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Note

High-performance liquid chromatography of cyclohexanone oxime in urine and plasma

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Cyclohexanone oxime (Fig. 1) is a chemical intermediate used in the production of nylon 6. In the synthesis of nylon 6, cyclohexanone oxime undergoes the Beckmann rearrangement to form the seven-membered heterocyclic caprolactam.

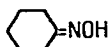

Fig. 1. Structure of cyclohexanone oxime, $C_6H_{11}ON$.

Caprolactam is then hydrolyzed to the straight-chain ε-aminocaproic acid which polymerizes to form the linear polymer. Toxicology data on cyclohexanone oxime is scarce, but the approximate seven-day LD_{50} in the male mouse, by intraperitoneal administration, has been reported[1] to be 250 mg/kg. Upon administration to the rat, cyclohexanone oxime appears to affect primarily the central nervous system. A synergistic central nervous system effect (as evaluated by loss of conditioned reflex) has been reported when cyclohexane, cyclohexanone, cyclohexanol and cyclohexanone oxime (four intermediates in the production of caprolactam) are administered simultaneously to the rat[2]. A decrease in the erythrocyte count and increase in blood methemoglobin levels in the rat after 6–10 week inhalation exposure to cyclohexanone oxime (0.1 or 1.0 mg/m^3) has also been reported[3]. Desquamation of the bronchial epithelium was also observed in the animals exposed to 1 mg/m^3, but no toxic effects were observed after exposure to 0.03 mg/m^3 (ref. 3).

Cyclohexanone oxime, along with cyclohexane, cyclohexanol and cyclohexanone, are also thought to be metabolites of the artificial sweetening agent sodium cyclamate[4–6]. In Drosophila tests, cyclohexanone oxime failed to cause sex-linked lethal mutations[7].

Several photometric[8], polarographic[9], thin-layer chromatographic[10,11], gas chromatographic[6,12] and liquid chromatographic[13] procedures for the determination of cyclohexanone oxime appear in the literature. However, most are indirect, relatively insensitive or unsuitable for the routine determination of trace quantities of the compound. Therefore, a sensitive, highly reproducible high-performance liquid chromatographic (HPLC) procedure for the determination of cyclohexanone oxime, suitable for use in water sample analysis, bioconcentration and metabolism studies, was developed.

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EXPERIMENTAL

Standards

Cyclohexanone oxime (purity 94.7%) was obtained from the Fibers and Plastics Company of Allied Chemical Corporation (Hopewell, VA, U.S.A.). Impurities in the material were water (*ca.* 5%) and cyclohexanone (*ca.* 0.2%). Cyclohexanone oxime was dissolved in the appropriate volume of acetonitrile to yield standards containing 0.005, 0.01, 0.05, 0.1 and 0.5 mg/ml. Standards were analyzed immediately after preparation. Samples (10 μl) were injected onto the column using microliter syringes (Glenco Scientific, Houston, TX, U.S.A.).

Equipment

Reversed-phase chromatography was performed using an LDC (Laboratory Data Control, Riveria Beach, FL, U.S.A.) constametric II-G HPLC system including an LDC Spectromonitor II variable-wavelength absorbance detector. Samples were injected onto the column using a Valco N60 fixed-volume loop injector. Separations were achieved with a Whatman (Clifton, NJ, U.S.A.) Partisil PXS 10/25 ODS-2 column (particle size, 10 μm; column dimensions, 25 cm $\times$ 4.5 mm I.D.). A guard column (Whatman) packed with pellicular ODS (particle size, 25–37 μm) was attached preceding the analytical column. The elution rate was 1.0 ml/min, and cyclohexanone oxime was detected at a wavelength of 205 nm, with the absorbance detector at sensitivities of 0.01–1.28 absorbance units full scale (a.u.f.s.). Solvent programming (Gradient Master, LDC) was used to establish the optimum solvent ratio.

Elution solvent

The elution solvent consisted of glass-distilled acetonitrile (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) and micro-filtered distilled water. Acetonitrile–water (1:3, v/v) was found to give adequate resolution of cyclohexanone oxime from interfering 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 a flow-rate of 1 ml/min, using acetonitrile–water (1:3) as the elution solvent, cyclohexanone oxime eluted as a sharp, symmetrical peak (Fig. 2). The retention times were highly reproducible. Fifteen injections of cyclohexanone oxime over a period of 6 days gave a mean retention time of 574 sec, with a coefficient of variation of 0.54% (Table I).

Precision and sensitivity

Precision was evaluated by injecting, over a two-day period, ten 10-μl aliquots of standard solution containing 0.5 μg cyclohexanone oxime. Reproducibility of peak height was good, with a coefficient of variation of 1.1%, representing the combined errors of injection, detection and flow-rate fluctuation (Table I). Mean sensitivity of detection, mm peak height per ng of cyclohexanone oxime injected (Table I) and the chromatograms shown in Fig. 2 indicate that as little as 10 ng of cyclohexanone oxime can be detected and quantitated.

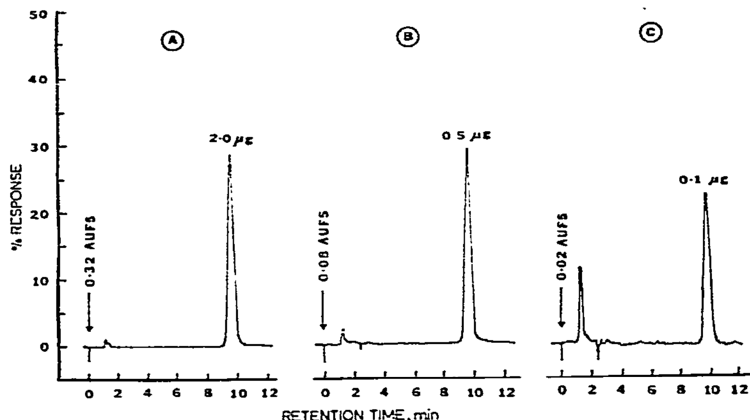

Fig. 2. HPLC chromatogram tracings of cyclohexanone oxime standards; (a) 2.0 μg, (b) 0.5 μg, (c) 0.1 μg. Elution solvent, acetonitrile–water (1:3); flow-rate 1 ml/min.

TABLE I

REPRODUCIBILITY OF RETENTION TIME AND PEAK HEIGHT FOR CYCLOHEXANONE OXIME BY HPLC

Retention time	
Injections, n^{*}	15
Range (sec)	565–579
Mean	574
Standard deviation (sec)	3.08
Coefficient of variation (%)	0.536
Peak height	
Injections, n^{**}	10
Range (mm)	85–88
Mean	86.95
Standard deviation (mm)	0.956
Coefficient of variation (%)	1.099
Sensitivity (mm peak height/ng)***	3.478

* Successive injections of cyclohexanone oxime standard (50 ng–5 μg) over a six-day period, using several separately prepared batches of the elution solvent.

** Successive injections of cyclohexanone oxime standard (0.5 μg, sensitivity 0.08 AUFS).

*** Calculated to maximum sensitivity, 0.005 AUFS.

Linearity

The relationship between peak height and quantity of cyclohexanone oxime injected was linear over a range of 50 ng–2.5 μg. In Fig. 3, the peak heights (converted to a common sensitivity) are plotted *vs.* the quantity of cyclohexanone oxime injected.

Recovery of cyclohexanone oxime from rat urine and plasma

For the determination of cyclohexanone oxime in urine, cyclohexanone oxime (dissolved in acetonitrile) was added to aliquots of urine (from untreated rats) to yield concentrations of 0.01, 0.05, 0.1 and 0.25 mg/ml. The spiked samples then were

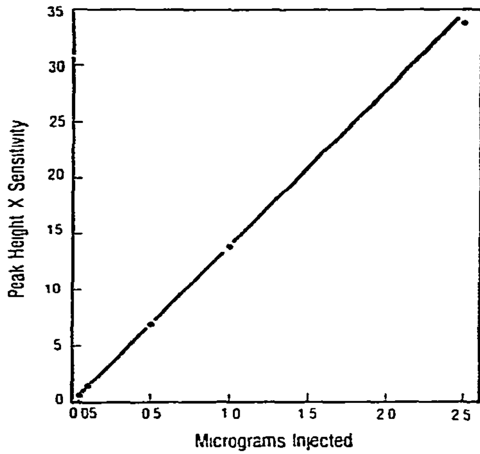

Fig. 3. Linearity, peak height *vs.* amount of cyclohexanone oxime injected (50 ng–2.5 μg).

centrifuged and the supernatants injected directly onto the high-performance liquid chromatograph for determination of cyclohexanone oxime.

For the determination of cyclohexanone oxime in plasma, cyclohexanone oxime (dissolved in acetonitrile) was added to aliquots of plasma (from untreated rats) to yield concentrations of 0.01, 0.05, 0.1 and 0.25 mg/ml. An equal volume of acetonitrile was added to each spiked sample to precipitate the plasma proteins. The

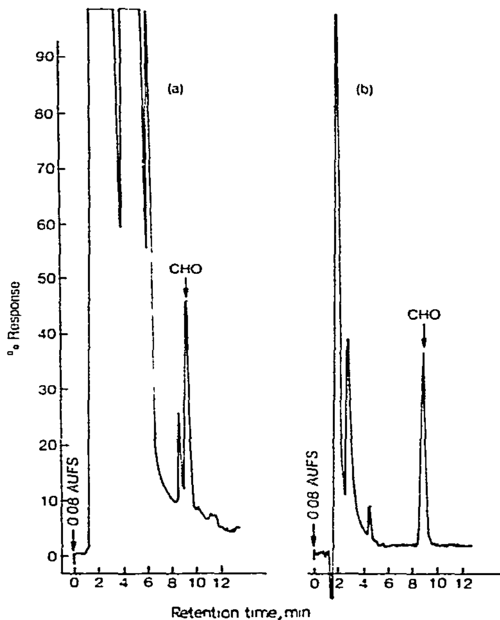

Fig. 4. Chromatogram tracings of urine (a) and plasma (b) spiked to 50 μg/ml with cyclohexanone oxime (CHO). Spiked plasma samples were diluted 1:1 with acetonitrile to precipitate the proteins. The peak (retention time 8.5 min) immediately preceding CHO was not observed in all urine samples.

TABLE II

RECOVERY OF CYCLOHEXANONE OXIME FROM SPIKED URINE AND PLASMA SAMPLES

Recovery given as mean ± standard error.

Cyclohexanone oxime added (mg/ml)	*Recovery (%)*	
	Urine	*Plasma*
0	–	–
0.01	96.9 ± 1.76	104.5 ± 2.56
0.05	102.5 ± 2.87	102.6 ± 1.86
0.1	103.3 ± 2.84	99.8 ± 0.947
0.25	98.7 ± 0.998	97.8 ± 2.10

samples were centrifuged (5 min at 1000 *g*), and the supernatants were removed. The supernatants were then injected onto the high-performance liquid chromatograph for determination of cyclohexanone oxime.

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

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