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Research Article

Stability-Indicating Reverse Phase HPLC Method for the Determination of Cefazolin

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Abstract

Purpose: The aim of the present study was to establish the inherent stability of cefazolin through stress studies under a variety of ICH recommended test conditions and, also to develop a stability indicating assay.

Methods: A stability-indicating HPLC assay method was developed and validated for cefazolin using an isocratic RP-HPLC method which employed an SS Wakosil II- C₁₈ column (250 mm × 4.6 mm i.d., 5 μm) with a mobile phase consisting of phosphate buffer (pH 6.8) and methanol (5:2 v/v), and UV detection at 254 nm at a flow rate of 1 ml/min. The stress testing of cefazolin was carried out under acidic, alkaline, neutral, oxidation and thermal conditions.

Results: The drug peak was well resolved from the peaks of the degradation products. The proposed method was validated for sensitivity, selectivity, linearity, accuracy, precision and solution stability. From the degradation studies it was found that the drug was thermally stable but unstable in acidic, alkaline, neutral and oxidative conditions. The response of drug was linear in the concentration Range of 1 - 50 μg/ml with the number of theoretical plates, and tailing factor being 1341 and 1, respectively. Limit of detection and limit of quantification were 0.1 and 0.2, μg/ml respectively while recovery ranged from 95 - 100%. Method precision and precision of the system were within the limits of acceptance criteria.

Conclusion: This study presents a simple and validated stability-indicating HPLC method for the estimation of cefazolin in the presence of degradation products. The developed method is specific, accurate, precise and robust. All the degradation products formed during forced degradation studies were well separated from the analyte peak.

Keywords: Cefazolin; Stability-indicating assay; Reversed-phase HPLC

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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 re-test or expiration dating periods [1]. Stability testing of an active substance or finished product provides evidence on how the quality of a drug substance or drug product varies with time. It is 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 excipients in the drug product. The results are applied in developing manufacturing processes and selecting proper packaging, storage conditions, product's shelf life and expiration dates [2-4]. Because the distribution environment is highly variable, products must be distributed in a manner that ensures product quality will not be adversely affected. The effect of possible temperature and humidity fluctuations, outside of labelled storage conditions, during transportation of drug products can be evaluated on the basis of the stability analysis of the drug [4,5].

Determination of cefazolin by various methods has been described but the stability of cefazolin has only been evaluated with electrophoresis [6], calorimetry [7] and gamma radiations [8]. A number of high performance liquid chromatographic (HPLC) methods have been developed for the cephalosporins in plasma [9-13] but only a few stability-indicating HPLC methods for drug analysis has been reported [14,15]. The aim of the present study was to establish the inherent stability of cefazolin through stress studies under a variety of ICH-recommended tests conditions [2] and

to develop a stability-indicating assay for the drug [16].

Cefazolin sodium [17] is (6R, 7R)-3-(((5-Methyl-1, 3,4-thiadiazol-2-yl) thio -methyl)-8-oxo-7-2-(1H-tetrazol-1-yl) acetamido)-5-thia-1-azabicyclo (4.2.0) oct-2-ene-2-carboxylate with molecular formula $C_{14}H_{14}N_8O_4S_3$. It is a first generation cephalosporin and it inhibits cell wall biosynthesis. Cephalosporins are bactericidal and have the same mode of action as other beta-lactam antibiotics (such as the penicillins). Modified cephalosporins have a much wider activity than the penicillins, and also show less toxicity and better stability [18].

EXPERIMENTAL

Materials

Pure cefazolin was received as a gift from Karnataka Antibiotics Pharmaceuticals Ltd, Bangalore, India. Cefazolin injection (Azolin Injection, 500mg, Biochem-Pharmaceutical Industries, Mumbai, India) was purchased from local drug market. HPLC grade methanol was purchased from Qualigens Fine Chemicals, Mumbai, India. Millipore water was obtained with Millipak 0.22 μ m filter. Buffer materials and all other chemicals were of analytical-reagent grade.

Instrumentation and chromatographic conditions

The HPLC system consisted of model 10AT Shimadzu- SPD10A detector an SS Wakosil II- C_{18} column (250 mm \times 4.6 mm i.d., 5 μ m). Separation was carried out by isocratic elution with a mixture of phosphate buffer (pH 6.8) and methanol in the ratio of 5:2 (v/v) at a flow rate of 1 ml/min. The detector was monitored at 254 nm and the injection volume was 100 μ l.

Preparation of standard and sample solutions

Standard preparation: A standard stock solution of 1 mg/ml of cefazolin in the mobile phase was prepared in a 10 ml volumetric flask. Working standard solutions were prepared by diluting the stock solution with the mobile phase to obtain cefazolin concentrations ranging from 0.1 – 100 µg/ml.

Sample preparation: Cefazolin sodium injection of 500 mg was dissolved in 2 ml water for injection and from this solution, a working sample solution of 2µg/ml was prepared with the mobile phase in a 10 ml volumetric flask and filtered through a 0.22 µm nylon syringe filter.

Degradation studies

Standard cefazolin with a concentration of 1 mg/ml was used in all the degradation studies. Standard cefazolin solutions were subjected to stress conditions in 0.1N HCl and 0.1N NaOH at of temperature of 80 °C at time intervals. After completion of the degradation processes, the solutions were neutralized and diluted with mobile phase. For neutral studies, standard cefazolin of 1 mg/ml was dissolved in water and subjected to stress conditions at a temperature of 80 °C at time intervals. For the oxidative studies, the standard cefazolin solution was subjected to stress conditions in 3 and 30% hydrogen peroxide at room temperature for 30 min. After the solutions had been subjected to the various stress conditions, 0.1 ml of the degraded solutions were withdrawn at the various intervals and subjected and analysed after suitable dilution to 10 µg/ml with mobile phase. The chromatographic analysis of the degraded solutions (100 µl each) were carried out under the same chromatographic conditions.

Method validation

Analytical method validation was carried out as per ICH method validation guidelines [16].

The validation parameters addressed were specificity, precision (inter-day and intra-day), linearity, accuracy, limit of detection, limit of quantitation, robustness and stability of cefazolin in mobile phase. A standard plot was constructed for cefazolin in the range of 0.1 – 50 µg/ml. Accuracy was determined by fortifying the mixture of pre-analysed standard of three known concentrations of the drugs with the marketed sample.

RESULTS

Development and optimization of the stability-indicating HPLC method

An isocratic method was found suitable to optimise the separation of major degradation products formed under various stress conditions. The best resolution was achieved with an initial run of a mixture of phosphate buffer (pH 6.8) and methanol in the ratio of 5:2 (v/v) at a flow rate of 1 ml/min; the retention time was observed to be 5.72 min.

Degradation behavior

HPLC data on samples (powder and solution combination) obtained on stress testing (alkaline, acidic, neutral, thermal and oxidative conditions) are indicated in Table 1.

Acidic conditions

The standard drug cefazolin at 80 °C in 0.1 M HCl was degraded in 5 min. Within 10 min 87.5 % drug was degraded. The chromatogram showed major degradation products at retention times (RT) of 2.96 min and 3.93 min with the standard peak at 5.9 min.

Alkali conditions

The drug was highly degraded in 0.1 M NaOH at 80 °C, being degraded within 10 min. Three major degradation peaks at RT of 2.00, 2.26, and 2.96 min were observed while

Table 1: Degradation data for cefazolin

Stress condition	Degradation time (min)	Area of peak*	Degradation (%)	Active drug present after degradation (%)
Standard drug	-	1683293	-	-
Acidic	10	108335	87.53	12.46
Alkaline	10	412316	61.88	38.10
Neutral	30	1088441	17.01	82.98
Neutral	60	746143	33.29	66.11
Oxidative (3% H ₂ O ₂)	20	1712823	70.33	29.88
Oxidative (30 %H ₂ O ₂)	30 min	393803	95.6	5.36
Thermal	36 hr	1180032	29.89	70.10

*Mean of three measurements

Table 2: Recovery data for cefazolin (n = 3)

Standard cefazolin (µg/ml)	Test cefazolin(µg/ml)	Total conc. (µg/ml)	Peak area*	Analysed total amount (µg/ml)	Recovery of standard (µg/ml)	Recovery of standard (%)
2	2	4	534115	3.9	1.9	95
4	2	6	761349	5.8	3.8	95
6	2	8	1102675	8	6	100

Neutral conditions

The degradation of standard cefazolin solution was highly labile in neutral medium (water) at room temperature (27 ± 2 °C) with 33.3 % of the drug decomposed in 60 min. The major degradation product appeared at RT of 2.50 min and the standard peak at 5.97 min.

Oxidative degradation

The degradation of cefazolin in 3 % hydrogen peroxide (oxidative conditions) at different time interval of 20 min was 29.9 %, giving rise to one degraded peak at RT of 2 min with the hydrogen peroxide and standard peaks occurring at RT of 2.98 and 5.90 min, respectively. In 30 % hydrogen peroxide, 95.6 % drug was degraded in 10 min with the degradation peak occurring at RT of 1.97 min. Hydrogen peroxide and

standard peaks occurred at 3.01 and 5.95 min, respectively. After 20 min, the drug was completely degraded.

Thermal degradation

Under thermal degradation conditions, standard cefazolin solution was stable for 24 h. exposition at 50 °C. After 48 h of thermal stress, 29.89 % of cefazolin was degraded, while 70.10 % (did not any degraded peaks) was recovered.

Validation of method

System suitability

The retention time of cefazolin was 5.67 min. Efficiency and tailing factor at 5 % of the height of the main peak were determined, giving $N = 1341.12$, and tailing factor = 1.

Linearity

Ten-point calibration curves were obtained in a concentration range of 1 to 50 µg/ml for cefazolin based on three independent determinations performed at each concentration. The response for the drug was linear and the calibration equation was $y = 291171x - 26955$ with $R^2 = 0.9989$.

Limit of detection (LOD) and limit of quantitation (LOQ) for cefazolin

The limit of detection and limit of quantitation for cefazolin were 0.1 and 0.3 µg/ml, respectively.

Accuracy

Recovery ranged from 95 – 100 %. The recovery study data are given in Table 2.

Precision

Method precision and precision of the system were within the limits of acceptance criteria. Assessment of the system precision showed that the relative standard deviation was 0.8033 and 0.5856 % for 4 and 2 mcg, respectively. The data for inter- and intra-day precision are given in Table 3.

Ruggedness

The ruggedness of the developed method was established by determining cefazolin in the same chromatographic system and the

same column by two analysts on different days. The assay result indicated that the method had a high precision. Additionally, good separation, effective separation of the drug from its degraded product was always achieved which suggests that the method was selective for all the components tested.

Solution stability

The stability of cefazolin solution was established by keeping the solution at room temperature for 24 h. The result showed no significant change in concentration, thus confirming the stability of the drug in the solvent used for the analysis.

DISCUSSION

In this study, the inherent stability of cefazolin was established based on tests utilising ICH recommended stress conditions. The drug degraded extensively in alkaline, acidic, neutral and oxidative conditions. Mild degradation was seen in thermal conditions without any degraded products. Drug peak was well resolved from the peak of its degradation product as well as those of the excipients in cefazolin sodium injection formulation. The developed method was simple, accurate, precise, specific, selective and rugged and, therefore, it should be suitable for the analysis of cefazolin and its degraded products of the drug product in the industry.

Table 3: Inter and intra-day precision

Actual conc. (µg/ml)	Inter-day measured conc. (µg/ml)		Intra-day measured conc. (µg/ml)	
	µg/ml	RSD (% , n = 3)	µg/ml	RSD (% , n = 3)
1	0.9	1.8153	0.9	0.6509
2	2.0	1.9191	2.0	0.9329
4	3.9	1.7236	3.9	1.3516
6	5.7	0.5500	5.6	1.2570
8	7.7	1.6507	8.2	1.9843
10	10.2	0.6146	10.1	1.0525

CONCLUSION

This study presents a simple and validated stability-indicating HPLC method for the estimation of cefazolin in the presence of its degradation products. The developed method is specific, accurate, precise and robust. All the peaks of the degradation products formed during forced decomposition studies were well separated from the analyte peak. This demonstrates that the developed method was specific and stability-indicating. The method was successfully applied to the analysis of a commercial cefazolin injection formulation, as no interference due to excipients or other components present was observed.

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