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CAPILLARY GAS CHROMATOGRAPHY OF ETHYLENETHIOUREA, A DEGRADATION PRODUCT OF ETHYLENEBISDITHIOCARBAMATES

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SUMMARY

Optimization of the determination of ethylenethiourea (ETU) using capillary columns was performed. The direct analysis of ETU in practical samples at residue levels was found to be difficult. Analysis of ETU via derivatization as S-butyl-ETU, S-benzyl-ETU and trifluoracetylated ETU was successful. The overall recoveries at different concentration levels and the detection limits in grape, wine and wheat were determined.

INTRODUCTION

Ethylenethiourea (ETU) is a toxicologically significant decomposition product formed during the chemical or biological degradation of ethylenebisdithiocarbamates (EBDCs). The EBDCs constitute an important group of agricultural fungicides that are used on seeds and crops throughout the growing season. The group includes nabam, maneb, mancozeb, metiram and zineb. Fishbein[1] has published a review of the toxicology of ETU, which may produce goiterogenic, oncogenic and teratogenic effects after being applied to laboratory animals.

ETU residues can be determined by polarographic[2] or radioisotope methods[3], but chromatographic methods predominate, mainly gas–liquid chromatography (GLC), possibly because of its selectivity and sensitivity. A review of these methods was published by Newsome[4]. GLC with packed columns require derivatization of ETU prior to analysis, although one method has been described that permits the determination of ETU directly[5]. GLC is reliable in the microgram range; column chromatographic pre-cleaning of the ETU concentrate is needed.

The aim of our work was the optimization of the determination of ETU by capillary GLC (direct analysis of ETU and the ETU derivatives 2-butylthio-2-imidazoline, 2-benzylthio-2-imidazoline and 2-benzylthio-1-trifluoroacetyl-2-imidazoline) without any pre-cleaning step.

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EXPERIMENTAL

Chemicals

The solvents used were of analytical-reagent grade and were distilled prior to use.

ETU was analytical-reagent grade material obtained from the Research Institute of Agrochemical Technology (Bratislava, Czechoslovakia). Standard solutions were prepared in methanol.

2-Butylthio-2-imidazoline (S-butyl-ETU) reference standard material was synthesized according to Onley[5] and 2-benzylthio-2-imidazoline (S-benzyl-ETU) according to Newsome[6]. The standards were checked by melting point, elemental analysis and IR spectroscopy. Standard solutions were prepared by dissolving 0.5–10 mg of the solid in 10 ml of solvent [S-butyl-ETU in toluene, S-benzyl-ETU in chloroform for flame-ionization detection (FID) and acetone with thermionic nitrogen–phosphorus specific detection (NPSD)]; solutions of lower concentration were prepared by dilution.

2-Benzylthio-1-trifluoro-2-imidazoline (trifluoroacetylated S-benzyl-ETU) was prepared directly as a standard solution according to Newsome[6].

Samples

Samples of 100 g of wheat, grapes and wine were taken for analysis. The wheat and grapes had been treated several times with Dithane M-45 during the agricultural season. The wine had been produced from the treated grapes.

The general methods[5,6] for the extraction and derivatization of ETU in crop materials were used with small modifications (salting-out procedures). No precleaning steps were used.

The determination of the recovery was performed using control samples with different levels of ETU.

Apparatus

A Carlo Erba Model 2350 gas chromatograph, equipped for FID, NPSD with a potassium chloride pellet in the nitrogen mode with the introduction of make-up gas, electron-capture detection (ECD) with make-up gas (^{63}Ni-type ECD) and micro-ECD, was used. A stream splitter was employed with glass capillary columns. In all measurements, nitrogen was used as the carrier gas. The chromatograms were obtained isothermally at 180 and 190°C, depending on the type of analysis.

All glass capillary columns were prepared from soft soda-lime glass, the surface being roughened with gaseous hydrogen chloride[7] or hydrochloric acid[8]. The surface was further modified with different modes of deactivation (high-temperature silylation[9], polysiloxane degradation by "baking"[8], immobilization of siloxane phases by irradiation[10], Carbowax 20M[11]). Columns were coated dynamically or statically with OV-101, SE-54, OV-17, Carbowax 20M and OV-275 stationary phases. Columns were tested with standard substances; those on which symmetrical peaks of at least one derivative were obtained are given in Table I.

TABLE I

COLUMNS USED

Column No.	*Length [m]*	*Inner diameter (mm)*	*Deactivation*	*Stationary phase*	*Concentration (%)*	*Coating method*
1	18.0	0.25	Carbowax 20M	OV-101	0.6	Static
2	25.0	0.25	–	OV-275	0.1	Static
3	49.0	0.30	High-temp. silylation	SE-54	0.2	Static
4	5.6	0.26	Carbowax 20M	OV-17	10.0	Dynamic
5	15.8	0.26	Carbowax 20M	Carbowax 20M	5.0	Dynamic

RESULTS AND DISCUSSION

The direct analysis of highly polar ETU at trace concentrations was found to be difficult owing to the adsorption of ETU, in spite of the use of capillaries with different modes of surface treatment, producing unsymmetrical peaks (Fig. 1). Symmetrical peaks were obtained only by using columns deactivated and coated with Carbowax 20M (Fig. 1). Only a thin film of stationary phase could be used, otherwise the time of analysis would be very long.

Analyses of actual samples (wheat, wine) at the studied residue concentrations of ETU (1.0–0.01 ppm) was not possible owing to problems in isolating ETU. In the final extract residue, in spite of using salting-out procedure, there were too many compounds to dissolve the residue in a small volume of methanol or ethyl acetate. Hirvi *et al.*[12] described the direct analysis of ETU with fortified samples at the following ETU concentrations: urine, 100 mg/kg (100 ppm); plums, tomatoes and apples, 10 mg/kg (10 ppm). As the range of allowable ETU residue concentrations proposed by the FAO is 0.1–0.01 ppm, these analysis have no practical value. In some crop samples it would be possible to consider a pre-cleaning step to remove undesirable components, although in many instances an easier method would be derivatization, which on the other hand may increase the errors connected with the decomposition of EBDCs to ETU during the preparation of the derivative[13].

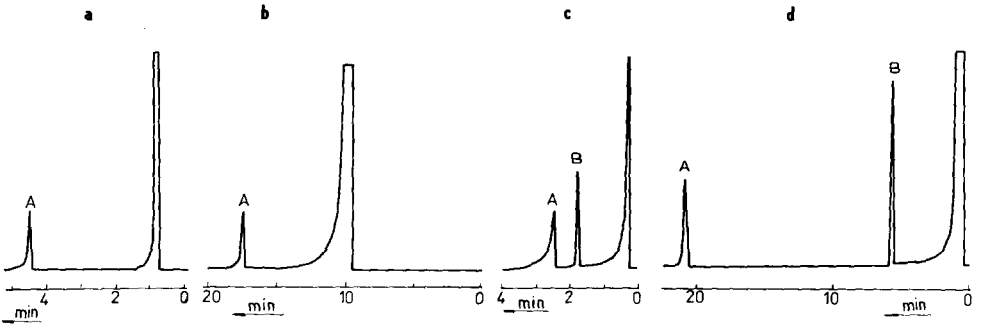


Fig. 1. Chromatograms of (A) ETU and (B) S-benzyl-ETU. (a) Column 1, 190°C, nitrogen pressure 1.0 atm; (b) column 3, 190°C, 0.36 atm; (c) column 4, 190°C, 0.1 atm; (d) column 5, 190°C, 1.0 atm.

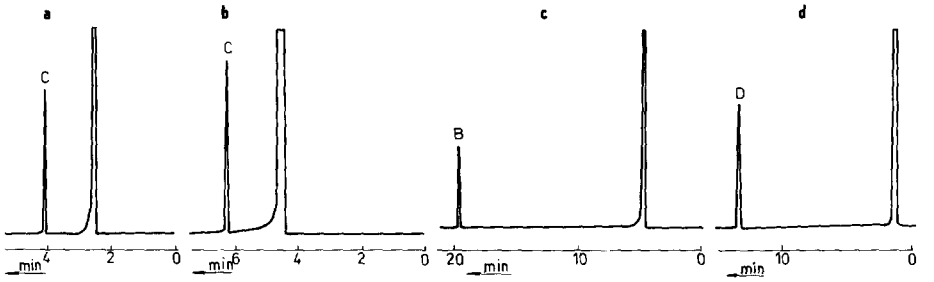


Fig. 2. Chromatograms of ETU derivatives: (B), S-benzyl-ETU, (C) S-butyl-ETU and (D) trifluoroacetylated S-benzyl-ETU. (a) Column 1, 180°C, nitrogen pressure 0.5 atm; (b) column 2, 180°C, 0.5 atm; (c) column 2, 190°C, 0.5 atm; (d) column 1, 190°C, 0.5 atm.

The analysis of ETU derivatives standards was satisfactory on several columns (Figs. 1 and 2).

The overall ETU recoveries with actual check samples at containing different levels of additions are given in Table II. The recoveries, with the exception of S-butyl-ETU, were found to be good. The low recovery of S-butyl-ETU confirms that not all derivatization procedures are suitable for the analysis of one sample (wine). The reasons for the low S-butyl-ETU recovery were not investigated. It could be caused by incomplete derivatization (matrix of sample), low stability of the derivative in wine or incomplete extraction.

The determination of ETU as derivatives was performed by the calibration and standard additions methods. For peak-area measurements, the height multiplied by the width at half-height (measured with a calibrated magnifying glass with a read-out precision of ±0.05 mm) was used. The sample (0.5–2.0 μl) was injected with a 10-μl Hamilton syringe by the washed-out plug of solvent technique. In quantitative analyses we studied the linear response range of the detectors used, the limit of detection and the reproducibility of the measurements.

The detection limits of ETU and the derivatives studied using various detectors are given in Table III.

The results of the analyses of practical samples of ETU derivative extracts are given in Figs. 3–6. As in wheat and wine producted from treated grapes at our limit

TABLE II

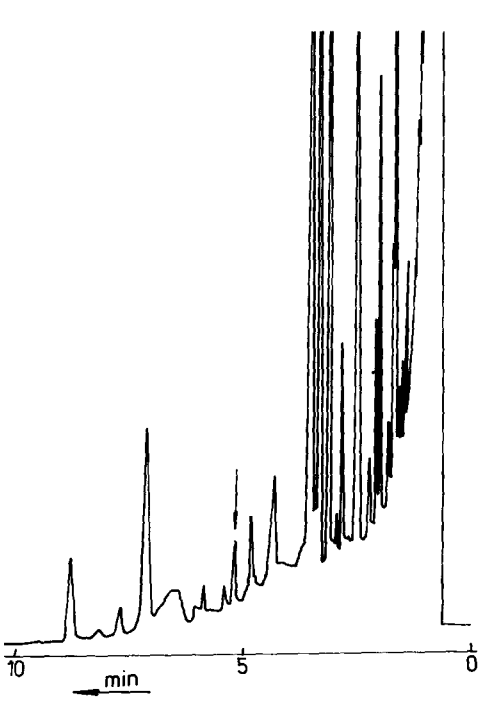
OVERALL ETU RECOVERIES FOR PRACTICAL SAMPLES WITH VARIOUS RANGES OF ETU CONCENTRATION

Sample	*ETU added (mg/kg)*	*Compound determined*	*Recovery (%)*
Grapes	0.018–0.734	S-Benzyl-ETU	90.7 ± 2.7
Wine	0.056–0.902	S-Butyl-ETU	33.0 ± 3.8
	0.045–0.902	S-Beznyl-ETU	91.4 ± 3.3
	0.003–0.100	Trifluoroacetylated S-benzyl-ETU	92.4 ± 5.4
Wheat	0.004–0.226	S-Benzyl-ETU	91.6 ± 3.2

TABLE III

LIMITS OF DETECTION OF ETU DETERMINED BY ANALYSIS OF ETU AND ETU DERIVATIVES (3 × NOISE LEVEL) WITH A SPLITTING RATIO OF 1:15

Compound analysed	*Detection*	*ETU detection limit*	
		ng	*mg/kg*
ETU	FID	3.210	–
S-Butyl-ETU	FID	0.839	$0.126 \cdot 10^{-2}$
S-Benzyl-ETU	FID	0.181	$0.201 \cdot 10^{-3}$
S-Benzyl-ETU	NPSD	0.341	$0.378 \cdot 10^{-3}$
Trifluoroacetylated S-Benzyl-ETU	ECD	0.195	$0.216 \cdot 10^{-3}$
Trifluoroacetylated S-benzyl-ETU	Micro-ECD	0.050	$0.550 \cdot 10^{-4}$



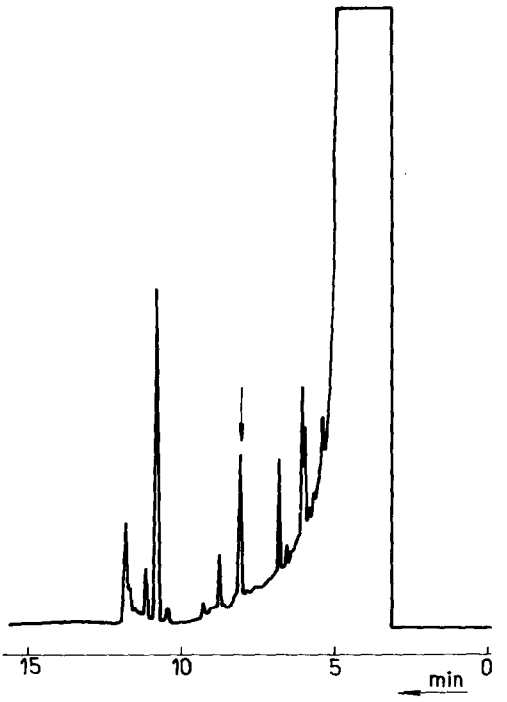


Fig. 3. Chromatogram of an extract of a treated grape sample (derivatization on S-benzyl-ETU) on column 1 at 190°C and a nitrogen pressure of 1.0 atm; 100 g of grapes were taken for extraction and derivatization; 1 μl was injected from the final volume of 0.5 ml of chloroform; splitting ratio, 1:80. The amount of ETU determined using FID was 0.056 mg/kg.

Fig. 4. Chromatogram of an extract of wine with added ETU (0.112 mg/kg) after derivatization on S-butyl-ETU on column 1 at 180°C and a nitrogen pressure of 0.4 atm; 100 ml of wine were taken for extraction and derivatization; 2.0 μl were injected from the final volume of 0.3 ml of toluene; splitting ratio, 1:80; FID.

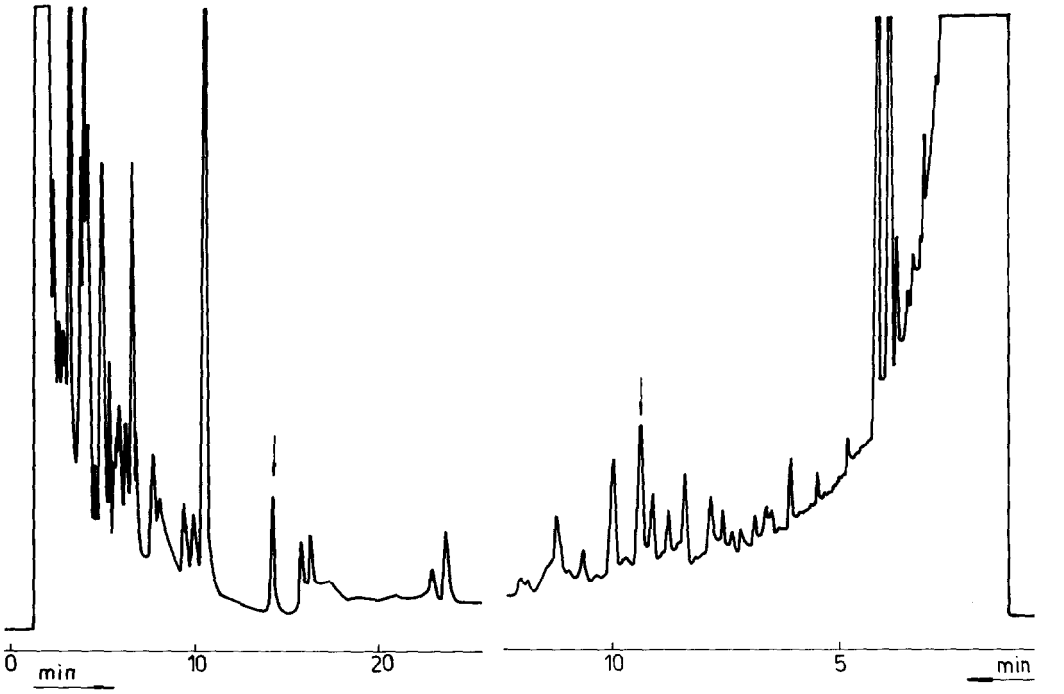


Fig. 5. Chromatogram of an extract of wine with added ETU (0.02 mg/kg) after derivatization on trifluoroacetylated S-benzyl-ETU on column 1 at 190°C and a nitrogen pressure of 0.43 atm; 100 ml of sample were taken for extraction and derivatization; 1 μl was injected; the final residue was dissolved in 0.45 ml of benzene; splitting ratio, 1:15; ECD.

Fig. 6. Chromatogram of an extract of a wheat sample with added ETU (0.0092 mg/kg) after derivatization on S-benzyl-ETU on column 1 at 190°C at a nitrogen pressure of 0.45 atm; 100 g of wheat were taken for extraction and further derivatization; 1.4 μl were injected from the final volume of 0.2 ml of chloroform; splitting ratio, 1:15; FID.

of detection no ETU was found, chromatograms obtained with fortified ETU samples are given.

In the quantitative analysis of practical samples, where there were many other compounds present in addition to the substances of interest, the peak position was always checked by the standard additions method.

From the results obtained it can be concluded that the proposed method is suitable for the determination of trace amounts of ETU in practical samples. It is not necessary to use any pre-cleaning step, which is a great advantage of this method over the use of packed columns, as a result of the high separation efficiency of capillary columns. The limits of detection of all the derivatives are at least 10 (S-butyl-ETU) to 100 (trifluoroacetylated S-benzyl-ETU) times lower than the allowable level of ETU residues in food.

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