Difference Spectroscopic and Reverse Phase HPLC Methods for the Estimation of Cefdinir in Pharmaceutical Dosage Forms

P. B. SHAH* AND K. PUNDARIKAKSHUDU[1]

Shri B. M. Shah College of Pharmacy, Modasa-383 315. [1]Shri S. K. Patel College of Pharmaceutical Education and Research, Kherva, Mehsana-382 711, India.

Two simple efficient and reproducible difference spectroscopic and reverse phase high performance liquid chromatographic methods have been developed for the estimation of cefdinir in pharmaceutical dosage forms. Difference spectroscopic method is based on the measurement of absorbance of cefdinir at maxima 265 nm and minima 230 nm. The measured value is the amplitude of maxima and minima between two eqimolar solutions of the analyte in different chemical forms, which exhibit different spectral characteristics. Beer's law was obeyed in the concentration range of 10 to 35 μg/ml. The second method, a High Performance Liquid Chromatography, was developed for the estimation of

***For correspondence**

E-mail: pbshah23@rediffmail.com

cefdinir, using 50 mM ammonium acetate (pH 3.0±0.1 adjusted with 10% phosphoric acid) and methanol (80:20% v/v) as the mobile phase with flow rate of 1 ml/min, and measuring the response at 285 nm. An external standard calibration method was employed for quantitation. Beer's law was obeyed in the concentration range of 15 to 125 μg/ml. The results obtained in the analysis of dosage forms agree well with the labelled contents.

Cefdinir is chemically [6R-[6α,7β(Z)]]–7-[[(2–amino–4–thiazolyl) hydroxyimino) acetyl] amino]–3–ethyl–8–oxo–5–Thia–1–azabicyclo-(4.2.0.)-oct–2–one–2–carboxylic acid.[1] It is a broad-spectrum oral cephalosporin active against Gram-positive and Gram-negative bacteria[2]. Literature survey reveals that HPLC methods have been reported for the estimation of cefdinir and its related impurities[3,4]. So far only one method has been reported for the estimation of cefdinir from pharmaceutical dosage forms[5].

We report here, two simple and reproducible methods, viz., (1) difference spectroscopic and (2) reverse phase HPLC methods for the analysis of cefdinir from pharmaceutical dosage forms. The methods were validated by employing suitable statistical methods. In difference spectroscopic method, the absorbance is measured at maxima 265 nm and minima at 230 nm. The measured value is the amplitude of maxima and minima between two eqimolar solutions of the analyte in different chemical forms, which exhibit different spectral characteristics. The method is advantageous over others, as it achieves the spectrophotometric isolation of the drug; moreover, interference due to additives can be nullified as can be proved by no change in isobestic points[6]. The second method, a high performance liquid chromatography (HPLC), was developed, using 50 mM ammonium acetate (pH 3.0±0.1 adjusted with 10% phosphoric acid) and methanol (80:20% v/v) as the mobile phase with flow rate of 1 ml/min and measuring the response at 285 nm.

All the reagents used were of analytical grade. A stock solution of cefdinir (1 mg/ml) was prepared by dissolving 100 mg of the drug in 100 ml 0.1 M phosphate buffer (pH 7.0). Spectral and absorbance measurement were made on Shimadzu-1601 UV/Vis Spectrophotometer by using 1 cm matched quartz cells.

Aliquots of cefdinir stock solution (1 mg/ml) of the drug ranging from 0.1 to 0.35 ml were transferred to two sets of a series of 10 ml volumetric flask. One set of cefdinir solutions was diluted with 0.5N HCl to volume, and second set of cefdinir solutions was diluted with buffer to volume. Difference spectrum was recorded by placing same concentration of acidic and buffer solution in sample and reference cell respectively. The amplitude was plotted versus concentration (10-35 μg/ml), calibration curve was constructed, and the regression equation was calculated (Table 1).

Not less than 20 capsules were weighed and emptied. A quantity of powder equivalent to 10 mg of cefdinir was than extracted with 10 ml buffer. The solution was prepared and analyzed as described above. The amount of cefdinir present in the sample solution was computed from the calibration curve (Table 2).

For HPLC method, all the chemicals used were of HPLC grade. A stock solution of cefdinir (1 mg/ml) was prepared by dissolving 100 mg of the drug in 100 ml 0.1 M phosphate buffer (pH 7.0). The HPLC system consisted of a model Hitachi-Merck L-7110 chromatographic pump with 20 μl loop and a model L-7420 (Hitachi-Merck) UV/Vis Detector. Integration was carried out by using the software MSN (Hitachi-Merck). Analysis was carried out on a Lichrospher RP C_{18} (4 x 250 mm, 5 μ) column. The mobile phase used was 50 mM ammonium acetate (pH 3.0±0.1 adjusted with 10% phosphoric acid) and methanol (80:20% v/v). The flow rate was set to 1 ml/min. The prepared mobile phase was filtered through a 0.45 μm pore-size membrane filter and ultrasonically degassed prior to use. The UV detection was set at a wavelength of 285 nm.

Aliquots of 0.15 to 1.25 ml of standard solution (1 mg/ml) of cefdinir were transferred to 10 ml of volumetric flasks and diluted to volume with mobile phase. A 20 μl each of solution was injected into the chromatographic system and

TABLE 1: OPTICAL CHARACTERISTICS

Parameters	Difference Spectroscopic Method	HPLC method
λ(nm)	265[a] 230[b]	285
Beer's law limits (μg/ml)	10 - 35	15 - 125
Regression equation (Y = a + bX)		
Slope (b)	0.0266	61698
Intercept (a)	-0.0551	-352970
Correlation coefficient	0.9933	0.9995
% Range of error		
0.05 level confidence limit	0.1390	0.0834
0.01 level confidence limit	0.2300	0.1380

[a]Absorption maxima, [b]Absorption minima, Y=a+bX, where "X" is concentration in mg/ml and Y is absorbance units.

TABLE 2: ANALYSIS OF CEFDINIR FORMULATIONS BY PROPOSED METHODS

Pharmaceutical formulations	Labeled Amount (mg)	Amount estimated (mg)		% Recovery*	
		Difference spectroscopic method	HPLC method	Difference spectroscopic method	HPLC method
1	300	293.0	299.5	98.7 ± 0.43	99.8 ± 0.10
2	300	301.0	299.0	99.0 ± 0.51	99.8 ± 0.10
3	300	304.0	300.0	101.6 ± 1.59	99.7 ± 0.10
4	300	302.5	299.0	101.6 ± 0.85	99.7 ± 0.13
5	300	302.0	299.5	100.0 ± 1.18	99.9 ± 0.09

*Values are mean±SEM of five determinations. Formulation 1 is capsules (300 mg) of ALDINIR (Alembic), formulation 2 is capsules (300 mg) of ZEFDINIR (German Remedies), formulation 3 is capsules (300 mg) of ADCEF (Torrent), formulation 4 is capsules (300 mg) of OCEPH (Zuventus) and formulation 5 is capsules (300 mg) of SEFDIN (Unichem).

chromatogram was recorded. The peak area was plotted versus concentration and calibration curve was constructed and the regression equation was calculated (Table 1).

Not less than 20 capsules were weighed and emptied. A quantity of powder equivalent to 10 mg of cefdinir was than extracted with 10 ml buffer and filtered through 0.45 µ. The solution was prepared and analyzed as described above. The amount of cefdinir present in the sample solution was computed from the calibration curve (Table 2).

The difference spectroscopic method is based on the nullification of UV absorbance at the wavelength corresponding to the point of intersection of drug spectra in acidic and basic media[7]. The technique of difference spectroscopy is as convenient and precise as conventional spectroscopy but offers the advantage of increased specificity. The methodology requires that a drug exists in two forms that differ in their absorption spectra, which have been obtained using temperature difference, pH difference, solvent perturbation and concentration, but mainly generated by pH effects. The pH chosen must quantitatively form single species with at least 99% spectral purity. 0.5N HCl and phosphate buffer (pH 7.0) were chosen to generate pH difference. The peak maxima were obtained at 265 nm and minima at 230 nm (Fig. 1). The amplitude, which is the sum of magnitude of absorbance at the above two wavelengths, was selected for the measurement. The isobestic points (points representing zero absorbance corresponding to cutting points of acidic and alkaline spectra) were recorded at 244 nm and 287 nm, which were identical irrespective of the pH of solution in reference cell. There is no change in isobestic points, which reveals that there is no interference by additives.

In HPLC method, mobile phase used was 50 mM ammonium acetate (pH 3.0±0.1 adjusted with 10% phosphoric acid) and methanol (80:20% v/v). The

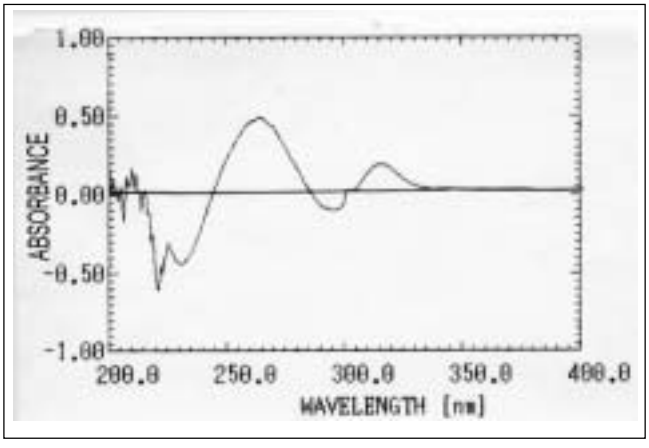

Fig. 1: Difference spectra of cefdinir.
X axis is wavelength in nm and Y axis is absorbance

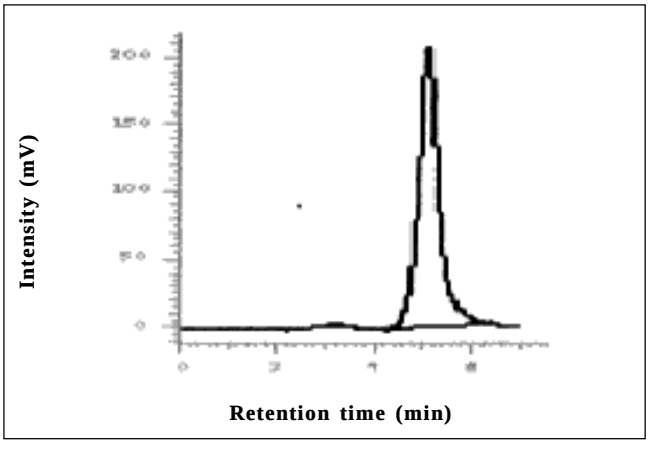

Fig. 2: Chromatographic pattern of cefdinir.
X axis is retention time in min and Yaxis is intensity in mV. Brand Names of formulations

retention time was found to be 5.08 min (Fig. 2). Beer's law was obeyed in the range of 15-125 µg/ml (Table 1). Precision of the method was established by five repeated analysis of the sample.

To evaluate the recovery of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by the proposed methods. The percentage

recoveries thus obtained are given in Table 2. Interference studies revealed that the common excipients and other additives usually present in dosage forms did not contribute in the proposed methods. The proposed methods are simple, sensitive, precise, reproducible and accurate and hence can be used for the routine determination of cefdinir in bulk as well as in pharmaceutical preparations as alternative to the existing methods.

ACKNOWLEDGEMENTS

The authors are thankful to Torrent Research Center, Bhatt, Ahmedabad, for providing a gift sample of cefdinir.

REFERENCES

1. Budavari, S., Eds., In; The Merck Index, 12th Edn., Merck and Co. Inc., Whitehouse Station. NJ, 1996, 1971.
2. Mine, Y., Wantanabe, Y., Matsumoto, Y., Kuno, K., Hantano, K., Higashi, Y., and Kuwahara, S., **Chemotherapy**, 1989, 37, 122.
3. Okamoto, Y., Itoh, K., Namiki, Y., Matsushita, J., Fujioka, M., and Yasada, T., **J. Pharm. Bio. Med. Anal.** 1996, 14, 739.
4. Cohen, C. and Michael, N., **Dig. Microbiol. Infect. Dis.**, 1994, 18, 31.
5. Gandhimathi, M., Suganthi, A., Ravi, T.K. and Minu B., **Indian J. Pharm. Sci.**, 2004, 66, 248.
6. Smith, R.V. and Stewart, J.T., In; Text book of Biopharmaceutic Analysis, Lea and Febiger, Philadelphia, 198, 138.
7. Beckett, A.H. and Stelnlake, J.B., In; Practical Pharmaceutical Chemistry, 2001, 4, 293.

Accepted 15 February 2006
Revised 30 March 2005
Received 29 May 2004
Indian J. Pharm. Sci., 2006, 68 (1): 90-93