Oriental Journal of Chemistry Vol. **24**(1), 153-156 (2008)

Development and validation of RP-HPLC method for the determination of Cefazolin

N. LALITHA*, VARUN PAWAR, S.B. PURANIK, P.N. SANJAY PAI and G.K. RAO

Al-Ameen College of Pharmacy, Near Lalbagh Main Gate, Bangalore - 560 027 (India)

(Received: March 08, 2008; Accepted: April 12, 2008)

ABSTRACT

A RP-HPLC assay method has been developed and validated for estimation cefazolin. An isocratic RP-HPLC method developed on a SS Wakosil II- C_{18} column (250 mm × 4.6 mm i.d., 5 µm) with a mobile phase of phosphate buffer (pH 6.8) and methanol (5:2 v/v) and UV detection at 254 nm at flow rate 1 ml/min. The response of drug was linear form the conc. ranges 1-100 µg/ml the no. of theoretical plates and tailing factor were 3745.04 and 1 respectively. Limit of detection and Limit of quantification were found to be 0.1µg/ml and 0.2µg/ml respectively. The percentage recovery ranges from 96-105%. Method precision and precision of the system was within the limits of acceptance criteria. The method was found to be robust in changed chromatographic conditions. The established method can be applied to the assay of marketed cefazolin injection formulation.

Key words: Cefazolin; Reversed-phase HPLC.

INTRODUCTION

Cefazolin sodium[1] 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 1st generation cephalosporin, inhibits cell wall biosynthesis. In the literature survey determination of cefazolin was given by various methods viz HPLC,UV, electrophoresis[2], calorimetry[3], TLC[8], and gamma radiations[5]. The aim of the present study was to develop and validate simple, rapid HPLC method for estimation of as per ICH guidelines.

EXPERIMENTAL

Material

Pure cefazolin was obtained from Karnataka Antibiotics Pharmaceuticals Ltd, Bangalore, India. Cefazolin Injection was purchased from local market. HPLC grade methanol was purchased from Qualigens Fine Chemicals, Mumbai, India. Millipore water was obtained from the Millipak 0.22 µm filter. Buffer materials and all other chemicals were of analytical-reagent grade.

Instrumentation and chromatographic conditions

An HPLC system consisted model 10AT Shimadzu- SPD10A detector, the column used was a SS Wakosil II- 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). The flow rate was 1 ml/min. The wavelength was monitored at 254 nm and the injection volume was 100 µl.

Preparation of standard and sample solutions

Standard preparation

Standard stock solution of 1 mg/ml of cefazolin in mobile phase was prepared in 10 ml

volumetric flask. Working standard solutions of conc. ranging from 0.1–100 µg/ml were prepared from the stock solution with the mobile phase as a diluent.

Sample preparation

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

Method validation

The analytical method validation was carried out as per ICH method validation guidelines. The validation parameters addressed were specificity, precision, linearity, accuracy, and limit of detection, limit of quantitation, robustness and ruggedness.

RESULTS AND DISCUSSION

Development and optimization of the HPLC method

An isocratic RP-HPLC method was developed and validated. The retention time of cefazolin was observed at 5.7 min (Fig. 1), the method was found to be suitable for the assay of the marketed formulation and the percentage assay was 98.67 % w/w which lies with in the acceptable criteria.

Validation of the method

System suitability

System suitability parameters were calculated to evaluate the chromatographic efficiency of the method. HETP and tailing factor at 10 % height of the main peak were determined giving the following data, N = 3745.04, Tailing factor = 1.

Linearity

Linearity of cefazolin was obtained in a concentration range from 1 to 100 µg /ml for cefazolin; three independent determinations were performed for each concentration. The response for the drug was linear and the calibration equation was y = 291171x - 269557 with R^2 = 0.9989 (Fig.2).

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

The Limit of Detection and Limit of Quantitation for cefazolin was found to be 0.1 µg/ml and 0.2 µg/ml respectively.

Accuracy

Accuracy was determined by fortiying the mixture of pre analysed standard of three known conc. of the drugs with drugs with the marked samples. The percentage recovery ranges from 96–105%. The results showed no interference from excipients for the proposed method, thus making the method simple, less time consuming and suitable for routine quantitative estimation of cefazolin sodium injection formulation.

Precision

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

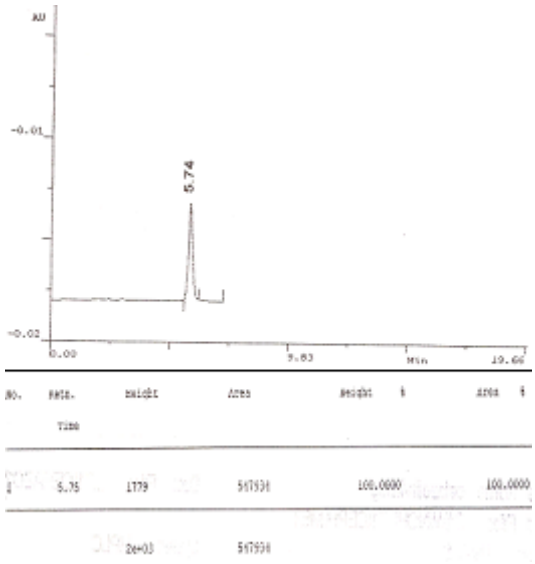

Fig. 1: Chromatogram of Standard Cefazolin

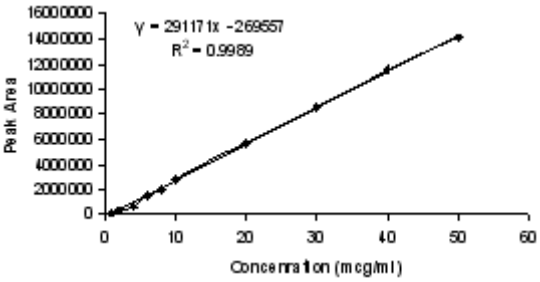

Fig. 2: Linearity Curve for Cefazolin

Table 1: Data of Robustness study of Cefazolin

Parameters for Robustness	Columns		Wavelengths (nm)		Organic phase conc. (Buffer:methanol)		Flow Rate (ml/min)	
	Wakosil	**Accuracil**	**256**	**252**	**4:1**	**9:1**	**0.8**	**1.2**
Peak height (mm)	40	36	36	37	34	41.5	36	36.5
Retention Time (mm)	45.5	46	45	45.5	49	44.5	35	51.5
Symmetry	1.16	1.33	1.33	1.33	1.4	2	1.5	1.5
Theoretical Plates	3745.04	3826.96	3661.14	3745.04	4343.36	4874.71	3014.49	6527.89
HETP (mm)	0.267	0.261	0.273	0.267	0.23	0.245	0.331	0.153
Peak Area	1557804	1556068	1485937	1486927	1440495	1536280	1417658	1407464
Conc. (mcg/ml)	6.27	6.26	6.02	6.03	5.87	6.20	5.79	5.75
% Assay	104.59	104.49	100.48	100.54	97.88	103.36	96.57	95.99

system precision the standard deviation was found to be 4399 and 1654 respectively, relative standard deviation was found to be 0.8033% and 0.856% respectively.

Ruggedness

The ruggedness was established by assaying cefazolin in the same chromatographic system and the same column by two analysts on a different day. The percentage assay of standard by two analysts was found to be 98.56% and 99.6%. The results indicated that the method was rugged.

Robustness

The robustness was established by assaying cefazolin in the different chromatographic parameters like columns, detection wavelength, flow rate and concentration of organic phase. The percentage assay of standard drug under changed chromatographic conditions was found be in the range of acceptance criteria (Table 1), hence the method was found robust.

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.

CONCLUSION

The study presents a reverse phase HPLC estimation of cefazolin with UV-detector. Method is simple, economical and less time consuming that the other prescribed methods. The method was validated and found specific, accurate, precise, rugged and robust. The method could be applied with success even to the analysis of marketed products cefazolin injection formulation, as no interference was observed due to excipients or other components present.

ACKNOWLEDGEMENTS

Highly thankful for Karnataka Antibiotics and Pharmaceuticals Ltd Bangalore, for providing the gift samples, and to all my research colleagues for their support in the work.

REFERENCES

1. USP-27-NF, The United States Phamacopoeial Convection Inc. Rockville, MD, 328 (2004).
2. Beverly, N., Brenda, C., Stephen, S., *J. Pharm. Biomed. Anal.* **14**: 73 (1995).
3. Pikal, M.J., Dellerman, K.M., *Int. J. Pharmaceu*. **50**: 233 (1989).
4. Bolos, J., Galindo, C., Gallardo, M. and Pico, A.R., *Int. J. Pharmaceu.* **40**: 175 (1987).
5. Jacobs, G.P., *Int. J. Pharmaceu.,* **17**: 29 (1983).
6. www.statisticaloutsourcingservices.com/methval