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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Cefoperazone and Sulbactam in Parenteral Preparation

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Abstarct: A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Cefoperazone and Sulbactam in parenteral preparation. The separation was carried out using a mobile phase consisting of Phosphate Buffer pH 3.5 adjusted with ortho phosphoric acid and Acetonitrile (35:65). The column used was Kromasil C8, 5µ, 15 cm × 4.6 mm id with flow rate of 1 ml / min using PDA detection at 215 nm. The described method was linear over a concentration range of 50 - 250 µg/ml and 100 - 500 µg/ml for the assay of Sulbactam and Cefoperazone respectively. Ornidazole (50µg/ml) was used as internal standard. The retention times of Sulbactam, Cefoperazone and Ornidazole were found to be 2.3, 4.2 and 5.1min respectively. Results of analysis were validated statistically and by recovery studies. The limit of quantification (LOQ) for Cefoperazone and Sulbactam were found to be 20 and 10 µg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Cefoperazone and Sulbactam bulk drug and in its pharmaceutical dosage form.

Keywords: Sulbactam, Cefoperazone and Ornidazole.

Introduction

Chemically Cefoperazone (CFN) is (6R,7R)-7-[[(2R)-[[(4-Ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino](4-hydroxy phenyl) acetyl] amino]-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid [1]. It is a third generation broad spectrum cephalosporin for parenteral administration used as a bactericidal and mainly used in the treatment of various bacterial infections caused by Gram-positive and Gram-negative micro organisms [2,3]. Sulbactam[4] (SBM) is chemically (2S,5R)-3,3-Dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane -2-carboxylic acid 4,4-dioxide.It is a penicillanic acid sulphone with (Glactamase inhibitory properties. It generally has only weak antibacterial activity, except against *N.gonorrhoea* and *N,meningitides* but it is an irreversible inhibitor of several p-lactamases 1,2. Sulbactam may therefore enhance the activity of many p-lactam antibiotics against bacteria that are normally resistant because of the production of p-lactamases, such as *staphylococci sp., N.gonorrhoea* and some enterobacteriaceae[5].

Cefoperazone official in USB, BP and not present in IP. The USP[6] and BP[7] describe HPLC method for estimation of Cefoperazone. The literature survey revealed that HPTLC[8], HPLC[9] and Spectrophotmetric methods[10] reported for its determination. There is no method has been reported for the simultaneous estimation of Cefoperazone and Sulbactam in combined dosage form. Hence we attempted to develop a simple, accurate, and economical analytical

method. This paper describes validated RP-HPLC for simultaneous estimation of CFT and SBM in combination using Phosphate Buffer pH 3.5 adjusted with ortho phosphoric acid and Acetonitrile (35:65). The column used was Kromasil C8, 5µ, 15 cm × 4.6 mm id with flow rate of 1 ml / min using PDA detection at 215 nm.

Materials and methods

Standard bulk drug sample Sulbactam, Cefoperazone and Ornidazole were provided by Micro Laboratories Ltd., Bangalore. Parenteral preparations in combined dosage form were procured from the local market. All other reagents used were of HPLC grade.

Instrumentation

HPLC (Shimadzu LC-20AT) method was developed using Kromasi C8, 5µ, 15 cm × 4.6 mm id. Mobile phase selected for this method using combination of Phosphate Buffer pH 3.5 adjusted with ortho phosphoric acid and Acetonitrile (35:65) acid that was filtered through 0.45-micron membrane filter. The column used was Kromasil C8, 5µ, 15 cm × 4.6 mm id with flow rate of 1 ml / min using PDA detection at 215 nm.

Preparation of Stock Solution

Method was developed using Ornidazole as internal standard. Standard stock solutions of pure drugs were made separately in mobile phase containing 50 - 250 µg/ml and 100 - 500 µg/ml for the assay of Sulbactam and Cefoperazone and 50 µg /ml of Ornidazole and filtered through a 0.45µ membrane filter. A volume of 20 µL of each sample was injected into column. All measurements were repeated six times for each concentration and the peak area and retention time was recorded. Mean retention times of Sulbactam, Cefoperazone and Ornidazole were found to be 3.1, 4.1 and 5.9min respectively. The calibration curve was constructed by plotting peak area ratio of analytes to internal standard Vs the corresponding drug concentration. **[Table – 1].**

Analysis of formulation

Weight equivalent to 100 mg/ml of Cefoperazone were measured and transferred to a 100 ml volumetric flask .It was dissolved in the mobile phase and filtered through a membrane filter (0.45µ). The sample solution was suitably diluted and used for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the 20 µl fixed - sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. A representative chromatogram has been given in **Figure-1**. The peak area ratios of each of the drugs to the internal standard were calculated and the amount of each drug present per formulation was estimated from the respective calibration curves. The result of analysis reported in **[Table – 2].** The stability of the sample in mobile phase was analyzed after 24 hrs; it was found no change in analytical parameters. In the RP-HPLC method, system suitability **[Table-3]** was applied to a representative chromatograph to check various parameters such as efficiency, resolution and peak tailing which was found to be complying with BP requirements [11].

Recovery studies

Recovery studies were carried out by adding known quantities of standard at different levels to the pre-analyzed sample to study the linearity, accuracy and precision of the proposed methods. The recovery studies also reveals whether there is a positive or negative influence on the quantification parameters by the additives usually present in dosage forms. The recovery study data are presented in **[Table 4]**.

Results and Discussion

The developed RP-HPLC method for simultaneous estimation of Cefoperazone and Sulbactam from combined dosage form utilizing Phosphate Buffer pH 3.5 adjusted with ortho phosphoric acid and Acetonitrile (35:65. Detection of eluent was carried out using PDA detector at 215 nm. The method was developed using Ornidazole as internal standard. The run time per sample is just 6 min. The excipients in the formulation did not interfere in the accurate estimation of Cefoperazone and Sulbactam. The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application and the results are shown in **[Table -3]**. Since none of the methods is reported for simultaneous estimation of Cefoperazone and Sulbactam from combined dosage form, this developed method can be used for routine analysis of two components in parenteral formulation.

Table – 1: Peak Areas of Calibration Curve

Internal Standard Peak area (μgml^{-1})	Cefoperazone			Sulbactam		
	Concentration (μgml^{-1})	Peak area	Response factor	Concentration (μgml^{-1})	Peak area	Response factor
246810	50	20427	0.082	25	47463	0.192
	100	40855	0.165	50	94980	0.384
	200	81728	0.331	100	189910	0.769
	300	122582	0.496	150	284810	1.153
	400	163493	0.662	200	379692	1.538
	500	204302	0.827	250	474802	1.923

Table – 2: Analysis of Formulations

Drugs	Labelled Amount (mg)	Amount taken for assay (μg/ml)	Amount found(mg)	% label claim
Sulbactam	500	75	74.98± 1.888	99.97
Cefoperazone	1000	150	149.66 ±2.678	99.97

Table – 3: Recovery Studies

Drugs	Label claim (mg)	Fortified amount (mg)	* % Recovery
CFN	500	150	99.96 ± 0.768
SBM	1000	300	100.52 ±0.620

Table – 4: System suitability & Validation parameters

Validation Parameters	SBM	CFN
Linearity range (µg / ml)	50 - 250	100 - 500
r	0.9997	0.9994
LOD (ng /ml)	10	20
LOQ (ng /ml)	50	100
Intra day (% RSD)*	0.718	0.915
Inter day (% RSD)*	0.381	0.261
Repeatability (% RSD)*	0.435	0.253
Recovery	99.96 %	100.52 %
Peak purity index	1.0000	1.0000
Resolution factor (Rs)	-	5.383
Asymmetry factor (A_s)	0.95	
No. of theoretical plates (N)	6952	6671
Capacity factor *(k)*	-	2.301
High equivalent to theoretical plates (HETP)	21.575	22.482
Tailing factor	1.327	1.423
Selectivity factor (α)	3.639	

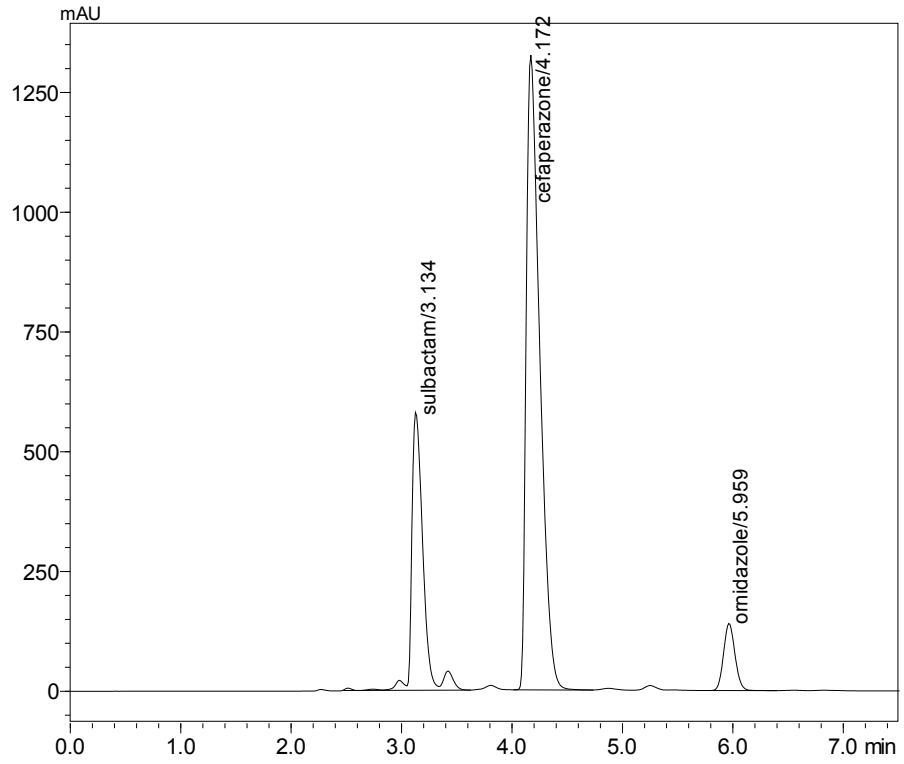

Fig.1: RP-HPLC Chromatogram of tablet sample with internal standard (SBM, CFN& IS)

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