A Glass Capillary Gas–Liquid Chromatography Method for Determining Ethylenethiourea without Derivatization

Ethylenethiourea, a toxic metabolite of ethylenebis(dithiocarbamate) fungicides, was detected with high sensitivity and selectivity, without preparation of derivatives, by a high-resolution glass capillary GLC method. The method was applied to analytical ethylenethiourea research problems.

Ethylenebis(dithiocarbamates) (EBDC), including Maneb, Zineb, Mancozeb, Nabam and Amobam, form an important class of fungicides for controlling crop diseases.

Studies on the decomposition of EBDC have shown ethylenethiourea (ETU) to be a primary metabolite in environmental degradation and in test organism (Engst and Schnaak, 1974; Marshall, 1977; Watts et al., 1974). ETU in turn has been found both toxic and to have carcinogenic effects and teratogenic properties (Engst and Schnaak, 1974). Simple, rapid, sensitive, and reliable methods of determining ETU are thus needed (Engst 1977).

ETU can be determined by chromatographic methods (Engst and Schnaak, 1974; Onley and Yip, 1971), by polarography (Engst and Schnaak, 1974), and by a radioisotope method (Graham and Bornak, 1973). The known volatile ETU derivatives which can be determined by gas–liquid chromatography (GLC) are 2-butylthio-2-imidazoline (Onley and Yip, 1971; Onley et al., 1977), 2-benzylthio-1-trifluoroacetyl-2-imidazoline (Newsome, 1972), 2-benzylthio-1-pentafluorobenzoyl-2-imidazoline (Nash, 1974), 2-(*m*-trifluoromethylbenzylthio)-2-imidazoline, and 2-(*m*-trifluoromethylbenzylthio)-1-trifluoroacetyl-2-imidazoline (King, 1977).

Derivatives of ETU have been detected by GLC when present in 0.005 ppm concentration (Nash, 1974; Newsome, 1972). Although ETU as a volatile derivative, can be detected with a high degree of sensitivity, the preparation of derivative slows down the analysis and increases the risk of error, for instance, because of the decomposition of EBDC to ETU during preparation of the derivative (Pease and Holt, 1977) or incomplete formation of the derivative.

Large amounts of ETU, without derivatization, have previously been analyzed by GLC (Bontoyan and Looker, 1973) and very recently Otto et al. (1977) have described a GLC method which does not require a prior derivatization. Since in the GLC methods packed columns were used, a column chromatographic precleaning of the ETU concentrate is needed and the method is reliable in the microgram range (Otto et al., 1977).

In this work, which forms a part of a larger study on ETU, we describe a highly sensitive method for determining ETU as such by high-resolution glass capillary GLC. The method is tested with various samples and is compared with methods involving derivatizations.

EXPERIMENTAL SECTION

GLC Measurements. For GLC measurements, high-resolution glass capillary GLC columns were constructed according to the method of Grob and Grob (1976). FFAP, Carbowax 20M, OV-17, and OV-101 liquid phases were successfully tested as phases for determining ETU. During hundreds of injections the columns were found to be stable up to 260 °C. When hydrogen was used as the carrier gas at 2 mL/min ETU eluted from the column at about 200 °C within a few minutes (Figure 1). Hydrogen as the carrier gas has the advantage of increasing the separation power of GLC but for safety purposes it can be replaced by helium.

Table I. Detectable Amounts (ng) of ETU and Its Derivatives in Glass Capillary GLC

	^{63}Ni ECD	NPSD	FID
ETU	0.01	0.02	0.5
2-benzylthio-1-trifluoroacetyl-2-imidazoline	0.005		
2-benzylthio-1-pentafluorobenzoyl-2-imidazoline	0.0005		

The stability of ETU in GLC experiments was confirmed by replacing the detector with a dry-ice trap. The undecomposed structure of the trapped ETU was confirmed by mass spectroscopy [MS *m/e* 102 (100%), 73 (20), 45 (20), 30 (45)]. The sensitivities in Table I were obtained with a flame ionization detector (FID), nitrogen-phosphorus-selective detector (KBr-type NPSD), and electron capture detector (^{63}Ni-type ECD).

Applications of the Glass Capillary GLC Method for Determining ETU. EBDC compounds reportedly decompose during storage and small amounts of ETU are formed (Bontoyan and Looker, 1973). When 200 mg of Maneb (Dithane M-22, Rohm & Haas Co.) was slurried in 10 mL of ethyl acetate and 1 μL of the filtered solution injected into the GLC, 0.02 μg of ETU was detected. This concentration of 0.1% of ETU in Maneb corresponds well with the percentages reported by Bontoyan and Looker (1973). When Maneb was carefully washed with ethyl acetate, no signal of ETU was obtained from the Maneb solution, which confirms that ETU is not formed from Maneb in the glass-covered injector of GLC at the temperature (200 °C) used. Because of its toxic properties, ETU has been subjected to toxicological studies and further studies are required (Engst, 1977). In this work the glass-capillary GLC method was applied to detect ETU in animal materials: 0.5 mg of ETU was dissolved in rats' urine (5 mL) and after 5 h the urine was evaporated in vacuum and the solid residue was dissolved in 5 mL of ethyl acetate. ETU as such, without precleaning the extract, was detected by glass-capillary GLC with a recovery of 90%. Figure 1b gives an example of detecting 5 ppb ETU in the liver of rats. The rats were fed with 1 mg/kg of ETU and the liver homogenated after 5 h with methanol (1 g/10 mL). The filtered methanol extract was evaporated to dryness and the solid residue dissolved in 5 mL of ethyl acetate. One microliter of ethyl acetate solution was injected into the GLC.

Determination of the residues of ETU in foodstuffs proved to be somewhat more complicated. Twenty-four hours after the addition of 0.2 mg of ETU to 20-g samples of apples, plums, and tomatoes, ETU was extracted with 50 mL of methanol and the methanol solution was evaporated in vacuum. The solid residue was dissolved in 20 mL of ethyl acetate and ETU was determined as such. The recovery was found to be less than 10% and no higher values were obtained even when the pH of the methanol was adjusted to 9–13. Using 1 mg of 2-^{14}C-labeled ETU added to 100 g of apples, making a methanol homogenate and preparing an ethyl acetate extract from the filtered methanol homogenate, it was found by TLC

0021-8561/79/1427-0194$01.00/0

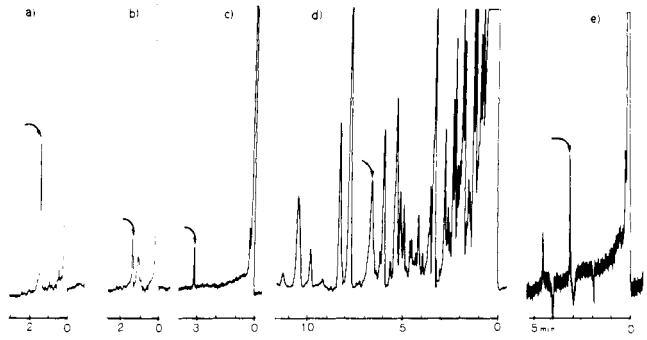

Figure 1. Glass capillary GLC chromatograms obtained from: (a) 10 ng of ETU in 1 μL of ethyl acetate, a 20-m Carbowax 20M column at 190 °C; (b) 1 ppb ETU in ethyl acetate, isolated from rats liver, ECD, a 20-m Carbowax 20M column at 190 °C; (c) 1 ng of ETU standard in 1 μL of ethyl acetate, a 30-m FFAP column at 180 °C, NPSD; (d) 2-benzylthio-1-pentafluorobenzoyl-2-imidazoline, isolated from plums, a 30-m OV-17 column at 170 °C, ECD. (e) 0.01 ng of 2-benzylthio-1-trifluoroacetyl-2-imidazoline in 1 μL of ethyl acetate, a 25-m OV-101 column at 180 °C, ECD.

and liquid scintillation counting experiments that most of the ETU was not in a free form detectable by GLC but possibly bounded with proteins or in a form of a metal complex having no retention in TLC with the solvent system used.

When the dried methanol extract was boiled in 50 mL of ethanol for 30 min, ETU was liberated, which could be also seen by TLC [R_f 0.48 for ETU using silica gel plates and EtOAc–MeOH–NH_4OH (90:6:6) solvent system]. When the ethanol solution was evaporated to dryness and the solid residue dissolved in 5 mL of ethyl acetate, the recovery of ETU was by glass capillary GLC found to be 80–90%. EBDC compounds can be decomposed to ETU in 80% yield (Marshall, 1977) and this can be used as a method to determine quantitatively the residues of EBDC compounds. The glass capillary GLC method could be successfully adapted also for this kind of analytical work. One milligram of Maneb was decomposed at 90 °C for 120 min in a water solution of pH 9. The water solution was evaporated in vacuum, the residue was dissolved in 5 mL of ethyl acetate, and ETU was determined with glass-capillary GLC with a recovery of 75%.

MATERIALS AND METHODS

ETU was synthesized from ethylenediamine and carbon disulfide (Allen et al., 1946), and the product (mp 198 °C) had, according to ^1H NMR and MS, a purity higher than 95%.

2-^{14}C-labeled ETU (sp act. 5.7 μCi/mmol) was synthesized from ^{14}C-labeled carbon disulfide (provided by Amersham, England), sp act. 62 mCi/mmol. 2-Benzylthio-1-trifluoroacetyl-2-imidazoline and 2-benzylthio-1-pentafluorobenzoyl-2-imidazoline were prepared according to the methods described by Newsome (1972) and Nash (1974).

In TLC experiments silica gel plates and a solvent system ethyl acetate–methanol–25% ammonium hydroxide (90:6:6) were used.

Measurements of radioactive ETU were carried out using a LKB Ultrobeta 1210 liquid scintillation counter. In the GLC experiments a Carlo Erba 2300 instrument was used.

The GLC glass capillary columns were prepared from a soda glass and had a length of 20–35 m and an inner diameter of 0.3 mm. The chromatograms were obtained isothermally at 170–200 °C. At the detection limits, the signal-to-noise ratio was 3:1.

DISCUSSION

A high-resolution glass capillary GLC was proved to be a very sensitive, simple, and time sparing way of detecting ETU. Precleaning of the sample or the extract is not necessarily needed and a few picograms could be detected. For higher sensitivities, the preparation of fluoronated derivative is recommended.

The GLC columns described in this work can be constructed according to the instructions in the literature cited and commercial columns are available as well.

LITERATURE CITED

Allen, C., Edens, C., VanAllen, J., *Org. Synth.* **26**, 34 (1946).
Bontoyan, W., Looker, J., *J. Agric. Food Chem.* **21**, 338 (1973).
Engst, R., *Pure Appl. Chem.* **49**, 675 (1977).
Engst, R., Schnaak, W., *Residue Rev.* **52**, 45 (1974).
Graham, W., Bornak, W., *Anal. Chem.* **45**, 623 (1973).
Grob, K., Grob, G., *J. Chromatogr.* **125**, 471 (1976).
King, R., *J. Agric. Food Chem.* **25**, 73 (1977).
Marshall, W., *J. Agric. Food Chem.* **25**, 357 (1977).
Nash, R., *J. Assoc. Off. Anal. Chem.* **57**, 1015 (1974).
Newsome, W., *J. Agric. Food Chem.* **20**, 967 (1972).
Onley, J., Yip, G., *J. Assoc. Off. Anal. Chem.* **54**, 165 (1971).
Onley, J., Guiffrida, L., Ives, N., Watts, R., Storherr, R., *J. Assoc. Off. Anal. Chem.* **60**, 1105 (1977).
Otto, S., Keller, W., Drescher, N., *J. Environ. Sci. Health B* **12**, 179 (1977).
Pease, H., Holt, R., *J. Agric. Food Chem.* **25**, 651 (1977).
Watts, R., Storherr, R., Onley, J., *Bull. Environ. Contam. Toxicol.* **12**, 2246 (1974).

Timo Hirvi
Heikki Pyysalo*
Karin Savolainen

Technical Research Centre of Finland
Food Research Laboratory
Biologinkuja 1, SF-02150
Espoo 15, Finland

Received for review April 7, 1978. Accepted October 12, 1978.

Enzymatic and High-Pressure Liquid Chromatographic Estimation of Glucose, Fructose, and Sucrose in Powders from Stored Onions

The enzymatic estimate of sucrose was always higher than that from high-pressure liquid chromatography because of the presence of oligosaccharides. For glucose and fructose, there was no significant difference between the results of both methods.

During enzymatic analysis of glucose, fructose, and sucrose in stored onions, we obtained some evidence that the specificity of sucrose estimation could be affected by the presence of soluble oligosaccharides (Bacon, 1957;

0021-8561/79/1427-0195$01.00/0