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Development And Validation Of RP-HPLC Method For Analysis Of Cefixime Trihydrate And Sulbactam Sodium In Their Combination Tablet Dosage Form

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Abstract: Cefixime Trihydrate and Sulbactam Sodium belong to a group of Anti-bacterial drugs. A Simple, Rapid, Specific and economic Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed for assaying both the drugs in combinational dosage form. Method involves elution of Cefixime Trihydrate and Sulbactam Sodium in Hyper ODS2, Column C_{18}, 250 x 4.6 mm (5 µm) using mobile phase composition of a mixture of 45 ml Acetonitrile and 55 ml of water, pH 3 adjusted with OPA at flow rate 1ml/min and analytes were monitored at 225 nm.Method has been validated according to ICH (International Conference on Harmonization) Guideline. Method shows good linearity over the range of 40-240 µg/ml for cefixime trihydrate and 100-350 µg/ml for sulbactam sodium. All the validation parameters were within the range. The developed method was successfully applied to estimate the amount of Cefixime Trihydrate and Sulbactam Sodiumin Tablet and synthetic mixture.
Keywords: Cefixime Trihydrate, Sulbactam Sodium, RP-HPLC, Validation.

Introduction:

Cefixime Trihydrate (CEF) is chemically (6R,7R)-7-[2-(2-amino-1,3-thiazol-4-yl)-2-[(carboxymethoxy) imino]acetamido]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid[1] (Fig. 1). It is a third-generation cephalosporin antibacterial drug and used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis, lower respiratory-tract infections such as bronchitis and urinary tract infections [2]. It is official in British Pharmacopoeia (BP) and United States Pharmacopoeia (USP), BP[1] describe High Performance Liquid Chromatography (HPLC) and USP[3] also describe HPLC method. Literature survey also reveals Spectrophotometric Methods[4], RP-HPLC[5], HPTLC[6], Colorimetric[7], Spectroflourimetry methods[8] for determination of CEF with other drugs. Sulbactam Sodium (SUL) is chemically Sodium (2S, 5R)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate 4,4-dioxide[1] (Fig. 1)is beta-lactamase inhibitor, enhance the activity of penicillins and cephalosporins against many resistant strains of bacteria. It is official in British Pharmacopoeia (BP) and United States Pharmacopoeia (USP), BP[1] describe High Performance Liquid Chromatography (HPLC) and USP[3] also describe HPLC method. Literature survey also reveals Spectrophotometric Methods[9] and RP-HPLC[10] for determination of SUL with other drugs. The combined dosage form of CEF and SUL 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 CEF and SUL in their combined dosage form. Literature survey does not reveal any simple

Spectrophotometric or other method for simultaneous estimation of CEF and SUL in combined dosage form. Hence aim of work is to develop simple, sensitive, specific, accurate, precise and economical Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method for routine analysis of CEF and SUL in their combined dosage form.

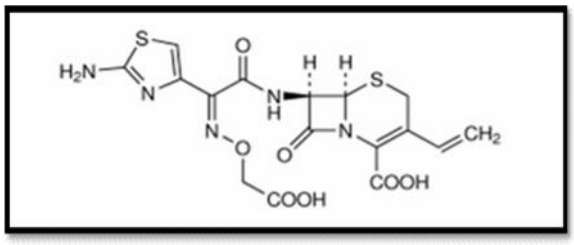

Fig. 1: Structure of Cefixime Trihydrate

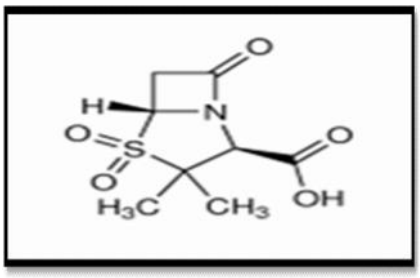

Fig. 2: Structure of Sulbactam Sodium

In present study Simple, Rapid, Specific and economic RP-HPLC method for estimation of Cefixime Trihydrate and Sulbactam Sodium in their combined pharmaceutical dosage form is reported.

Material And Method:

1.1 Chemicals And Reagents:

CEF and SUL were kindly given as a gratis sample by Relax Pharmaceuticals, Makarpura, Baroda, Gujarat and Intracin Pharmaceutical Pvt. Ltd., Nadiad, Gujarat respectively. The market formulation CEFLA (CEF 200 mg and SUL 125 mg) was procured from local market which is manufactured by H & Care Pharmaceutical Enterprises, Chandigarh, India.Acetonitrile (HPLC Grade) and Water(HPLC Grade) were obtained from RFCL limited, New Delhi. Other reagents O-phosphoric acid of analytical gradewas purchased from SD Fines chemicals, Bombay.

1.2 RP-HPLC Instrumentation And Conditions:

The HPLC(Analytical technologies limited) system consisted ofP2230 plus HPLC pump,Rhenodyne valve with 20µl fixed loop, UV 2230 plus detector, Analchrom 2006 Software, The chromatographic separation achieved on a Hyperchrom ODS-BP Column, (5µm, 200mm x 4.6mm i.d.) using a mobile phase consisted of 45 ml Acetonitrile and 55 ml of water, pH 3 adjusted with OPA, at flow rate 1ml/min and analytes were monitored at 225 nm.

1.3 Preparation Of Stock And Standard Solution:

1.3.1 Stock solution of CEF:

A 100mg of standard CEF accurately was weighed and transferred to a 100 ml volumetric flask and dissolved in 50 ml ACN: Water (50:50). The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with ACN: Water (50:50) to give a solution containing 1000 µg/ml CEF. From this solution 2.5 ml was transfer to 25 ml volumetric flask. The volume was adjusted to the mark with the ACN: Water (50:50) to give a solution containing 100 µg/ml CEF.

1.3.2 Stock solution of SUL:

A 100 mg of standard SUL was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 50 ml ACN: Water (50:50). The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with ACN: Water (50:50) to give a solution containing 1000 µg/ml SUL. From this solution 2.5 ml was transfer to 25 ml volumetric flask. The volume was adjusted to the mark with the ACN: Water (50:50) to give a solution containing 100 µg/ml SUL.

1.3.3 Calibration Standard solutions of CEF and SUL:

Appropriate volume of aliquot from CEF and SUL stock solution was transferred to same volumetric flask of 10 ml capacity. The volume was adjusted to the mark with mobile phase to give a solution containing 40, 80, 120, 160, 200 and 240 µg/ml CEF and 100, 150, 200, 250, 300 and 350µg/ml SUL.

1.4 Preparation Of Sample Solution For Tablet Assay:

Twenty tablets were weighed and finely powdered. The powder equivalent to 200 mg CEF and 125 mg SUL was accurately weighed and transferred to volumetric flask of 100 ml capacity. 50 ml of ACN: Water (50:50) was transferred to volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with ACN: Water (50:50).

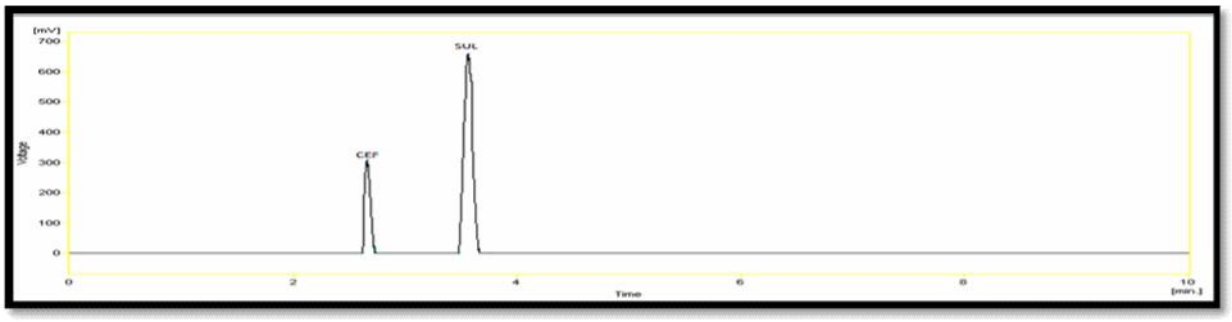

Figure 2: representative chromatogram obtained for standard solution 200 µg/ml of CEF and 300 µg/ml SUL.

The above solution was filtered through whatmann filter paper (0.45µ). 2.5 ml of aliquot was taken and transferred to volumetric flask of 25 ml capacity and volume was made up to the mark with the ACN: Water (50:50) to give a solution containing 200 µg/ml CEF and 125 µg/ml SUL. This solution was used for the estimation of CEF and SUL.

2. Result And Discussion:

2.1 HPLC Method Development And Optimization:

Hyperchrom ODS-BP 5µm, 200mm x 4.6mm i.d. Column (Analytical technologies limited) maintained at ambient temperature was used for the separation and the method validated for estimation of CEF and SUL in their combined tablet dosage form. The composition, pH, Flow rate of mobile phase changed to optimize the separation condition. A mobile phase consisting of 45 ml Acetonitrile and 55 ml of water, pH 3 adjusted with OPA with gradient elution was selected for use for further studies after several preliminary investigatory chromatographic runs (Table-1). Under described conditions, all peaks were well defined and free from tailing.

2.2 Validation Of HPLC Method:

2.2.1 Linearity:

Linearity was established by least square linear regression analysis of the calibration curve. The constructed calibration curves were linear over the range of 40-240 µg/ml for CEF and 100-350 µg/ml for SUL. Peak area of CEF and SUL were plotted versus their respective concentrations and linear regression analysis was performed on the resultant curves. Typically, the regression equations were: **y =** 4.4742 x + 211.548(R^2 = 0.9982), **y =** 12.3430 x - 335.6564 (R^2 = 0.9992) for CEF and SUL respectively (Table-2).

2.2.2 LOD and LOQ:

LOD and LOQ were performed on samples containing concentrations of analytes, based on calibration method. Standard solution of CEF and SUL were injected in six replicate (Table 2). Average peak area of six analyte was plotted against concentration. LOD and LOQ were calculated using following equation,

$LOD=(3.3 \times \sigma) / S$

$LOQ = (10 \times \sigma) / S$

Table 1: Various Mobile phases tried for optimization

Mobile Phase	Proportion (v/v)	Detection Wavelength (nm)	Mobile Phase
Acetonitrile : Water	50:50	225	Acetonitrile : Water
Acetonitrile :Water, pH 7	50:50	225	Acetonitrile :Water, pH 7
Acetonitrile : Water, pH 6	50:50	225	Acetonitrile : Water, pH 6
Acetonitrile :Water, pH 5	50:50	225	Acetonitrile :Water, pH 5
Acetonitrile :Water, pH 4	50:50	225	Acetonitrile :Water, pH 4
Acetonitrile :Water, pH 3	50:50	225	Acetonitrile :Water, pH 3
Acetonitrile :Water, pH 3 (proposed mobile phase)	45:55	225	Acetonitrile :Water, pH 3 (proposed mobile phase)

Table 2: Statistical data for CEF and SUL by RP- HPLC method

Parameter	CEF	SUL
Linear Range (µg/ml)	40 – 240	100 – 350
Slope	4.474	12.342
Intercept	211.54	335.65
Standard deviation of slope	0.095	0.178
Standard deviation of intercept	14.818	43.028
Limit of Detection (µg/ml)	10.93	11.50
Limit of Quantitation (µg/ml)	33.12	34.86

2.2.3 System suitability:

System suitability parameters can be defined as a test to ensure that the method can generate results of acceptable accuracy and precision. System suitability parameters like Retention time, Resolution, theoretical plates, tailing factor were calculated and compared with standard values to ascertain whether the proposed RP-HPLC method for the estimation of CEF and SUL in pharmaceutical dosage form was validated or not. Results are shown in Table-3.

2.2.4 Accuracy:

A known amount of each standard powder (80%, 100%, and 120%) was added to the synthetic mixture of excipients and subsequently diluted to yield a starting concentration of 64 µg/ml, 80 µg/ml and 96 µg/ml for CEF and 120 µg/ml, 150 µg/ml and 180 µg/ml for SUL. The observed % recovery was ranging from 99.16-99.73% for CEF and 99.21-99.82% for SUL (Table-4).

2.2.5 Precision:

The Interday intraday variability data are summarised in Table-4. They were assessed by using standard solutions to produce solutions of three different concentrations of each drug. Intraday precision investigated by injecting three replicate sample of each of sample of three different concentrations. Intraday precision were assessed by injecting same three samples over three consecutive days (Table-4).

Table 3: system suitability parameters:

Parameter	CEF	SUL	Range	Inference
Retention time(minutes) (Rt) ±S.D.	2.66±0.005	3.56±0.010	-	-
Peak width (minutes) ±S.D.	0.113±0.020	0.176±0.008	-	-
Resolution(Rs)	------- 6.20 --------		>2	Criteria met
Tailing factor ± S.D.	1.20±0.062	1.21±0.063	<2	Criteria met
Theoretical Plates (Plates/Meter)	9,356	6,258	Above 2000	Criteria met

Table 4: Summary of Validation Parameters of RP-HPLC

Parameters	CEF	SUL
Recovery %	99.16-99.73	99.21-99.82
Repeatability (RSD, n=6)	0.0036	0.0026
Precision(CV)		
Intra-day (n=3)	0.23-0.38	0.16-0.26
Inter-day (n=3)	0.51-0.93	0.42-0.63
Specificity	Specific	Specific
Solvent suitability	Solvent suitable for 48 hrs	Solvent suitable for 48 hrs

Table 5: Assay Results of Marketed Formulation

Formulation	Actual concentration µg/ml		Amount obtained µg/ml		% CEF±S.D.	% SUL±S.D.
	CEF	SUL	CEF	SUL		
Tablet	200	125	1990.8	1243.7	99.31±0.44	99.24±0.47

2.2.6 Repeatability:

Standard mixture solutions of CEF (40, 80, 120, 160, 200 & 240 µg/ml) and VAL (100, 150, 200, 250, 300&350 µg/ml) were prepared and chromatograms were recorded. Area was measured of the same concentration solution six times and C.V. was calculated (Table-4).

2.3 Assay:

Validated method was applied for the determination of CEF and SUL in commercially available CEFLA tablets. The result of assay undertaken yielded 99.31% and 99.24% of label claim for CEF and SUL respectively (**Table-5).**

Conclusion:

A Simple, Rapid, Specific and economic Reverse phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed and validated for routine analysis of CEF and SUL in API and combinational dosage forms. The proposed method has ability to separate these drugs from excipients found in tablet dosage form.

Acknowledgement

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