Asian Journal of Chemistry Vol. 16, Nos. 3–4 (2004), 1495–1499

Reverse Phase HPLC Determination of Cephalexin in Tablets

J.V.L.N.S. RAO*, G. HARITHA, K. VENUGOPAL, M. DHANANJAYA and K.E.V. NAGOJI
Department of Pharmaceutical Sciences, Andhra University
Visakhapatnam-530 003, India
E-mail: jvlns@yahoo.com

A reverse phase HPLC method is described for the determination of cephalexin in pharmaceutical dosage forms. Chromatography was carried out on an ODS column using a mixture of methanol and water (50 : 50 v/v) as the mobile phase at a flow rate of 0.9 mL/min. Benazepril was used as an internal standard and the detection was done at 230 nm. The retention time of the drug was 3.78 min. The method produced linear responses in the concentration range of 0.05–80 μg/mL of cephalexin. The method was found to be applicable for determination of the drug in tablets.

Key words: Cephalexin, Estimation, Tablets, HPLC.

INTRODUCTION

Cephalexin[1-4] (5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid, 7-[(amino-phenyl acetyl) amino]-3-methyl-8-oxo-monohydrate) is a recent semi-synthetic antibiotic derived from cephalosporin-C. A literature survey reveals the reports of spectrophotometric[5-7], fluorimetric[8] and HPLC[9-13] methods for the determination of cephalexin in pharmaceutical dosage forms and in biological fluids. In the present investigation, the authors propose a simple, sensitive and reproducible method for the determination of cephalexin.

EXPERIMENTAL

Chemicals and solvents: HPLC grade methanol (E. Merck, India) and water (Qualigens) were used for preparing the mobile phase. Pure samples of cephalexin (Ranbaxy) and benazepril hydrochloride (Novartis) and commercial samples of tablets containing cephalexin, namely, Cefacure (Orchid), Phexin (Glaxo-Smith-Kline) and Zeecef (Zeelab) were employed in the study.

Chromatographic conditions: A Shimadzu LC-10AT high pressure liquid chromatographic instrument provided with an SPD-10A UV-Vis detector, an ODS column (250 × 4.6 mm I.D.), 25 μL Hamilton injecting syringe and monitored by Windows-based single channel software was employed in the study. Freshly prepared 50 : 50 v/v mixture of methanol and water was used as the mobile phase. Both methanol and water were filtered through 0.45 μ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at

1 mL/min. The column temperature was maintained at 25 ± 1°C. The detection was carried out at 230 nm.

Estimation of Cephalexin: About 50 mg of cephalexin was weighed accurately and transferred into a 50 mL volumetric flask and dissolved in 25 mL of the mobile phase. The solution was sonicated for 15 min and then the volume made up with a further quantity of the mobile phase to get a 1 mg/mL solution. Subsequent dilutions of this solution ranging from 0.05 to 100 μg/mL were made in 10 mL volumetric flasks after addition of 0.1 mL of a 100 μg/mL benazepril solution as an internal standard to each flask. 20 μL of the solution was injected each time into the column. Each of the dilutions was injected 5 times into the column and the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug and the internal standard were noted. Using these area values, the mean ratio of peak area of the drug to that of the internal standard for each dilution was calculated. The regression of the drug concentrations over these ratios was computed. This regression equation was later used to estimate the amount of cephalexin in pharmaceutical dosage forms.

To check the intra-day and inter-day variation of the method, solutions containing 8, 12 and 20 μg/mL of cephalexin were subjected to the proposed HPLC analysis. The drug recovery studies were carried out by adding known amounts of cephalexin to the preanalyzed drug samples and then analyzing them by the proposed HPLC method.

Estimation of the drug in tablet dosage forms: Three commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate cephalexin in tablet formulations. For this, 20 tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 50 mg of cephalexin was transferred to a 50 mL volumetric flask containing 25 mL of the mobile phase. The contents of the flask were allowed to stand for 6 h with intermittent sonication to ensure complete solubility of the drug and then filtered through a 0.45 μ membrane filter. Appropriate volume of this filtrate equivalent to 10 μg/mL of the drug along with 0.1 mL of the benazepril hydrochloride solution were taken in a 10 mL volumetric flask. The contents of the flask were made up to volume with the mobile phase and mixed well. 20 μL of the solution was then injected into the column. The mean peak area ratio of the drug to that of the internal standard of five such determinations was calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive, precise and accurate HPLC method for the analysis of cephalexin in pharmaceutical dosage forms. For this, a binary mixture of methanol and water in 50 : 50 v/v proportion was found to be the most suitable mobile phase as the chromatographic peaks obtained with this system were better defined and resolved and all almost free from tailing. Under the above-mentioned chromatographic conditions, the retention times

obtained for cephalexin and the internal standard were 3.78 and 6.72 min respectively. A model chromatogram is shown in Fig. 1.

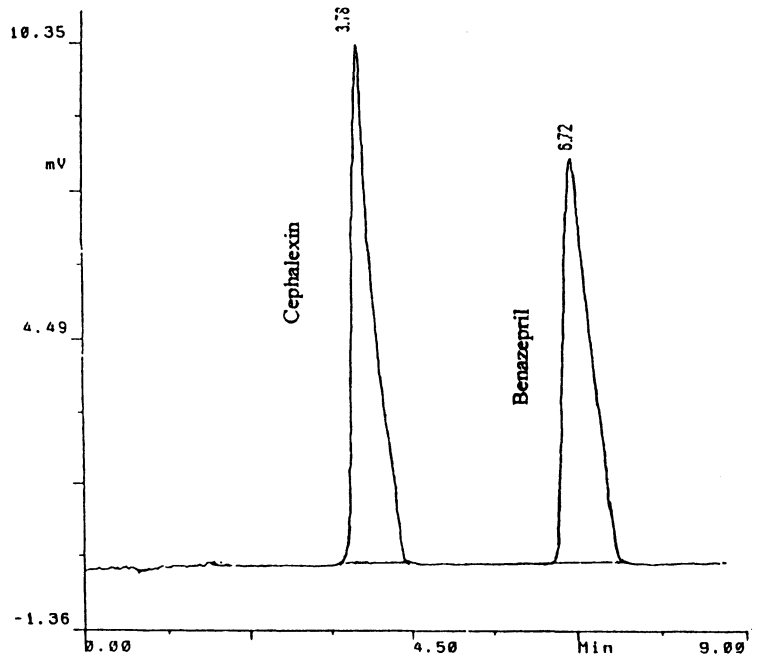

Fig. 1. A model chromatogram for cephalexin and benazepril

The ratios of the peak area of cephalexin to that of the internal standard for different concentrations set up as above were calculated. The peak areas of both the drug and the internal standard were reproducible as indicated by low coefficient of variation (1.22%) shown in Table-1. A good linear relationship (r = 0.9990) was observed between the concentration of cephalexin and the

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Concentration of cephalexin (μg/mL)	Mean peak area ratio (n = 5)	Coefficient of variation (%)
0.05	0.0588	0.08
0.10	0.1176	0.73
0.50	0.5289	0.96
1.00	1.3085	1.22
5.00	5.7126	0.01
20.00	21.9265	1.02
40.00	44.0810	0.68
80.00	87.6481	0.83

Regression equation (from 0.05 to 290 μg/mL
Y = 0.00157 + 0.44166x (r = 0.9990)

respective ratio of peak areas. The mathematical expression obtained from the regression curve constructed by linear regression fitting was $y = 0.10034 + 0.09542x$ (where 'y' is the ratio of area under the curve of the drug to that of the internal standard and 'x' is the concentration of cephalexin). The regression characteristics are given in Table-2.

TABLE-2
REGRESSION CHARACTERISTICS OF THE PROPOSED HPLC METHOD

Parameters	Value
Standard deviation on slope (S_b)	0.00549
Standard deviation on intercept (S_a)	0.00280
Standard error of estimation (S_e)	0.00431
Relative standard deviation (%)*	1.24300
% Range of error at 95% confidence limit	1.31470
% Range of error at 99% confidence limit	1.94510
Slope (a)	1.09540
Intercept (b)	0.10034
Correlation coefficient (r)	0.99900

* Average of six replicates

The intra- and inter-day drug variation studies by the proposed HPLC method showed low coefficient of variation as shown in Table-3. The drug content in the tablets was quantified using the proposed analytical method. The mean amount of cephalexin obtained in two different brands of tablet dosage forms is shown in Table-4. This reveals that the method is quite precise. High recoveries of above 99.8% of cephalexin from the preanalyzed samples of pure drug and its formulations indicate appreciable accuracy of the proposed HPLC method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets.

TABLE-3
INTRA- AND INTER-DAY PRECISION OF THE PROPOSED METHOD

Concentration of cephalexin monohydrate (μg/mL)	Observed concentration of cephalexin monohydrate (μg/mL)			
	Intra-day		Inter-day	
	Mean (n = 5)	RSD (%)	Mean (n = 5)	RSD (%)
8	7.96	1.04	8.02	0.89
12	12.04	0.52	11.98	1.67
20	25.01	1.29	24.95	0.94

TABLE-4
ASSAY OF CEPHALEXIN IN TABLET DOSAGE FORMS

Brand name of the tablet	Labelled amount (mg) of drug	Mean (± s.d.) amount (mg) found by the proposed method (n = 5)	Mean (± s.d.) labelled amount (%) (n = 5)
Cefacure	125	124.0 ± 0.11	99.8 ± 0.53
Phexin	250	252.6 ± 0.44	101.0 ± 0.78
Zeecef	500	501.2 ± 0.67	100.2 ± 0.21

Conclusion

It can be concluded that the proposed HPLC method is sensitive and reproducible for the analysis of cephalexin in pharmaceutical dosage forms in a short analysis time. The method was duly validated by evaluation of the required parameters.

REFERENCES

1. Indian Pharmacopoeia, p. 152, 619 (1996).
2. British Pharmacopoeia, p. 1230, 7760 (1993).
3. J.E.F. Reynolds (Ed.), Martindale, The Extra Pharmacopoeia, Royal Pharmaceutical Press, p. 128 (1993).
4. G.A. Saleh, H.F. Askal, I.H. Refaat and N.A. Mohmed, *S.T.P. Pharma Sci.*, **12**, 133 (2002).
5. S. Jain, P.R. Mishra and N.K. Jain, *Eastern Pharmacist*, **41**, 115 (1998).
6. A.A. Alwarthan, S. Abdel-Fattah and N.M. Zahran, *Talanta*, **39**, 703 (1992).
7. J. Wu, S. Lian and F. Kong, *Zhongguo Yiyuan Yaoxue Zazh*, **12**, 267 (1992).
8. C. Cheng and J. He, *Huaxi Yaoxue Zazhi*, **15**, 314 (2000).
9. W. Wang and P. Li, *Fenxi Ceshi Xuebao*, **18**, 81 (1999).
10. S.A. Coran, M. Bambagiotti-Alberti, V. Giannellini, A. Baldi, G. Picchioni and F. Paoli, *J. Pharma Biomed. Anal.*, **18**, 271 (1998).
11. N.M. Najib, M.S. Suleiman, Y.M. El-Sayed and M.E. Abdulhameed, *J. Clin. Pharm. Therap.*, **12**, 419 (1987).

(*Received*: 27 November 2003; *Accepted*: 7 April 2004) AJC-3392