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A Stability-Indicating HPLC Method for Cefoperazone

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Abstract

Stability indicating HPLC assay method for the standard drug cefoperazone was developed using reverse phase appurassil C-18, 250 X 4.6mm. 5µm column, in the mobile phase phosphate buffer (pH 6.8) and methanol (5:2) at flow rate 1mLmin^{-1} with UV detection at 254 nm. The retention time was found to be 2.67 min. Validation of an analytical method was established by laboratory studies. The proposed method was found to be linear at concentration of 1 to 10 µgmL^{-1} (R^2=0.9899). The limit of detection and limit of quantification was 0.2 µgmL^{-1} and 0.4 µgmL^{-1} respectively and the method was found to be specific. Method precision and precision of the system was found to be within the limits of the acceptance criteria. Relative Standard deviation for precision of the method and precision of the system was found to be 0.49% and 0.7451% respectively. The percentage recovery ranges from 95 –106 % The results indicate that there is no interference from excipients for the proposed method, thus making the method more simple, less time consuming and suitable for routine quantitative estimation of cefoperazone sodium injection formulation. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.

Keywords:

Cefoperazone; stability-indicating assay; reversed-phase HPLC

1. Introduction

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods [1]. Stability testing of an active substance or finished product provide evidence on how the quality of a drug substance or drug product varies with time influenced by a variety of environmental factors such as temperature, humidity and light. Knowledge from stability studies enables understanding of the long-term effects of the environment on drugs. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product. Results are applied in developing of manufacturing processes, selecting proper packaging, storage conditions, product's shelf life and expiration dates [2, 3, 4]. Because the distribution environment is highly variable, products must be distributed in a manner that ensures the product quality will not adversely affected. The effect of possible temperature and humidity fluctuations, outside of labeled storage conditions, during transportation of drug products, can be evaluated on the basis of the stability analysis for that drug [4,5].

Cefoperazone has been found to be determind by High performance liquid chromatography (HPLC) [6], ESR spectroscopy [7], Electrochemical [8], Thin layer chromatography [9], colorimetric and AAS [10] according to the literature. But there is no stability indicating method discussed for cefoperazone. The aim of the present study was to

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establish inherent stability of cefoperazone through stress studies under variety of ICH recommended test conditions [2] and to develop stability indicating assay [11].

Cefoperazone sodium [12] is 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-{[[[(4-ethyl-2,3-dioxo-1-piperazinyl)carbonyl]amino](4-hydroxy-phenyl)acetyl]amino}-3-[[(1-methyl-1*H*-terazol-5-yl)thio]methyl]-8-oxo-,monosodium salt with molecular formula $C_{25}H_{26}N_9NaO_8S_2$. It is a 3rd generation cephalosporin, active against gram negative bacteria but less active against gram positive bacteria. Beta-lactums came with the revelation that modified cephalosporin have much wider activity than penicillin with less toxicity and better stability [13].

2. Experimental

2.1 Materials

Pure cefoperazone was obtained as gift samples from Karnataka Antibiotics Pharmaceuticals Ltd, Bangalore, India. Cefoperazone Injection was purchased from local market. HPLC grade methanol was purchased from Qualigens fine chemicals, Mumbai, India Was used for preparing the mobile phase Millipore water was obtained from the Millipak 0.22µm filter. Buffer materials and all other chemicals used were of analytical-reagent grade.

2.2. Instrumentation and chromatographic conditions

The HPLC system used was 10AT Shimadzu- SPD10A model with UV detector, the column used was accuracil C-18 column (250 mm × 4.6 mm i.d., 5 µm). The separation was carried out under isocratic elution with phosphate buffer (pH 6.8) and methanol in the ratio of 5:2 (v/v) as the mobile phase and the injection volume was 100 µL. The flow rate was 1 $mLmin^{-1}$, the column temperature was ambient and the elutents were monitored at wavelength 254 nm.

2.3. Preparation of standard and sample solutions

2.3.1. Standard preparation

Standard stock solutions of 1 $mgmL^{-1}$of cefoperazone in mobile phase were prepared in 10 mL volumetric flask. Working solutions were prepared by diluting the stock solutions with the mobile phase to contain 0.1–10 $\mu gmL^{-1}$of standard cefoperazone. These solution were used to obtain the calibration graph.

2.3.2. Sample preparation

Cefoperazone sodium injection powder of 250 mg was dissolved in 2 mL water for injection; from this solution working sample solution of 2 μgmL^{-1} was prepared with the mobile phase in 10 mL volumetric flask and filtered through a 0.22 µm nylon filter.

2.4. Degradation studies

Standard cefoperazone at a concentration of 1 mg mL^{-1} was used in all the degradation studies. The standard cefoperazone were subjected to stress conditions in 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} NaOH, at the temperature 80 °C at different time intervals, after completion of the degradation processes, the solutions were neutralized and diluted with mobile phase. For the neutral studies standard cefoperazone of 1 mg mL^{-1} was dissolved in water and subjected to stress condition at the temperature 80 °C at different time intervals. Thermal degradation were performed by exposing solid standard drug to dry heat at 50 °C for 45 days. Further 0.1 mL of

these degraded solutions were withdrawn periodically and subjected to analysis after suitable dilution with mobile phase to get 10 μgmL^{-1}. 100 µL of this degraded solution were injected using the same chromatographic conditions.

2.5. Method validation

The analytical method validation was carried out as per ICH method validation guidelines [11]. The validation parameters addressed were specificity, precision (inter-day and intra-day), linearity, accuracy, and limit of detection, limit of quantitation, robustness and stability of cefoperazone in mobile phase. Standard plots were constructed for cefoperazone in the range of 0.1–10 μgmL^{-1}. Accuracy was determined by fortifying the mixture of pre-analysed standard of three known concentrations of the drugs with the marketed sample.

3. Results and discussion

3.1. Development and optimization of the stability-indicating HPLC method

An isocratic method was found necessary to optimize the separation of major degradation products formed under various stress conditions. The best resolution was achieved with initial run of phosphate buffer (pH 6.8) and methanol in the ratio of 3:1 (v/v) at the flow rate of 1 $mLmin^{-1}$, the retention time was observed at 6.22 min. The mobile phase ratio was changed to 5:2 (v/v) at the same flow rate, a retention time 2.68 min was obtained. The method worked well with the mixture of degradation solutions and was even applicable to injection formulations.

3.2. Degradation behavior

HPLC studies on the combination under different stress conditions indicated the following degradation behaviour (Table 1).

Table 1. Degradation study of Cefoperazone

Stress Conditions	Degradation Time	Area of Peak	% Degradation	% of active drug present after degradation
Standard Drug	-	1683293	-	-
Acidic	10 min	566169	48.44	51.55
Alkaline	10 min	88225	93.14	6.85
Neutral	20 min	598046	45.11	44.88
Thermal	46 h	178869	2.28	97.7

3.2.1. Acidic condition

The standard drug cefoperazone at 80^{o}C in 0.1 M HCl was found to be degrade 48.44% at 10 min. The chromatogram showed major degradation product at retention time (RT) 1.81 min with cefoperazone peak at 2.61 min (Fig. 1).

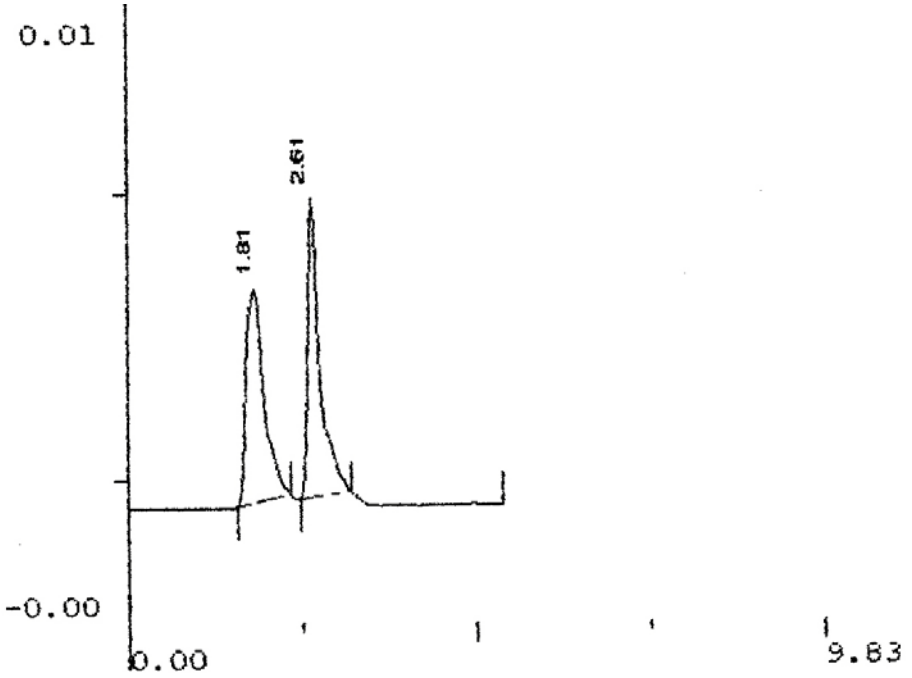

Fig. 1. Chromatogram of degraded Standard solution of Cefoperazone under acidic various conditions.

3.2.2. Degradation in alkali

The drug was found to be highly degrade in alkaline hydrolysis of 0.1 M NaOH at 80^{o}C and degraded to an extent of 93.14% within 10 min. The major degradation peak at RT 1.83 min was identified (Fig.2).

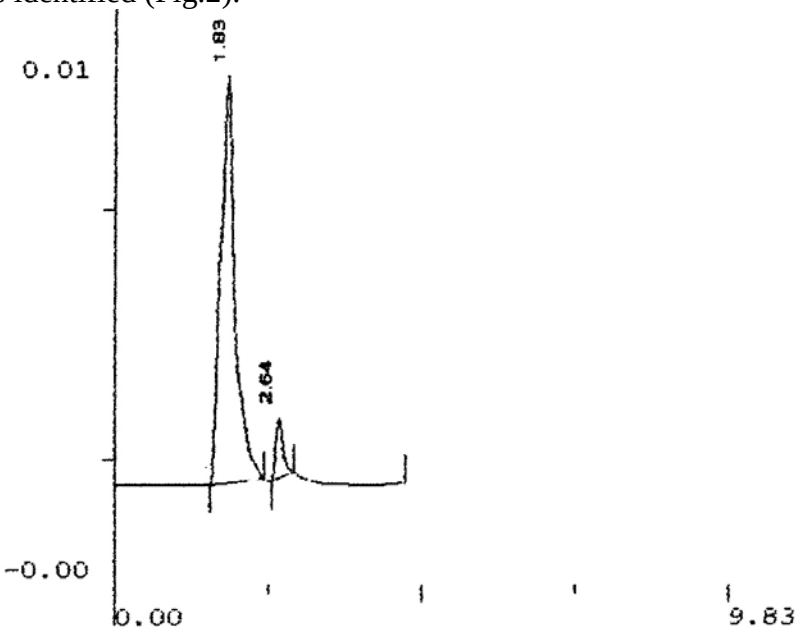

Fig. 2. Chromatogram of degraded Standard solution of Cefoperazone under alkaline condition

3.2.3. Neutral degradation

The degradation drug was found to be highly labile to neutral medium (water) at 80^{o}C and 45.11 % of the drug decomposed in 20 min, the major degradation products appeared at RT 1.83, 2.08. After 30 min 78.52% of the drug was degraded. (Fig.3).

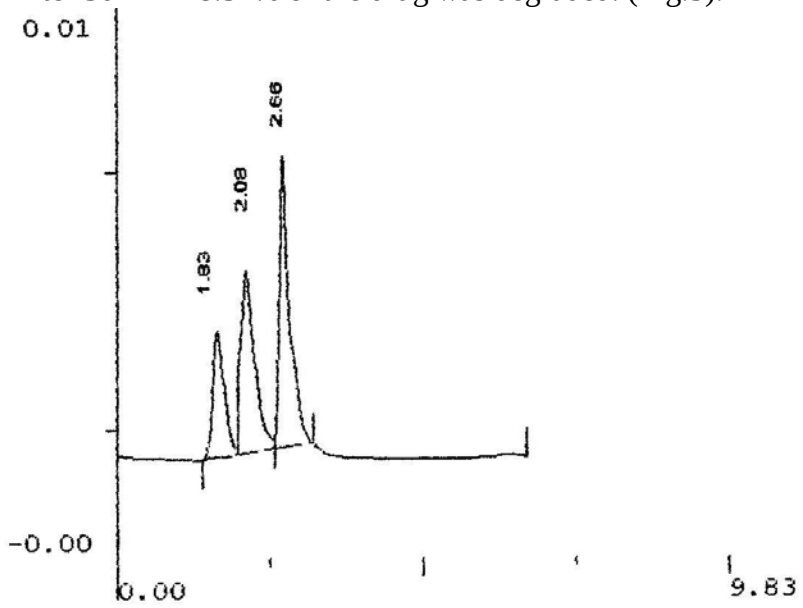

Fig.3. Chromatogram of degraded Standard solution of Cefoperazone under neutral condition

3.2.4. Thermal degradation

In thermal degradation, negligible degradation was seen on subjecting the drug to dry heat at 50°C for 45 days.(Fig.4).

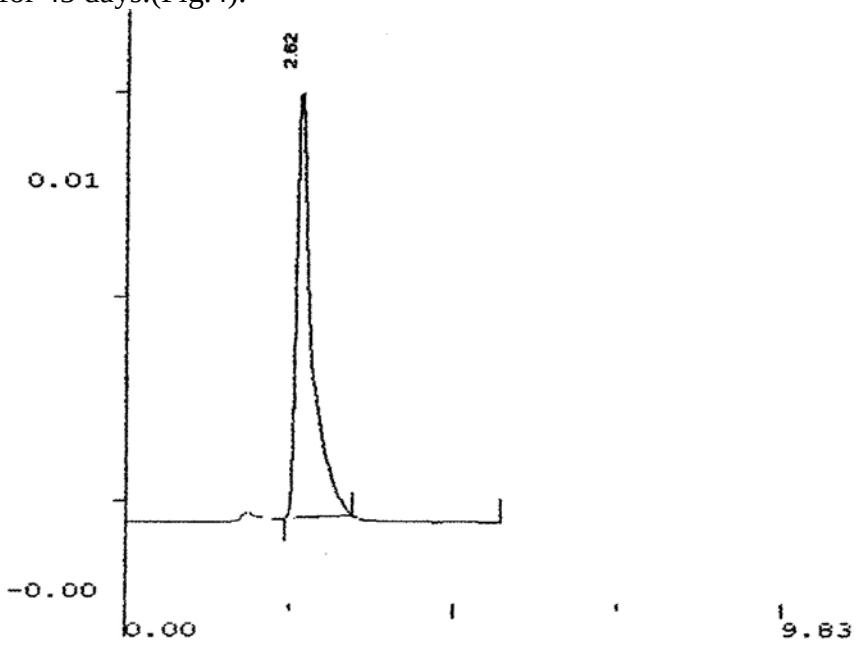

Fig. 45. Chromatogram of degraded Standard solution of Cefoperazone under thermal condition at 48 days

3.3. Validation of method

3.3.1 System suitability

A system suitability test was performed to evaluate the chromatographic parameters (number of theoretical plates, asymmetry of the peak) before the validation runs. Three replicate injections of the standard solution and three injections of the solution prepared for the specificity procedure were used. The retention time of cefoperazone was found to be 2.68 min. Efficiency and tailing factor at 5% height of the main peak were determined giving the following data, N = 71.99, tailing factor = 1.

3.3.2. Linearity

Six-point calibration curves were obtained in a concentration range from 1 to 10 μgmL^{-1} for cefoperazone, three independent determinations were performed at each concentration. The response for the drug was linear and the calibration equation was y = 123835 x-56623 with R^2 = 0.9899. The linearity curve of cefoperazone is shown in Fig. 5.

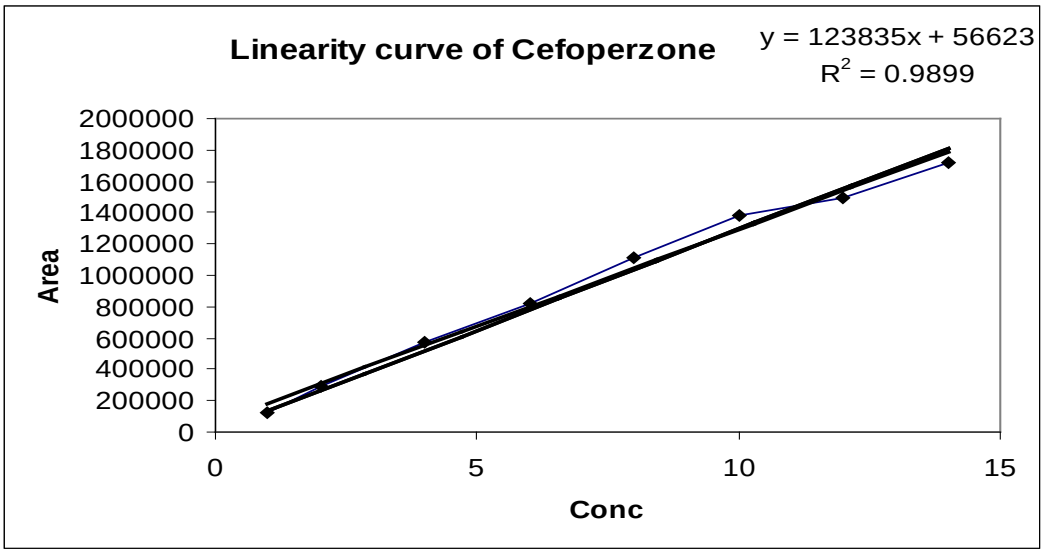

Fig. 5. Linearity Curve for Cefoperazone

3.3.3. Limit of detection (LOD) and limit of quantitation (LOQ) for Cefoperazone

The Limit of Detection and Limit of Quantitation for cefoperazone was found to be 0.2 μgmL^{-1} and 0.4 $\mu g\ mL^{-1}$ respectively.

3.3.4. Accuracy

The percentage recovery ranges from 95–106%. The results show that there is no interference from excipients for the proposed method, thus making the method simple, less time consuming and suitable for routine quantitative estimation of cefoperazone sodium injection formulation. The recovery study data is given in Table 2.

3.3.5. Precision

Method precision and precision of the system was found to be within the limits of acceptance criteria. For the analytical method and system precision the standard deviation was

found to be 4546 and 11301 respectively, relative standard deviation was found to be 0.49% and 0.7451% respectively. The data for inter and intra day precision is given in Table 3.

Table 2. Recovery study data of Cefoperazone

SI No.	Std Cefoperazone μg-mL^{-1}	Sample Cefoperazone μg-mL^{-1}	Total Conc.	Peak area	Total amount from standard graph μg-mL^{-1}	Recovery of standard	% Recovery of standard
1	2	2	4	541729	3.9	1.9	95
2	4	2	6	801934	5.9	3.9	97.5
3	6	2	8	1116003	8	6	106

Table 3. Inter and intra-day precision

Actual conc. (μg-mL^{-1})	**Intra-day Measured conc.**			**Inter-day Measured conc.**		
	(μgmL^{-1})	**± S.D.**	**R.S.D.% (n = 3)**	**(μgmL^{-1})**	**± S.D.**	**R.S.D.% (n = 3)**
1	0.90	2580.6	1.8153	0.9	915.36	0.6509
2	2	5407.6	1.9191	2	2653.48	0.9329
4	3.9	9533.87	1.7236	3.9	7521.84	1.3516
6	5.7	4423.42	0.55	5.6	9999.57	1.2570
8	7.7	19281.07	1.6507	8.2	22871.14	1.9843
10	10.2	8774.35	0.6146	10.1	14865.61	1.0525

3.3.6. Ruggedness

The ruggedness was established by determining cefoperazone using the same chromatographic system and the same column by two analysts on a different day. The assay result indicated that the method was capable with high precision. Additionally, good separations were always achieved which suggested that the method was selective for all components under the test.

3.3.7. Solution Stability

The stability of solution under study was established by keeping the solution at room temperature for 24 hr. The result showed no significant change in concentration and thus confirms the stability of the drug in the solvent used for the analysis.

4. Conclusion

This study presents a simple and validated stability-indicating HPLC method for estimation of cefoperazone in the presence of degradation products. The developed method is specific, accurate, precise and robust. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrating that the developed method was specific and stability-indicating**.** The method could be applied with success even to the analysis of marketed products cefoperazone injection formulation, as no interference was observed due to excipients or other components present.

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References

1. FDA (1998) Draft Guidance for Industry: Stability Testing of Drug Substances and Drug Products, FDA. Rockville, MD.
2. International Conference on Harmonisation (2000) Guidance for Industry, Q1A(R2): Stability Testing of New Drug Substances and Products, IFPMA. Geneva.
3. Grimm W, Carstensen J T and Rhodes C T (2000) Drug Stability, Principles and Practices, Marcel Decer, Inc. New York, p 415.
4. Dean D A, Carstensen J T and Rhodes C T (2000) Drug Stability, Principles and Practices, Marcel Decer, Inc. New York, p 515.
5. ICH Harmonized Tripartite Guideline (2003) Q1F: Stability Data Package For Registration Applications in Climatic Zones III and IV, London.
6. Steven A S, Thomas M F and James S T (1984) High Pressure Liquid Chromatographic Method for Analysis of Cephalosporins. Antimicrob. Agents Chemother. 26:652.
7. Basly J P, Basly I and Bernard M (1998) ESR spectroscopy applied to the study of the pharmaceuticals radiosterilization; cefoperazone. J. Pharm. Biomed. Anal.18:93.
8. Ogorevc B, Gomi S (1991) Electrochemical analysis of cephalosporin antibiotics. J. Pharm. Biomed. Anal. 9:225.
9. Nabi S A, Laiq E and Islam A (2004) Selective Separation and determination of Cefalosporins by TLC on Stannic Oxide Layers. Acta. Chromatographica. 14:92.
10. Salem H, Askal H (2002) Colourimetric and AAS determination of cefalosporins using Reineck's salts. J. Pharm. Biomed. Anal. 29:347.
11. www.statisticaloutsourcingservices.com/methval
12. USP-27-NF (2004) The United States Phamacopoeial Convection Inc. Rockville, MD, p. 336.
13. Deshpande AD, Baheti KG and Chatterjee NR (2004) Degradation of β-lactum antibiotics. Current Sci. 87: 1684.
14. Bakshi M, Singh S (2004) HPLC and LC-MS studies on stress degradation behaviour of tinidazole and development of a validated specific stability- indicating HPLC assay method. J. Pharm. Biomed. Anal. 26:1011.
15. Xu QA, Trissel LA (2003) Stability- Indicating HPLC Methods for Drug Analysis, Pharmaceutical Press. London, p 106.
16. Martinez L G, Falco P C, Cabeza A S (2002) Comparison of several methods used for the determination of Cephalosporins. Analysis of cefalexin in pharmaceutical samples. J. Pharm. Biomed. Anal. 29: 405.
17. Al-Rawithi S, Hussein R, Raines D A, AlShowaier I and Kurdi W (2001) Sensitive assay for the determination of cefazolin or ceftriaxone in plasma utilizing LC. J. Pharm. Biomed. Anal. 22: 281.

18. Sorensen L K, Snor L K (2000) Determination of cefalosporins in raw bovine milk by high-performance liquid chromatography. J. Chromatogr A. 882:145.

19. Signs S A, File T M and Tan J S (1984) High-pressure Liquid Chromatographic Method for Analysis of Cefalosporins. Antimicrob. Agents Chemother. 26:652.

20. Joshi S (2002) HPLC separation of antibiotics present in formulated and unformulated samples. J. Pharm. Biomed. Anal. 28:795.