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Note

High-pressure liquid chromatography of caprolactam and its metabolites in urine and plasma

PETER D. UNGER and MARVIN A. FRIEDMAN
Allied Chemical Corporation, Corporate Medical Affairs, Morristown, N.J., 07960 (U.S.A.)
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Caprolactam (Fig. 1) is a cyclic amide chemically synthesized in a multi-step process from phenol. The last stage of the synthesis usually involves the Beckmann rearrangement of cyclohexanone oxime to the seven-membered ring. In the synthesis of nylon 6, caprolactam is hydrolyzed to the straight-chain ε-aminocaproic acid which then spontaneously polymerizes via amide linkages into the linear polymer. U.S. production of nylon 6 is estimated at over $0.5 \cdot 10^9$ kg per year, the applications of which include tire cord, apparel, carpeting, plastics and packaging films.

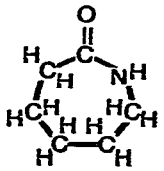

Fig. 1. Structure of caprolactam, $C_6H_{11}ON$.

A moderate order of toxicity has been reported for caprolactam, with acute oral lethalities of >1 g/kg body weight in the rat[1–3] and rabbit[2]. Other effects attributed to caprolactam include pulmonary[4] and skin[5,6] irritation in humans and sensitization in guinea pigs[7,8]. After administration to the rat of a lethal dose of caprolactam (>900 mg/kg, intraperitoneal) toxic effects include stupor and bleeding from the nostrils, followed by clonic convulsions which progress to tonic convulsions and death[11]. Chronic and subchronic administration of caprolactam to rodents produced no pathological changes[9–11]. Greene *et al.*[12], using a variety of bacterial and mammalian cell screens, have recently reported that caprolactam shows no mutagenic activity.

Several gas[13–15], liquid[16] and gel permeation[17–20] chromatographic procedures exist for the determination of caprolactam, but most were developed primarily for the determination of caprolactam in mixtures of oligomers from extracts of the polymer. The present study, therefore, was undertaken to develop a sensitive and reliable high-pressure liquid chromatographic (HPLC) procedure for the determination of caprolactam in biological samples, adaptable to pharmacokinetic and metabolic studies.

EXPERIMENTAL

Standards

Commercial grade caprolactam (purity >99%) was obtained from the Fibers Division of Allied Chemical Corp. (Hopewell, Va., U.S.A.). Caprolactam was dissolved in distilled water to yield a stock standard containing 1 mg/ml. Aliquots of the stock standard were dissolved in the appropriate volume of distilled water to yield caprolactam standards containing 0.1, 0.01 and 0.001 mg/ml.

Equipment

Reversed-phase chromatography was performed using an LDC Constametric IIG HPLC system including an LDC Spectromonitor III variable-wavelength absorbance detector (Laboratory Data Control, Riviera Beach, Fla., U.S.A.). Samples were injected onto the column using a Rheodyne model 7120 sample injector (Rheodyne, Berkeley, Calif., U.S.A.). Separations were achieved with two Lion Technology (Dover, N.J., U.S.A.) FNP 018 reversed-phase columns (particle size, 10 μm; column dimensions, 25 cm × 4.6 mm I.D.) connected in series. A guard column (Whatman, Clifton, N.J., U.S.A.) packed with pellicular octadecylsilane (particle size, 25–37 μm) was attached preceeding the analytical columns. The elution rate was 2 ml/min, and caprolactam was detected at a wavelength of 210 nm, with the absorbance detector at sensitivities of 0.005–1.0 absorbance units full scale (AUFS). Solvent programming (Gradient Master, LDC) was used to establish optimum solvent ratios.

Elution solvent

The elution solvent consisted of glass-distilled acetonitrile (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.) and micro-filtered distilled water (Milli-RO and Milli-Q Water Purification System, Bedford, Mass., U.S.A.). Acetonitrile–water (11:89) was found to give adequate resolution of caprolactam from interferring peaks in urine and plasma samples. The elution solvent was degassed under vacuum before use, and kept under nitrogen during chromatography.

RESULTS AND DISCUSSION

Retention time

At the flow-rate of 2 ml/min, using acetonitrile–water (11:89) as the elution solvent, caprolactam eluted as a sharp, symmetrical peak (Fig. 2). The peaks appearing between three and four minutes on the chromatograms were found to be due to the distilled water in which the standards were dissolved. The retention time was highly reproducible, 20 injections of caprolactam standards of varying concentrations over a 5-day period gave a mean retention time (t_R) of 576 sec, with a coefficient of variation of 1% (Table I).

Precision and sensitivity

Precision was evaluated by injecting, over a 3-day period, ten 9.5-μl aliquots of standard solution containing 95 ng caprolactam. Reproducibility of peak height was good, with a coefficient of variation of 1.03%, representing the combined errors

of injection, detection and flow-rate fluctuation (Table I). Mean sensitivity of detection, mm peak height per ng of caprolactam (Table I) and the chromatograms shown in Fig. 2 indicate that 5–10 ng of caprolactam can easily be detected and quantitated.

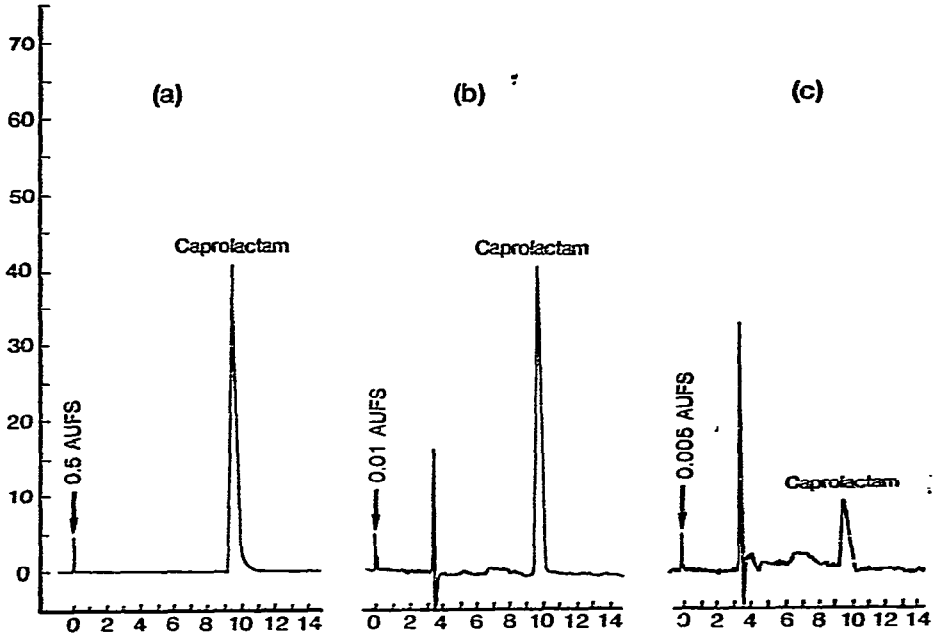

Fig. 2. HPLC chromatogram tracings of caprolactam standards; (a) 0.95 μg, (b) 95 ng, (c) 9.5 ng. Elution solvent, acetonitrile–water (11:89); flow-rate, 2 ml/min.

TABLE I

REPRODUCIBILITY OF RETENTION TIME AND PEAK HEIGHT FOR CAPROLACTAM BY HPLC

Retention time	
Injections, N*	20
Range (sec)	564–582
Mean	576
Standard deviation (sec)	5.78
Coefficient of variation (%)	1.00
Peak height	
Injections, N**	10
Range (mm)	95.5–98.5
Mean	96.6
Standard deviation (mm)	0.994
Coefficient of variation (%)	1.029
Sensitivity (mm peak height/ng)***	2.034

* Successive injections of caprolactam standards (9.5 ng–0.95 μg) over a 5-day period, using several separately-prepared batches of the elution solvent.

** Successive injections of caprolactam standard (95 ng) over a 3-day period.

*** Calculated to maximum sensitivity, 0.005 AUFS.

Linearity

The relationship between peak height and amount of caprolactam injected was linear over a range of 9.5 ng–9.5 μg (Fig. 3). Fig. 3 contains a logarithmic graph of peak heights (converted to a common sensitivity) plotted against the quantity of caprolactam injected. Injections (5–9.5 μl) of each caprolactam standard solution were made over a two-day period using microliter syringes (Hamilton, Reno, Nev., U.S.A.).

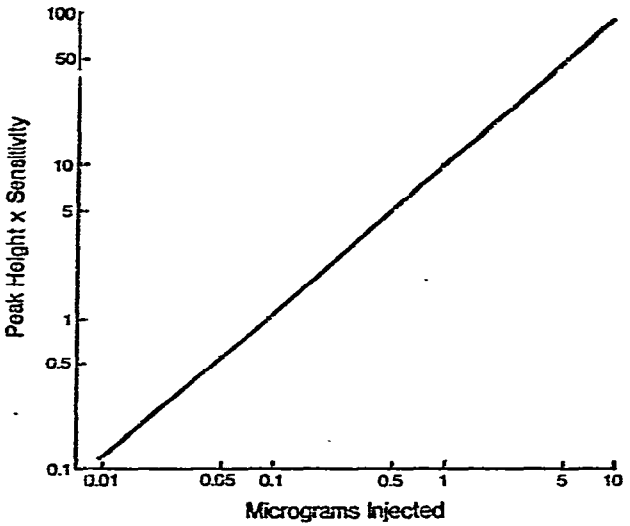

Fig. 3. Linearity, peak height *vs.* amount of caprolactam injected (9.5 ng–9.5 μg).

BIOLOGICAL APPLICATIONS

Due to the nature of mobile and stationary phases used in reversed-phase chromatography, aqueous (such as biological) samples may be loaded directly onto the column, provided that adequate measures are taken to protect the column from particulate matter and components in the samples which might bind irreversibly to the packing material. If a chromatographic procedure is to be adapted to biomonitoring and metabolic studies, the advantage of direct analysis becomes important in terms of convenience and speed. Furthermore, in the analysis of biological fluids for metabolites, reversed-phase chromatography allows a view of the total metabolic profile, rather than the selective glimpses provided by extraction with organic solvents followed by normal-phase chromatography.

Determination of caprolactam in rat urine and plasma

For the determination of caprolactam in urine, caprolactam (dissolved in distilled water) was added to aliquots of untreated rat urine to yield concentrations of 0.05, 0.1, 0.5 and 1 mg/ml. The spiked urine samples were then injected directly onto the HPLC for caprolactam determination.

For the determination of caprolactam in plasma, caprolactam (dissolved in

distilled water) was added to aliquots of untreated rat plasma to yield concentrations of 0.05, 0.1, 0.5 and 1.0 mg/ml. An equal volume of acetonitrile was added to each spiked sample to precipitate to plasma proteins. Following centrifugation (5 min at *ca.* 1000 *g*), the supernatants were carefully removed using pasteur pipettes. The supernatants were then injected onto the HPLC for determination of caprolactam.

As indicated in Table II, determination of caprolactam in both urine and plasma was quantitative over the concentration range investigated. Chromatogram tracings of spiked urine and plasma samples are shown in Fig. 4a and 4b, respectively.

TABLE II

RECOVERY OF CAPROLACTAM FROM SPIKED URINE AND PLASMA SAMPLES

Caprolactam added (mg/ml)	*Recovery (%)* *	
	Urine	*Plasma*
0	—	—
0.05	95.56 ± 1.44	98.72 ± 3.80
0.1	100.08 ± 1.99	97.30 ± 1.56
0.5	96.47 ± 5.06	98.5 ± 1.35
1.0	93.57 ± 5.65	99.60 ± 2.98

* Recovery given as mean ± standard deviation.

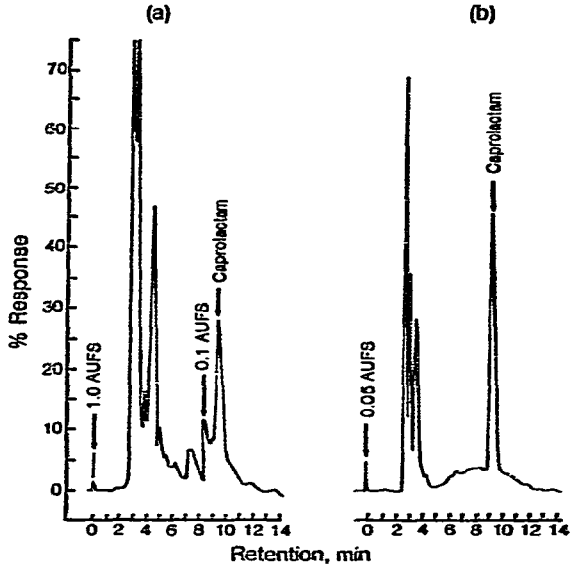

Fig. 4. Chromatogram tracings of urine spiked to 50 μg/ml (a), and plasma spiked to 100 μg/ml (b). Spiked plasma samples were diluted 1:1 with acetonitrile to precipitate the proteins. The large peak (t_R, 7.5 min) immediately preceeding caprolactam was not observed in all urine samples.

Determination of urinary metabolites

For the determination of urinary metabolites of caprolactam, male Fischer 344 rats were dosed (by oral intubation) with 1 μCi [carbonyl-^{14}C]caprolactam

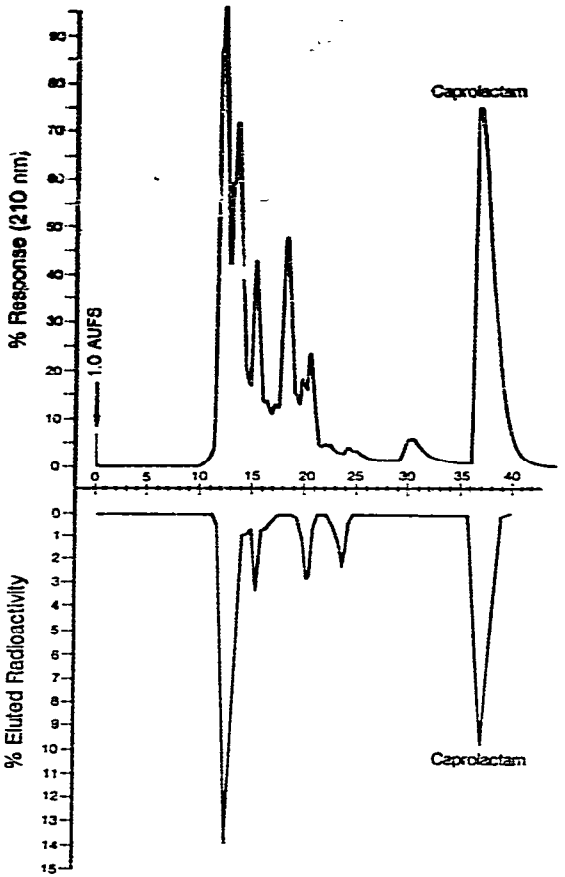

Fig. 5. Chromatogram tracing of 6-h urine of rat orally dosed with 1.5 g/kg of [carbonyl-^{14}C]caprolactam (a), and elution profile of radioactivity (b). Flow-rate, 0.5 ml/min.

(1.5 g/kg body weight). Six hours after dosing, the urine was collected, centrifuged to remove particulate matter, and analyzed by HPLC. The flow-rate was reduced to 0.5 ml/min to allow better resolution of the early-eluting metabolites. The eluate was collected at 24-sec intervals and radioassayed. Fig. 5a shows an HPLC chromatogram of the urine and the radioactivity contained in each of the corresponding vials of the eluate (Fig. 5b). As shown in this figure, the radioactivity administered as [^{14}C]caprolactam was excreted primarily as a major metabolite (t_R, 12.5 min) and as the parent compound. Three lesser metabolites were also observed, with t_R values of approximately 15.5, 20.5 and 23.5 min.

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