antibiotic. The combination provides a broad spectrum of antibacterial activity against ß-lactamase producing bacteria.[2].

Literature survey revealed that some analytical methods have been used for individual estimation of cefoperazone and sulbactam. A spectrophotometric method has been described for estimation of ampicillin and sulbactam [3]. A capillary electrophoretic method has been described for determination of ampicillin and sulbactam [4]. An isocratic LC

method with UV detector has been described for simultaneous determination of amoxicillin sodium and sulbactam sodium [5]. A RP-HPLC method for quantification of sulbactam in plasma, urine and tissue is also described [6]. A colorimetric method has been described for estimation of cefoperazone using Ce (IV) / Fe (III) in acid medium [7]. A HPLC method has been described for determination of cefazolin, cefoperazone, ceftriofur in raw bovine milk [8]. An isocratic RP-HPLC method with acid mobile phase has been described for simultaneous estimation of sulbactam and cefoperazone [9].

EXPERIMENTAL

(i)Chemicals: Sulbactam, cefoperazone, ornidazole were procured from Aurabindo Laboratories, Hyderabad. Sodium phosphate (AR Grade), Acetonitrile (HPLC Grade),Water (HPLC Grade)were purchased from Merck (India)Ltd, Mumbai, India. The 0.45µm and 0.2µm nylon filters were purchased from Advanced Micro Devices pvt. Ltd. Injection vial were purchased from Indian market, containing sulbactam 500mg and cefoperazone 500mg.

(ii)Equipments: Analysis was performed on a chromatographic system of shimadzu LC-20 AT HPLC equipped with a manual injector (20µl), variable wave length UV-visible detector. The chromatographic separation was achieved on phenomenex phenyl hexyl column (250mmX4.6mm, 5µm) analytical column.

(iii)Standard solutions and calibration graphs: Standard stock solutions of sulbactam (1mg/ml), cefoperazone (1mg/ml), ornidazole (1mg/ml) were prepared in mobile phase in different volumetric flasks. To study the linearity range of each component serial dilutions were made by adding the standard stock solutions in the different weights of sulbactam in the range of 10-50µg/ml of sulbactam and cefoperazone in the range of 10-50µg/ml of cefoperazone. A graph was plotted as concentration of drugs versus peak area ratio.

(iv)Sample preparation: Injection vial containing 500mg of each cefoperazone and sulbactam was taken and the contents were extracted with 50ml of mobile phase. This was then filtered and diluted to 10mg/ml. The final concentration was made to 25µg/ml of sulbactam, cefoperazone and 20µg/ml of internal standard with mobile phase.
Blood was collected from human volunteer in a clean tube containing anticoagulant and shaken well. Plasma was separated by centrifugation. Four clean tubes were taken and to each 0.5 ml of plasma and 0.5 ml of 200 µg/ml drug solution was added. For extracting drug four different types of solutions were used such as 1% tri chloro acetic acid, acetonitrile, 1% H_2SO_4 and diethyl ether. Drugs were extracted by centrifugation process. Clear supernatant solution separated and filtered through 0.2µ filter and 20µl of solution was injected to the column and chromatograms were recorded.

RESULTS AND DISCUSSION

Validation of method:

(i)Specificity: The specificity of the HPLC method is illustrated in Fig. 3 where complete separation of sulbactam and cefoperazone was noticed in presence of excipients. In addition there was no any interference at the retention time of sulbactam and cefoperazone in the chromatogram of placebo solution. This shows that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

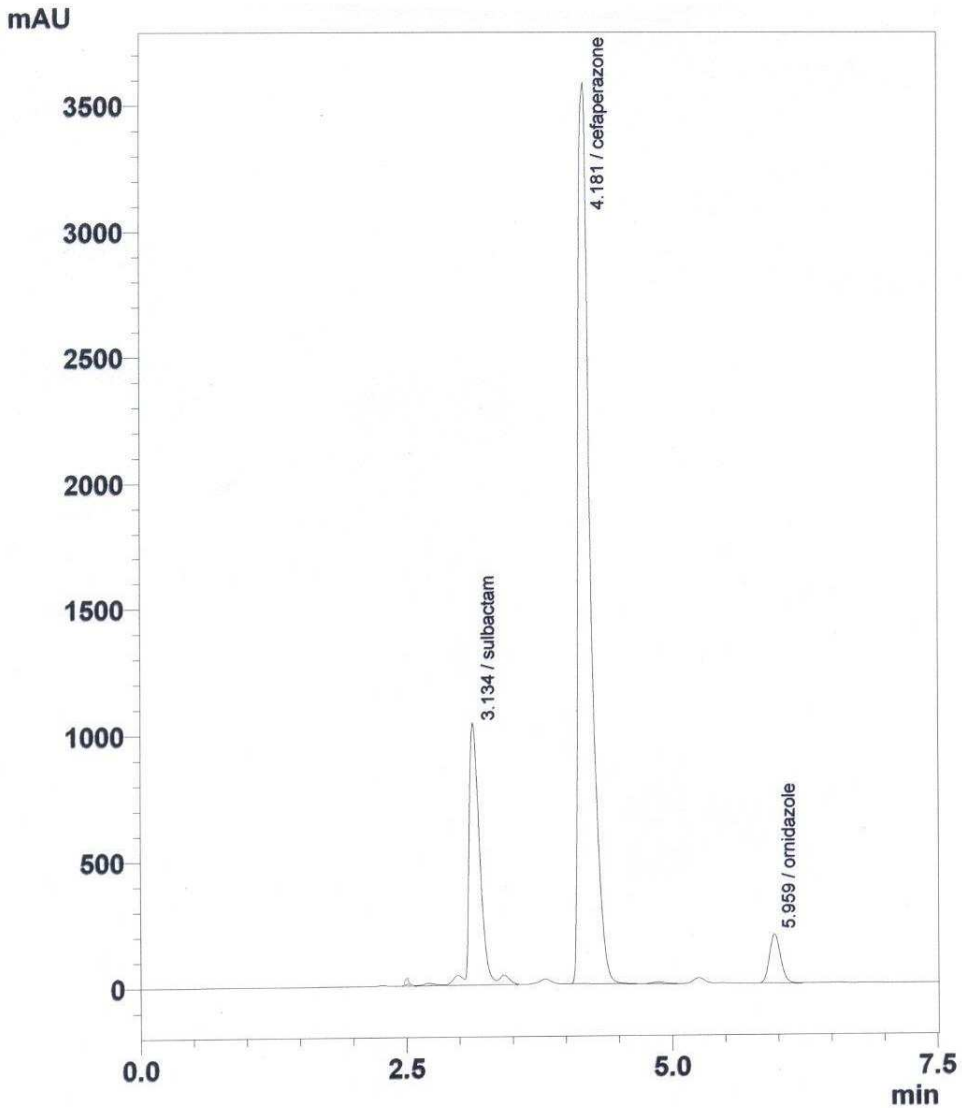

Figure 3

A typical chromatogram of sample solution containing 25µg/ml of sulbactam and 25µg/ml of cefoperazone.

(ii) Accuracy: Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1). The mean percentage recoveries obtained for sulbactam and cefoperazone were 84.7 and 80.56%, respectively.

Table 1
Results of Recovery analysis of sulbactam and cefoperazone

S. No.	Compound	Wt. spiked µg	Wt.recovered µg	% Recovery	%RSD(n=3)
1.	Sulbactam	50	42.35	84.7	0.13
2.	Cefoperazone	50	40.28	80.56	0.71

(iii)Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analyses of the same working solution. The relative standard deviation (R.S.D.) obtained for sulbactam and cefoperazone was 1.51 and 0.29%, respectively (Table 2).

Table 2
System suitability parameters

Parameters	Sulbactam	Cefoperazone
Theoretical plates	34855	39048
USP resolution	5.44	8.36
Peak symmetry	1.02	1.04
Capacity factor	2.66	2.45
%RSD	1.51	0.29

The intra- and inter-day variability or precision data are summarized in Table 3. The intra-day precision of the developed LC method was determined by preparing the sample solutions and nine determinations with three concentrations and three replicate each. The results indicated the good precision of the developed method (Table 3).

Table 3
Intra- and inter-day precision data (n=9)

Concentration	%RSD	
	Intra-day	Inter-day
Sulbactam (µg/ml)		
10	0.040	0.053
20	0.013	0.743
30	0.483	0.511
cefoperazone(µg/ml)		
10	0.525	0.559
20	0.146	0.184
30	0.053	0.084

(iv)Linearity: Linearity was determined for sulbactam in the range of 10–50µg/ml; and for cefoperazone, 30–180µg/ml. The correlation coefficient (‘*r*’) values for both the drugs were >0.999. Typically, the regression equation for the calibration curve was found to be y=0.1031x−0.06 for sulbactam and y = 0.391x+0.35 for cefoperazone.

(v)Limit of detection (LOD) and limit of quantitation (LOQ): 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.

(vi) Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Robustness of the method was investigated under a variety of conditions including changes of pH of the mobile phase, flow rate, percentage of acetonitrile in the mobile phase. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 4).

Table 4
Results of robustness study

Factor	Level	Retention time		%RSD	
		sulbactam	cefoperazone	sulbactam	cefoperazone
pH of mobile phase	3.4	3.02	4.03	0.42	1.07
	3.6	3.19	4.28	0.98	0.86
Flow rate(ml/min)	0.9	3.31	4.32	1.05	0.72
	1.1	2.93	3.88	0.52	0.63
% of acetonitrile	60	3.46	4.35	0.75	0.81
	70	2.82	3.78	1.25	0.68

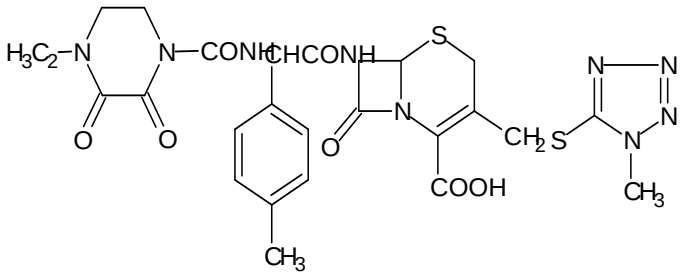

Figure 1
Structure of Cefoperazone

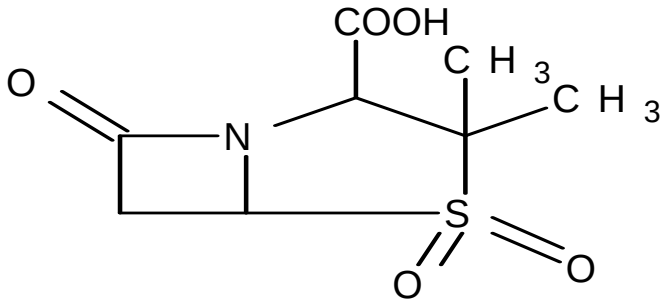

Figure 2
Structure of Sulbactam

CONCLUSION

A simple, specific, linear, precise, and accurate RP-HPLC method has been developed and validated for quantitative determination of sulbactam and cefoperazone in formulation. The method is very simple and specific as both peaks are well separated from its impurities and excipient peaks with total runtime of 7.5min, which makes it especially suitable for routine quality control analysis work.

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