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Letter to the Editor

High-performance liquid chromatographic determination of urinary mercapturic acids in rat

Sir,

Toluene and xylene are widely used as organic solvents in industry. The major metabolites of toluene and xylene in urine are hippuric acid and methylhippuric acid, respectively [1,2]. Some reports [3–5] have been published on the determination of hippuric acids in urine by high-performance liquid chromatography (HPLC). Recently, the mercapturic acids (N-acetyl-S-benzyl-L-cysteine, N-acetyl-S-xylyl-L-cysteine) originating from toluene and xylene have been detected in rat urine by a thin-layer chromatographic method [6]. The urinary excretion of the mercapturic acids was determined by calculation of the SH concentration according to the Ellman method [7] after alkaline hydrolysis of urine.

In this letter, we describe a direct HPLC procedure for the determination of mercapturic acids in urine from rats administered toluene or *o*-xylene.

EXPERIMENTAL

Apparatus and chromatographic conditions

A liquid chromatograph consisting of a Waters Assoc. (Milford, MA, U.S.A.) Model 600 constant-volume pump, a U6K injection system and 490 UV absorbance detector (250 nm) was used. The analytical column was stainless-steel (150×3.9 mm I.D.) packed with μBondasphere C_{18}, 100 Å (5 μm) (Waters Assoc.) and operated at ambient temperature. The mobile phase was acetonitrile–1% aqueous acetic acid (25:75 or 35:65, v/v). The flow-rate was 0.4 or 0.5 ml min^{-1}.

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Chemicals

N-Acetyl-S-benzyl-L-cysteine (Tol-M) and N-acetyl-S-(*o*-xylyl)-L-cysteine (Xyl-M) were prepared according to the report method [6]. All other chemicals were of analytical-reagent grade.

Animals and urine samples

Male Slc Wistar rats (4 weeks old) were housed in individual stainless-steel metabolism cages with free access to water and food. Toluene and *o*-xylene were given i.p. in doses of 1, 2 or 3 mmol/kg body mass, respectively (administered in 2.5 ml of olive per 1 kg of body mass).

Urine samples were collected daily during 2 days and stored at $-20\,^{\circ}C$ until the analysis.

Sample preparation

N-Acetyl-S-benzyl-L-cysteine (Tol-M). Urine samples were centrifuged at 1200 *g* for 5 min. Aliquots (0.5 ml) of the supernatant were transferred into glass-stoppered tubes and diluted about 5-fold with water. After acidification of the diluted solution to pH 1–2 with 3 *M* hydrochloric acid, the solution was saturated with ammonium chloride and 4.0 ml of ethyl acetate were added. The layers were shaken vigorously for 2 min and separated by centrifugation at 1200 *g* for 5 min.

Aliquots (2.5–3 ml) were transferred into glass-stoppered tubes and shaken with 1 *M* sodium hydrogencarbonate (1 ml). Aliquots of the aqueous layer (0.8 ml) were reacidified to pH 1–2 and saturated with ammonium chloride. Ethyl acetate (4 ml) was added and re-extracted. The ethyl acetate layer was evaporated to dryness and reconstituted with the HPLC mobile phase (1 ml). A 15-μl sample of the solution was analysed by HPLC.

N-Acetyl-S-(o-xylyl)-L-cysteine (Xyl-M). Urine samples were centrifuged at 1200 *g* for 5 min. Aliquots of the supernatant (0.5 ml) were diluted to about 2 ml and passed through Sep-Pak C_{18} cartridges.

After washing with 6 ml of water, the contents adsorbed on the cartridge were eluted with 3 ml of methanol. Evaporation of methanol gave a residue, which was reconstituted with the HPLC mobile phase (2 ml); 10 μl of the solution were analysed by HPLC.

RESULTS AND DISCUSSION

Mercapturic acid can be determined by HPLC of urine samples as described above. The plot of the detector response in arbitrary units of peak height for mercapturic acid versus concentration (5–110 μg ml for Tol-M; 10–800 μg ml for Xyl-M) was linear. The reproducibility and accuracy of this simple method were determined for urine using spiked urine samples; a 91.0–95.0% recovery was obtained for the concentration range of 100–200 μg Tol-M in 0.5 ml of

TABLE I

URINARY EXCRETION OF MERCAPTURIC ACIDS FROM RATS ADMINISTERED TOLUENE OR *o*-XYLENE INTRAPERITIONEALLY

Compound	Dose (mmol/kg)	Urinary mercapturic acid excretion[a] (% of dose)		
		First day	Second day	Total
Toluene	1	1.6±0 7		1.6±0.7
	2	0 9±0.5		0.9±0.5
	3	0.9±0.1		0.9±0.1
o-Xylene	1	27.0±2.4	1.1±1.4	28.0±5.3
	2	24.7±6.9	1.5±0.8	26.2±12.7
	3	27.6±1.9	1.0±0.2	28.6±1 8

[a]Each figure represents the mean ±S.D. for three rats.

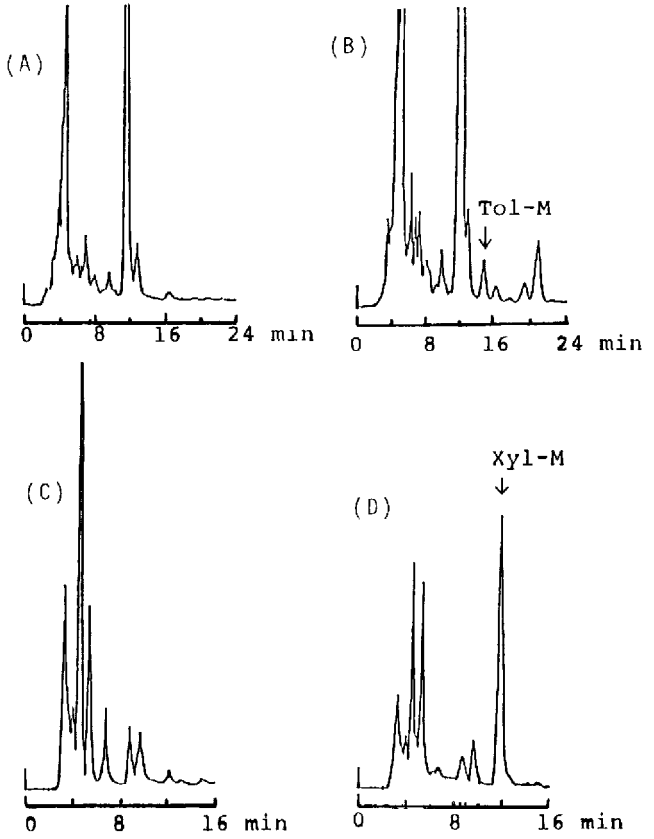

Fig. 1. Typical chromatograms of urine samples from toluene- or *o*-xylene-treated rats. (A) Chromatogram of a normal urine sample (B) Chromatogram of a urine sample from a toluene-treated rat (3 mmol/kg). (C) Chromatogram of a normal urine sample. (D) Chromatogram of a urine sample from an *o*-xylene-treated rat (3 mmol/kg). (A) and (B)· eluent, acetonitrile–1% acetic acid (25 75, v/v); flow-rate, 0.5 ml min^{-1}; detector sensitivity, 0.02 a.u.f.s. (250 nm) (C) and (D): eluent, acetonitrile–1% acetic acid (35:65, v/v); 0.1 a.u.f.s (250 nm).

normal urine and a 94.7–96.1% recovery for 200–300 μg of Xyl-M in 0.5 ml of normal urine.

Table I shows the results of the urinary excretion of mercapturic acids from rats after i.p. administration of toluene or *o*-xylene (1, 2 and 3 mmol/kg). Fig. 1 shows typical chromatograms of urine samples of toluene- or *o*-xylene-treated rats. The level of mercapturic acid in urine of a toluene-treated rat is much lower than that of a *o*-xylene-treated rat (Table I). The results in Table I are in good agreement with those obtained by the Ellman method [6].

In general, the Ellman method [6,7] used for the measurement of mercapturic acid consists of two steps: qualitative analysis of mercapturic acid by thin-layer chromatography and quantitative determination of mercapturic acid by spectrophotometry after alkaline hydrolysis. The determination of mercapturic acid by this method is indirect and the measurement takes a relatively long time.

The proposed method permits the determination of mercapturic acids directly and rapidly by using HPLC with UV detection (250 nm). The method can also be used for the determination of other aromatic mercapturic acids in urine.

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