Table I—Values Observed on Day of Experiment [a]

Parameter	Value
Animal weight, g	225 ± 38
Amount of mixture eaten, g/kg[b]	57.1 ± 7.2
Potassium oxonate ingested, g/kg	2.86 ± 0.36
Uric acid ingested, g/kg	1.14 ± 0.14

[a] Values are reported as mean ± *SD*. [b] Mixture consisted of ground laboratory ration containing 5% potassium oxonate and 2% uric acid (w/w each).

Accumulation to a plateau would be expected to occur with a zero-order administration process (19). The absorption of uric acid from the rat intestine, however, has been reported to be first order (20). Therefore, gastric emptying rather than intestinal absorption may be rate limiting. Reported data (16, 17) for untreated control animals trained by this technique are consistent with a zero-order stomach emptying rate for the first 10–12 hr following the daily feeding. This animal model will be used to test the efficacy of light and riboflavin in the reduction of serum uric acid.

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ACKNOWLEDGMENTS

Supported in part by Biomedical Sciences Support Grant 26-16994-0650 from the University of Texas at Austin.

The authors thank Mr. Paul Godley and Mr. Joseph Lew for their assistance.

High-Performance Liquid Chromatographic Determination of Cephacetrile

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Received June 6, 1978, from the *Research Laboratories, Pierrel SpA, Via Degli Artigianelli 10, 20159 Milan, Italy* Accepted for publication October 19, 1978.

Abstract □ A rapid and accurate quantitative determination of cephacetrile in finished bulk and dosage forms is reported. The high-performance liquid chromatographic method is free of interference by acetyl hydrolysis products and synthesis by-products. The assay can be performed in about 15 min, affording <0.7% coefficients of variation within and between days. The chromatographic results are in good agreement with the microbiological assay requested by the "Code of Federal Regulations" for certification of cephacetrile sodium.

Keyphrases □ High-performance liquid chromatography—analysis, cephacetrile in various dosage forms □ Cephacetrile—high-performance liquid chromatographic analysis in various dosage forms □ Antibacterials—cephacetrile, high-performance liquid chromatographic analysis in various dosage forms

Cephacetrile (I) sodium [7-cyanoacetamido-3-acetoxymethyl-3-cephem-4-carboxylic acid, sodium salt] is a new cephalosporin antibacterial for parenteral use (1). The approved methods of analysis for certification are the microbiological agar diffusion assay and the hydroxylamine colorimetric assay (2).

This report outlines a more selective, rapid, and accurate method for the quantitative determination of cephacetrile in bulk drugs and pharmaceutical formulations using high-performance liquid chromatography (HPLC). The method is free of interference by acetyl hydrolysis products and synthesis by-products. Furthermore, the desacetyl derivative (II) of cephacetrile and lactone (III) can be detected accurately up to 0.1%.

EXPERIMENTAL

Materials—Cephacetrile FDA reference standard (microbiological potency of 885 μg/mg, lot 9/72) was used as received. Desacetyl derivative (II) of cephacetrile was prepared by enzymatic hydrolysis with acetyl esterase (3), while lactone (III) was synthesized by reacting trifluoroacetic acid with cephacetrile. Cephalosporins (IV*a* and IV*b*) were obtained by reaction of V with the proper acid chloride in acetone in the presence of urea (4), while IV*c* was prepared by reaction of methanesulfonyl chloride with the silyl derivative of V in dichloroethane (Scheme I). Cefazolin was Pierrel working standard. Methanol[1], acetic acid[2], and 1 *N* NaOH[2] were analytical reagent grade.

Apparatus—A liquid chromatograph[3], equipped with a 20-μl valve

[1] E. Merck, Darmstadt, West Germany.
[2] C. Erba, Milan, Italy.
[3] Model 1084 A, Hewlett-Packard, Böblingen, West Germany.

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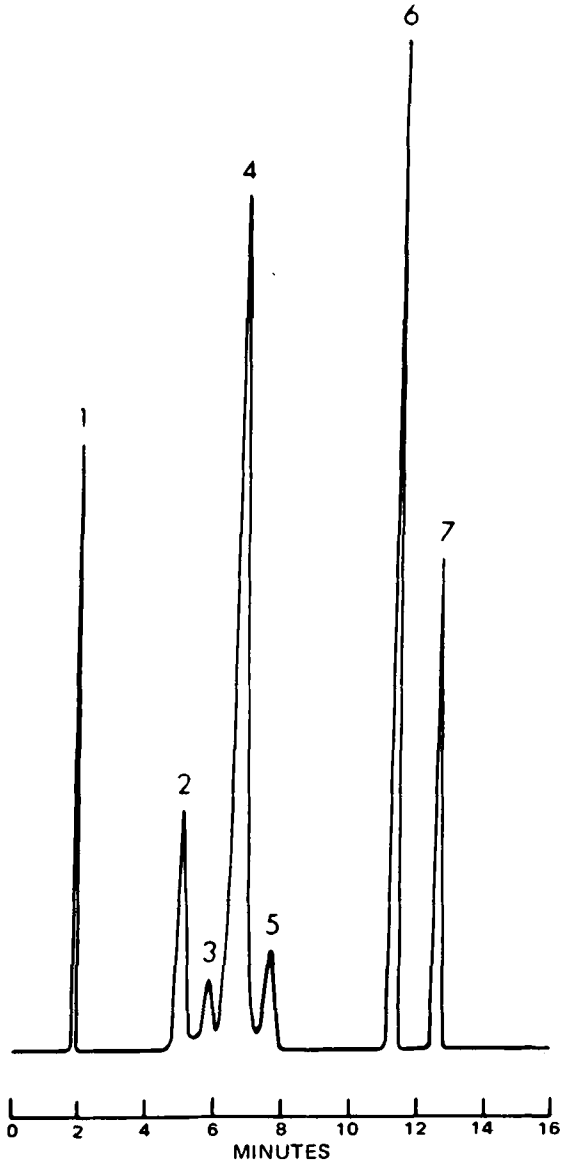


Figure 1—*High-performance liquid chromatogram of an artificial mixture of cephalosporins (I–IV). Key: 1, II; 2, III; 3, IVc; 4, I; 5, IVa; 6, cefazolin; and 7, IVb.*

and loop injector[4] and a fixed-wavelength UV detector (254 nm), was used. The prepacked[5] stainless steel column (25 × 0.45 cm i.d.) was operated at 40° at a flow rate of 2 ml/min.

Mobile Phase—An acetic acid–sodium acetate pH 4.2 buffer (0.01 ionic strength) and methanol were used as the mobile phase. The elution (5% of methanol) was isocratic for 5 min; then a linear gradient was programmed, reaching 30% of methanol in 7 min.

Standard Solutions—*Internal Standard Solution*—Approximately 25 mg of cefazolin was weighed accurately in a 50-ml volumetric flask and dissolved and diluted to volume with the pH 4.2 buffer.

Cephacetrile Standard Solution—Approximately 15 mg of the reference standard was weighed accurately in a 25-ml volumetric flask and dissolved and diluted to volume with the pH 4.2 buffer. Fresh standard solutions were prepared daily.

Standard Calibration Solution—Five standard calibration solutions were prepared daily by adding 1, 2, 3, 4, and 5 ml of cephacetrile standard solution and 3 ml of internal standard solution to five 10-ml volumetric flasks and then diluting to volume with the pH 4.2 buffer.

Sample Analyses—Approximately 15 mg of cephacetrile samples was accurately weighed in a 25-ml volumetric flask, dissolved with the pH 4.2 buffer, and diluted to volume. A 5.0-ml aliquot was pipetted into a 10-ml volumetric flask together with 3.0 ml of the internal standard solution and diluted to volume. Aliquots (20 μl) of the solution were injected onto the chromatograph.

[4] Model 7120, Rheodyne, Berkeley, Calif.

[5] Lichrosorb RP8, 10 μm, Brownlee Laboratories, Berkeley, Calif.

Table I—Same-Day Reproducibility of HPLC Cephacetrile Sodium Assay

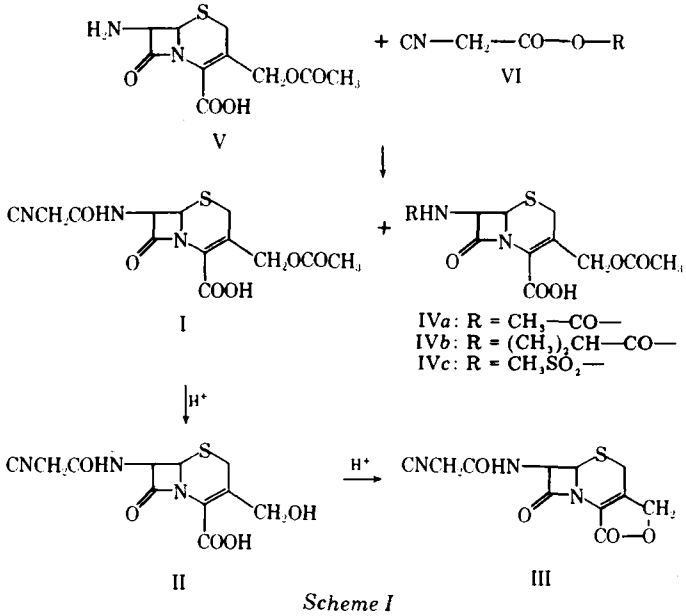
Sample	Cephacetrile, % purity[a] Run 1	Run 2	Run 3	Mean	*CV*, %
		Day 1			
1	85.8	85.7	85.8	85.77	0.13
2	85.2	84.6	85.1	84.97	0.38
3	76.1	77.0	76.4	76.5	0.6
4	77.2	77.7	77.5	77.47	0.32
5	78.5	78.6	78.1	78.4	0.33
Pooled standard error = 0.3%					
		Day 2			
1	85.4	86.0		85.7	0.5
2	87.1	86.9		87.0	0.16
3	77.7	77.4		77.55	0.27
4	79.8	79.3		79.55	0.44
5	79.7	79.9		79.8	0.18
Pooled standard error = 0.34%					
		Day 3			
1	85.5	85.2		85.35	0.25
2	87.5	87.6		87.55	0.08
3	75.7	76.1		75.9	0.37
4	80.1	79.8		79.95	0.27
5	80.3	80.3		80.3	0
Pooled standard error = 0.23%					

[a] Calculated as free acid (molecular weight 339.3).

RESULTS AND DISCUSSION

Synthesis—Cephacetrile is synthesized (Scheme I) by reaction of 7-amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (V) with mixed anhydrides (VIa–VIc) of cyanoacetic acid, both carboxylic (3) and sulfonic[6]. Crude cephacetrile is accompanied by variable amounts of by-products (IV), as shown by TLC. Moreover, II and III, arising from I through the acid hydrolysis removal of the acetyl group, can be formed during the workup of the reaction mixture and, therefore, be present in the finished product after a long storage. The described HPLC method is a rapid and accurate tool for isolating and measuring cephacetrile.

Evaluation of Chromatographic Systems—Reversed-phase chromatography was chosen because of the stability of the resins over time and a wide pH range, easy preparation, and low cost of mobile phases. The retention times of products (I–IV) can be changed by varying the amount and type of the organic modifier and the buffer pH. With cephacetrile as the reference peak, at a constant concentration of the organic



Scheme I

[6] Unpublished data.

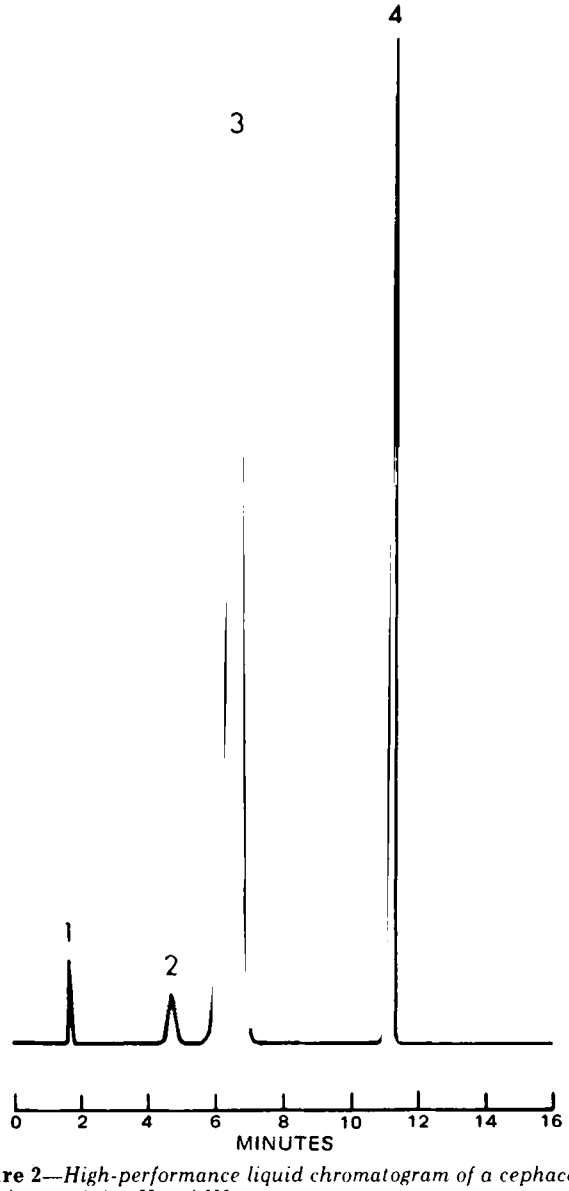


Figure 2—*High-performance liquid chromatogram of a cephacetrile sample containing II and III.*

solvent, III was rapidly eluted while the retention time of IV*a* was increased by lowering the pH from 7.2 phosphate buffer to 4.2 acetate buffer. The elution of IV*c* was not affected by changing the pH and type of the organic modifier, while IV*b* was eluted only by using a proper gradient. Methanol allowed a better partition between the components than acetonitrile. Cefazolin was selected as the internal standard because of its efficient separation from I–IV.

Figure 1 shows a chromatogram of an artificial mixture of cephalosporins I–IV and cefazolin. The peaks of the compounds were well resolved in less than 15 min. Figure 2 shows a typical chromatogram of a cephacetrile sample containing desacetyl (II) and lactone (III) impurities.

Table II—Comparison of Analysis by HPLC and Official Methods for Certification [a] of Cephacetrile Sodium

	Percent Purity (Acid)			
Sample	HPLC[b]	Microbiological[c] Method	Hydroxylamine[c] Method	Perchloric Acid[c] Method
A	85.6	82.5	91.9	89.6
B	85.3	87.3	94.7	89.6
C	87.0	85.7	92.2	91.6
D	86.3	86.1	90.8	89.1
E	78.8	77.8	88.9	90.9
F	82.7	85.3	92.7	91.3
G	79.3	81.6	88.5	90.9
H	82.3	81.6	87.4	90.3
I[d]	70.5	73	71.2	80.8
L[d]	70.9	72.5	78.2	83.5
M[d]	76.6	80.4	87.7	94.3
Average	80.48	81.3	87.6	89.3

[a] Reference 2. [b] Mean of three determinations. [c] Mean of two determinations. [d] Lyophilized salts containing 10% mannitol (w/w).

Reproducibility and Accuracy—The calibration curve was linear in the range of cephacetrile content examined [correlation coefficient (5) of 0.99993]. The within-day standard error was less than 0.4% (Table I). The pooled standard error from different days was less than 0.5%.

Specificity—As shown in Fig. 1, the hydrolysis products (II and III) and the potential impurities (IV*a*–IV*c*) cannot interfere with the quantitative HPLC determination of cephacetrile. While the desacetyl derivatives and lactones do not interfere with the *Bacillus subtilis* assay of cephalosporins, if present in a quantity lower than 15% (6), they do interfere with the colorimetric hydroxylamine assay mandated by the "Code of Federal Regulations." A comparison of the analyses of I by HPLC, the microbiological determination, the hydroxylamine colorimetric method, and the perchloric acid titration is given in Table II. Samples were selected from freshly crystallized salts (Samples A–D), aged salts (E–H), and sterile lyophilized forms (I–M) to show the dependability of the HPLC analysis. The deviation between the HPLC method and the microbiological determination was less than 1%. The other two official assays were less accurate.

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ACKNOWLEDGMENTS

The authors thank Dr. Chiarani and his staff for microbiological determinations and Prof. G. Pagani, University of Milan, for reviewing this manuscript.