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Determination of ethylenethiourea in water samples by gas chromatography with alkali flame ionization detection and mass spectrometric confirmation

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ABSTRACT

A gas chromatographic method with alkali flame ionization detection and mass spectrometric confirmation is described for the determination of ethylenethiourea (ETU) in water samples. The method is based on the extraction of ETU with dichloromethane in the presence of thiourea and sodium L-ascorbate. The limit of detection is less than 0.1 μg/l in water. The average recovery in groundwater is 71%. Several hundred samples of groundwater and river water were analysed over a 2-year period.

INTRODUCTION

Ethylenethiourea (ETU) is a toxicologically important metabolite of the widely used ethylenebisdithiocarbamate (EBDC) fungicides formed during biological and chemical degradation. EBDCs are frequently used for the control of diseases in seeds and crops throughout the growing season [1]. In 1987, more than 2300 tonnes EBDCs were used in Netherlands. The group includes such fungicides as maneb, mancozeb, nabam and zineb. A review of the toxicology of ETU, which may produce teratogenic, oncogenic and goiterogenic effects after being applied to laboratory animals, has been published [2]. EBDCs degrade in the presence of moisture, oxygen and/or biological systems and several degradation products are formed, including ETU. The reactions leading to ETU formation have been described previously [3]. Most of the ETU occurring in crops and environmental samples arises, however, from EBDC formulations, which contain 0.02–5% of ETU [4]. ETU is a relatively stable and very polar metabolite and in the areas where EBDC fungicides are used, its possible occurrence in groundwater and river water is a major concern for the safety of drinking water.

Residue analysis of ETU has been conducted in different matrices such as fruits and plant tissues by various methods, including high-performance liquid chromatography (HPLC) with non-selective UV absorption detection [5–8] and selective electrochemical detection [9–12] and gas chromatrography (GC) with derivatization to achieve sensitive detection [13–17]. The HPLC and GC methods share the disadvantages of laboriousness and/or insufficient sensitivity and/or specificity, except for one HPLC method [5] which was applied at the μg/l level.

However to reach the desired 0.1 μg/l water level which is required by an EEC Directive for drinking water [18], also with this method preconcentration of the water sample and extraction steps are necessary. We have developed a method for the determination of ETU without derivatization in water samples after extraction with dichloromethane and gas chromatography with alkali flame ionization detection and confirmation by mass spectrometry. This method is easily applicable to the 0.1 μg/l ETU level water in required by the EEC Directive [18]. Several hundred water samples were successfully analysed with this method.

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EXPERIMENTAL

Reagents and apparatus

ETU (99%) was obtained from Promochem (Wesel, Germany). A standard solution containing 1% (v/v) of diethylene glycol was prepared in methanol and appeared to be stable for at least 3 months if stored at 4°C in the dark.

Thiourea (99%), diethylene glycol (99%) and sodium L-ascorbate (99%) were obtained from Aldrich (Brussel, Belgium). A 0.05% (v/v) solution of diethylene glycol in methanol was used.

All other chemicals were of analytical-reagent grade and were checked for the absence of interfering impurities by means of control determinations. Evaporation of water samples was carried out at 50°C (water-bath) using a vacuum rotary evaporator.

Gas chromatography

A Carlo Erba MEGA 8000 gas chromatograph equipped with an alkali flame ionization (nitrogen–phosphorus) detector was used. The instrument was equipped with a wide-bore fused-silica capillary CP-WAX 52 CB column (10 m × 0.53 mm I.D., film thickness 2.0 μm) (Chrompack, Middelburg, Netherlands). Helium was used both as the carrier gas (constant pressure 25 kPa) and as the make-up gas for the detector (16 ml/min). The temperature of the injection port was 250°C and that of the detector 260°C. A 4-μl volume of the sample extract was injected splitless on to the column at 130°C. After 20 s the carrier gas splitting was restarted and then an oven temperature programme was started as follows: initially 130°C, increased at 10°C/min to 250°C, held for 20 min and then cooled to the initial temperature of 130°C.

Gas chromatography–mass spectrometry

The gas chromatograph–mass spectrometer (Carlo Erba MEGA 5000–QMD 1000) was equipped with a CP-WAX 52 CB fused-silica capillary column (12 m × 0.27 mm I.D., film thickness 0.22 μm) (Chrompack). Helium was used as the carrier gas (constant pressure 30 kPa). The injection port temperature was 250°C. The oven temperature programme was started at 50°C (held for 1 min), then increased at 25°C/min to 240°C and held there for 15 min. A 1-μl sample volume was injected under splitless conditions; 40 s after injection the splitting valve was opened.

The ion source temperature of the mass spectrometer was 200°C. The spectra were recorded under electron impact (EI) conditions (70 eV), with a scan range of m/z 25–130 and a scan rate of 0.5 s.

Determination

A 10-mg amount of thiourea and 1 g of sodium L-ascorbate were dissolved in 500 ml of water. The mixture was concentrated by a vacuum rotary evaporator at 50°C to *ca.* 30 ml (± 5 ml). The concentrate was transferred to a separating funnel with 2 × 5 ml of distilled water and saturated with 14 g of sodium chloride. The mixture was then extracted once with 100 ml and twice with 50 ml of dichloromethane. The extract was dried by passing it through a funnel containing *ca.* 25 g of anhydrous sodium sulphate prewetted with dichloromethane. The sodium sulphate was washed twice with 10 ml of dichloromethane. To the combined dichloromethane extract, 10 ml of a 0.05% (v/v) solution of diethylene glycol in methanol were added and the mixture was concentrated with a rotary evaporator at 40°C to *ca.* 5 ml. To the concentrate 10 ml methanol were added and the mixture was concentrated to *ca.* 5 ml. The concentrate was transferred quantitatively with 2 × 0.5 ml of methanol into a graduated test-tube and further concentrated to 0.5 ml (corresponding to 1 l of water per ml of methanol extract) by using a gentle stream of dry nitrogen at 50°C. This extract was examined by gas chromatography. Quantification was achieved by comparing the peak height of ETU with those of standard solutions of comparable concentration.

RESULTS AND DISCUSSION

Extraction procedure and recovery experiments

The extraction of ETU was based on a procedure described by Otto *et al.* [19]. To achieve a detection limit of 0.1 μg/l of ETU or less in groundwater or river water, required for water samples used as a source of drinking water [18], a concentration and extraction procedure is necessary. Because ETU is poorly soluble in organic solvents but readily soluble in water (2%, w/v) at 30°C, direct extraction of ETU with dichloromethane or a more polar organic solvent (ethyl acetate) was not feasible. Before con-

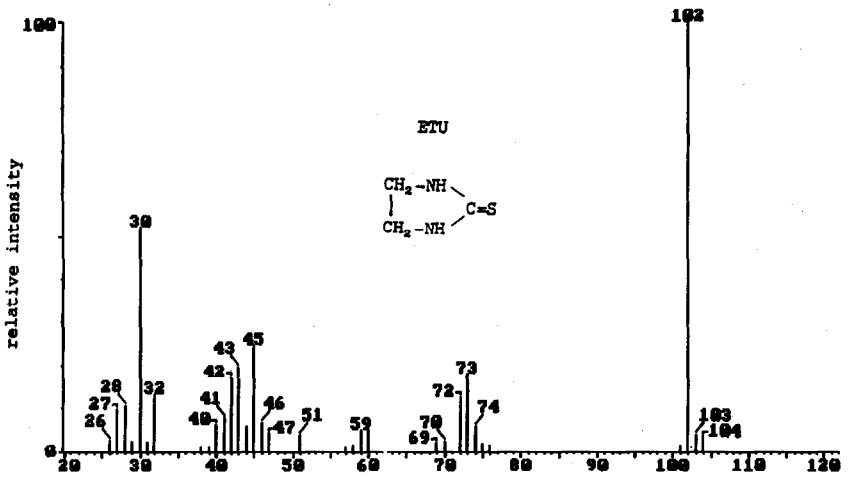

Fig. 1. EI mass spectrum of 10 ng of ETU.

centrating and extracting the water sample, thiourea and sodium L-ascorbate were added. The beneficial function of these actions cannot be fully explained, but it is assumed that thiourea and sodium L-ascorbate protect ETU against oxidation, complex formation with heavy metals [19] and adsorption on active glassware surfaces. By just extracting the concentrate with dichloromethane and concentrating the extract obtained to 0.5 ml, a considerable loss of ETU was observed, possibly caused in part by adsorption of ETU on active glassware surfaces. The recovery for spiked water samples at the residue level was 20–60%. By adding a mixture of diethylene glycol in methanol before concentrating the dichloromethane extract, the recovery of ETU in spiked water samples increased considerably and the repeatability was acceptable. Diethylene glycol is used as "keeper" and seems also to protect ETU

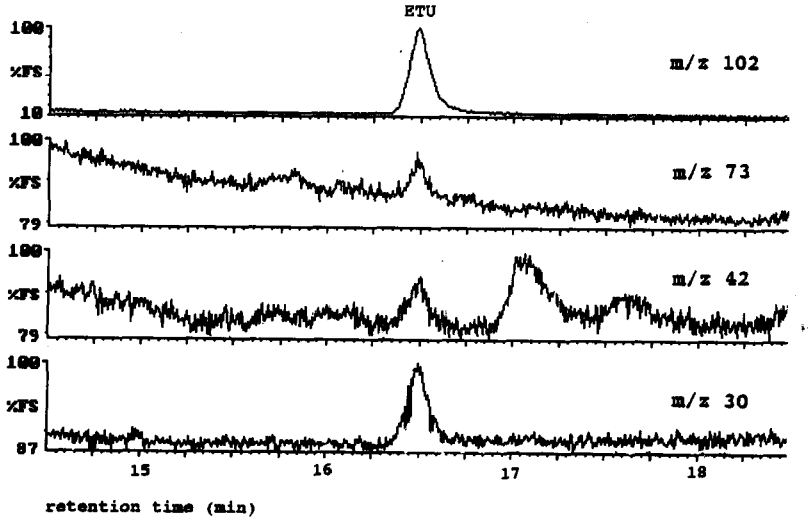

Fig. 2. SIM mass chromatogram obtained for a sample of groundwater fortified with 0.1 μg/l of ETU. Injection volume, 1 μl.

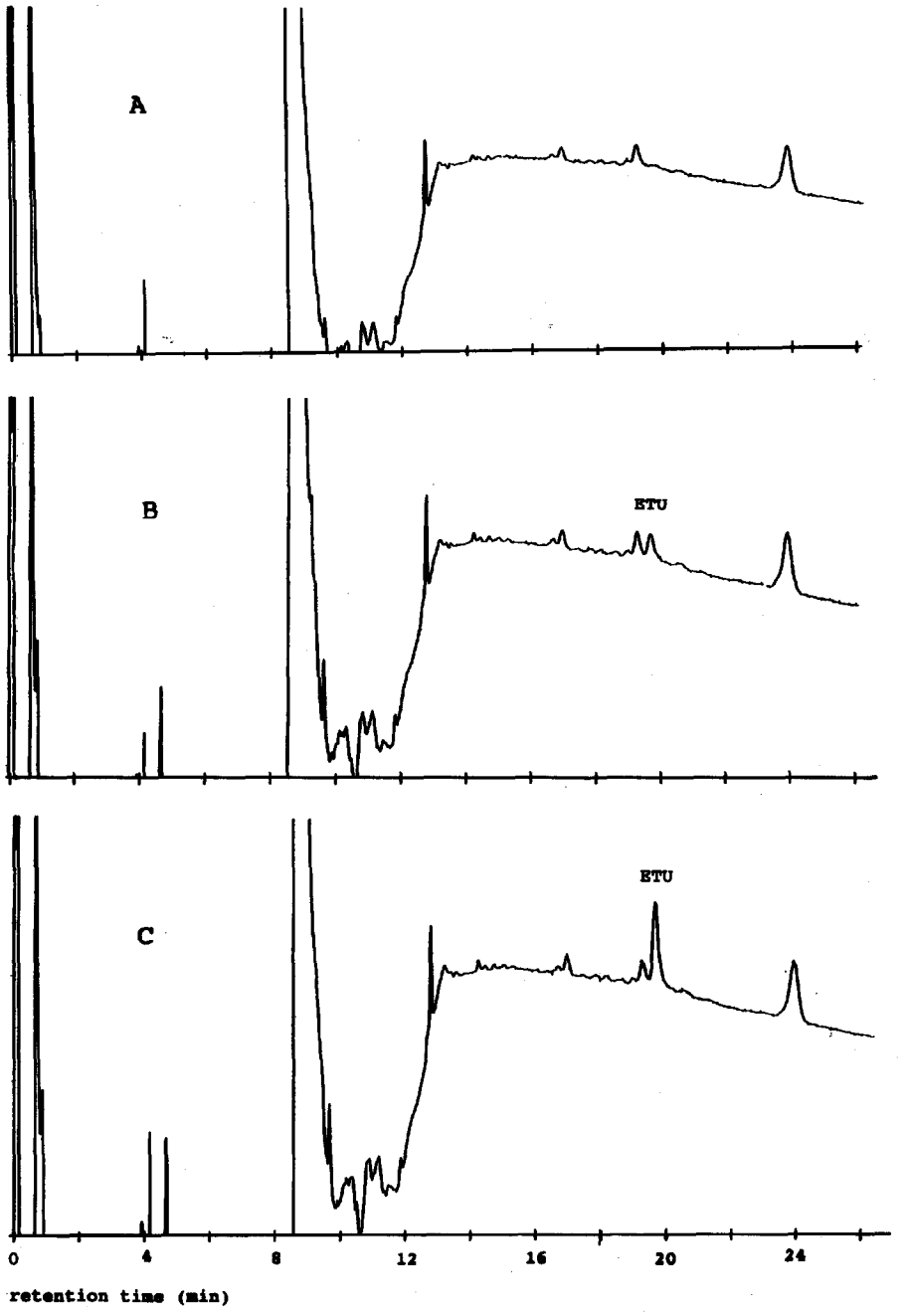

Fig. 3. Chromatograms obtained with CP-WAX 52 CB fused-silica GC column. (A) 4 ml of groundwater; (B) 4 ml of groundwater fortified with 0.2 μg/l of ETU; (C) 4 ml of groundwater fortified with 0.7 μg/l of ETU. Injection volume, 4 μl.

against adsorption on active parts of the glassware used. With diethylene glycol in the final extract also a better peak shape (no tailing) was obtained.

Recovery experiments were carried out by adding known amounts of ETU to 500 ml of groundwater. The results are given in Table I.

TABLE I

RESULTS OF RECOVERY EXPERIMENTS

ETU added to water (μg/l)	n	Recovery (%) (mean ± S.D.)	R.S.D. (%)
0.23	4	70 ± 15	21
1.0	13	72 ± 11	15
6.2	4	70 ± 8.2	12

Gas chromatography and gas chromatographic–mass spectrometric confirmation

The response of the alkali flame ionization detector to ETU is linear up to at least 15 ng and the minimum determinable amount is *ca.* 0.2 ng (see Fig. 3B). Generally in residue analysis, if possible positive samples should be confirmed by mass spectrometry. The identity of ETU was verified in this manner. From the EI mass spectrum the following ions were chosen for selective ion monitoring (SIM): *m/z* 102 (100%), *m/z* 73 (20%), *m/z* 42 (20%) and *m/z* 30 (55%). A typical EI mass spectrum of ETU is shown in Fig. 1. For quantification the area of the molecular ion at *m/z* 102 was used. The response was linear up to at least 2 ng and the minimum determinable amount was *ca.* 0.05 ng (see Fig. 2). A Typical SIM mass chromatogram of groundwater fortified with 0.1 μg/l of ETU is shown in Fig. 2.

TABLE II

INTERLABORATORY STUDY OF THE DETERMINATION OF ETU IN GROUNDWATER SAMPLES

Sample series	Sample location	ETU content (μg/l)	
		TNO	RIVM
A	1	34	53
A	2	12	17
A	3	3.1	3.2
A	4	34	38
B	5	0.1	0.1
B	6	0.1	0.1
B	7	<0.1	<0.03
B	8	0.1	0.1
B	9	0.1	0.15

Interlaboratory studies

Two series of groundwater samples were analysed both by the TNO Nutrition and Food Research Laboratory and by the RIVM Laboratory (Bilthoven, Netherlands). The latter institute used a similar extraction–concentration procedure but the determination of the ETU was performed by a column-switching RPLC procedure with UV detection at 233 nm [5]. The results are presented in Table II and show a reasonably satisfactory agreement between the two methods.

The method described has been successfully applied in the investigation of ETU in groundwater and river water samples over the past 2 years. Fig. 3 shows typical gas chromatograms of control and fortified samples of groundwater analysed by the method described.

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REFERENCES

1 *Gewasbeschermingsgids*, Plantenziektenkundige Dienst, Wageningen, Netherlands, 12th ed., 1991.
2 L. Fishbein, *J. Toxicol. Environ. Health*, 1 (1976) 713.
3 Ch. Lentza-Rizos, *Rev. Environ. Contam. Toxicol.*, 115 (1990) 1–37.
4 W. R. Bontoyan, D. J. Looker, T. E. Kaiser, P. Giang and B. M. Olive, *J. Assoc. Off. Anal. Chem.*, 60 (1977) 1105.
5 E. A. Hoogendoorn, P. van Zoonen and U. A. Th. Brinkman, *Chromatographia*, 31 (1991) 285.
6 J. Pflugmacher and W. Ebing, *Z. Lebensm.-Unters.-Forsch.*, 178 (1984) 90.
7 P. A. Greve and H. A. Herbold, *Meded. Fac. Landbouwwet. Rijksuniv. Gent*, 40 (1983) 933.
8 P. Kurtitio, T. Vartiainen and K. Savolainen, *Anal. Chim. Acta*, 212 (1988) 297.
9 M. Koniger, G. Engelhardt, A. Schmitt and P. R. Wallnofer, *Dtsch. Lebensm. Rundsch.*, 85 (1989) 5.
10 J. L. Prince, *J. Agric. Food Chem.*, 33 (1985) 93.
11 R. T. Krause and Y. Wang, *J. Liq. Chromatogr.*, 11 (1988) 349.
12 R. T. Krause, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 975.
13 J. H. Onley, L. Giuffrida, N. F. Ives, R. R. Watts and R. W. Storherr, *J. Assoc. Off. Anal. Chem.*, 60 (1977) 1105.
14 N. A. Smart, *Analyst*, 112 (1987) 1559.
15 W. H. Newsome, *Bull. Environ. Contam. Toxicol.*, 11 (1974) 174.

16 R. G. Nash, *J. Assoc. Off. Anal. Chem.*, 58 (1975) 566.
17 E. Matisova, J. Chovancova and T. Buzinkaiova, *J. Chromatogr.*, 286 (1984) 331.
18 *Gifap Bulletin*, Vol. 11, Groupement Internationale des Associations Nationales des Fabriques de Produits Agrochimiques, Brussel, 1985, pp. 6.12 and 6.13.
19 S. Otto, W. Keller and N. Drescher, in *Rückstandanalytik von Pflanzenschutzmitteln*, VCH, Weinheim, 11th ed., 1991, pp. 389-1–389-7.