the elixir had significantly greater bioavailability than did the tablet. The incomplete systemic availability of oral acetaminophen could be explained either by incomplete absorption or presystemic biotransformation, (*e.g.*, first-pass hepatic extraction or metabolism in the epithelium and/or lumen of the GI tract), or by a combination of these two factors (11). Differentiation between these two possible mechanisms could be achieved through an examination of route-dependent differences in the pattern of drug metabolism, as described by Harris and Riegelman (12). In a previous study of systemic availability (13), Rawlins *et al.* reported the bioavailability of acetaminophen tablets in a 500-mg dose to be only 63% compared with 79% in our study of a 650-mg dose. Bioavailability increased to 89 and 87% following 1,000- and 2,000-mg doses, respectively (13). The discrepancy in results of the two studies may be due to saturation of presystemic biotransformation at doses >500 mg.

The comparative bioavailability studies of two widely used acetaminophen tablets suggest that they have essentially similar systemic availability and therefore should be therapeutically equivalent.

REFERENCES

(1) B. Ameer and D. J. Greenblatt, *Ann. Intern. Med.*, **87**, 202 (1977).

(2) N. F. Billups and S. M. Billups, "American Drug Index 1981," J. B. Lipincott, Philadelphia, Pa., 1981.

(3) B. Ameer, D. J. Greenblatt, M. Divoll, D. R. Abernethy, and L. Shargel, *J. Chromatogr.*, **226**, 224 (1981).

(4) D. W. Marquardt, *J. Soc. Ind. Appl. Math.*, **11**, 431 (1963).

(5) R. A. Usanis, "NLIN—Nonlinear Least Squares Estimation of Parameters (Library Services Series Document No. LSR-089-1)," Triangle Universities Computation Center, Research Triangle Park, N.C., 1972.

(6) J. C. K. Loo and S. Riegelman, *J. Pharm. Sci.*, **59**, 53 (1970).

(7) M. Gibaldi, and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975.

(8) D. J. Greenblatt and J. Koch-Weser, *N. Engl. J. Med.*, **293**, 702, 964 (1975).

(9) G. Levy, *Arch. Intern. Med.*, **141**, 279 (1981).

(10) J. B. Sotiropoulus, T. Deutsch, and F. M. Plakogiannis, *J. Pharm. Sci.*, **70**, 422 (1981).

(11) C. F. George, *Clin. Pharmacokinet.*, **6**, 259 (1981).

(12) L. Harris and S. Riegelman, *J. Pharm. Sci.*, **58**, 71 (1969).

(13) M. D. Rawlins, D. B. Henderson, and A. R. Hijab, *Eur. J. Clin. Pharmacol.*, **11**, 283 (1977).

ACKNOWLEDGMENTS

Presented in part at the APhA Academy of Pharmaceutical Sciences meeting in Las Vegas, April 1982.

The authors are grateful for the assistance of Lawrence J. Moschitto, Jerold S. Harmatz, Dr. Dean S. MacLaughlin, and the staff of the Clinical Study Unit, New England Medical Center Hospital. Supported by Grants RR-24040, RR-05830-1, and MH-34223 from the United States Public Health Service.

Simultaneous Determinations of Cefsulodin and Cefotiam in Serum and Bone Marrow Blood by High-Performance Liquid Chromatography

KEIKO YAMAMURA *x, MAKOTO NAKAO *, JUN-ICHIRO YAMADA *, and TOSHIHISA YOTSUYANAGI ‡

Received March 17, 1982, from the **Pharmacy Department, Nagoya University Branch Hospital, Higashi-ku, Nagoya 461* and the ‡*Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467 Japan.* Accepted for publication August 9, 1982.

Abstract □ A high-performance liquid chromatographic method is described for the simultaneous determinations of cefsulodin and cefotiam in serum and bone marrow blood samples. After extraction with acetonitrile, the cephalosporins were applied to a reverse-phase column with an internal standard, cefazolin; the mobile phase was a mixture of 0.005 *M* tetrabutylammonium phosphate and methanol (35:65, v/v). The method yielded satisfactory resolutions for these agents, and the results were compared with those obtained using the microbiological method. The statistical analysis of the relationship between the methods gave a good correlation for all of these agents and samples. The concentrations of cefsulodin and cefotiam, concurrently administered by the intravenous route to patients subjected to artificial total joint prosthesis, in serum and bone marrow blood collected at 0.5 and 1 hr postinjection were almost equivalent.

Keyphrases □ Cefsulodin—simultaneous determination with cefotiam in serum and bone marrow blood, high-performance liquid chromatography □ Cefotiam—simultaneous determination with cefsulodin in serum and bone marrow blood, high-performance liquid chromatography □ High-performance liquid chromatography—simultaneous determination of cefsulodin and cefotiam in serum and bone marrow blood

Cefsulodin, sodium 4-carbamoyl-1-[(6*R*,7*R*)-2-carboxy-8-oxo-7- [(2*R*) -2-phenyl-2-sulfoacetamido] -5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methylpyridium hydroxide, a potent cephalosporin derivative, is superior to sulbenicillin and carbenicillin and comparable to gentamicin in activity against *Pseudomonas aeruginosa*. The drug is stable to *P. aeruginosa*-specific cephalosporinase (1–3). Cefotiam, (6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)-acetamido]-3-[[[1-[2-(dimethylamino)ethyl]-1*H*-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid dihydrochloride, also shows broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria (4). Combined administration of these agents is frequently used to treat systemic infections in which a broader anti-infective spectrum is needed. A simple, specific high-performance liquid chromatographic (HPLC) method was developed which determines both cefsulodin and cefotiam in biological fluids. A comparison is made with the previously used microbiological method.

EXPERIMENTAL

Materials—Cefsulodin[1] and cefotiam[2] were used as received. Cefazolin[3], employed as an internal standard, was used as received. HPLC-quality methanol[4] and acetonitrile[5] were used. Tetrabutylammonium

[1] Tilmapor, Ciba-Geigy, Basle, Switzerland.
[2] Halospor, Ciba-Geigy, Basle, Switzerland.
[3] Cefamezin, Fujisawa Pharmaceutical Co., Osaka, Japan.
[4] Wako Chemical Co., Osaka, Japan.
[5] Tokyo Kasei Chemical Co., Tokyo, Japan.

0022-3549/83/0800-0958$01.00/0

Table I—Recoveries of Cefsulodin, Cefotiam, and Cefazolin from Serum

Drug	Concentration, μg/ml	Recovery[a], %
Cefsulodin	100	103.0 ± 2.7
	50	98.0 ± 1.2
	10	98.5 ± 3.2
	5	98.1 ± 2.0
Cefotiam	100	99.6 ± 2.1
	50	102.1 ± 4.1
	10	99.9 ± 1.3
	5	98.5 ± 1.8
Cefazolin	100	101.8 ± 2.3
	50	103.2 ± 1.1
	10	99.4 ± 3.2
	5	98.8 ± 3.3

[a] Recovery calculated as (serum value/water control value) × 100. Each value is the mean ± *SD* of six determinations.

phosphate[6] was used as a constituent of the mobile phase. All other reagents were of analytical grade.

Serum and Bone Marrow Blood Samples—Cefsulodin (1 g) and cefotiam (2 g) were administered concurrently by intravenous injection to 16 patients immediately before beginning surgery. Serum and bone marrow blood samples were collected from each subject at 0.5 and 1 hr after injection (a total of 64 samples). Samples were centrifuged immediately at 3000 rpm for 15 min. The serum fractions were separated and stored frozen at −60° until assayed.

Chromatographic Conditions—An HPLC apparatus[7] equipped with a UV-absorbance detector[8] and a reverse-phase column[9] (10 μm, 30 cm × 3.9-mm i.d.) were used. Peak areas were obtained by a data analyzer[10]. The mobile phase was a mixture of methanol–water (35:65, v/v) and 0.005 *M* tetrabutylammonium phosphate. The flow-rate was 1.5 ml/min with an operating pressure of 220 kg/cm². The column eluate was continuously monitored at 280 nm, with a sensitivity of 0.08 AUFS and a chart speed of 5 mm/min.

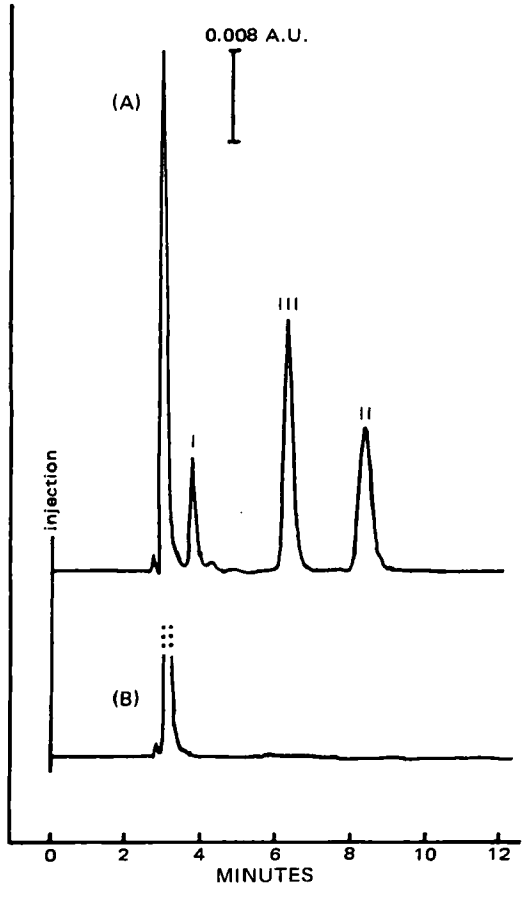

Figure 1—*Chromatograms of cefsulodin (I), cefotiam (II), and cefazolin (internal standard, III) in human serum, at concentrations of 25 μg/ml for I, 40 μg/ml for II, and 50 μg/ml for III. Key: (A) spiked serum sample; (B) serum blank. Chromatograph was run at 0.08 AUFS with a chart speed of 5 mm/min.*

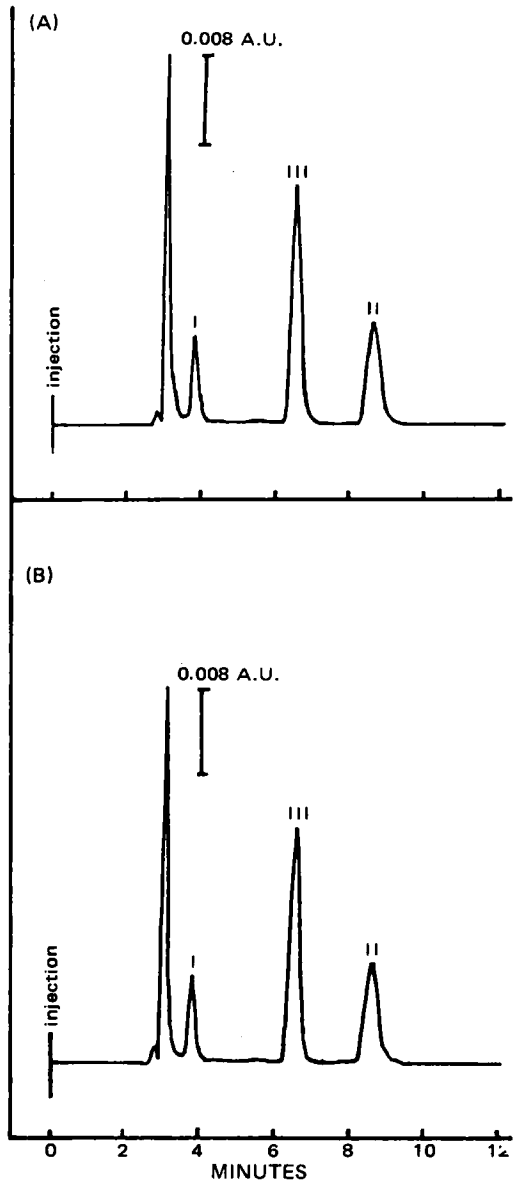

Figure 2—*Typical chromatograms of human serum (A) and bone marrow blood (B) samples collected from a patient 0.5 hr after intravenous injection of both drugs. Key: (I) cefsulodin; (II) cefotiam; (III) cefazolin.*

Assay Procedures—A 0.2-ml serum sample was mixed with acetonitrile (0.2 ml) containing a known amount of the internal standard on a vortex mixer for 10 sec and then contrifuged at 12,000 rpm for 5 min. The supernatant was passed through a membrane filter (0.45 μm)[11] and a 10-μl aliquot was injected into a port of the HPLC column.

Calibration Curves of Cefsulodin, Cefotiam, and Cefazolin—Calibration curves were constructed using a known concentration of the drugs (10–100 μg/ml) dissolved in human serum. The relationship between the concentration (ordinate) and the peak area (abscissa) showed good linearity for each: respective intercepts, slopes, and correlation coefficients, 0.558, 0.0106, and 0.998 for cefsulodin; 0.030, 0.0088, and 0.999 for cefotiam; and 0.558, 0.0059, and 0.998 for cefazolin (internal standard). The ratios (R) of the slopes of cefsulodin and cefotiam to that

[6] Waters Associates, Milford, Mass.
[7] Model 520, GASUKURO KOGYO, Tokyo, Japan.
[8] UVIDEC 100-II, JASCO, Tokyo, Japan.
[9] μBondapak C_{18}, Waters Associates, Milford, Mass.
[10] C-R1A CHROMATOPAC, Shimadzu, Kyoto, Japan.
[11] TM-2P, Toyo Scientific Inc., Tokyo, Japan.

Table II—Concentrations of Cefsulodin and Cefotiam in Serum and Bone Marrow Blood Determined by the HPLC Method

Sample	Time[b], hr	Concentration[a], μg/ml	
		Cefsulodin	Cefotiam
Serum	0.5	44.2 ± 20.8	79.8 ± 33.1
	1	34.1 ± 16.8	46.4 ± 14.5
Marrow	0.5	39.6 ± 16.8	76.1 ± 34.7
	1	30.1 ± 13.4	44.4 ± 22.1

[a] Mean ± *SD* of 16 subjects. [b] Blood samples were collected with 0–10-min variance.

of the internal standard were 1.80 and 1.49, respectively, from which the sample concentration (C_a) was calculated as: C_a = (peak area ratio between sample and internal standard) × (concentration of internal standard)/R.

Microbiological Assay—The microbiological assay was performed according to a modified method of Fugono *et al.* (5). The concentrations of cefsulodin and cefotiam were determined by the cup plate method (6), using *P. aeruginosa*[12] and *Proteus mirabilis*[13] as test organisms. The minimum inhibitory concentrations of these agents are: 1.56 and >100 μg/ml against *P. aeruginosa* for cefsulodin and cefotiam, respectively,and >100 and 0.01 μg/ml against *P. mirabilis* for cefsulodin and cefotiam, respectively, with an inoculation of 10^6 cells/ml (7, 8). The culture medium was prepared from an agar base[14] containing 0.1% sodium acetate (w/v). A series of dilutions for the calibration curve were made with normal human serum.

RESULTS AND DISCUSSION

Recovery and Separation of Cephalosporins—Recovery of cefsulodin, cefotiam, and cefazolin from injected solutions in serum was established by comparing the peak areas for samples with those from water solutions in the range of 5–100 μg/ml. As shown in Table I, the recoveries were regarded as 100% for these cephalosporins.

The liquid chromatograms of cefsulodin, cefotiam, and the internal standard in serum are shown in Fig. 1. The retention times were found to be 3.8, 6.3, and 8.3 min, respectively. The peaks of the control serum had low retention times and were completely separated from those of the antibacterial agents. Also, the separation of peaks among cefsulodin, cefotiam, and the internal standard was established with satisfactory resolutions for simultaneous determination.

Determinations of Cephalosporins in Serum and Bone Marrow Blood—The HPLC method was applied for the simultaneous determinations of cefsulodin and cefotiam in serum and bone marrow blood samples. Figure 2 shows typical chromatograms of serum and bone marrow blood samples collected from a patient 0.5 hr after intravenous injection. There was little difference in the retention time between serum and bone marrow blood samples that originated from different sources of body fluids.

Table II indicates the average concentrations and standard deviations of cefsulodin and cefotiam in serum and bone marrow blood samples that were collected from 16 subjects. There were no significant differences in the concentrations between the different samples for each agent, although there was a tendency for the average concentration in serum to be slightly higher than that in bone marrow blood. The results indicate that very fast movement of these agents from the peripheral to the bone marrow blood was always established.

Correlation of the HPLC and Microbiological Methods—To examine the reliability of the HPLC method, the same biological samples containing cefsulodin and cefotiam were also assayed by the microbiological method in general use, and the correlation between both the methods was studied. The great difference in the minimum inhibitory concentration enabled these agents to be determined individually even when concurrently administered, even though cefsulodin and cefotiam are categorized in the same family of cephalosporins.

The calculation of the correlation coefficient and regression analysis were conducted for both serum and bone marrow blood samples irrespective of the sampling time. The statistical values were: in cefsulodin, y(HPLC) = 0.91x(microbiological) + 1.71, r = 0.974 (n = 32) in serum and y = 0.91x − 1.52, r = 0.961 (n = 32) in bone marrow blood; in cefotiam, y = 1.07x −8.99, r = 0.971 (n = 32) in serum and y = 1.01x − 4.05, r = 0.978 (n = 32) in bone marrow blood.

The intercepts of the regression lines of cefsulodin gave values closer to the origin than those of cefotiam, while the regression coefficients were very close to unity for each agent, indicating that the sensitivity of the HPLC procedure described here was almost equivalent to the microbiological method. Therefore, the HPLC method is accurate enough to use within the putative range of effective concentrations in biological fluids as well as enabling the simultaneous determination of these cephalosporins. The HPLC method, furthermore, was more rapid and easier to perform than the microbiological method.

[12] IFO 12582, Institute of Fermentation of Osaka, Osaka, Japan.
[13] IFO 3849, Institute of Fermentation of Osaka, Osaka, Japan.
[14] Diagnostic Sensitivity Test, Code CM 261, Oxoid Ltd., England.

REFERENCES

(1) K. Tsuchiya, M. Kondoh, and H. Nagatomo, *Antimicrob. Agents Chemother.*, **13**, 137 (1978).

(2) K. Tsuchiya and M. Kondo, *Antimicrob. Agents Chemother.*, **13**, 536 (1978).

(3) M. Kondo and K. Tsuchiya, *Antimicrob. Agents Chemother.*, **14**, 151 (1978).

(4) K. Tsuchiya, M. Kida, M. Kondo, H. Ono, M. Takeuchi, and T. Nishi, *Antimicrob. Agents Chemother.*, **14**, 557 (1978).

(5) T. Fugono and K. Maeda, *Chemotherapy (Tokyo)*, **27**(s-2), 120 (1979).

(6) "Commentary of Antibiotics Standard of Japan (KIJUN KAISETSU)," Ministry of Public Health and Welfare of Japan, YAKUGYO JIHO, Tokyo, 1978, p. 593.

(7) S. Goto, M. Ogawa, A. Tsuji, Y. Kaneko, and S. Kuwahara, *Chemotherapy (Tokyo)*, **27**(s-2), 1 (1979).

(8) T. Nishino and T. Iwahi, *Chemotherapy (Tokyo)*, **27**(s-3), 45 (1979).