this purpose. The base of the gauge was measured for the chord length (L). The gauge was then set inside the vessel with the ends of the gauge base resting on the curved surface of the vessel. The distance (h) between the base and the bottom of the vessel was taken from the gauge rule. The cylindrical diameter of the vessel was then taken 2 to 3 cm above opposite points where a tangent to the curved bottom of the vessel coincides with the vessel wall. Inside calipers were used for this purpose.

Table VII shows the measurements taken from the vessels and the comparison of the theoretical diameters calculated from these measurements with the actual diameters. The glass vessels possess a flatter curvature than a sphere of the same diameter. The curvature of the plastic vessels closely approximates a sphere of the same diameter. When the results from plastic vessels differ from those obtained from glass vessels, the results from the plastic vessels are more correct, because the plastic vessels conform more closely to the USP specifications.

CONCLUSIONS

Differences in the bottom curvature of dissolution vessels can cause bias in the dissolution results obtained from prednisone tablets. Vessels with a curvature which is less (flatter) than that of a sphere cause a high bias. Vessels with a curvature that is greater (steeper) than that of a sphere cause a low bias. The plastic vessels are more uniform than glass vessels and possess a curvature that more closely approximates the curvature specified in the USP. As such, they are preferable to the glass vessels for use in the dissolution test when the drug is not adsorbed and the vessel is not attacked by the dissolution medium.

The dissolution rate is controlled by the velocity of the liquid passing over the tablet. The liquid velocity at any point in a stirred vessel is controlled by the stirring rate and the geometry of the system. An idealized geometry is defined in the USP. Minor variations from this idealized geometry, such as those discussed in this paper and previously (7), change the liquid velocity in the vicinity of the tablet and, thus, the dissolution results. If the reproducibility of the test is to be improved, equipment must be made available which allows the analyst to adhere to this idealized geometry as closely as possible.

REFERENCES

(1) R. D. Kirchhoefer, *J. Assoc. Off. Anal. Chem.*, **59**, 367 (1976).

(2) "The United States Pharmacopeia," 20th rev., Mack Publishing Co., Easton, Pa., 1980, p. 959.

(3) "Fourth Supplement to USP XIX and NF XIV," The United States Pharmacopeial Convention, Inc., Rockville, Md., 1978, p. 141.

(4) "The United States Pharmacopeia," 20th rev., Mack Publishing Co., Easton, Pa., 1980, p. 655.

(5) D. P. Page, D. C. Cox, M. L. Dow, M. A. Kreienbaum, P. A. McCullen, T. W. Moore, and L. K. Thornton, *FDA By-Lines*, **10**, 57 (1980).

(6) J. F. Brower, *J. Assoc. Off. Anal. Chem.*, **60**, 27 (1977).

(7) D. C. Cox and W. B. Furman, *J. Pharm. Sci.*, **71**, 451 (1981).

(8) W. J. Dixon and F. J. Massey, Jr., "Introduction to Statistical Analysis," McGraw-Hill, New York, N.Y., 1969, pp. 175–181.

(9) F. Langenbucher, *J. Pharm. Sci.*, **58**, 1265 (1969).

ACKNOWLEDGMENT

The authors thank John C. Black for drawing the figure.

HPLC Determination of D and L Moxalactam in Human Serum and Urine

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ABSTRACT □ A high-pressure liquid chromatographic procedure was developed to determine the D and L isomers of moxalactam in human plasma and urine. After protein precipitation with hydrochloric acid the sample was extracted with ethyl acetate. It was then back extracted into tromethamine buffer (pH 8.0) and washed with octanol. Extraction recovery from plasma ranged from 73–81%. An aliquot of the tromethamine buffer was then injected onto a C_{18}-μBondapak column. The mobile phase was 3% acetonitrile in 0.05 *M* ammonium acetate pH 6.5 buffer. Samples were quantitated by UV detection at 275 nm and 0.01 aufs. The lower limit of detection was 0.5 μg/ml for each isomer. Preliminary stability studies were performed to assess proper sample handling and storage conditions. The procedure was evaluated in a clinical setting to demonstrate its applicability to the study of moxalactam pharmacokinetics in critically ill patients.

Keyphrases □ Moxalactam—determination in human plasma and urine by high-pressure liquid chromatography, D and L isomers □ High-pressure liquid chromatography—determination of moxalactam in human plasma and urine, D and L isomers □ Anti-infectives—moxalactam, high-pressure liquid chromatographic determination, D and L isomers

Moxalactam is a new oxycephalosporin derivative undergoing clinical trials in the United States and Europe. *In vitro* experiments have demonstrated that moxalactam is active against a broad spectrum of microorganisms, including resistant Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Bacteroides fragilis*, some indole-positive *Proteus* species (1), β-lactamase-producing strains of *Enterobacteriaceae* (2), and clinical isolates shown to be cephalosporin resistant (3). Its expanded spectrum of activity compared to conventional β-lactam antibiotics is attributed to replacement of the thio group at the 1 position of the dihydrothiazine nucleus with an oxygen moiety (4).

The pharmacokinetics of moxalactam elimination usually have been evaluated employing standard microbiological techniques (5, 6). However, a more specific analytical procedure was required for pharmacokinetic studies of this compound in critically ill patients. A high-pressure liquid chromatographic (HPLC) assay developed recently, although more specific than microbiological techniques, had limited applicability in the critical care setting[1]. A more specific HPLC analysis was required for studies in seriously ill patients. The present report describes a suitable HPLC procedure for the quantitation of both moxalactam isomers in patient plasma and urine.

[1] D. J. Miner, D. L. Coleman, A. M. Shephend, and T. Hardyn, Antimicrob. Agents Chemother., in press.

0022-3549/82/0400-0399$01.00/0

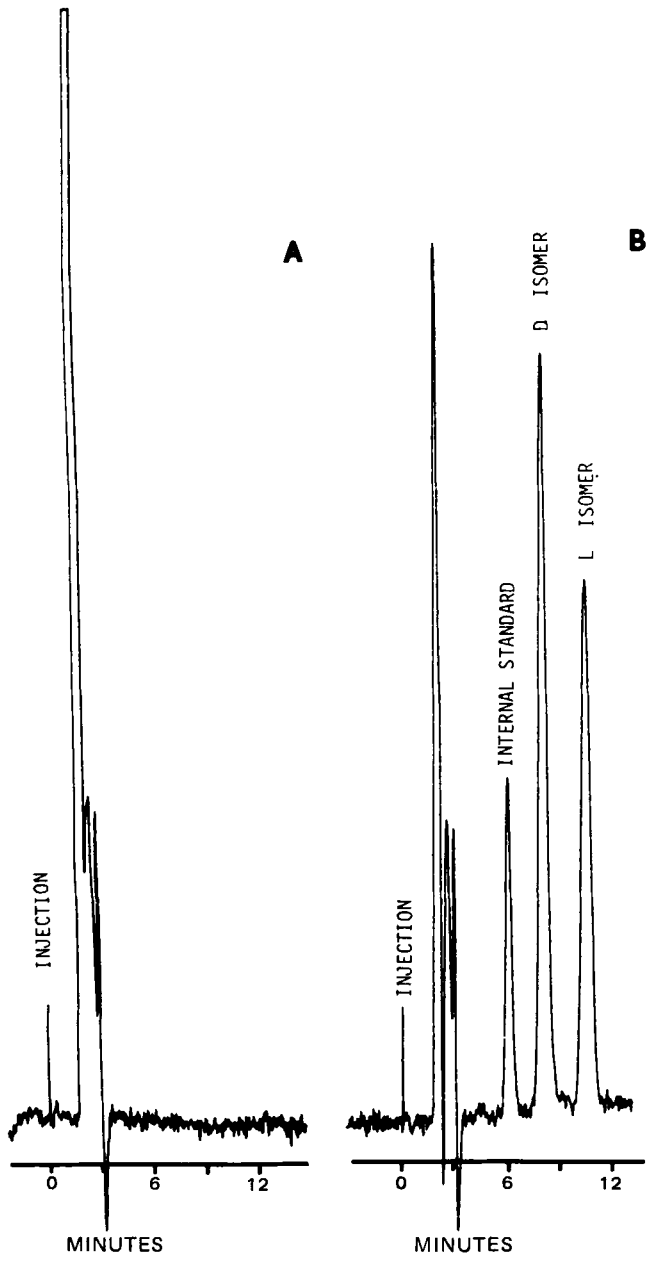

Figure 1—*Chromatograms obtained after extracting 0.5 ml of a blank patient plasma (A) and a moxalactam plasma standard (B). The peak heights of each isomer correspond to a plasma concentration of 10 μg/ml.*

EXPERIMENTAL

Reagents—All chemicals and reagents were analytical grade unless otherwise indicated. Moxalactam was supplied as a lyophylized powder containing a 1:1 mixture of D and L isomers[2]. Allopurinol[3], ammonium acetate, potassium chloride, acetic acid, hydrochloric acid, sodium hydroxide, octanol, tromethamine, glass-distilled acetonitrile[4], methanol, and ethyl acetate[5] were used as received.

Preparation of Stock Solutions—Initial stock solutions of moxalactam were prepared in water and subsequent dilutions made with blank human plasma. Due to the poor aqueous solubility of the internal standard (allopurinol), the drug was first dissolved in 0.1 *N* NaOH and then diluted with water to a final working concentration of 1.5 mg/ml.

Ammonium acetate buffer (0.05 *M*) was adjusted to pH 6.5 with acetic acid. Tromethamine–HCl pH 8.0 and KCl–HCl pH 1.0 buffers (0.05 *M*) were prepared as standard laboratory reagents (7). All buffers were stored at 5° and allowed to reach ambient temperature prior to use.

Extraction Procedure—Two hundred microliters of concentrated hydrochloric acid was added to 0.5 ml of plasma or dilute urine (typically 1:100) containing 0.1 ml of the internal standard solution (allopurinol, 1.5 mg/ml). The mixture was then vortexed, resulting in precipitation of almost all protein present in the sample. After centrifuging at 5000 rpm for 5 min[6], a 0.6-ml portion of the clear supernate was transferred to a 12 × 75-mm polypropylene tube containing 0.5 ml of KCl–HCl pH 1.0 buffer. Three milliliters of HPLC grade ethyl acetate was then added, and the solution was vortexed for 60 sec. After centrifuging[7] at 3000 rpm for 5 min, a 2.9 ml aliquot of the upper ethyl acetate phase was transferred to a second tube containing 0.5 ml of tromethamine–HCl pH 8.0 buffer. This mixture was vortexed for 30 sec, and then centrifuged. The upper organic phase was aspirated and discarded. A 0.4-ml aliquot of the lower aqueous phase was transferred to another tube and washed with 3 ml of octanol by rotation[8] for 1 min. The upper organic phase was discarded after centrifugation, and a 50-μl portion of the lower aqueous phase was used for HPLC analysis. When necessary, the aqueous phase could be stored at 5° for as long as 24 hr prior to HPLC quantitation.

HPLC Conditions—A high-pressure liquid chromatograph[9] equipped with a variable wavelength UV detector[10] and a 10-mv recorder[11] were used. Separation occurred on a C_{18} reverse phase column[12] with a 3% acetonitrile/0.05 *M* ammonium acetate mobile phase at a flow rate of 1.5 ml/min. The column effluent was monitored at 275 nm, and an attenuation of 0.01 aufs was used in all chromatographic procedures.

Recovery Studies—Recovery experiments were performed by adding equivalent amounts of moxalactam to blank plasma and to a solution of mobile phase. Samples were extracted according to the described procedure, except that the internal standard was introduced just prior to injection according to the external standardization method. Recovery was calculated by comparing moxalactam peak-height ratios for the extracted plasma samples with those obtained from direct injection of the mobile phase standard.

Recovery studies were carried out at two concentrations, with five replicate determinations at each concentration. Within- and between-day variation was evaluated at 10 and 80 μg of moxalactam/ml. To assess within-day variation, five aliquots of a single plasma sample were extracted at each concentration. Additional samples were assayed daily over 13 days to assess between-day variation.

Stability Studies—To determine the effects of temperature on the stability of D and L moxalactam, 1.0-ml aliquots of spiked plasma were incubated at −30, 5, 25, and 38°. Samples were periodically assayed for each isomer for up to 40 days. Semilogarithmic plots of each isomer

Table I—Analytical Recovery of D and L Moxalactam from Human Plasma

	Moxalactam, μg/ml	Mean Recovery, %	±*SD*[a]	*CV*, %[b]
D isomer	5.0	81.3	2.6	3.2
(n = 5)	40.0	75.2	2.7	3.5
L isomer	5.0	79.6	5.3	6.7
(n = 5)	40.0	72.8	4.3	5.9

[a] *SD* = standard deviation. [b] *CV* = coefficient of variation, calculated as *SD*/mean.

Table II—Within-Day and Between-Day Variation in the Determination of D and L Moxalactam in Human Plasma

	Within-Day			Between-Day		
	Mean, μg/ml[a]	*SD*[b]	*CV*, %[c]	Mean, μg/ml[a]	*SD*	*CV*, %
D isomer	4.8	0.13	2.8	4.8	0.23	4.7
	41.5	1.5	3.6	39.6	0.60	1.5
L isomer	4.7	0.18	3.9	4.8	0.37	3.6
	41.2	1.3	3.2	39.7	0.70	1.8

[a] n = 5. [b] *SD* = standard deviation. [c] *CV* = coefficient of variation, calculated as *SD*/mean.

[2] Eli Lilly Research Laboratories, Indianapolis, Ind.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] Burdick & Jackson Laboratories, Muskegon, Mich.
[6] Fisher Micro-Centrifuge, Pittsburgh, Pa.
[7] Sorvall GLC-1 Centrifuge, Newton, Conn.
[8] Fisher Roto-Rack, Fisher Scientific Co., Pittsburgh, Pa.
[9] Perkin-Elmer Series II HPLC, Perkin-Elmer Co., Norwalk, Conn.
[10] Schoeffel Variable Wavelength Detector Model 770, Schoeffel Instruments, Westwood, N.J.
[11] Houston Omniscribe Recorder, Houston Instruments, Austin, Tex.
[12] μBondapak-C_{18}, Waters Associates, Milford. Mass.

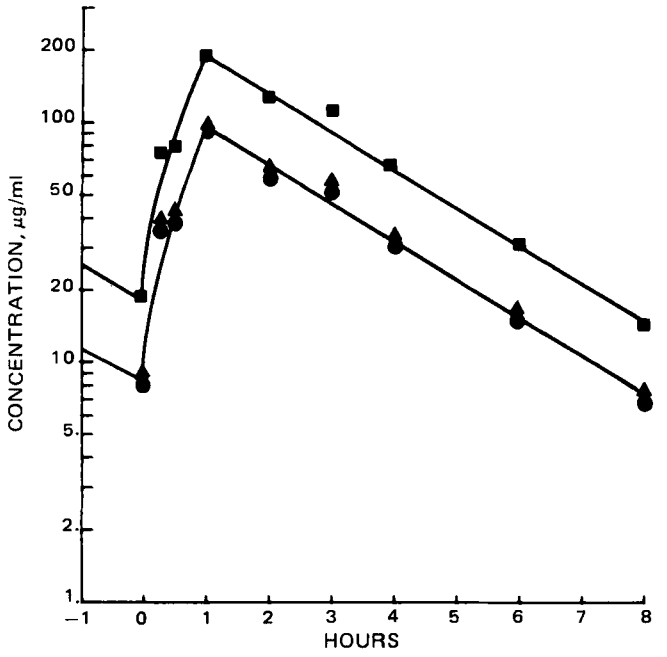

Figure 2—*Plasma concentration versus time profiles for moxalactam (■), its D isomer (▲), and L isomer (●) after intravenous infusion of moxalactam.*

concentration *versus* time were constructed to determine the rate and order of moxalactam degradation. The data were evaluated by means of an Arrhenius plot, and the line of best fit was determined by linear regression analysis.

Pharmacokinetic Study—After getting informed consent, a 10-day course of moxalactam therapy was started in a 60-year old female patient diagnosed as having an abdominal abscess secondary to invasive large bowel cancer. The pathogens involved included *Escherichia coli* and enterococcus, both of which were susceptible to moxalactam *in vitro*. This patient had normal hepatic and renal function, and therefore was given 2 g of intravenous moxalactam every 8 hr. The pharmacokinetic study was conducted on day 6 of therapy to allow steady-state concentrations to be achieved. Plasma and urine samples were obtained throughout a dosing interval and stored at −30° prior to quantitation of D and L moxalactam. The serum concentration *versus* time profiles for moxalactam and each isomer were evaluated by means of nonlinear regression analysis.

RESULTS

Chromatography—Representative chromatograms for a blank patient sample (A) and a spiked plasma standard (B) are presented in Fig. 1. No other peaks were evident in the blank plasma chromatogram even though the patient was receiving numerous medications. Chromatogram B was obtained after extracting 0.5 ml of plasma containing a total moxalactam concentration of 20 μg/ml. A 50-μl aliquot of the final buffer solution was injected onto the column. Allopurinol, the internal standard, had a retention time of 6.0 min, while the D and L isomers of moxalactam had retention times of 7.8 and 10.5 min, respectively. The lower limit of detection for each isomer was 0.5 μg/ml when a 50-μl aliquot of the final buffer phase was injected onto the column.

Calibration curves for each individual isomer were prepared daily by plotting peak-height ratio (isomer/internal standard) *versus* concentration for series of prepared plasma or urine standards. These plots were linear over the 2.5–50-μg/ml concentration range. Samples that exceeded the range of standards were diluted prior to extraction.

Recovery Experiments—The results of the recovery experiments are given in Table I. The overall recovery for both isomers ranged from 73 to 81% over an approximate 10-fold increase in concentration. Although these values are less than quantitative, the recovery was adequate for routine analysis of patient samples. When increased sensitivity was required, the volume ultimately injected onto the column can be increased to as much as 150 μl without deviation from linearity.

Both isomers demonstrate low between- and within-day variation at each of the concentrations evaluated (Table II). The coefficient of variation ranged from 2.8 to 3.9% for the within-day experiments, while the between-day studies yielded coefficients of variation ranging from 1.5 to 4.7%.

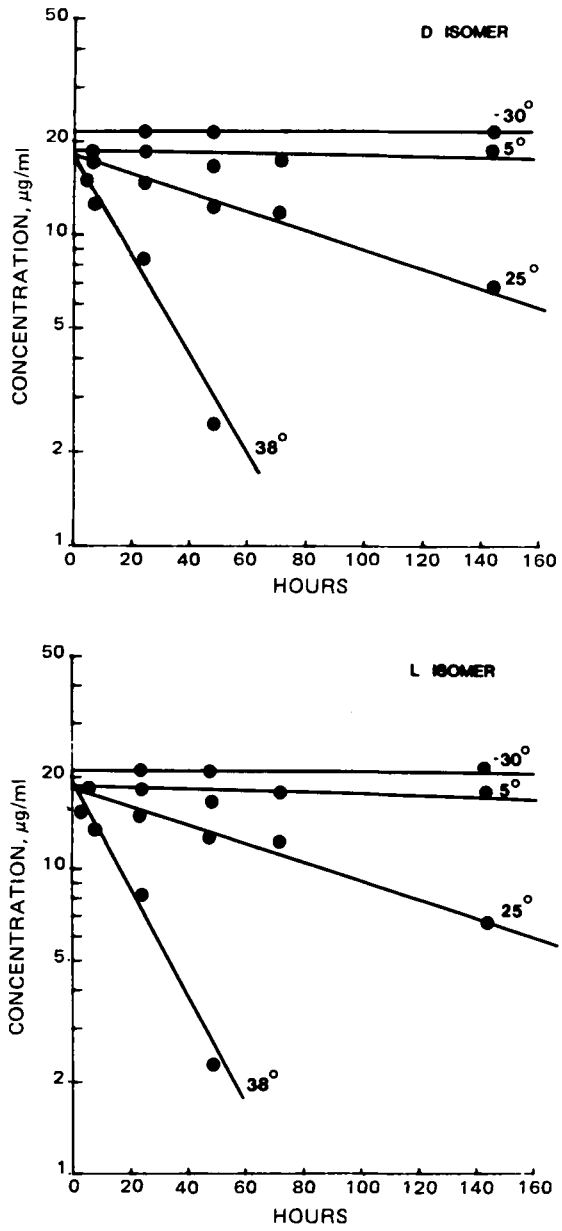

Figure 3—*Effects of temperature on the degradation of D and L moxalactam in human plasma.*

Pharmacokinetic Analysis—Figure 2 is the serum concentration *versus* time profile for moxalactam and its individual isomers in the patient. The subject received 2.0 g of moxalactam by intravenous infusion over 1 hr. The moxalactam concentration is given as the sum of the D and L isomers. The zero plasma sample was obtained immediately before the start of the infusion. The peak concentrations of 95 μg/ml for both isomers were noted at the completion of the infusion period, and concentrations decreased monoexponentially to a minimum of 7 μg/ml. There were no discernible differences in the concentration profile of each isomer.

The data for moxalactam and its isomers were fitted to a one-compartment model with zero order input, by means of nonlinear least-squares regression analysis. Total body clearance of moxalactam calculated as dose/*AUC* was 55.3 ml/min. Total moxalactam and the separate isomers showed similar elimination rates, with half-lives of 1.86, 1.9, and 1.85 hr, respectively.

Stability Studies—Moxalactam is a relatively unstable compound subject to degradation *in vitro*. To assess proper sample handling and storage conditions, the effect of temperature on the degradation rate was evaluated. Figure 3 shows the effect of temperature on the stability of each moxalactam isomer. No differences were observed in the degradation rate for the individual isomers from the two figures. The degradation rate

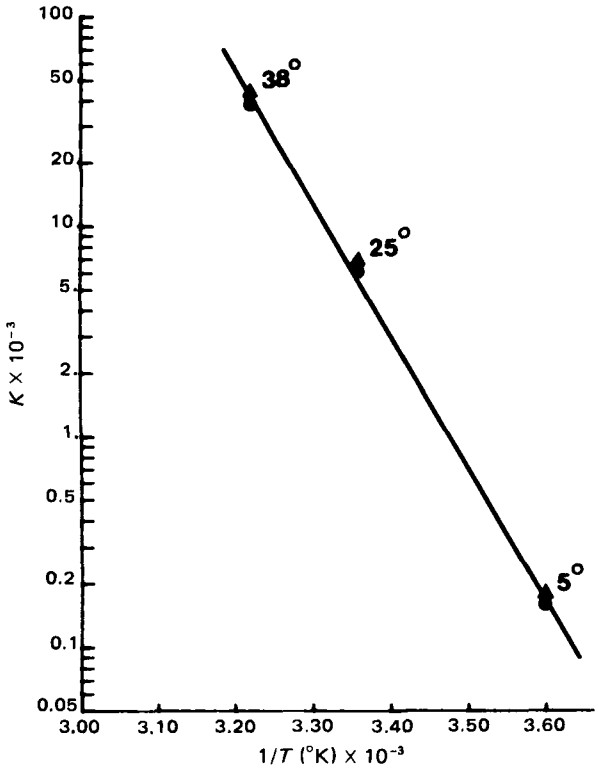

Figure 4—*Arrhenius plot of moxalactam degradation in human plasma. Key:* ▲ *D isomer;* ●, *L isomer.*

is consistent with an apparent first-order process, with the most rapid rate observed at the elevated temperature. Plasma samples were stable over the 40-day test period if frozen at −30°.

An Arrhenius plot was constructed by plotting the apparent first-order rate constant for 10% degradation (K) as a function of the reciprocal of temperature. The data for each isomer are presented in Fig. 4. The relationship was linear over the temperature range of 5–38°, with the slope yielding an activation energy of 29.1 kcal/mole, a value consistent with hydrolysis reactions.

DISCUSSION

Serum moxalactam concentrations have been routinely determined in healthy volunteers using standard microbiological assays (5, 6). The limitation of these assays in a clinical setting is well known, with the most frequently encountered problem being assay interferences from concurrent medications. Additional interferences may arise due to metabolic and/or degradative products of many drugs, or from endogenous substances possessing antibacterial activity. These procedures also cannot measure the individual isomers of moxalactam, whose quantitation may be clinically relevant, as was the case with warfarin (8) and propranolol (9).

An HPLC procedure was reported recently for the determination of D and L moxalactam in serum and urine[1]. This procedure has several short-comings. The extraction procedure, although adequate for samples obtained from select healthy volunteers, was unable to remove interfering substances in samples from critically ill patients. In most instances, extraneous peaks and interferences made quantitation of moxalactam impossible. This method also lacks a suitable internal standard; thus, reproducibility and precision are difficult to achieve. Finally, the previous method requires a separate chromatographic procedure utilizing PIC[13] reagents for the determination of moxalactam in urine.

The present procedure has advantages over existing techniques in that it is more specific than previous methods as well as useful for quantitating both the D and the L isomers of moxalactam. It was tested rigorously during the course of a clinical trial in critically ill patients hospitalized for a variety of disease states including renal and hepatic failure. These patients were receiving numerous concurrent medications and no interferences were seen in any of these samples. Some compounds that were specifically tested and found not to interfere were: cephalothin, cefamandole, cefazolin, cephapirin, cephalexin, caffeine, cimetidine, theophylline, tobramycin, clindamycin, furosemide, dicloxacillin, methicillin, amoxicillin, ampicillin, penicillin G, nafcillin, and trimethoprim.

The effect of disease state on the preferential elimination of one of the isomeric forms of some compounds, such as warfarin and propranolol, is well known. Although the clinical significance of discriminating moxalactam D and L isomers is unknown, this procedure is capable of addressing the question in the course of moxalactam clinical trials.

REFERENCES

(1) T. Yoshida, S. Matsuura, M. Mayama, Y. Kameda, and S. Kuwahara, *Antimicrob. Agents Chemother.*, **17**, 302 (1980).

(2) H. Neu, N. Aswapokee, K. Fu, and P. Aswapokee, *ibid.*, **16**, 141 (1979).

(3) R. N. Jones, P. C. Fuchs, H. M. Sommers, T. L. Gavan, A. L. Barry, and E. H. Gerlach, *ibid.*, **17**, 750 (1980).

(4) K. Matsumoto, Y. Uzuka, T. Nagatake, and H. Shishido, in "Proceedings of 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy," J. D. Nelson and C. Grassi, Eds., vol. 1, American Society for Microbiology, Washington, D.C., p. 112.

(5) J. Kurihara, K. Matsumoto, Y. Uzuka, H. Shishido, T. Nagatake, H. Yamada, T. Yoshida, T. Oguma, Y. Kimura, and Y. Tochino, in *Ibid.*, p. 110.

(6) J. N. Parsons, J. M. Romano, and M. E. Levison, *Antimicrob. Agents Chemother.*, **17**, 226 (1980).

(7) "Documenta Geigy Scientific Tables," K. Diem and C. Lenter, Eds., Ciba-Geigy Limited, Basle, Switzerland, 1970, p. 280.

(8) L. Goding and B. West, *J. Med. Chem.*, **12**, 517 (1969).

(9) A. Barrett and V. Cullum, *Br. J. Pharmacol.*, **34**, 43 (1968).

ACKNOWLEDGMENTS

Supported in part by NIGMS Grant 20852 from the National Institutes of Health, and in part by a grant from Eli Lilly Company.

[13] Pair ion chromatography.