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Column liquid chromatography and microbiological assay compared for determination of cefadroxil preparations

Mei-Chich Hsu, Ya-Wen Chang and Yu-Tzer Lee

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, 161-2 Kuen Yang St., Nankang, Taipei 11513 (Taiwan)

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ABSTRACT

A reversed-phase column liquid chromatographic method was developed for the assay of cefadroxil in bulk drugs and pharmaceutical preparations. An equation was derived showing a linear relationship between peak-area ratios of cefadroxil to dimethylphthalate (internal standard) and the cefadroxil concentration over a range of 0.02–0.8 mg/ml (r = 0.9999). Standard addition recoveries were generally greater than 97.7%. The coefficients of variation in the within-day assay were between 0.36 and 0.65, and in the between-day assay was 0.71%. The column liquid chromatographic assay results were compared with those obtained from a microbiological assay, which indicated that the proposed method is a suitable substitute for the microbiological method for potency assays and stability studies of cefadroxil preparations.

INTRODUCTION

The present official assay method of the U.S. *Code of Federal Regulations* [1] describes two official methods for potency assay of cefadroxil: a microbiological method and hydroxylamine assay. The regulations state that the results obtained from the microbiological method shall be conclusive. The greatest disadvantage of the microbiological, and chemical methods in current use is their lack of specificity. This deficiency has prompted the search for an alternative method which is fast, simple and selective, *e.g.* column liquid chromatography (LC). Several LC methods for the determination of cefadroxil in biological fluids [2–4] and to separate cephalosporin compounds [5–8] have been reported.

In order to establish whether an LC method is acceptable, it is important to determine whether it is robust enough for assaying samples kept under extreme conditions. Degradation in the sample should be equally reflected by microbiological and LC assays. This paper describes a comparison of a proposed LC method with a microbiological assay for the determination of cefadroxil in commercial formulations. Further, cefadroxil was kept at elevated temperatures as part of an accelerated degradation experiment and assayed by microbiological and LC methods.

Correspondence to: Dr. Mei-Chich Hsu, National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, 161-2 Kuen Yang St., Nankang, Taipei 11513, Taiwan.

EXPERIMENTAL

Apparatus

A Waters Model 600E consisting of two solvent pumps, a Model 484E UV detector and a Model 746 data module were employed during the study. Samples were introduced through a Model U6K injection valve (Waters Chromatography Division, Milford, MA, USA). The mobile phase was pumped through a reversed-phase column (μBondapak C_{18}, 30 cm × 3.9 mm I.D., particle size 10 μm; Waters P/N 27324) The mobile phase was aceto-

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nitrile–0.01 *M* phosphate buffer solution, pH 4.5 (60:40, v/v). The mobile phase was filtered (0.45-μm Millipore filter) and degassed with an ultrasonic bath prior to use. Flow-rate was 1.0 ml/min. The detector was set at 254 nm. Chromatography was performed at room temperature. Injections of 10 μl were made of all solutions to be analysed.

Reagents and materials

Acetonitrile, LC grade was supplied by J. T. Baker (Phillipsburg, NJ, USA). Potassium phosphate, monobasic, reagent grade, was supplied by Wako (Osaka, Japan). Dimethylphthalate, reagent grade, was supplied by E. Merck (Darmstadt, Germany). Cefadroxil was NLFD house standard (National Laboratories of Foods and Drugs, Taipei, Taiwan). Different lot numbers of cefadroxil bulk drugs were kindly donated by local manufacturers. Capsules and powders to make solutions of cefadroxil to be taken orally were obtained from commercial sources. Double-deionized water was used for all solution preparations.

Standard solutions

Internal standard dimethylphthalate (3 g) was dissolved in 100 ml of acetonitrile–water (1:1). Exactly 0.5 ml of this internal standard solution were added to 5.0 ml of a 1 mg/ml cefadroxil standard solution and the volume was made up to 50.0 ml with 0.1 *M* phosphate buffer solution (pH 4.5).

Sample preparation

To accurately weighed samples of bulk drugs, homogeneous capsule contents or powders for oral solution formulations (equivalent to 50 mg of cefadroxil) were added 50 ml of 0.1 *M* (pH 4.5) phosphate buffer solution. Exactly 0.5 ml of internal standard solution was added to 5.0 ml of 1 mg/ml cefadroxil sample solution and the volume was made up to 50.0 ml with 0.1 *M* phosphate buffer solution (pH 4.5).

TABLE I

RECOVERY OF CEFADROXIL FROM VARIOUS COMMERCIAL COMPOSITES

Manufacturer	Added (mg)	Found (mg)	Average recovered (%)
Capsule (250 mg)			
A	1.0	0.99	99.5
B	1.0	0.99	
C	1.0	1.01	
Capsule (500 mg)			
B	1.2	1.19	99.8
D	1.2	1.21	
E	1.2	1.21	
F	1.2	1.21	
G	1.2	1.21	
H	1.2	1.20	
I	1.2	1.21	
J	1.2	1.19	
K-1	1.2	1.18	
K-2	1.2	1.18	
K-3	1.2	1.21	
K-4	1.2	1.21	
L	1.2	1.20	
M	1.2	1.17	
N	1.2	1.20	
Powders for oral solution (125 mg/5 ml)			
B	2.4	2.48	103.1
C	2.4	2.54	
I	2.4	2.48	
K	2.4	2.39	

Solution for linearity response

Ten solutions of cefadroxil, which ranged in concentration from 0.02 to 0.8 mg/ml, were prepared. Each solution was chromatographed six times.

Solutions for recovery studies

Different amounts of cefadroxil standard and appropriate amounts of internal standard solution were combined. Each solution was chromatographed in triplicate.

Microbiological assay procedure

Bacillus subtilis (Culture Collection and Research Center, Taiwan) was used in the microbiological assay. According to the cup plate method, standards and test drugs were diluted to 1.0 mg/ml (potency) with 0.1 *M* phosphate buffer solution (pH 4.5) and then diluted to 30.0 and 7.5 μg/ml with 0.1 *M* phosphate buffer solution (pH 4.5) on the day of analysis. Five 9.0-cm-diameter Petri dishes were used for each sample. After incubation for 16–18 h, the zone diameter was measured by a zone analyser (Model ZA-F; Toyo, Tokyo, Japan).

RESULTS AND DISCUSSION

The linearity of the relationship between peak-area ratio (cefadroxil *vs.* internal standard) and cefadroxil concentration was verified by injection of ten solutions containing 0.2–8 μg of cefadroxil and 3 μg of dimethylphthalate. A straight line with a correlation coefficient of 0.9999 ($y = 11.3444x + 0.0010$) was obtained when the ratios of the area counts of the cefadroxil divided by the area counts of the internal standard were plotted against concentration of cefadroxil.

Reproducibilities for both within-day assays and between-day assays were evaluated. The coefficients of variation, on the basis of peak-area ratios for six replicate injections in the within-day assay, were between 0.36 and 0.65% at the cefadroxil amount of 1 μg. The coefficient of variation in the between-day assay ($n = 6$) was 0.71% at the same amount.

The results of standard addition recovery studies of cefadroxil from sample composites of commercial preparations and powders for oral solution are shown in Table I. The average recoveries were 99.5% for 250-mg capsules, 99.8% for 500-mg capsules and 103.1% for powders for oral solution. These data indicate that the proposed LC method is relatively unaffected by the sample matrix.

Typical chromatograms of the cefadroxil commercial dosage forms are shown in Fig. 1. The retention time was about 2.4 min for cefadroxil and 4.7 min for the internal standard. Excipients from commercial formulations did not interfere.

When samples of capsule and powders for oral solution formulations were heat degraded, the resulting mixtures yielded chromatograms containing additional peaks, none of which interfered with the interpretation and measurement of the chromatographic peaks for cefadroxil and dimethylphthalate, as shown in Fig. 1. In addition, a decrease in peak

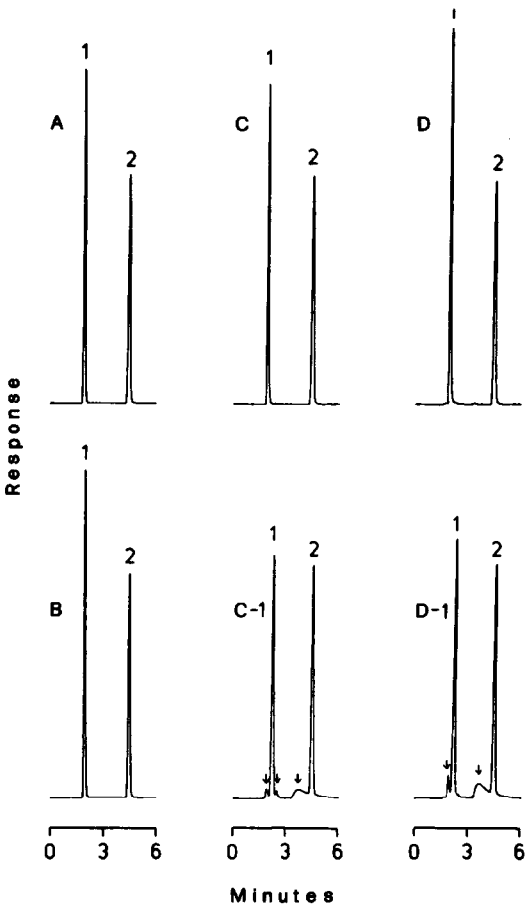

Fig. 1. Chromatograms of cefadroxil preparations: (A) house standard; (B) bulk drug substance; (C) capsule; (C-1) degraded capsule; (D) powder for oral solution; (D-1) degraded powder for oral solution. Peaks: 1 = cefadroxil; 2 = dimethylphthalate. Arrows indicate degradation compounds.

TABLE II

COMPARISON OF MICROBIOLOGICAL AND LC ASSAYS FOR CEFADROXIL

The potency was determined as micrograms per milligram for bulk drug and as a percentage of the declared amount for dosage forms. Values for the microbiological assay are averages of five determinations; values for LC are averages of three determinations.

Sample	Found in	
	Microbiological assay	LC
Bulk drug		
House standard	934.8	934.8
Brand C	909.9	915.3
Brand D	915.0	923.7
Brand E	918.6	923.7
Brand F	884.8	915.3
Brand G	896.1	915.3
Brand H	940.8	898.3
Brand I	918.4	915.3
Brand J	927.1	906.8
Dosage form, declared		
Brand A, 250 mg/capsule	105.1	104.4
Brand B		
250 mg/capsule	94.9	97.4
500 mg/capsule	94.0	95.6
125 mg/5 ml powders for oral solution	102.4	106.1
Brand C		
250 mg/capsule	105.9	107.8
125 mg/5ml powders for oral solution	110.3	113.9
Brand D, 500 mg/capsule	102.0	107.0
Brand E, 500 mg/capsule	102.7	104.4
Brand F, 500 mg/capsule	99.5	101.7
Brand G, 500 mg/capsule	103.9	102.6
Brand H, 500 mg/capsule	108.5	111.3
Brand I		
500 mg/capsule	103.3	100.9
125 mg/5ml powders for oral solution	107.4	104.4
250 mg/5ml powders for oral solution	105.2	103.5
Brand J, 500 mg/capsule	97.5	100.0
Brand K, 125 mg/5 ml powders for oral solution	108.2	119.2
Brand K-1, 500 mg/capsule	102.0	105.2
Brand K-2, 500 mg/capsule	97.3	99.1
Brand K-3, 500 mg/capsule	107.0	107.0
Brand K-4, 500 mg/capsule	101.3	99.1
Brand L, 500 mg/capsule	98.4	97.4
Brand M, 500 mg/capsule	95.0	103.5
Brand N, 500 mg/capsule	94.5	98.3

height (and/or peak area) with increase in temperature and time can be observed.

A number of samples of bulk drug substance and commercial preparations of fourteen brands were analysed for cefadroxil content by LC. These samples were also assayed by the microbiological method. The results are shown in Table II. A *t*-test was applied to the data: analysis showed no significant difference at the 99% confidence level for any of the preparations when assayed by the microbiological or LC methods.

A study was initiated to ascertain the suitability

TABLE III

COMPARISON OF PERCENTAGE POTENCY OF CEFADROXIL FORMULATION AS DETERMINED BY MICROBIOLOGICAL AND LC METHODS

Formulation	Percentage of declared concentration in	
	Microbiological assay	LC
Capsule (250 mg)	111.0	104.2
	91.6	93.0
	87.8	84.0
	63.2	69.0
Capsule (500 mg)	112.2	116.4
	104.3	104.3
	102.7	104.4
	101.6	100.7
	102.3	99.2
	100.5	96.8
	94.6	98.4
	90.1	91.2
	83.8	85.2
	69.0	75.6
	58.7	61.8
	36.8	40.4
	32.8	37.6
	22.2	26.7
	15.7	20.6
Powders for oral solution (125 mg/5 ml)	106.7	110.5
	101.2	101.3
	89.7	86.6

of the proposed method for stability studies. Samples were made to a concentration of 1 mg/ml and stored in temperature-controlled cabinets (ambient or 55–150°C). Samples were taken from the cabinets periodically for microbiological and LC assays. The assay values, expressed as a percentage of the level claim, are given in Table III. The 22 paired values in Table III have a correlation coefficient of 0.994. This value indicates that no significant difference was found in the assay values obtained by the two analytical methods for degraded or non-degraded samples.

This study demonstrates the applicability of the proposed LC method for the potency determination of cefadroxil in bulk drug, capsules and powders for oral solution formulations. The method can be successfully used for routine quality control and stability assays and offers advantages in speed, simplicity and reliability.

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