THE DETERMINATION OF CEPHRADINE AND CEPHALEXIN BY REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

MARGARET A. CARROLL, E. RODERICK WHITE,
ZSUZSA JANCSIK and JOHN E. ZAREMBO

Smith, Kline and French Laboratories, 1500 Spring Garden Street,
Philadelphia, PA 19101, U.S.A.

(Received for publication February 15, 1977)

The application of reverse phase high-performance liquid chromatography to the separation and analysis of cephradine and cephalexin is demonstrated. The procedure has been applied to chemicals, pharmaceutical formulations and reaction solutions. The preparation of samples is simple and rapid. Chromatographic conditions are described for both pellicular and small particle columns. The feasibility of determining cephradine and cephalexin in physiological fluids has also been demonstrated.

Cephradine and cephalexin are members of the cephalosporin family, an important new class of antibiotics. As shown in Fig. 1, these compounds have a β-lactam and a fused six-member sulfur-containing ring and differ by only one double bond. Cephalexin contains the phenylglycine moiety attached to the nitrogen at the 7-position, while in the case of cephradine the dihydrophenylglycine moiety is attached.

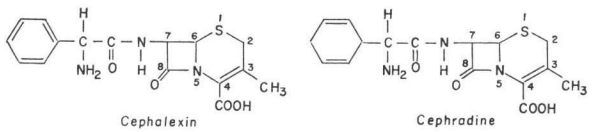
Both cephradine and cephalexin, as well as other cephalosporin antibiotics, are frequently determined by microbiological assay or chemical methods, such as iodometric titrations, hydroxylamine assay or the ninhydrin reaction[1]. These chemical methods are based on functional groups common to both compounds and do not distinguish between cephradine and cephalexin. However, the high-performance liquid chromatographic techniques presented here provide more accurate, convenient, rapid and specific methods of analysis for these compounds. In a previous paper[2], we described the application of reverse phase high-performance liquid chromatography to many cephalosporins and other antibiotics. Varian used a variation of our approach for the separation of 7-ADCA and cephalexin using a small particle column[3]. This paper presents the application of reverse phase chromatography to the separation and quantitation of cephradine and cephalexin in various media.

Experimental

Reagents and Materials

Distilled water was used for preparing all solutions. Cephalexin, cephradine, and cephaloglycin

Fig. 1. Structures of cephalexin and cephradine.



* Presented, in part, at the 26th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 3~7, 1975

chemicals and cephalexin formulations were obtained from commercial sources. Cephradine formulations were obtained "in house." Methanol and ammonium carbonate were reagent grade and were obtained from Arthur H. Thomas Co., Philadelphia, Pennsylvania. Human serum (Validate^{+m}) was obtained from Warner Lambert Company, Morris Plains, New Jersey.

Apparatus

The instruments used in this study include Dupont Models 820 and 830 (E. I. duPont de Nemours & Co., Inc., Instrument Products Division, Wilmington, Delaware) and instruments assembled in our laboratory from commercially available components which have been described previously[2]. All instruments were equipped with UV detectors (254 nm). Samples were injected with either a 10- or 100-μl syringe (C-160 Series, Precision Sampling Co., Baton Rouge, Louisiana) or with a 20-μl valve and loop injector (Cat. No. 190 A 48 Sample Injection Valve Module, Chromatronix, Inc., Berkeley, California). All separations were carried out at room temperature.

Columns and Column Packings

Octadecylsilyl (ODS) Sil-X-11 packing was obtained from Perkin-Elmer Corporation, Norwalk, Connecticut. Columns (1 m×2.1 mm i.d.) prepared from this material were packed using standard dry-pack procedures[4]. μ Bondapack C_{18} was obtained as a pre-packed column (30 cm×4 mm i.d.) from Waters Associates, Milford, Massachusetts. Small particle columns (30 cm×4.6 mm i.d. and 15 cm×4.6 mm i.d.) were prepared in our laboratory by slurry packing[5] Lichrosorb SI 100 (10 μm) and then coating the silica with C_{18} by an "in situ" technique which is similar to that described by GILPIN *et al.*[6]. All column tubing used in this study was 6.3 mm O.D. stainless steel. The 1 meter columns used in the Dupont 820 and the self-built instruments were straight, and the columns for the Dupont 830 were "U" shaped.

Chromatographic Procedures

Mixtures of methanol and ammonium carbonate in various proportions were used as the mobile phase. The injection volumes were 10 μl for small particle columns and 20 μl for pellicular columns. Calculations were based on peak height measurements.

Preparation of Standard Solutions

Internal standard solution: A solution of cephaloglycin was prepared containing 0.5 mg/ml in methanol - water (1 : 9). The solution was prepared fresh daily.

Cephalexin standard: Approximately 5 mg of reference standard cephalexin was accurately weighed into a 50-ml volumetric flask, and dissolved in and diluted to volume with water. A 2-ml aliquot was pipetted into a 25-ml volumetric flask, 10.0 ml of internal standard solution added, and the solution diluted to volume with water.

Cephradine standard: Approximately 4.5 mg of reference standard cephradine was accurately weighed into a 25-ml volumetric flask. About 12 ml of water was added to dissolve the sample, 10.0 ml of internal standard solution was added, and the solution was diluted to volume with water.

Preparation of Samples

Chemicals: Chemicals were prepared in a similar manner to the standards.

Capsules (250 and 500 mg): The average capsule fill of 20 capsules was determined. An accurately weighed sample of the well-mixed powder, equivalent to about 65 mg of cephradine, was transferred to a 50-ml volumetric flask and dissolved in and diluted to volume with water. A 3-ml aliquot was pipetted into a 25-ml volumetric flask, 10.0 ml of internal standard solution added, and the solution was then diluted to volume with water.

Oral suspension (125 and 250 mg/3.3 g): An accurately weighed sample of dry suspension, equivalent to about 65 mg of cephradine, was transferred to a 50-ml volumetric flask, and dispersed in and diluted to volume with water. The preparation of the sample was continued as described for "Capsules."

Reaction solutions: An aliquot of reaction solution was accurately weighed and diluted with water to an expected concentration of approximately 0.2 mg/ml.

Determination of Cephradine and Cephalexin in Urine

Aliquots of human urine were spiked with cephradine at a concentration of 0.7 mg/ml. Three ml aliquots were pipetted into a 10-ml volumetric flask, 2.0 ml of internal standard solution (2 mg/ml)

was added and the solution diluted to volume with water. This solution contained 0.2 mg/ml of cephradine. Two-ml aliquots of cephalexin (0.5 mg/ml) were diluted in a similar manner to a final concentration of 0.1 mg/ml.

Determination of Cephradine in Serum

One-ml aliquots of reconstituted human serum were spiked with 5 μg of cephradine and 100 μl of serum were injected with a syringe directly on the column using a stop flow technique.

Fig. 2. Separation of a mixture of cephalosporins on a small particle column.
Column: 30 cm×4 mm i.d., μ Bondapak C_{18}; Mobile phase: methanol - 0.03% ammonium carbonate (5: 95); Pressure: 1,000 psi; Flow: 0.9 ml/min.; Sensitivity: 0.02 AUFS

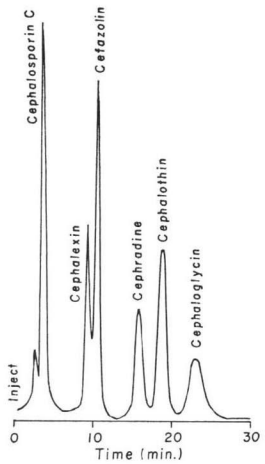


Fig. 3. Effect of varying ammonium carbonate concentration of the mobile phase.
Column: 30 cm×4 mm i.d., μ Bondapak C_{18}; Mobile phase: methanol - ammonium carbonate (8: 92); Pressure: 800 psi; Flow: 0.8 ml/min.; Sensitivity: 0.08 AUFS

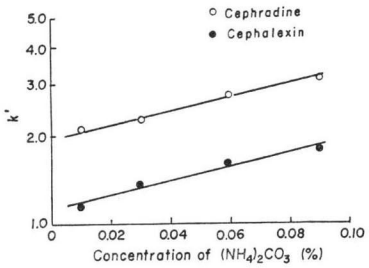


Fig. 4. Effect of varying the amount of organic modifier in the mobile phase.
Column: 30 cm×4 mm i.d., μ Bondapak C_{18}; Mobile phase: methanol - 0.03% ammonium carbonate; Pressure: 1,000 psi; Flow: 0.9 ml/min.; Sensitivity: 0.08 AUFS

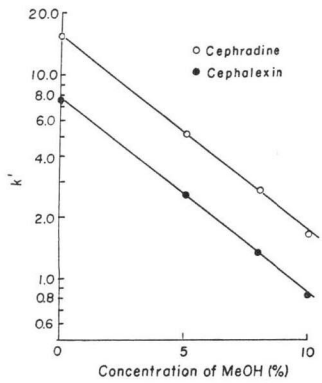


Fig. 5. Quantitation of cephradine in an oral suspension on a small particle column.
Column: 30cm×4mm i.d., μ Bondapak C_{18}; Mobile phase: methanol - 0.03% ammonium carbonate (8:92); Pressure: 1,000 psi; Flow: 0.9 ml/min.; Sensitivity: 0.08 AUFS

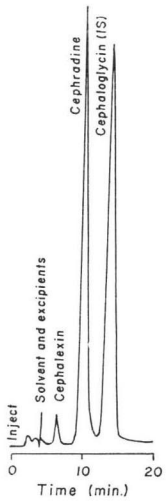


Fig. 6. Quantitation of cephradine in an oral suspension on a pellicular column.
Column: 1m×2.1mm i.d., Sil-X-11 ODS; Mobile phase: methanol - 0.5% ammonium carbonate (5:95); Pressure: 1,000 psi; Flow: 0.6 ml/min.; Sensitivity: 0.08 AUFS

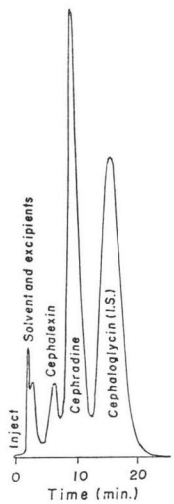


Table 1. Multiple determinations of a single sample of cephradine capsule fill and oral suspension using a small particle column

	Capsules, mg/cap (Claim 250 mg/cap)		Oral suspension, mg/3.3 g (Claim 250 mg/3.3 g)	
	Cephalexin	Cephradine	Cephalexin	Cephradine
	8.26	265.4	11.30	276.6
	7.92	261.5	10.93	273.4
	8.00	262.5	10.93	278.9
	8.27	261.7	10.67	275.9
	8.02	261.2	10.81	273.5
Average	8.09	262.5	10.93	275.7
σ	0.16	1.7	0.23	2.3
$RSD_{(1\sigma)}$	1.9%	0.7%	2.1%	0.8%

Results and Discussion

Although cephradine and cephalexin are ionic compounds and can also be separated by ion-exchange chromatography, the use of reverse phase chromatography offers several advantages. The resins are permanently bonded and are stable over wide ranges of pH, temperature and for extended periods of time. The mobile phases

Table 2. Multiple determinations of a single sample of cephalexin capsule fill on a pellicular column

Claim, mg/cap	Found, mg/cap
250	250.8
	255.6
	252.0
	250.2
	255.5
Average	252.8

$\sigma=2.6$, $RSD_{(1\sigma)}=1.0\%$

Fig. 7. Quantitation of cephalexin in capsules on a pellicular column.
Column: 1 m×2.1 mm i.d., Sil-X-11 ODS; Mobile phase: methanol - 0.5% ammonium carbonate (2: 98); Pressure: 1,500 psi; Flow: 1.3 ml/min; Sensitivity: 0.08 AUFS

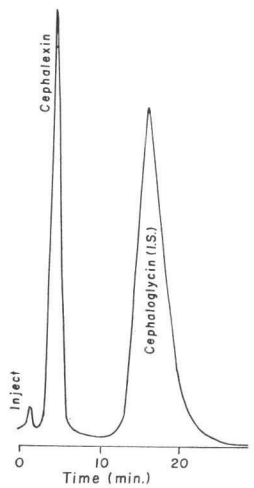


Fig. 8. Quantitation of cephradine in urine samples on a pellicular column.
Column: 1 m×2.1 mm i.d., Sil-X-11 ODS; Mobile phase: methanol - 0.5% ammonium carbonate (2: 98); Pressure: 1,500 psi; Flow: 1.3 ml/min.; Sensitivity: 0.02, 0.08 AUFS

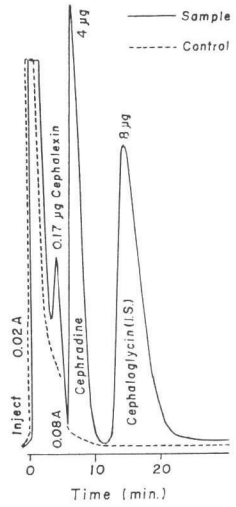


Fig. 9. Quantitation of cephradine in serum on a small particle column.
Column: 15 cm×4.6 mm i.d., "*in situ*" C_{18} on Lichrosorb SI 100; Mobile phase: 0.06% ammonium carbonate; Pressure: 1,500 psi; Flow: 1.2 ml/min.; Sensitivity: 0.02 AUFS

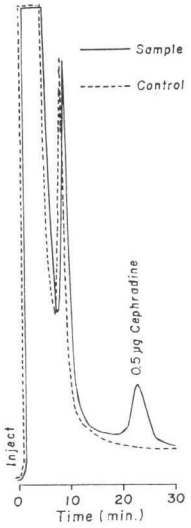


Table 3. Analysis of spiked cephradine and cephalexin urine samples on a pellicular column

Cephradine, μg/ml			Cephalexin, μg/ml		
Calcd.	Found	Recovery, %	Calcd.	Found	Recovery, %
664	663	99.8	469	461	98.3
656	661	100.8	468	467	99.8
698	702	100.6	491	476	96.9
654	657	100.4	446	443	99.4
689	697	101.2	506	497	98.3
	Average=100.6			Average=98.5	
	σ=0.5			σ=1.1	
	$RSD_{(1\sigma)}$=0.5%			$RSD_{(1\sigma)}$=1.1%	

are inexpensive and easy to prepare. Ionic strength and pH are not as critical in reverse phase chromatography as they are in ion-exchange. Another cephalosporin compound with similar physical and chemical characteristics can easily be selected as the internal standard. Fig. 2 shows the elution pattern of a number of cephalosporins obtained on a small particle C_{18} column. As can be seen from the figure, cephaloglycin is an ideal internal standard for this assay.

Table 4. Analysis of spiked cephradine serum samples on a micropack column

Calcd., μg/ml	Found, μg/ml	Recovery, %
5.6	5.2	92.9
5.6	5.4	96.4
5.1	4.7	92.2
5.4	5.4	100.0
5.1	4.9	96.0
5.1	4.8	94.1
Average		94.9

σ=2.5, $RSD_{(1\sigma)}$=2.6%

The retention times of cephradine and cephalexin can be changed by varying the amount of organic modifier and the concentration of the ammonium carbonate. Increasing the polarity of the mobile phase by the addition of ammonium carbonate increases the retention time. Fig. 3 is a semi-logarithmic plot of the capacity factor, k'*, as a function of the concentration of the ammonium carbonate. An increase in ammonium carbonate concentration from 0.01 to 0.09% increased k' (cephradine) from 2.1 to 3.1 and k' (cephalexin) from 1.1 to 1.8. However, these changes in k' are small in comparison to those obtained by varying the concentration of organic modifier. Fig. 4 shows a similar semi-logarithmic plot of k' for cephradine and cephalexin as a function of methanol concentration in the mobile phase. By varying the methanol content from 0 to 10%, k' (cephradine) is varied, conversely to the salt concentration, from 15 to 1.7, k' (cephalexin) from 7.5 to 0.83. Thus, a change in organic modifier concentration is the principal means by which k' is varied. The mobile phase selected for the small particle column, methanol - 0.03% ammonium carbonate (8: 92), elutes the two compounds and the internal standard within 13 minutes (Fig. 5) with a resolution factor of 1.6 for cephalexin and cephradine, and 0.95 for cephradine and cephaloglycin.

This assay can also be accomplished on a pellicular packing (Fig. 6) using a mobile phase of methanol - 0.5% ammonium carbonate (5: 95). An assay is completed in 22 minutes. Cephalexin and cephradine are not completely separated and have a resolution factor of 0.7. Precision (1σ) for cephradine is 1.2%, for low levels (~3%) of cephalexin 2.3%.

* $k'=(t_R-t_o)/t_o$ where t_R=retention of compound and t_o=retention time of an unretained component.

chemicals and cephalexin formulations were obtained from commercial sources. Cephradine formulations were obtained "in house." Methanol and ammonium carbonate were reagent grade and were obtained from Arthur H. Thomas Co., Philadelphia, Pennsylvania. Human serum (Validate^{+m}) was obtained from Warner Lambert Company, Morris Plains, New Jersey.

Apparatus

The instruments used in this study include Dupont Models 820 and 830 (E. I. duPont de Nemours & Co., Inc., Instrument Products Division, Wilmington, Delaware) and instruments assembled in our laboratory from commercially available components which have been described previously[2]. All instruments were equipped with UV detectors (254 nm). Samples were injected with either a 10- or 100-μl syringe (C-160 Series, Precision Sampling Co., Baton Rouge, Louisiana) or with a 20-μl valve and loop injector (Cat. No. 190 A 48 Sample Injection Valve Module, Chromatronix, Inc., Berkeley, California). All separations were carried out at room temperature.

Columns and Column Packings

Octadecylsilyl (ODS) Sil-X-11 packing was obtained from Perkin-Elmer Corporation, Norwalk, Connecticut. Columns (1 m×2.1 mm i.d.) prepared from this material were packed using standard dry-pack procedures[4]. μ Bondapack C_{18} was obtained as a pre-packed column (30 cm×4 mm i.d.) from Waters Associates, Milford, Massachusetts. Small particle columns (30 cm×4.6 mm i.d. and 15 cm×4.6 mm i.d.) were prepared in our laboratory by slurry packing[5] Lichrosorb SI 100 (10 μm) and then coating the silica with C_{18} by an "in situ" technique which is similar to that described by GILPIN *et al.*[6]. All column tubing used in this study was 6.3 mm O.D. stainless steel. The 1 meter columns used in the Dupont 820 and the self-built instruments were straight, and the columns for the Dupont 830 were "U" shaped.

Chromatographic Procedures

Mixtures of methanol and ammonium carbonate in various proportions were used as the mobile phase. The injection volumes were 10 μl for small particle columns and 20 μl for pellicular columns. Calculations were based on peak height measurements.

Preparation of Standard Solutions

Internal standard solution: A solution of cephaloglycin was prepared containing 0.5 mg/ml in methanol - water (1 : 9). The solution was prepared fresh daily.

Cephalexin standard: Approximately 5 mg of reference standard cephalexin was accurately weighed into a 50-ml volumetric flask, and dissolved in and diluted to volume with water. A 2-ml aliquot was pipetted into a 25-ml volumetric flask, 10.0 ml of internal standard solution added, and the solution diluted to volume with water.

Cephradine standard: Approximately 4.5 mg of reference standard cephradine was accurately weighed into a 25-ml volumetric flask. About 12 ml of water was added to dissolve the sample, 10.0 ml of internal standard solution was added, and the solution was diluted to volume with water.

Preparation of Samples

Chemicals: Chemicals were prepared in a similar manner to the standards.

Capsules (250 and 500 mg): The average capsule fill of 20 capsules was determined. An accurately weighed sample of the well-mixed powder, equivalent to about 65 mg of cephradine, was transferred to a 50-ml volumetric flask and dissolved in and diluted to volume with water. A 3-ml aliquot was pipetted into a 25-ml volumetric flask, 10.0 ml of internal standard solution added, and the solution was then diluted to volume with water.

Oral suspension (125 and 250 mg/3.3 g): An accurately weighed sample of dry suspension, equivalent to about 65 mg of cephradine, was transferred to a 50-ml volumetric flask, and dispersed in and diluted to volume with water. The preparation of the sample was continued as described for "Capsules."

Reaction solutions: An aliquot of reaction solution was accurately weighed and diluted with water to an expected concentration of approximately 0.2 mg/ml.

Determination of Cephradine and Cephalexin in Urine

Aliquots of human urine were spiked with cephradine at a concentration of 0.7 mg/ml. Three ml aliquots were pipetted into a 10-ml volumetric flask, 2.0 ml of internal standard solution (2 mg/ml)

particle columns include improved resolutions, sharper peaks, greater retention and shorter analysis time.

References

1) FLYNN, E. H.: Cephalosporins and Penicillins. Academic Press, New York and London, p. 610, 1972
2) WHITE, E. R.; M. A. CARROLL, J. E. ZAREMBO & A. D. BENDER: Reverse phase high speed liquid chromatography of antibiotics. J. Antibiotics 28: 205~214, 1975
3) LOTSCHER, K. M.; B. BRANDER & H. KERN: Liquid chromatographic analysis of antibiotics. Varian Instrument Applications 9: 12~15, 1975
4) KIRKLAND, J. J.: Performance of Zipax controlled surface porosity support in high-speed liquid chromatography. J. Chromat. Sci. 10: 129~137, 1972
5) MAJORS, R. E.: High performance liquid chromatography on small particle silica gel. Anal. Chem. 44: 1722~1726, 1972
6) GILPIN, R. K.; J. A. KORPI & C. A. JANICKI: On column preparation of bonded phases for high pressure liquid chromatography. Anal. Chem. 46: 1314~1316, 1974