International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-1, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Development of a Stability Indicating HPLC Method for Simultaneous Estimation of Ceftriaxone and Sulbactam in Sterile Powder for Injection

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Manuscript No: IJPRS/V4/I1/00044, Received On: 20/03/2015, Accepted On: 27/03/2015

ABSTRACT

A stability indicating HPLC method was developed for the simultaneous determination of Ceftriaxone and Sulbactam in pharmaceutical dosage form. Efficient chromatographic separation was achieved on BDS hypersil C_{18}, 250mm × 4.6mm, 5µ (particle size), Thermo scientific stationary phase with simple combination of a mobile phase containing phosphate buffer (pH 3.5): methanol (60:40 v/v) and quantification was carried out using UV detection at 277 nm at a flow rate of 1.0 ml/min with injection volume of 20µl. This method is capable to detect both the drug components of Ceftriaxone and Sulbactam in presence of their degradation products with detection level of 0.05%. Ceftriaxone and Sulbactam in their combination drug product were exposed to acidic, alkaline, oxidative, thermal and photolytic stress conditions, and the samples were analyzed. Peak homogeneity data of Ceftriaxone and Sulbactam is obtained using standard photo detector, in the stressed sample chromatograms, demonstrating the specificity. The method showed excellent linearity over a range of 10-30% and 5-15% for Ceftriaxone and Sulbactam. The correlation coefficient for Ceftriaxone and Sulbactam were 0.999 and 0.998 respectively. The relative standard deviation was always less than 2%. The proposed method was found to be suitable and accurate for quantitative determination and the stability study of Ceftriaxone and Sulbactam in pharmaceutical preparations. The developed HPLC method was validated with respect to linearity, range, accuracy, precision and robustness.

KEYWORDS

High Performance Liquid Chromatography, Method validation, Stability Indicating Study, Ceftriaxone, Sulbactam

INTRODUCTION

The key intermediate for semisynthetic production of a large number of cephalosporin is 7-aminocephalosporanic acid, which is formed by hydrolysis of cephalosporin C produced by fermentation[1]. Among the cephalosporins, Ceftriaxone (CFTX) is a semisynthetic cephalosporin of the third generation with high antibacterial activity, which is widely used to treat bacterial infections caused by susceptible, usually Gram-positive organism, meningitis caused by aerobic Gram-negative bacteria and other medical conditions. CFTX is chemically known as Disodium (6R, 7R) -3[(acetyloxy) methyl] -7-[(2Z) – (2-amino- 4-thiazolyl) (methoxy amino) – acetyl] amino] -8-oxo-5-thia-1-azabicyclo [4.2.0.] Oct -2-ene- 2 carboxylic acid. It is official in IP, BP and USP.[2,3,4]

Sulbactam (SLB) is (*R*)-3, 3- dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid 4, 4-dioxide is a beta-lactamase inhibitor. SLB is an irreversible inhibitor of beta lactamase; it binds the enzyme and does not allow it to

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interact with the antibiotic. It is official in USP.[5] It is freely soluble in methanol and water.

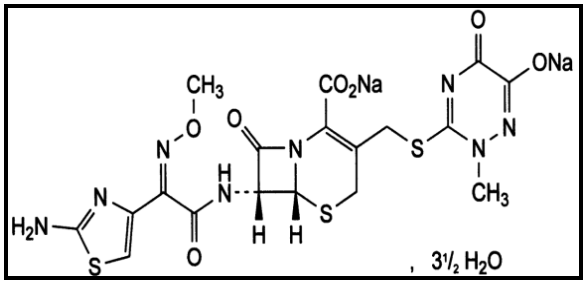

Figure 1: Ceftriaxone Chemical Structure

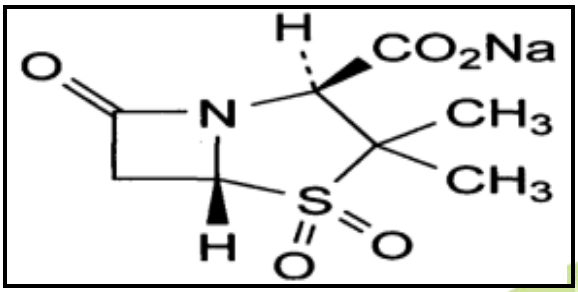

Figure 2: Sulbactam Chemical Structure

Their combination drug product Troxin-S injection (contain CFTX 1000 mg and SLB 500 mg) is a synergistic anti-microbial combination with marked *in-vitro* antibacterial activity against a broad spectrum of organisms. The combine formulation has been approved by Central Drug Standard Control Organization (CDSCO) in 21/09/2011.[6] A very few analytical methods have been reported for the analysis of CFTX and SLB individually and also in combination with other drugs.[7,8,9,10,11] The proposed method was validated with respect to specificity, linearity, accuracy, precision and robustness. In addition, stress testing of the drug was also conducted, as required by the International Conference on Harmonization (ICH)[12] to support the suitability of the method.

MATERIAL AND METHODS

Instrumentation

The chromatography was performed on a Shimadzu HPLC instrument (LC-20AT) equipped with standard photo detector and Spinchrom software, BDS hypersil C_{18} column (250mm, 4.6mm and 5µm**)** thermo scientific was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad) and a hot air oven (Grover, New Delhi, India) were used in the study.

Chemicals and Solvents:

CFTX and SLB standards and its marketed formulation TROXIN-S injection (containing CFTX sodium 1gm and SLB sodium 0.5gm) were kindly supplied as a gift sample from Intracin Pharmaceutical Pvt. Ltd., Nadiad, Gujarat, India.

HPLC grades Acetonitrile, Methanol, triple distilled water (Finar Chemicals Ltd., Mumbai, India) were used and AR grade Hydrochloric Acid, Sodium Hydroxide, Hydrogen Peroxide (Finar Chemicals Ltd., Mumbai, India), Ortho-phosphoric acid, Triethylamine and Potassium dihydrogen phosphate (Merck India Ltd.) were used. Whatman filter paper no. 41. (Whatman International Ltd., England) was used in the study.

Preparation of Solutions

Preparation of 0.1N HCl

A solution of 0.1N HCl was prepared by taking 0.86 ml concentrated HCl in 100 ml volumetric flask and diluted up to mark with water.

Preparation of 0.1 N NaOH

A solution of 0.1N NaOH was prepared by dissolving 0.4 gm NaOH pallets in 100 ml water.

Preparation of 3% H_2O_2

A solution of 3 % H_2O_2 was prepared by taking 3 ml of H_2O_2 in 100 ml volumetric flask and diluted up to mark with water.

Preparation of Solutions of CFTX and SLB

Accurately weighed CFTX (10 mg) and SLB (10 mg) were transferred to individual 10 ml volumetric flasks and diluted up to 10 ml with methanol to obtain a standard stock solution (1000µg/ml). For preparation of working standards, for CFTX 2 ml stock solution of CFTX was taken and dilute up to 10ml in 10ml volumetric flask (200µg/ml) and for SLB 1ml stock solution of SLB was taken and dilute up to 10 ml in 10ml volumetric flask (100µg/ml).

Preparation of Sample Solution

An accurately weighed amount of powder mixture equivalent to 1.5 gm of CFTX and SLB was transferred to 100 ml volumetric flask, dissolved with in methanol. The content was filtered through Whatman filter paper and diluted up to mark with methanol. This solution contains 10,000 µg/ml of CFTX and 5000 µg/ml of SLB. From the above solution, 2 ml was transferred to 100 ml volumetric flask and diluted up to mark with methanol to get the concentration of 200 µg/ml of CFTX and 100 µg/ml of SLB.

Analytical Method Development

To optimize the HPLC parameters, several mobile phase compositions were tried. Satisfactory results were obtained from given chromatographic condition for CFTX and SLB.

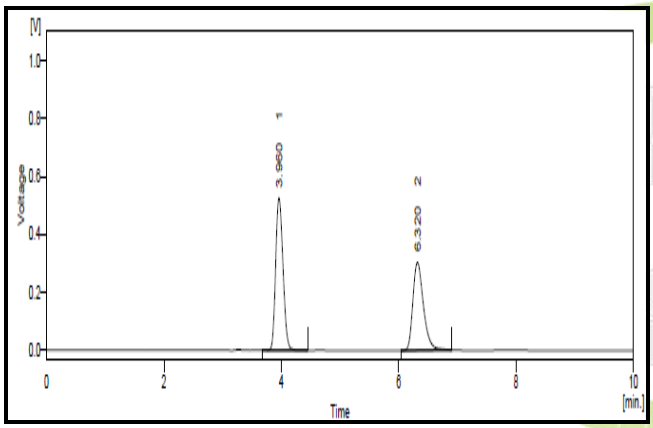

Figure 3: Chromatogram of CFTX (20µg/ml) and SLB (10µg/ml) at 277nm by HPLC method

Table 1: Optimized Chromatographic Condition

Parameters	Conditions
Mobile phase	Phosphate buffer (pH: 3.5): Methanol (60:40 v/v)
Stationary phase	BDS hypersil C_{18}, 250mm × 4.6mm, 5µ (particle size), Thermo scientific
Flow rate (ml/min.)	1
Run time (min.)	10
Volume of Injection (µL)	20
Detection wavelength (nm)	277
Retention time (min.)	CFTX: 3.96,SLB: 6.32

Analytical Method Validation

The developed chromatographic method was validated as per ICH guideline for following parameters.

System Suitability

As per USP-24, system suitability tests were carried out on freshly prepared standard stock solution of CFTX and SLB of both drugs under optimized chromatographic condition and parameters were studied to evaluate the suitability of the system. Results are shown in Table: 2.

Table 2: System Suitability Studies

Parameters	CFTX	SLB	Acceptance criteria
Theoretical plates	4432	5920	Not less than 2000
USP tailing factor	1.313	1.523	Not more than 2
Resolution	8.332		Not less than 2

Linearity and Range

The drug response was linear over the concentration range between 10-30 µg/ml for CFTX and 5-15 µg/ml for SLB. The results are shown in Figure: 4, 5 and Table 3.

Accuracy

Good recoveries of CFTX and SLB were obtained at various added concentrations by spiking standards like 80%, 100% and 120%. Results are shown in Table 3.

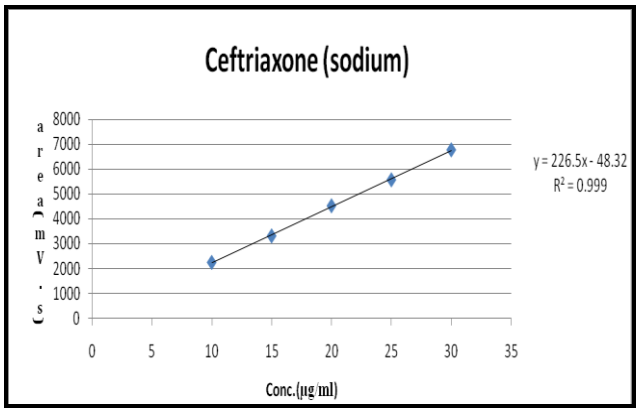

Figure 4: Calibration curve of CFTX

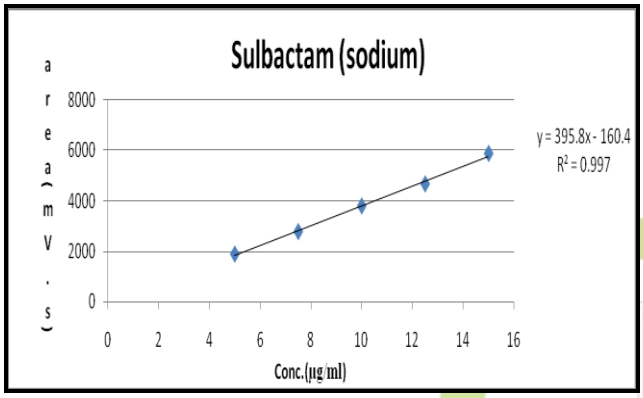

Figure 5: Calibration curve of SLB

Precision

The results of the repeatability, intra-day and inter-day precision experiments are shown respectively as given in Table 3. The developed method was found to be precise as the %RSD were < 2%.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the analytical procedure parameters [pH (±0.2), Flow rate (±0.2 ml) and proportion of mobile phase (±2.0 v/v]. The standard deviation of the peak is calculated for each parameter and the %RSD was found to be less than 2%. Results are shown in Table 3.

Force Degradation Study

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acidic, alkaline, oxidative, thermal and sunlight degradations.

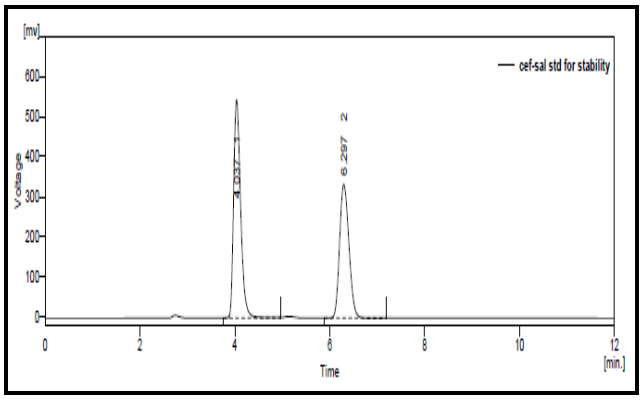

Figure 6: Chromatogram of combined CFTX and SLB standard mixture and blank at 277nm

Acidic Degradation

Forced degradation in acidic media was performed by keeping the standard solution in contact with 0.1N HCl for 4hour at room temperature. After 4hour the solution was neutralized with 0.1N NaOH and solution was diluted up to 10ml with mobile phase. Dilution was done to achieve the appropriate concentration 20µg/ml of CFTX and10 µg/ml of SLB. Result is shown in Figure 7.

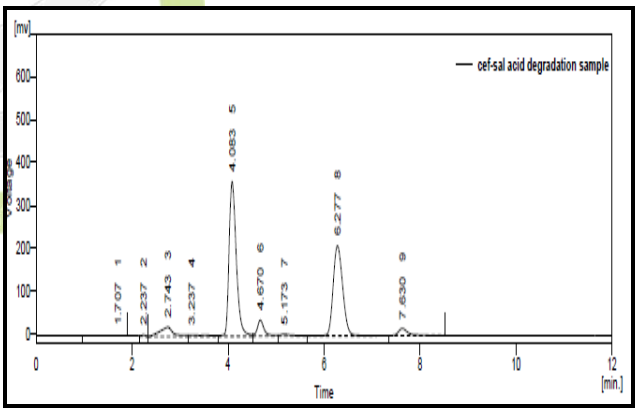

Figure 7: Chromatogram of combined CFTX and SLB mixture in acid degradation (0.1 N HCl, 4 hour)

Alkaline Degradation

Forced degradation in basic media was performed by keeping the standard solution in contact with 0.1N NaOH for 3 hour at room temperature. After 3hour the solution was neutralized with 0.1N HCl and solution was diluted up to 10ml with mobile phase. Dilution was done to achieve the appropriate

concentration 20µg/ml of CFTX and 10 µg/ml of SLB. Result is shown in Figure 8.

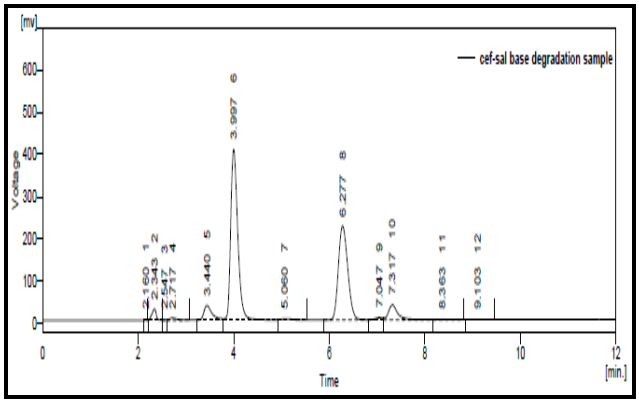

Figure 8: Chromatogram of combined CFTX and SLB mixture in base degradation (0.1 N NaOH, 3 hour)

Oxidative Degradation

Forced degradation in 3% H_2O_2 media was performed by keeping the standard solution in contact with 3% H_2O_2 for 1hour at room temperature. After 1hour, solution was diluted with mobile phase upto 10ml to achieve the appropriate concentration 20µg/ml of CFTX and 10 µg/ml of SLB. Result is shown in Figure 9.

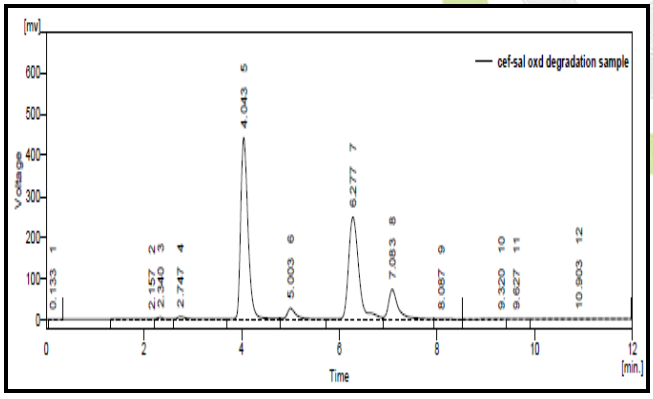

Figure 9: Chromatogram of combined CFTX and SLB mixture in oxidative degradation (3 % H_2O_2, 1 hour)

Thermal Degradation

Sample solution was exposed to temperature of 105°C for half an hour in an oven. Then after solution was diluted with mobile phase upto 10ml. From this solution, dilution was done to achieve the appropriate concentration 20 µg/ml of CFTX and 10 µg/ml of SLB. Result is shown in Figure 10.

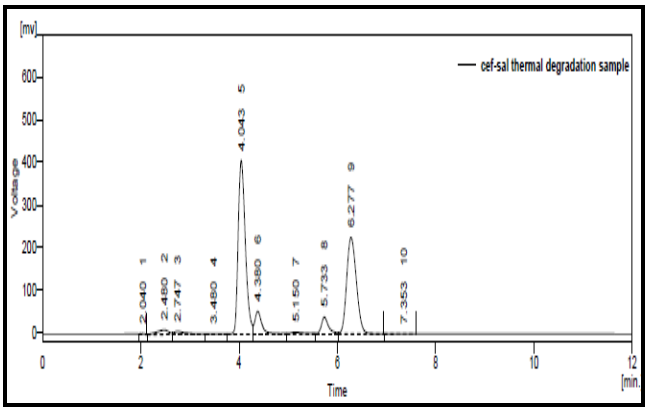

Figure 10: Chromatogram of combined CFTX and SLB mixture in thermal degradation at 277nm (105 °C, 30 min)

Sunlight Degradation

Sample solution was exposed in the sunlight for 2 hours. After 2 hour solution was diluted with mobile phase upto 10ml. From this solution, dilution was done to achieve the appropriate concentration 20 µg/ml of CFTX and 10 µg/ml of SLB. Result is shown in Figure 11.

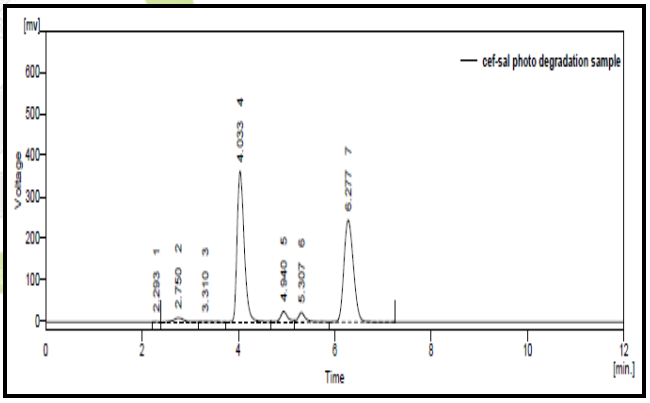

Figure 11: Chromatogram of combined CFTX and SLB mixture in sunlight degradation (2hours)

RESULTS AND DISCUSSION

Validation Parameters

The method was validated in compliance with ICH guidelines[12].

Force Degradation Studies

In the present investigation the CFTX and SLB were subjected to its stability studies as per ICH guideline[12]. The results of the forced degradation study of CFTX and SLB are summarized in Table 4 & 5**.**

Table 3: Regression analysis data and summary of validation parameter for the proposed method

Parameters		**CFTX**	**SLB**
Linear Range (n=3) (µg/ml)		10-30	5-15
Regression Equation		Y=226.5x - 48.325	Y=395.88x - 160.47
Slope		226.5	395.88
Intercept		48.325	160.47
Co-relation Co-efficient (R^2)		0.999	0.998
LOD (µg/ml)		0.694744981	0.828305172
LOQ (µg/ml)		2.105287822	2.510015672
Recovery (%)		99.56-99.61	99.52-99.93
Repeatability (% RSD NMT 2)		0.5179	0.8551
Intra-day (n=3) Precision (% RSD NMT 2)		0.48-1.15	1.11-1.37
Inter-day (n=3) Precision (% RSD NMT 2)		0.29-0.78	0.8-1.7
Robustness	pH (± 0.2)	0.867	0.931
	Flow rate (± 0.2 ml)	0.918	1.598
	Mobile phase Ratio (± 2 ml)	1.053	1.276
% Assay		98.60	100.98

n = number of replicate inject

Table 4: Results of forced degradation study of CFTX

Stress Conditions	**Time (hour)**	**Retention Time (min)**	**Area**	**% Area**	**Degradants (% area)**
0.1 N HCl	4	4.063	3847.95	80.83	30.87
0.1 N NaOH	3	3.993	4244.73	86.29	23.74
3 % H_2O_2	1	4.057	4707.07	84.56	15.43
Heat exposure	0.30	4.037	3910.24	70.24	29.75
Sunlight exposure	2	4.030	3864.68	69.12	30.57

Acid Degradation

CFTX and SLB were found to undergo acid degradation very rapidly. The reaction was occurred in 0.1 N HCl for 4 hours. showed extensive degradation for CFTX and SLB with additional peaks. % Degradation is higher. So, CFTX and SLB are acid labile in nature. Results are shown in Table 4 & 5.

Alkaline Degradation

CFTX and SLB were found to undergo base degradation very rapidly. The reaction was occurred in 0.1 N NaOH for 2 hrs 28 min. showed extensive degradation for CFTX and SLB with additional peaks. % Degradation is higher. So, CFTX and SLB are basic labile in nature. Results are shown in Table 4 & 5.

Oxidative Degradation

CFTX was found to undergo oxidative degradation very rapidly. The reaction with 3 % hydrogen peroxide showed degradation for CFTX and SLB with additional peaks. % degradation was higher in 3 % hydrogen peroxide. % Degradation for CFTX was about 15.44 % at 1 hour. % Degradation for SLB was about 18.64 % at 1 hour. So, CFTX and SLB are labile in nature for hydrogen peroxide. Results are shown in Table 4 & 5.

Thermal Degradation

CFTX and SLB were found to undergo thermal degradation very rapidly. The reaction with 105°C for 24 min showed extensive degradation for CFTX and SLB with additional peaks. % Degradation was higher in 105°C. So, CFTX and SLB are labile nature in thermal condition. Results are shown in Table 4 & 5.

Sun-Light Degradation

CFTX and SLB were found to undergo sunlight degradation very rapidly. The reaction for 2 hours showed extensive degradation for CFTX and SLB with additional peaks. % Degradation is higher. So, CFTX and SLB are labile nature in sunlight condition. Results are shown in Table 4 & 5.

Table 5: Results of forced degradation study of SLB

Stress Conditions	Time (hours)	Retention Time (min)	Area	% Area	Degradants (% area)
0.1 N HCl	4	6.273	2889.94	63.52	36.47
0.1 N NaOH	3	6.273	3116.52	68.50	31.50
3 % H_2O_2	1	6.273	3700.98	81.35	18.64
Heat exposure	0.30	6.273	2918.40	64.14	35.85
Sunlight exposure	2	6.273	3296.15	72.45	27.54

CONCLUSION

The isocratic HPLC method developed for the analysis of CFTX and SLB in their pharmaceutical preparations is simple, rapid and economic with less run time. The method has been validated and it has been shown that it is reliable, linear, accurate and precise as well as robust with minor variations in chromatographic parameters. Therefore, it can be applied for both routine analytical and quality control assay and it could be a very powerful tool to investigate stability of CFTX and SLB.

ACKNOWLEDGEMENT

Authors are grateful to Intracin Pharmaceuticals Pvt Ltd., Nadiad, Gujarat, India for providing gift sample and marketed formulation.

REFERENCES

1. Adiki, S. K., Arebi, A. A. & Melad, F. M. (2014). Development and validation of UV and RP-HPLC methods for the estimation of ceftriaxone sodium in pharmaceutical formulations. *Journal of Pharmaceutical Science and Innovation*, *3*(1), 87-90.
2. *Indian Pharmacopoeia, Volume II*, the Indian Pharmacopoeia Commission, Ghaziabad, 6th Edition, 1024-1025, 2010.
3. *British Pharmacopoeia. 6th Edition,* the Stationary Office, London Medicines and Healthcare Product Regulatory Agency, 408-409, 2009.
4. *United States Pharmacopoeia, Asian Edition*, the United States Pharmacopoeial Convention, Rockville, USA, 1697-1698, 2011.
5. *British Pharmacopoeia. 6th Edition.* The Stationary Office, London Medicines and Healthcare Product Regulatory Agency, 1938-1939, 2009.
6. Approval date of Ceftriaxone and Sulbactum. October 2011, cdsco.nic.in/new%20drug%20update.doc
7. Patel, F. M., Dave, J. B., & Patel, C. N. (2012). Spectrophotometric method for simultaneous estimation of cefuroxime sodium and sulbactum sodium in injection. *International Journal of Pharmaceutical Science and Research*, *3*(9), 3513-3517.
8. Rao, K. S., Rao, M. E., Patro, S. S., & Patnaik, A. K. (2008). Development and validation of new analytical method for ceftriaxone sodium in bulk and pharmaceutical dosage forms. *International Journal of Research and Reviews in Pharmacy and Applied Science*, 2, 556-565.
9. Gandhimathi, M., Saravanakumar, M., & Ravi, T. (2010). Validated iron pair HPLC method for simultaneous estimation of ceftriaxone sodium and Tazobactum sodium in dosage form. *International Journal of Pharmaceutical Bio Science*, 1, 17-22.
10. Shah, J., Rasul Jan, M., Shah, S., & Khan, N. (2013). Development and validation of HPLC method for simultaneous determination of ceftriaxone and cefaclor in commercial formulation and biological samples. *Journal of Mexican Chemistry and Soc, 57,* 314- 320.
11. Mahgoub, H. & Aly, F. M. (1998). UV-spectrophotometric determination of ampicillin sodium and sulbactum sodium in two-component mixtures. *Journal of Pharma and Biomed Analysis*, 1273-1278.
12. *The International Conference on Harmonization (ICH)*, Q1A (R2), Q1B, Q1C and Q2 (R1). Validation of Analytical Procedure: Text and Methodology: 2005.

