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Short Communication

Determination of cefpodoxime levels in chinchilla middle ear fluid and plasma by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method has been developed to determine cefpodoxime levels in chinchilla plasma and middle ear fluid (MEF) to be used in studying otitis media. Cefpodoxime and the internal standard, cefuroxime, were separated on an ODS column (250 × 2.1 mm I.D., 5 μm Hypersil), using a mobile phase of 25 m*M* acetate buffer (pH 4.3)/15 m*M* triethylamine–acetonitrile (92.5:7.5, v/v). Following elution of cefpodoxime and the internal standard, at 3.5 and 5.9 min respectively, the acetonitrile concentration was increased to 1:1 (v/v) in a step function to elute endogenous compounds retained on the column. Sample preparation involved protein precipitation with acetonitrile. This fast, efficient protein precipitation procedure together with UV detection allows a quantitation limit of 50 ng/ml with a 50-μl sample size. Recoveries (mean ± S.D., $n = 3$) at 0.1 μg/ml in MEF were 90.3 ± 2.9% and 88.6 ± 1.2% for cefpodoxime and cefuroxime respectively. Recoveries (mean ± S.D., $n = 3$) at 0.1 μg/ml in plasma were 72.1 ± 7.3% and 81.1 ± 1.1% for cefpodoxime and cefuroxime respectively. The method was evaluated with biological samples taken from chinchillas with middle ear infections after administering cefpodoxime proxetil.

1. Introduction

Cefpodoxime, a methoxyimino aminothiazolyl cephalosporin, is the active form of the orally absorbed prodrug cefpodoxime proxetil. This antimicrobial has a broad spectrum of activity against the bacteria that cause otitis media including organisms producing β-lactamase. In general, cefpodoxime is prescribed for episodes of acute otitis media in high risk patients and when amoxicillin resistant bacteria are suspected. The antimicrobial actions of cefpodoxime appear to be the result of binding to penicillin-binding protein which disrupts cell wall synthesis, causing cell lysis.

Antibiotic treatment of acute otitis media (AOM) has a failure rate of 5% to 10%. About 30% of the patients develop recurrent infection [1]. In an effort to explain these treatment

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failures and possibly prevent recurrence, we have conducted experimental studies on the effect of antibiotic penetration and antimicrobial treatment response in models of AOM [2,3]. To study antimicrobial penetration into and out of the middle ear, it is essential to develop analytical methods that allow us to precisely measure various antimicrobials at low concentrations in very small volumes of middle ear fluid (MEF) [4,5].

Concentrations of cefpodoxime in plasma, urine, and other biological fluids and tissues have been determined by microbiological [6] and HPLC [7–9] methods. There is good agreement between the results; however, the HPLC assays are more sensitive than the microbiological method [10].

2. Experimental

2.1. Chemicals

All chemicals were of analytical grade and included acetic acid, sodium hydroxide, and triethylamine (Sigma, St. Louis, MO, USA). Acetonitrile (Fisher, Fairlawn, NJ, USA) was HPLC grade. Cefuroxime (internal standard) (Sigma, St. Louis, MO, USA) was USP grade; cefpodoxime was a gift from Upjohn Company (Kalamazoo, MI, USA). Chinchilla plasma and MEF samples were obtained from the University of Minnesota Otitis Media Research Center.

2.2. Instrumentation and chromatography

Chromatography was done with a Hewlett-Packard 1090 L liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) and a Spectra Physics Model 100 UV detector set at 254 nm. The mobile phase was pumped at 0.35 ml/min through a Keystone Scientific (Bellefonte, PA, USA) 250 × 2.1 mm I.D. column and matching 10 × 2.1 mm I.D. precolumn, packed with 5 μm C_{18} Hypersil packing material and maintained at 40°C. The initial mobile phase consisted of 25 m*M* acetate buffer/15 m*M* triethylamine solution (adjusted to pH 4.3 with sodium hydroxide)–acetonitrile (92.5:7.5, v/v). Following elution of cefpodoxime and the internal standard, at 3.5 and 5.9 min respectively, the acetonitrile concentration was increased to 1:1 (v/v) in a step function to remove late eluting compounds retained on the column. Data was collected using Chrom-Perfect Direct data collection software (Justice Innovations, Palo Alto, CA, USA) and analyzed using proprietary software written with Visual Basic (Microsoft, Redmond, WA, USA).

2.3. Stock solutions and standards

Cefpodoxime was made up as a 1.0 mg/ml stock solution in methanol and distilled water (1:10, v/v). Cefpodoxime was further diluted with water to make working stock solutions of 20, 2, and 0.2 μg/ml. Cefuroxime was dissolved in methanol and water (1:10, v/v) to generate a 0.2 mg/ml stock solution that was further diluted with water to make working stocks of 40 μg/ml. Standards for the calibration curve were prepared from 0.05 μg/ml to 10 μg/ml by spiking pooled MEF or plasma with an appropriate amount of cefpodoxime.

2.4. Sample preparation

A 50-μl aliquot of plasma, MEF, or standard matrix was pipetted into a 12 × 75 mm disposable culture tube to which 50 μl of the 40 μg/ml internal standard solution was added. Protein precipitation was carried out by adding 2 ml of acetonitrile, vortex-mixing briefly, and centrifuging at 1500 *g* for 10 min. The acetonitrile was then transferred to a clean 10 × 75 mm disposable culture tube and evaporated to dryness under nitrogen in a 50°C water bath. The residue was then reconstituted in 75 μl of mobile phase and transferred to autoinjector vials; 25 μl were injected onto the column.

To test the assay in experimental conditions, 3 chinchillas were given 5 mg/kg of cefpodoxime proxetil by intramuscular injection. Samples of plasma and MEF were collected from 0.5 to 24 h after dosing. The log concentration *versus* time data was plotted and the half-life of elimination

was calculated from the linear portion of the curve for each animal.

3. Results

Fig. 1 shows the cefpodoxime level at each sample collection time point. In plasma, cefpodoxime had a mean ± S.D. disappearance half-life of 1.09 ± 0.26 h. Concentration of the drug in MEF was significantly lower than in plasma. Cefpodoxime disappearance half-life from the MEF was 13.7 h.

3.1. Recovery, precision, and accuracy

Recovery was determined by comparing the peak heights of treated plasma and lavage samples with the peak heights of standard injections of the same concentration. Recoveries (mean ± S.D., $n = 3$) at 0.1 μg/ml in MEF were 90.3 ± 2.9% and 88.6 ± 1.2% for cefpodoxime and cefuroxime respectively. Recoveries (mean ± S.D., $n = 3$) at 0.1 μg/ml in plasma were 72.1 ±

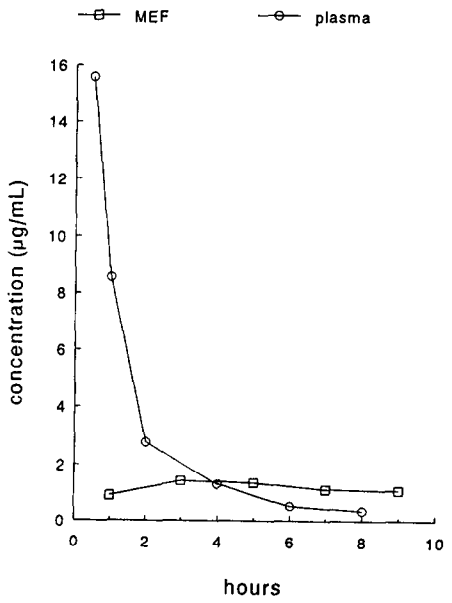

Fig. 1. Concentration–time profile of cefpodoxime in chinchilla plasma and middle ear fluid after an intramuscular injection of 5 mg/kg cefpodoxime proxetil.

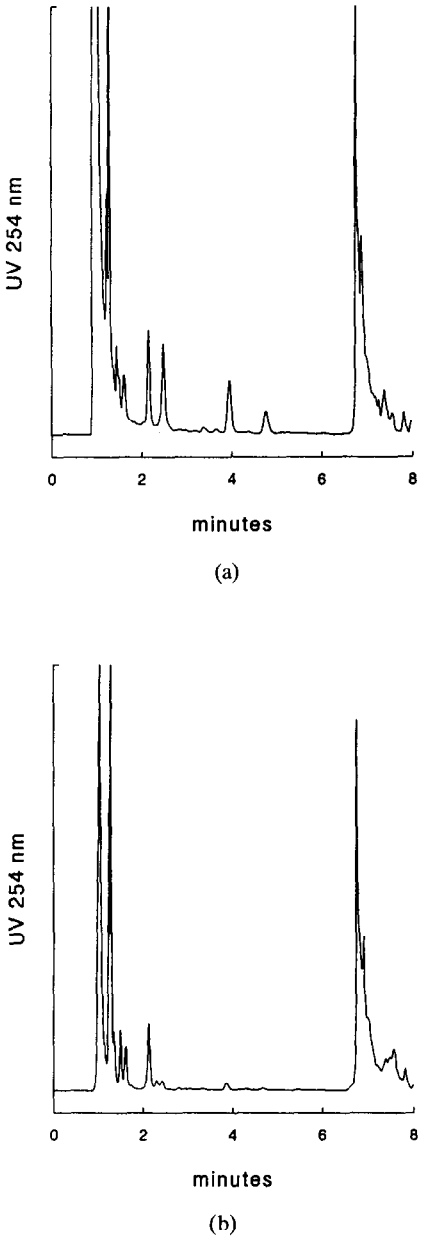

Fig. 2. Chromatograms of (a) blank chinchilla plasma, and (b) blank chinchilla MEF. Separation of cefpodoxime and cefuroxime, the internal standard, occurred on a Keystone ODS column (250 × 2.1 mm I.D., 5 μm Hypersil), using a mobile phase of 25 m*M* acetate buffer (pH 4.3)/15 m*M* TEA–acetonitrile (92.5:7.5, v/v). The retention times were 3.5 min for cefpodoxime and 5.9 min for cefuroxime. This fast, efficient protein precipitation procedure together with UV detection allows a limit of quantitation of 0.05 μg/ml with a 50-μl sample size; 25 μl were injected.

7.3% and 81.1 ± 1.1% for cefpodoxime and cefuroxime respectively.

Peak heights were used for quantitation. Linear regression of the peak height ratios *versus* the drug concentration were performed on the standard curve to determine the slope, intercept, variability, and strength of correlation. The limit of detection was 0.02 μg/ml at a signal-to-noise ratio of 5:1. The standard curve spanned a range from 0.05 to 10 μg/ml. A typical standard curve had a slope of 0.0579, an intercept of − 0.0009, and a correlation coefficient of 0.9991.

There was no interference from endogenous sample components with this method. See Fig. 2a, a chromatogram of blank chinchilla plasma, and Fig. 2b, a chromatogram of blank chinchilla MEF. Figs. 3a and b are chromatograms of chinchilla plasma and a chinchilla MEF, respectively, following administration of cefpodoxime.

Intra-day calibration curves consisted of three replicates at each calibration level. Inter-day calibration standards and quality control standards were assayed over five days. Analysis was done five times intra-assay and fourteen times inter-assay (Tables 1 and 2).

No degradation of QC samples was noticed over a two-week period. All real samples were run within two days of collection. Working stocks were made fresh each day.

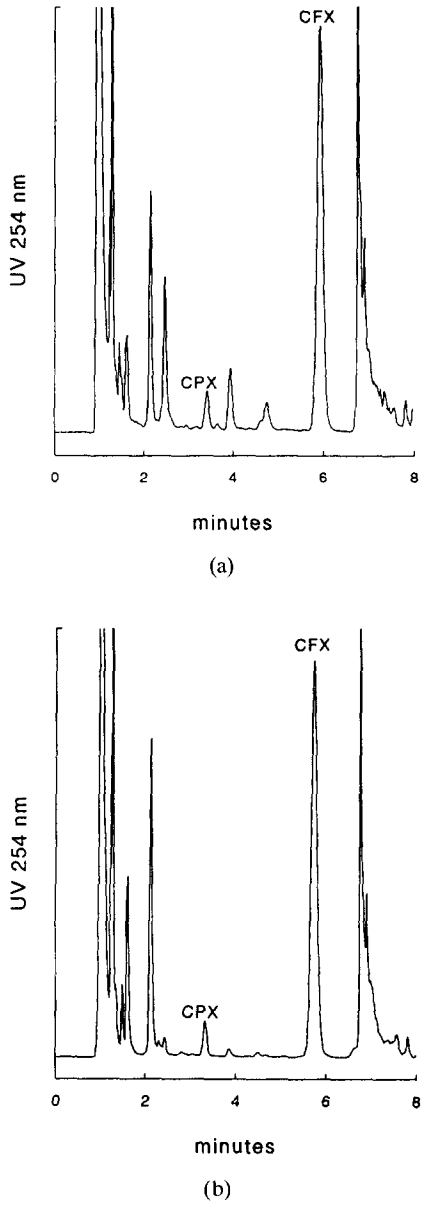

Fig. 3. Chromatograms of (a) chinchilla plasma collected 5 h after a 5 mg/kg intramuscular dose of cefpodoxime peroxetil (0.9 μg/ml cefpodoxime), and (b) chinchilla MEF collected 5 h after a 5 mg/kg intramuscular dose of cefpodoxime proxetil (1.6 μg/ml cefpodoxime). Conditions as in Fig. 2.

4. Conclusion

An HPLC method for analyzing cefpodoxime in microliter volumes of chinchilla plasma and MEF is described. This method is sensitive and rapid, yet requires as little as 50 μl of sample. It involves protein precipitation with acetonitrile and UV detection. We use this method to quantify cefpodoxime in chinchilla plasma and MEF, and are currently using it to study the efficacy and penetration of cefpodoxime in the middle ear of chinchillas with AOM.

5. Acknowledgment

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Table 1
Intra-day and inter-day precision of cefpodoxime determination in chinchilla plasma

Concentration added (μg/ml)	Concentration determined (mean ± S.D.) (μg/ml)	R.S.D. (%)	Accuracy (%)
Intra-assay n = 5%			
0.05	0.049 ± 0.004	7.86	−7.05
0.10	0.104 ± 0.005	5.07	1.08
0.50	0.481 ± 0.022	4.58	−0.65
1.5	1.485 ± 0.041	2.77	3.49
3.0	2.969 ± 0.077	2.59	4.06
5.0	4.837 ± 0.135	2.80	−6.39
10	10.671 ± 0.252	2.37	−3.56
Inter-assay n = 14			
0.05	0.051 ± 0.004	8.80	1.53
0.10	0.098 ± 0.009	9.28	−2.17
0.50	0.483 ± 0.019	4.06	−3.33
1.5	1.456 ± 0.046	3.15	−2.94
3.0	2.878 ± 0.117	4.07	−4.08
5.0	5.025 ± 0.218	4.34	0.50
10	11.049 ± 0.487	4.40	10.50

Analysis conditions as in Fig. 2. Quantitation was by a weighted linear calibration curve of peak-height ratios of cefpodoxime/cefuroxime *versus* concentration over the range of 0.05 to 10 μg/ml.

Table 2
Intra-day and inter-day precision of cefpodoxime determination in chinchilla middle ear fluid

Concentration added (μg/ml)	Concentration determined (mean ± S.D.) (μg/ml)	R.S.D. (%)	Accuracy (%)
Intra-assay n = 5			
0.05	0.049 ± 0.003	5.94	13.89
0.10	0.103 ± 0.004	3.91	−9.81
0.50	0.492 ± 0.013	2.62	−3.30
1.5	1.444 ± 0.060	4.15	4.46
3.0	2.903 ± 0.090	3.10	1.66
5.0	5.080 ± 0.151	2.97	5.39
10	10.543 ± 0.276	2.62	0.77
Inter-assay n = 14			
0.05	0.048 ± 0.004	7.36	−2.92
0.10	0.106 ± 0.005	4.52	6.02
0.50	0.506 ± 0.030	5.88	1.25
1.5	1.430 ± 0.049	3.45	−5.33
3.0	2.882 ± 0.080	2.77	−3.93
5.0	5.015 ± 0.136	2.72	0.30
10	10.462 ± 0.374	3.58	4.62

Analysis conditions as in Fig. 2. Quantitation was by a weighted linear calibration curve of peak-height ratios of cefpodoxime/cefuroxime *versus* concentration over the range of 0.05 to 10 μg/ml.

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