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Note

Simultaneous quantitation of thioureas in rat plasma by high-performance liquid chromatography

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Certain thioureas have been reported to be carcinogenic and teratogenic in mammals. Thiourea (TU) and tetramethylthiourea are carcinogens toward rats and mice[1–3]. Methylthiourea and ethylthiourea have been found to have teratogenic effects in rats[4]. 1,3-Dimethylthiourea induced conjunctivitis and dermatitis of the eyelids of textile workers[5]. Ethylenethiourea, which is a degradation product of fungicidal ethylenebisdithiocarbamates, has carcinogenic and teratogenic properties in rats or mice[6–9]. For monitoring these toxic thioureas and related compounds in animals, a selective and effective method is required.

Current methods for determining TU and ETU include thin-layer chromatography (TLC)[10,11] and gas–liquid chromatography (GLC)[11–14].

This paper describes a superior method for the isolation, identification and determination of thioureas in rat plasma by high-performance liquid chromatography (HPLC) without derivatization.

EXPERIMENTAL

Materials and reagents

Thiourea (TU) was obtained from Kanto Chemical (Tokyo, Japan), methylthiourea (MeTU), ethylenethiourea (ETU) and 1,3-diethylthiourea (1,3-DETU) from Tokyo Chemical (Tokyo, Japan), ethylthiourea (EtTU) from ICN Pharmaceuticals (Plainview, NY, U.S.A.) and 1,3-dimethylthiourea (1,3-DMTU) from Nakarai Chemicals (Kyoto, Japan). 1,1-Dimethylthiourea (1,1-DMTU) was synthesized from dimethylammonium chloride and ammonium thiocyanate by the method mentioned below. A standard solution of thioureas was prepared in distilled water. HPLC-grade methanol was obtained from Wako (Osaka, Japan). All other reagents were of analytical-reagent grade. The silica gel used for column chromatography was Kieselgel 60 (0.063–0.200 mm, 70–230 mesh) from E. Merck (Darmstadt, G.F.R.).

Synthesis of 1,1-dimethylthiourea

1,1-Dimethylthiourea was synthesized by modifying the method of Gebhart[15], which was devised for preparing 1-methyl-1-phenylthiourea.

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Ammonium thiocyanate (28.5 g, 0.375 mol) in distilled water (20 ml) was added to dimethylammonium chloride (20.25 g, 0.248 mol). The mixture was stirred for 50 h at 100°C, then rendered alkaline with 10% sodium hydroxide solution. The solution was extracted three times with 300 ml of ethyl acetate and the extract was washed three times with 25 m of distilled water. The organic phase was dried over anhydrous sodium sulphate and evaporated *in vacuo*. The residue was extracted with 100 ml of hot chloroform and, after evaporation to dryness, the residue was purified by recrystallization from ethanol to give 1,1-dimethylthiourea as colourless needles, m.p. 161°C, with the following properties: infrared, $\nu_{max.}^{KBr}$ 3390, 3280, 3190, 1625, 1545, 1420, 1365 cm^{-1}; UV, $\lambda_{max.}^{CH_3OH}$ 243 nm; mass spectrometry, *m/z* 104 (M^+, 100%), 90(0.2%), 88(2.9%), 76(3.0%), 74(0.3%), 73(2.1%), 71(6.1%), 60(21.1%), 44(63.8%); ^{1}H-nuclear magnetic resonance (C^2HCl_3), δ 3.3 (6H, s, 2 × CH_3), 5.77 (2H, broad s, NH_2). Elemental analysis: calculated for $C_3H_8N_2S$, C 34.62, H 7.69, N 26.92; found, C 34.48, H 7.69, N 26.81.

Apparatus

All analyses were carried out using a Jasco-Tri-Rotal high-performance liquid chromatograph (Japan Spectroscopic, Tokyo, Japan) equipped with a Uvidec-100 spectrophotometer monitoring the absorbance at 240 nm. The column (25 cm × 4.6 mm I.D.) was packed with ODS SC-02 (Japan Spectroscopic) and eluted with 5% methanol in water at a flow-rate of 0.8 ml/min (48 kg/cm^2) and ambient temperature. The detector sensitivity was set at 0.256 or 0.032 a.u.f.s.

Plasma

Adult male Wistar rats were anaesthetized with diethyl ether. The blood was collected with heparinated syringe and then centrifuged at 3000 rpm for 5 min. The supernatant was used for study.

Plasma extraction procedure

Plasma (1–2 ml) was shaken vigorously for 10 min with 5 ml of ethanol. The mixture was centrifuged at 3000 rpm at room temperature for 20 min, then the organic layer was carefully transferred to a flask and evaporated to dryness under a stream of nitrogen. The residue was dissolved in a small volume of chloroform and applied to a 125 × 15 mm I.D. silica gel column (10 g). After the column had been washed with 20 ml of chloroform and with 20 ml of 3% methanol in chloroform, 1,3-DETU, ETU, 1,3-DMTU and 1,1-DMTU were eluted with 60 ml of 3% methanol in chloroform, and TU, MeTU and EtTU with 100 ml of 10% methanol in chloroform.

These fractions were concentrated to about 1 ml *in vacuo* at 40°C, and then blown to dryness with a stream of nitrogen. The residue was dissolved in 2 ml of mobile phase and this solution was injected into the HPLC system.

RESULTS AND DISCUSSION

The wavelengths of maximal absorption for TU, MeTU, EtTU, 1,1-DMTU, 1,3-DMTU, 1,3-DETU, TMTU and ETU were 242, 240.5, 242.5, 243, 239, 242, 255.5 and 239.5 nm, respectively. For the simultaneous determination of these compounds, 240 nm was selected as a reasonable wavelength to monitor the chromatograms.

Normal- and reversed-phase partition systems were compared and the reversed-phase system (SC-02) with 5% methanol in water as the mobile phase at ambient temperature was found to be best for separating most of the thioureas.

The only exception was for TMTU, which gave a long retention time (112.0 min) and broad peak on the reversed-phase system. In contrast, a normal-phase system (SS-05) with 3% methanol in chloroform–*n*-hexane (80:20) as the mobile phase gave a sharp peak at a retention time of 4.5 min and was therefore suitable for the determination of TMTU.

A typical chromatogram obtained from a rat plasma sample is shown in Fig. 1. The retention times for TU, MeTU, ETU, EtTU, 1,3-DMTU, 1,1-DMTU and 1,3-DETU were 4.0, 5.2, 6.0, 8.9, 9.0, 11.2 and 40.6 min, respectively. Five of the tested compounds were clearly separated. However, EtTU and 1,3-DMTU could not be separated under these or any other conditions. However, these two thioureas can easily be separated by column chromatography on silica gel. When 3% methanol in chloroform was used as eluent in the silica gel column, ETU, 1,3-DMTU, 1,1-DMTU and 1,3-DETU could be eluted and separated from the other thioureas, which were eluted by 10% methanol in chloroform (Fig. 2).

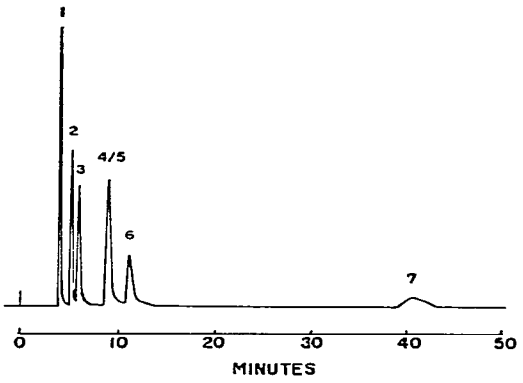

Fig. 1. Chromatogram of a standard mixture of thioureas. Column, SC-02 (25 cm × 4.6 mm I.D.); mobile phase, water–methanol (95:5); flow-rate, 0.8 ml/min; temperature, ambient; wavelength of detection, 240 nm; sensitivity, 0.256 a.u.f.s.; amounts injected, 200 ng of each compound. Peaks: 1 = TU; 2 = MeTU; 3 = ETU; 4 = EtTU; 5 = 1,3,-DMTU; 6 = 1,1-DMTU; 7 = 1,3-DETU.

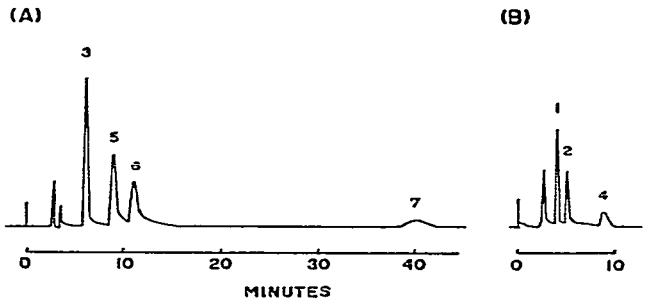

Fig. 2. Chromatogram of plasma spiked at 1 ppm. Operating conditions and peaks as in Fig. 1, except 0.032 a.u.f.s.; (A) 3% methanol in chloroform fraction, 50 ng of each compound injected; (B) 10% methanol in chloroform, 25 ng of each compound injected.

Calibration graphs were prepared for TU, MeTU, EtTU, ETU, 1,1-DMTU, 1,3-DMTU and 1,3-DETU by plotting the peak height or peak area against amount, as illustrated in Fig. 3. Linear relationships were obtained in the range 15–200 or 100–400 ng for each compound.

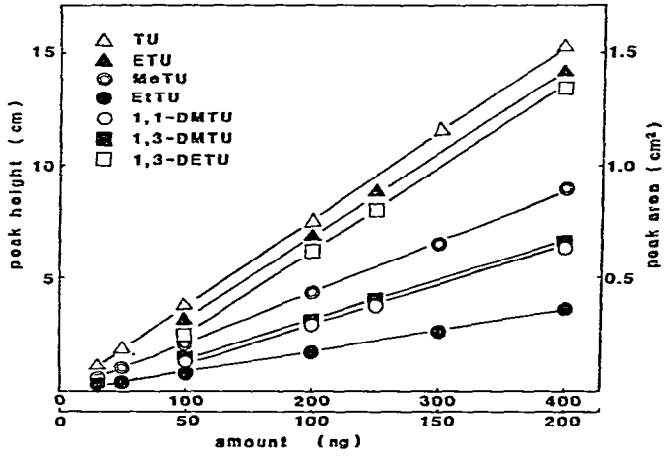

Fig. 3. Calibration graphs for determination of thioureas. Range of amounts: 15–200 ng for TU, MeTU and EtTU; 100–400 ng for ETU, 1,3-DMTU, 1,1-DMTU and 1,3-DETU. Peak area was used only for 1,3-DETU.

Table I gives the recovery data obtained for the plasma. The thioureas were recovered in high yield at concentrations from 1 to 100 ppm.

The detection limits were 0.05 ppm for 1,1-DETU and 0.02 ppm for the others (sample, 2 ml; injection volume, 100 μl).

TABLE I

RECOVERY OF THIOUREAS

Amount added (ppm)	*Recovery (%)**						
	TU	*MeTU*	*ETU*	*EtTU*	*1,3-DMTU*	*1,1-DMTU*	*1,3-DETU*
100	84	92	81	74	100	84	82
25	88	89	91	88	90	85	96
10	75	70	79	84	90	71	78
5	93	94	78	89	99	80	86
2.5	92	93	79	86	97	74	92
1	91	76	84	73	89	77	78
Mean	87	86	82	82	94	79	85

* Results are the means of duplicate determinations.

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