DRUGS, COSMETICS, FORENSIC SCIENCES

Simultaneous Liquid Chromatographic Analysis of the β-Lactam Antibiotics Cefazolin, Cefadroxil, Cephalexin, Ampicillin, and Cephradine in Solution

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A liquid chromatographic method was developed for the determination of nanogram quantities of 5 broad-spectrum structurally related β-lactam antibiotics (cefazolin, cefadroxil, cephalexin, cephradine, and ampicillin) in solution. The method uses a C_{18} reversed-phase column, UV absorption (240 nm) detection, and an aqueous mobile phase containing isopropyl alcohol and acetic acid. Relative resolution between the antibiotic peaks ranged from 1.7 to 5.9 for all peaks. Chromatographic retention times were 2.97, 3.92, 4.57, 5.37, and 6.56 min for cefazolin, cefadroxil, cephalexin, ampicillin, and cephradine, respectively. Accuracy, precision, linearity, and long term analytical reproducibility were determined by statistical analysis. Use of the proposed method to evaluate the degradation of cephradine solutions stored at room temperature illustrated its potential as a stability-indicating assay.

Cefazolin, cefadroxil, cephalexin, ampicillin, and cephradine (Figure 1) are semi-synthetic cephalosporin β-lactam antibiotics that are active against both Gram-positive and Gram-negative bacteria (1) and are widely used for the treatment of infections. However, the in vitro bactericidal activity of an antibiotic does not always correlate with its therapeutic efficacy for many reasons (2). In recent years, a growing market demand (1) has served to intensify synthesis efforts on the part of pharmaceutical companies, leading to the introduction and subsequent approval of a variety of β-lactams with higher potency and/or broader spectral range. Methods for the analysis of β-lactams include microbial assay (3), hydroxylamine assay (3), iodometric assay (1), immunoassay (4), nonaqueous titration for the acidic and basic functional groups (1), and liquid chromatography (LC; 1, 5). Of all these methods, LC has proven to be superior to the others in its specificity, stability-indicating ability, and simultaneous analysis (1).

The purpose of this investigation was to develop an accurate, reproducible, and rugged LC assay to determine the presence of cefazolin, cefadroxil, cephalexin, ampicillin, and cephradine combined in solution and to evaluate the method for its use in assessing the stability of admixture solutions containing these β-lactams. Although published methods are available for some of these antibiotics, the present method is more convenient and efficient for the assay of large numbers of samples.

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Experimental

Apparatus

(a) *Columns.*—C_{18} reversed-phase 250 × 3.2 mm, 3 μm chromosphere (Phenomenex, Torrance, CA), C_{18} reversed-phase 250 × 4.62 mm, 5 μm chromosphere (Phenomenex), and C_{18} reversed-phase 250 × 4.6 mm, 5 μm ODS (Beckman Instruments, Fullerton, CA).

(b) *Liquid chromatograph.*—UV/Vis spectrophotometer multiple wavelength detector (Hewlett-Packard, ChemStation 1050, Palo Alto, CA) solvent delivery system, autosampler injector, and data station.

(c) *Chromatographic parameters.*—Flow rate, 1 mL/min; detector wavelength, 240 nm. Mobile phase, 10% acetic acid solution in water–isopropyl alcohol–water (4 + 9 + 87).

Reagents

(a) *Antibiotics.*—Cephradine hydrate, cephalexin, cefazolin sodium salt, and cefadroxil (Sigma Chemical Co., St. Louis, MO). Ampicillin sodium, USP Reference Standard (U.S. Pharmacopeial Convention, Rockville, MD).

(b) *Isopropyl alcohol.*—Glass-distilled, suitable for LC (EM Science, Gibbstone, NJ).

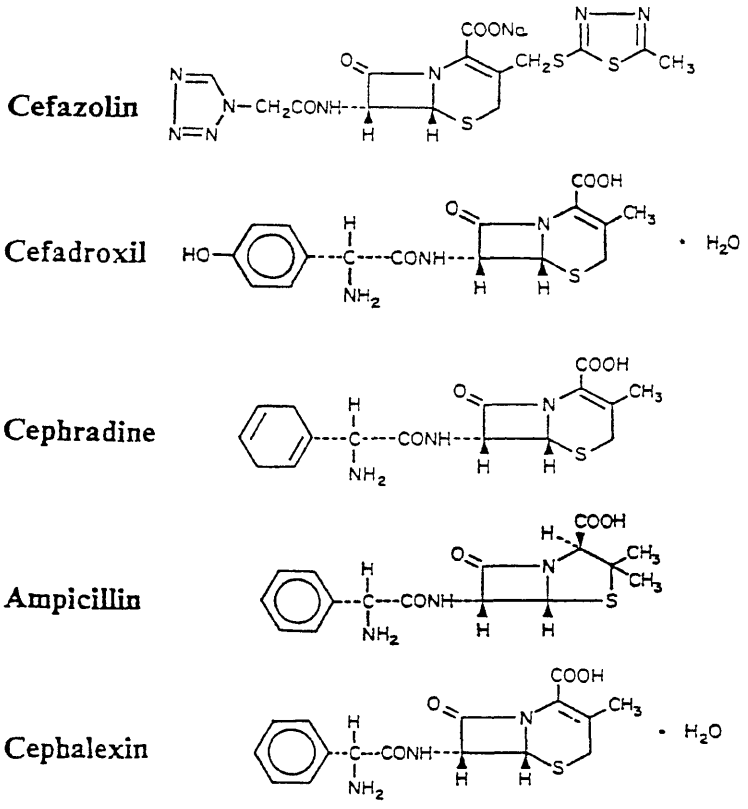

Figure 1. Chemical structures of ampicillin, cefazolin, cephradine, cephalexin, and cefadroxil.

(c) *Water.*—Milli-Q grade (MQ water, Waters Associates, Inc., Milford, MA).

Sample Preparation

A 20 mg portion of each antibiotic was weighed and dissolved quantitatively in 200 mL deionized water (these stock solutions can be stored at 0°–5°C for 5 days). Immediately before the analyses, working solutions were prepared by diluting each stock solution (1 + 9) with a refrigerated solution of 0.5% sodium chloride in MQ water (0.01 mg antibiotic/mL, or 10 μg/mL). The working solutions were prepared fresh every day and stored under refrigeration. The working admixture solution was prepared by carefully mixing 1 mL of each antibiotic stock solution and diluting the resultant solution (5 + 5) with 1% sodium chloride solution in MQ water. Immediately before starting the analyses, ca 2 mL of each antibiotic working solution and the admixture working solution were transferred to separate autosampler vials. The working solutions were diluted further for linearity and minimum quantitative studies.

LC Determination

The Chemstation was programmed to make one 5 μL injection of each of 11 vials. Each vial contained independently prepared samples of each antibiotic and the mixture. The precision of the assay was determined for each antibiotic. The same analysis was repeated once each week, for 7 weeks, with different concentrations each week (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, and 17.6 μg) to determine the week-to-week variation and the linearity of the method. To evaluate the short-term stability of reconstituted antibiotic solutions, the contents of each auto injector vial were allowed to stand at room temperature (25°C) for 3 days. Each solution was reassayed twice daily during this period.

Results

Acceptable separation was obtained between each pair of the 5 β-lactams as shown by the retention times and resolution in Figure 2. The relative standard deviations (RSD) of the retention times, peak areas, and peak heights are shown in Tables 1–3. The method was repeated with 3 different analytical colums and at different weeks. Table 4 shows that the method offers reasonable precision and is rugged. The method is stability-indicating for cephradine (Figure 3) and is linear in the range of 2.5–17.6 μg/mL. The linear correlation coefficients are shown in Table 4. The minimum

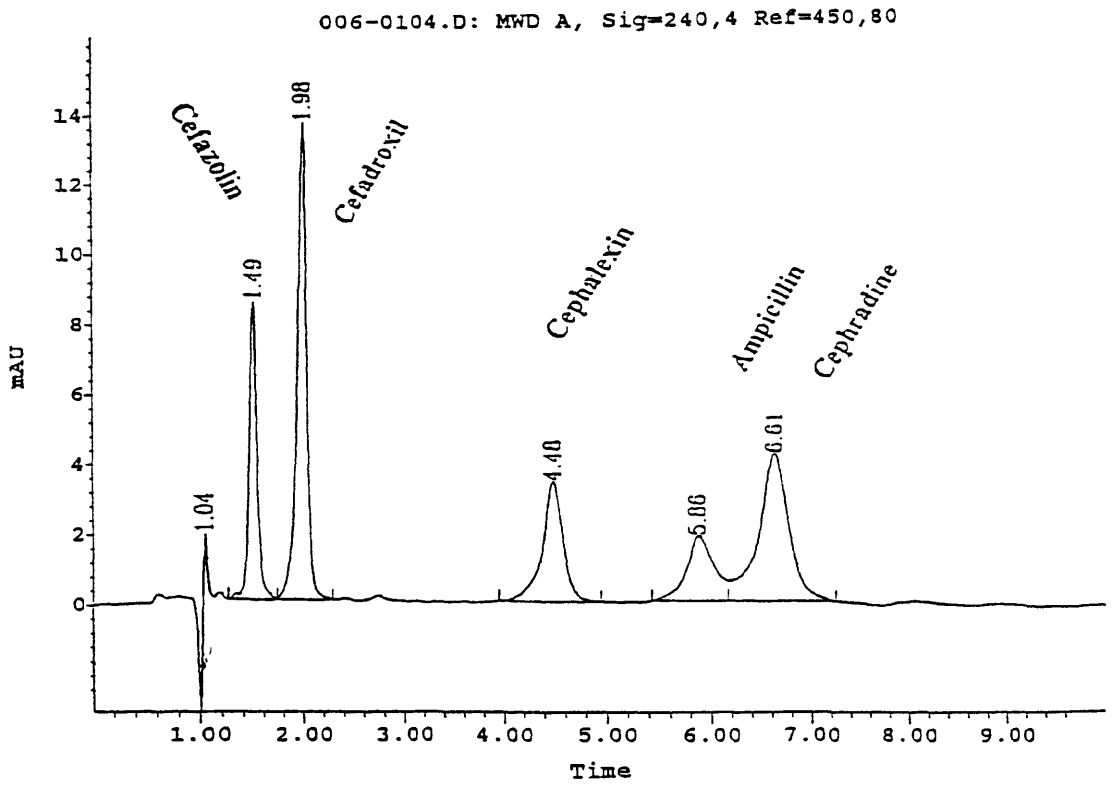

Figure 2. Separation of cefazolin, cefadroxil, cephalexin, ampicillin, and cephradine by LC method (*see* text for *conditions*).

Table 1. Precision of assay of cefazolin, cefadroxil, cephalexin, ampicillin, and cephradine (n = 11) by retention time, peak area, and peak height for 5 μL injection of 2.5 μg/mL solution

	Retention time			Mean peak response			
Compound	Min	RSD, %	Resolution (R)[a]	Area[b]	Area RSD, %	Height	Height RSD, %
Cefazolin	2.97	1.6		889	0.4	209	1.62
Cefadroxil	3.92	1.6	5.9	391	1.2	45	1.68
Cephalexin	4.57	1.4	2.8	480	1.1	53	0.00
Ampicillin	5.37	1.8	2.8	143	1.7	12	0.00
Cephradine	6.05	1.6	1.7	300	1.4	25	1.69

[a] Relative separation factors between each peak and its previous eluting peak.
[b] These data are also recorded in Table 4, column 1.

Table 2. Precision of assay of cefazolin, cefadroxil, cephalexin, ampicillin, and cephradine (n = 8) by retention time, peak area, and peak height for 5 μL injection of 5 μg/mL solution

	Retention time			Mean peak response			
Compound	Min	RSD, %	Resolution (R)[a]	Area[b]	Area RSD, %	Height	Height RSD, %
Cefazolin	2.88	0.6		1725	0.9	384	1.1
Cefadroxil	3.89	1.9	4.5	763	1.6	84	1.1
Cephalexin	4.49	1.0	2.8	931	1.8	92	1.1
Ampicillin	5.34	1.0	2.4	276	1.5	20	1.6
Cephradine	6.03	1.1	1.8	590	1.5	43	1.6

[a] Relative separation factors between each peak and its previous eluting peak.
[b] These data are also recorded in Table 4, column 2.

Table 3. Precision of assay of cefazolin, cefadroxil, cephalexin, ampicillin, and cephradine (n = 8) by retention time, peak area and peak height for 5 μL injection of 15 μg/mL solution

	Retention time			Mean peak response			
Compound	Min	RSD, %	Resolution (R)[a]	Area[b]	Area RSD, %	Height	Height RSD, %
Cefazolin	2.90	0.5		5353	1.4	805	1.5
Cefadroxil	3.93	0.6	4.3	2386	1.9	210	1.5
Cephalexin	4.50	0.5	2.4	2838	1.6	202	0.5
Ampicillin	5.33	0.5	2.2	838	1.5	43	1.3
Cephradine	6.05	0.7	1.4	1771	1.9	92	1.8

[a] Relative separation factors between each peak and its previous eluting peak.
[b] These data are also recorded in Table 4, column 6.

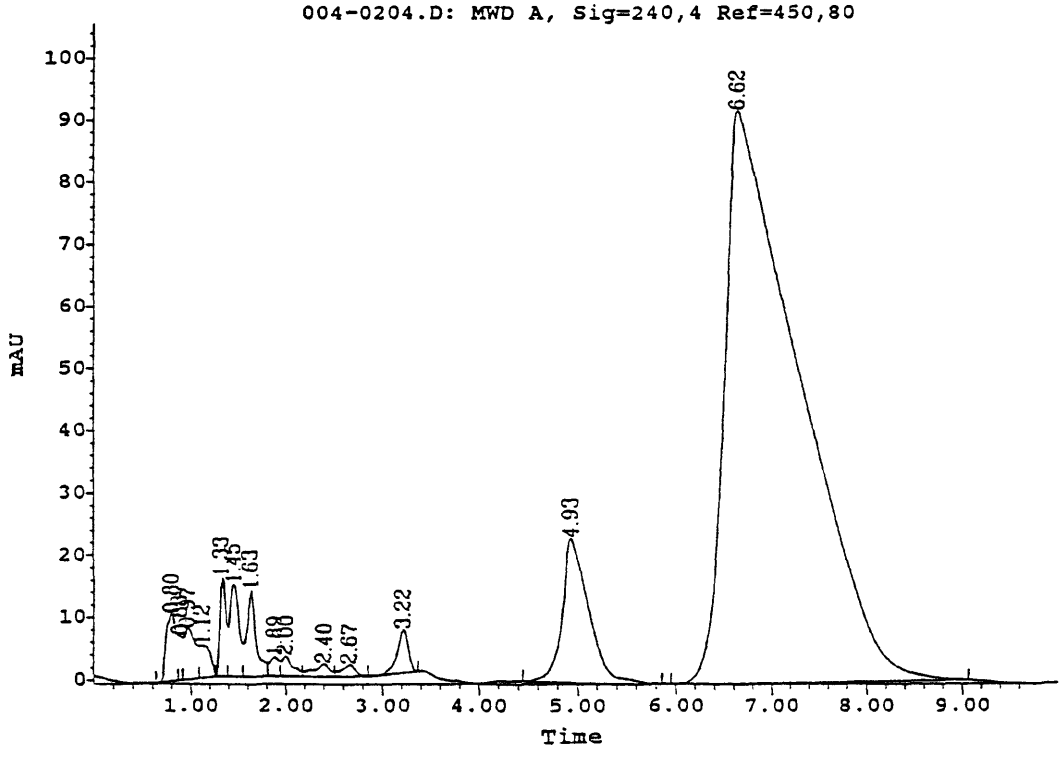

Figure 3. Chromatograms of cephradine standard (*see* text for conditions). This standard contains several measurable contaminants, demonstrating that the method is stability-indicating for cephradine.

Table 4. Calibration curve linearity of each antibiotic studied

Compound concentration (μg/mL)	Mean peak area response							
	2.5[a]	5.0[b]	7.5	10.0	12.0	15.0[c]	17.6	
Run time	at start	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	Linear correlation coefficient
Cefazolin	889	1725	2719	3635	4460	5352	6264	0.9998
Cefadroxil	391	763	1159	1613	2013	2386	2807	0.9997
Cephalexin	480	931	1398	1881	2394	2838	3392	0.9998
Ampicillin	143	276	408	545	719	838	1036	0.9984
Cephradine	300	590	890	1164	1491	1771	2152	0.9994

[a] These data are also presented in Table 1.
[b] These data are also recorded in Table 2.
[c] These data are also recorded in Table 3.

Table 5. Stability of cefazolin, cefadroxil, cephalexin, ampicillin, and cephradine solutions at refrigeration and room temperatures

	Percentage of assay after days refrigerated					Percentage of assay after days at room temperature		
Solution	1	2	3	4	5	1	2	3
Cefazolin,	100.0	100.5	99.0	99.3	101.7	100.0	95.4	94.2
Cefadroxil	100.0	99.4	101.9	100.8	98.1	100.0	96.6	90.9
Cephalexin	100.0	100.0	101.8	99.3	98.6	100.0	94.7	93.0
Ampicillin	100.0	99.1	101.5	99.8	101.1	100.0	95.5	92.8
Cephradine	100.0	100.5	99.4	99.0	98.8	100.0	88.7	62.1

levels of quantitation (10 to 1 signal-to-noise ratio) are cefazolin, 50 ng/mL; cefadroxil, 20 ng/mL; cephalexin, 40 ng/mL; ampicillin, 10 ng/mL; and cephradine, 20 ng/mL.

Short-term stability studies of the reconstituted antibiotic solutions showed that cephradine solution lost more than 38% potency during 3 days of storage at room temperature (Table 5). The other antibiotic solutions lost from 6 to 9% potency under similar storage conditions.

Conclusion

The proposed method provides a fast, accurate, and rugged assay with stability-indicating potential for these β-lactams in solution alone or in mixtures. It can also be used for a large number of samples.

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