RESEARCH ARTICLE

Novel HPLC analysis of cefadroxil in bulk formulation

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A rapid, accurate, and sensitive method has been developed and validated for the quantitative determination of cefadroxil (first generation) in bulk form. An Inertsil ODS, 4.60 × 250 mm^2, 5 μm analytical column was used with an eluting system consisting of a mixture of phosphate buffer (pH 6.5)-methanol 78-32% (v/v) at a flow-rate 1.5 ml/min. Detection was performed by UV-Vis detector at 210 nm, resulting in limit of detection of 0.06 ppm for cefadroxil per 20-μl injection. A linear relationship was observed up to 0.2 ppm for cefadroxil. Analysis time was less than 10 min. The statistical evaluation of the method was examined by means of within-day repeatability (n = 6) and day-to-day precision (n = 7) and was found to be satisfactory with high accuracy and precision. The method may be applied for the determination of the cefadroxil in bulk formulation in future.

Key words: *Cefadroxil, cephalosporins, HPLC, phosphate buffer*

INTRODUCTION

Cephalosporins are β-lactam antibiotics with the same fundamental structural requirements as penicillin. They are used for the treatment of infections caused by Gram-positive and Gram-negative bacteria. They act by inhibiting the synthesis of essential components of bacterial cell wall. They are among the safest and the most effective broad-spectrum antibactericidal antibiotics.[1,2] As only cephalosporin C is found naturally, the remaining semi-synthetic cephalosporins are derived from 7-amino-cephalosporanic acid, a product obtained from cephalosporin C hydrolysis.[3] Their composition is accomplished by β-lactam ring fusion with a dihydrothiazine ring differing in the nature of the substituents attached at the 3- and/or 7-positions of the cephem ring. The substitution at the 3-position affects the pharmacokinetic properties, whereas the substitution at the 7-position affects the antibacterial spectrum of the cephalosporins.

Cephalosporin antibiotics are divided into four generations: first, second, third, and recently, fourth generation compounds [Figure 1].

Various methods and reviews have been published covering the analysis of cephalosporins in biological matrices and pharmaceuticals. Among the published methods of determination of cephalosporins, thin layer chromatography, gas chromatography, high performance liquid chromatography (HPLC), column electrophorosis and microbiological assays are available. HPLC methods have been described for the determination of cephalosporins in biological fluids using different stationary phases, mobile phases with different buffer systems, mostly phosphates or ion pairing agents, detection mode, e.g., UV and electrochemical and sample preparation procedures.[3,4-12] One of the review describes general HPLC conditions for simultaneous separation of more than two cephalosporins.[13] Best sensitivity in pharmaceuticals is achieved with electrochemical detection in the range of parts per billion levels; however, analysis time is more than 20 min.[3]

As numerous new molecules belonging to this group of cephalosporin antibiotics continuously being developed, purity must require in each cephalosporins during their production. As cross-contamination may occur between these cephalosporins during their production, because of the use of same premises, a simple separation technique was required to determine contamination of one cephalosporin to another. A simple, rapid assay method for the determination of cefadroxil in bulk form is developed. The method gives good separation and is sensitive as well as linear over a wide range of concentration.

EXPERIMENTAL WORK

Materials and chemicals

Cepfadroxil is kindly gifted by Lupin Ltd. (Mandideep, Madhya Pradesh, India) and used for experiments without further purification. HPLC grade methanol was

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purchased from S.D. Fine Chem Ltd., Mumbai (India). For all the experimental work, the water was filtered through a 0.45-μm nylon HNWP 47-mm filter. Disodium hydrogen phosphate (AR grade) was purchased from S.D. Fine Chemicals (Mumbai) and was of analytical grade.

Apparatus

A Dionex LC-P680 Solvent Delivery Gradient Pump was used to deliver the mobile phase to the analytical column, Inertsil ODS 3V [250 × 4.6 mm, 5 μm (SC Science, Japan]. Sample injection was performed by ASI 100 automated sampler injection with 20-μl loop. Detection was achieved by an UVD170V UV-Vis detector at wavelength of 210 nm. CHROMELEON software version 6.60 was used for quantitative determination of eluted peaks. Dissolution of compounds was enhanced by sonication in transonic 460/H ultrasonic bath (Elma, Germany). Simadzu 1700A UV Spectrophotometer (Tokyo, Japan) was used for scanning of drugs components to select appropriate wavelength.

Chromatographic conditions

The mobile phase consisted of disodium hydrogen phosphate buffer (pH 6.5):methanol [72:28 (v/v)] was prepared and filtered through 0.45-μm nylon HNWP 47-mm filters and was degassed before use. Stock standard solutions of cefadroxil was prepared by dissolving an accurate weight of 10 mg of drug in 100 ml of mobile phase; and by an appropriate dilution, aliquots of 10 ppm were made for each drugs. A system suitability mixed solution (10 ppm) was also made by dilution with mobile phase. A 20-μl aliquot was injected into the column.

The flow rate of 1.5 ml/min was maintained using the column at ambient temperature. The preparation was injected (20 μl injection volume) and monitored at 210 nm.

Procedure

Preparation of mobile phase

Seven hundred twenty milliliters of disodium hydrogen phosphate (pH 6.5) was mixed with 280 ml of HPLC grade methanol, filtered through 0.45-μm nylon filter, and degassed before use.

Standard solution

Stock standard solutions of cefadroxil were prepared by dissolving an accurate weight of 10 mg of drug in 100 ml of mobile phase; and by an appropriate dilution aliquot of 10 ppm that was prepared for the drug. A system suitability solution (10 ppm) of drug was also prepared by dilution with mobile phase. A 20-μl aliquot was injected onto the column.

Method validation

Method validation was performed in terms of sensitivity and specificity, precision, and linearity.

Specificity and selectivity: The interference from endogenous compounds was investigated by the analysis of six injection of system suitability solution.

Precision and accuracy: Method validation regarding reproducibility was achieved by six replicate injections of standard solutions of 10 ppm. Intermediate precision study (day-to-day reproducibility) was conducted during routine operation of a system over a period of nine consecutive days. Statistical evaluation revealed relative standard deviation at different values of six injections. Within-day repeatability was studied by six replicate measurements at three concentration levels.

RESULTS AND DISCUSSION

Chromatography

A chromatogram obtained using the developed method conditions is illustrated in Figure 2. Retention time precision for 10-day period is summarized in Table 2.

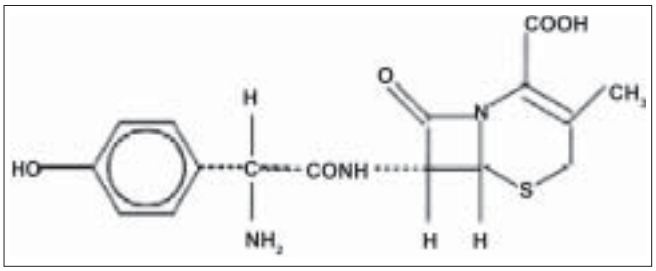

Figure 1: Structure of cefadroxil

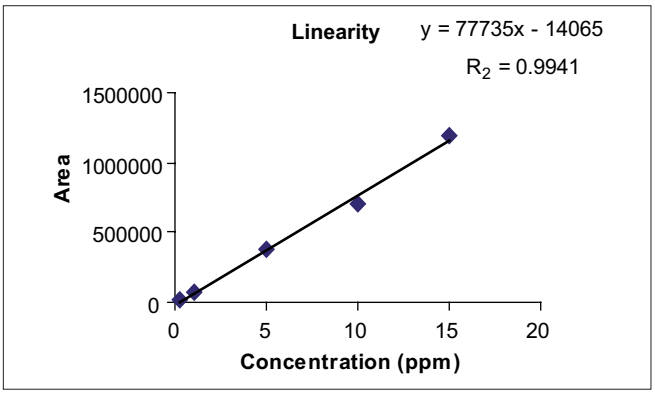

Figure 2: Linearity curve and retention time of cefadroxil

Table 1: Retention times, calibration data, and sensitivity of cephalosporin determination (*n* = 6)

Compound	Retention times, mean value ± SD	RSD (%)	LOD (ppm)	LOQ (ppm)	Regression equation	R^2
Cefadroxil	8.05 ± 2.28	0.02	0.06	0.2	$Y = 77735x - 14065$	0.9941

Y - peak area ratio; x - ppm

Table 2: Day-to-day (over a period of six consecutive days) and within-day (n = 6) precision and accuracy study for determination of cefadroxil

Added (ppm)	Within day			Day-to-day		
	Found ± SD (ppm)	RSD	Relative error%	Found ± SD (ppm)	RSD	Relative error (%)
Cefadroxil						
10	9.97 ± 0.15	0.015	–0.3	10.03 ± 0.03	0.34	0.3
20	19.9 ± 0.2	1.0	–0.5	20.1 ± 0.14	0.7	5
50	49.67 ± 0.53	1.0	–0.66	50.11 ± 0.43	0.86	0.22

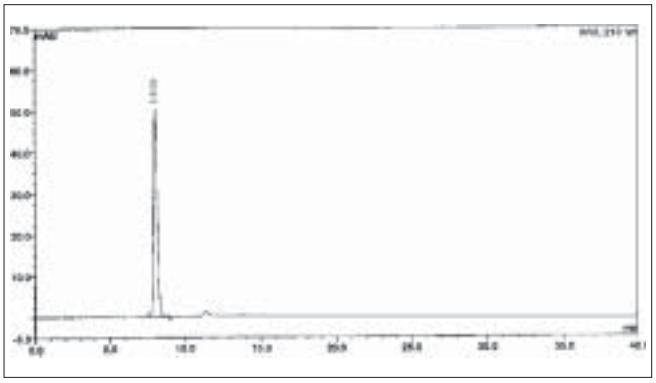

Figure 3: Linearity curves of cefadroxil

Validation of the method

Detection limit and quantitation limit

The detection limit of the method was investigated by injecting standard solutions of cefadroxil into the HPLC column. The limit detection is defined as that concentration of the analyte that will give a signal-to-noise (S/N) ratio of 3:1. The detection limit of cefadroxil was 0.06 ppm. The quantitation limit is defined as the concentration of related substance in the sample that give a signal-to-noise ratio of 10:1[14] of the analyte, producing the signal which is at least five times that of the baseline noise (S/N = 5), which were found to be 0.2 ppm for cefadroxil.

Linearity and range

Table 1 lists the mean HPLC area responses of cefadroxil at different concentrations. The linearity plot shown in Figure 3 calibration curves was obtained by least squares linear regression analysis of the peak area ratio of analyte versus analyte concentration. The method was linear up to 0.2 ppm for cefadroxil and 0.9941 correlation coefficient was obtained.

Table 2 summarizes the results of the method validation regarding accuracy, within-day and day-to-day precision assays. The measured concentration had relative standard deviation < 2% with relative error (inaccuracy) in the range of –0.66% to 5%.

Precision and accuracy

The precision of the method based on within-day repeatability was performed by replicate injections (n = 6) of three standard solutions covering different concentration levels: low, medium, and high. Statistical evaluation revealed relative standard deviations at different values (results shown in Table 2).

The reproducibility (day-to-day variation) of the method was established using the same concentration range as above. A triplicate determination of each concentration was conducted during routine operation of the system over a period of nine consecutive days. Reproducibility results are illustrated in Table 2.

Accuracy was determined and expressed as relative error and can be calculated by the equation:

$$\text{Relative error (\%)} = \frac{\text{Mean determine value} - \text{Theoretical value (added amount)}}{\text{Theoritical value}} \times 100$$

CONCLUSION

The aim of this study was to develop a simple fast and sensitive method for the determination of cefadroxil antibiotic agents in bulk form. Although the method is highly sensitive, it can be used for drug analysis in formulations as well as for analysis of cross-contamination between the cephalosporins in those premises where production of different cephalosporins are carried out.

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