Analysis of Ethylenethiourea in Urine by High-Performance Liquid Chromatography

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A method is described for the determination of ethylenethiourea (ETU) in urine. ETU is separated from the urine by a Gas-Chrom S column chromatographic cleanup, followed by reverse-phase high-performance liquid chromatographic (HPLC) analysis using an electrochemical detector. The minimum level of detection is 0.025 ppm of ETU in a 10-g sample of urine, and recoveries above 90% are obtained over a wide range of concentrations.

Ethylenebis(dithiocarbamates) are an important class of fungicides for controlling numerous crop diseases. Ethylenethiourea (ETU, 2-imidazolidinethione) can be produced as a degradation product of these fungicides during manufacture and storage. ETU has also been shown to be a human metabolite formed after absorption of the ethylenebis(dithiocarbamates) by inhalation or direct absorption through the skin. Since ETU has been shown to be oncogenic and teratogenic when fed to rats and mice (Graham et al., 1975; Ruddick and Khera, 1975), it is important that methodology be available to monitor worker's total exposure and absorption of ethylenebis(dithiocarbamates) by all routes. This can be achieved by analysis of the ETU levels in the urine of these workers.

This method is a simplified modification of the Onley-Yip ETU method (Onley et al., 1972, 1977; Nitz et al. 1982), which eliminates the *S*-butyl derivation step. ETU is measured directly by HPLC using an electrochemical detector. In this way, not only is the work up of the samples simplified but also a lower level of detection (0.025 vs. 0.05 ppm) is realized.

EXPERIMENTAL SECTION

Apparatus. The HPLC analyses were conducted by using a Hewlett-Packard Model 1080 HPLC equipped with a Du Pont Zorbax ODS 25 cm × 4.6 mm column and an Astra electrochemical detector, Model No. ED 110. The latter consists of a flow-through cell that contains a glassy carbon working electrode, a platinum counter electrode, and a silver/silver chloride reference electrode. The control unit contains all of the electronic circuitry necessary to produce a recorder output signal that corresponds linearly to sample concentration.

The samples were concentrated when necessary on a Fisher Scientific rotary evaporator and water bath or a Meyer N-EVAP evaporator, Model No. 111.

The glass chromatographic column was 300 mm × 22 mm i.d., with a Teflon stopcock.

Reagents. The solvents were distilled-in-glass chloroform, ethanol, and acetonitrile from Burdick and Jackson Laboratories.

The aluminum oxide used (Fisher Scientific catalog no. A-540) must be pretreated before using. A total of 450 g of aluminum oxide is placed in a beaker and 300 mL of methanol is added. The solution is mixed well and filtered through a Büchner funnel. The aluminum oxide is returned to the beaker and washed a second time with another 300 mL of methanol. This slurry is then filtered through a Büchner funnel. The methanol is discarded, and the aluminum oxide is air-dried to remove all traces of methanol. The aluminum oxide is placed in an oven and heated at 135–140 °C for 12 h and then stored in a closed container.

The Gas-Chrom S (45–60 mesh, Applied Science catalog no. 03005) must also be pretreated before using. A total of 350 of Gas-Chrom S is placed in a 500-mL beaker and 300 mL of methanol added. The solution is mixed well and the methanol filtered through a Büchner funnel. The Gas-Chrom S is returned to the beaker and washed a second time with another 300 mL of methanol. The slurry is then filtered through a Büchner funnel. The methanol is discarded and the Gas-Chrom S air-dried to remove the residual methanol. The Gas-Chrom S is heated in an oven at 130–145 °C for 12 h and then stored in a closed container.

The ethylenethiourea (2–imidazolidinethione) standard was obtained from the Aldrich Chemical Co., Milwaukee, WI.

Procedure. A total of 0.010 ± 0.0001 g of ETU standard was weighed into a 100-mL volumetric flask, dissolved in about 50 mL of distilled water, and made to the mark with distilled water. This stock solution contains 100 μg/mL ETU. To 3-100-mL volumetric flasks was added 0.25, 0.50, and 1.0 mL of the stock solution. Each was made to the mark with the mobile phase and mixed thoroughly. These standard solutions now contain 0.25, 0.50, and 1.0 μg/mL ETU, respectively. These are used to construct a calibration curve by plotting the concentration vs. the average peak height of duplicate injections of 30 μL of each (Figure 1).

Ten grams of Gas-Chrom S was added to a 500-mL round-bottomed flask and 10 g of urine. The flask was stoppered and shaken vigorously until the Gas-Chrom S is lump free. Fifty milliliters of eluting solution (4% ethanol in chloroform) was added, and the flask was stoppered and shake for 2 min. The resultant slurry was poured into a chromatographic column containing 4 g of Al_2O_3 held in place with $^1/_2$-in. glass wool plug. The sample flask was rinsed with an additional 3 × 50 mL of eluting solution, the solution being added to the column. The elute was collected in a 500-mL round-bottomed flask. The sample was concentrated on a rotary vacuum evaporator in a water bath held at 50 °C, to approximately 5 mL. The solution was transferred to a 10-mL Kimble tube, with 3 × 1 mL washings of chloroform. The tube was placed in a N_2 water bath and the solution evaporated to dryness. The residue was made to 1-mL volume with mobile phase, which consists of 3.28 g of sodium acetate, 0.2 mL of acetic acid, and 0.2 mL of acetonitrile made to 1 L with deionized water.

Thirty microliters of the sample was then injected into the chromatograph equilibrated at a flow rate of 1.0

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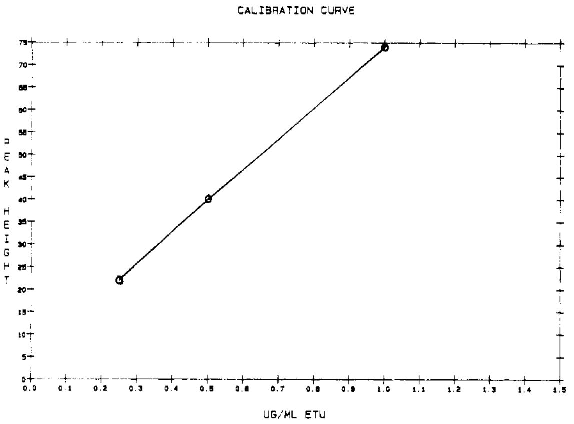

Figure 1. Calibration curve that plots concentration vs. average peak height.

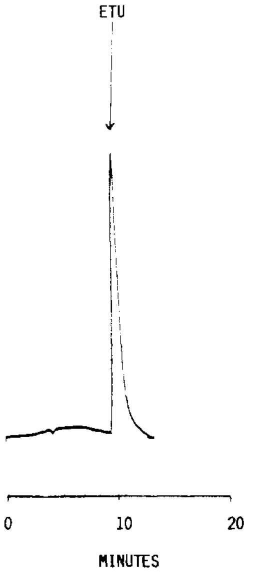

Figure 2. 1.0 μg/mL ETU standard.

Table I. ETU Recoveries from Urine

fortification, ppm	% recovery	1 SD, %
control	ND	
0.025	92	±8
0.050	94	±8
0.10	88	±7
	av: 91	±8

mL/min and column temperature of 28 °C. The electrochemical detector was operated at a potential of +1.0 V and adjusted according to the operating manual.

In addition to the urine samples to be evaluated, control samples from unexposed personnel and control samples of urine (10 g) fortified with 1 mL of 0.25, 0.50, and 1.0 μg/mL standard solution were also analyzed according to procedure.

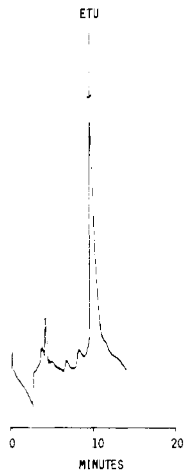

Figure 3. Urine sample fortified with 1.0 μg/mL ETU.

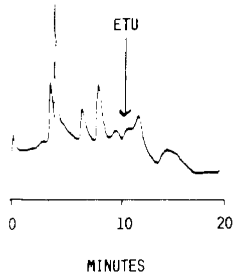

Figure 4. Urine control.

Calculations. The peak height of the ETU in the sample was measured, and calibration curves were determined from the μg/mL ETU present.

$$\text{ETU (ppm)} = (\mu\text{g/mL})/10\text{-g sample}$$

RESULTS AND DISCUSSION

As illustrated by the results in Table I and the HPLC scans shown (Figures 2–4), this method provides a rapid method of determination of ethylene thiourea (ETU) in urine. No derivatization is required since the ETU is detected directly by the electrochemical detector. In addition, the level of detection of 0.025 ppm in a 10-g sample of urine is lower than previously reported.

Registry No. ETU, 96-45-7.

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