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Note

Determination of ethylenethiourea in beverages without sample pretreatment using high-performance liquid chromatography and amperometric detection on a copper electrode

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Ethylenethiourea (ETU) is important as a degradation product of the widely employed ethylenebis(dithiocarbamate) fungicides. Whereas the fungicides themselves are not highly toxic, ETU poses a substantially higher risk to human health and has been found to be mutagenic and teratogenic in rats[1–8]. The formation of ETU in foodstuffs is greatly enhanced by their thermal treatment[9,10]. Moreover, ETU is also produced in humans through metabolism of the fungicides inhaled or absorbed through the skin[11]. Therefore, there is considerable interest in the routine determination of low concentrations of ETU in a wide variety of samples that are mostly characterized by highly complex matrices.

Paper[12] and thin-layer[13–16] chromatographic methods are simple but necessarily insufficiently sensitive and only semiquantitative. Gas chromatography[6,9,10,15,17–20] is sufficiently sensitive and selective, but requires derivatization and positive errors are often caused by thermal degradation of the parent fungicides and some matrix components during analysis[21]. High-performance liquid chromatography (HPLC) is considered to be the most suitable method for these analyses[22].

When UV photometric detection is used in HPLC, at 240 nm[10,22,23], 233 nm[24], 254 or 264 nm[25,26], complicated sample pretreatment involving, *e.g.*, extraction and adsorption chromatography, is usually required for suppression of interferences and analyte preconcentration. The sample pretreatment is simplified and the limit of detection lowered when ETU is detected amperometrically, employing its oxidation at a glassy carbon electrode[11,25]. A further improvement in the selectivity is achieved when ETU is detected at a dropping mercury electrode using the complex formation between mercury(II) ions and ETU[25,26], but the limit of detection is higher than in detection on a glassy carbon electrode[25].

Amperometric detection on a passivated copper electrode combines the advantages of the high sensitivity of solid electrode measurement and the selectivity of the complexation reaction between the analyte and copper(II) ions[27,28]. Therefore, we utilized this approach for the HPLC determination of ETU in some beverages without any sample pretreatment.

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EXPERIMENTAL

Apparatus

A HPP 5001 high-pressure pump, an ADLC 1 electrochemical detector (Laboratorní Přístroje, Czechoslovakia) with a tubular copper working electrode[29], a silver–silver chloride reference electrode and a stainless-steel counter electrode and a Rheodyne 7125 injector with 20- and 200-μl loops were used. The copper electrode was activated in the mobile phase at -0.3 V for 15 min and then maintained at a working potential of $+0.15$ V (*vs.* Ag/AgCl).

Two column types were used: (a) a glass column (150 × 4 mm I.D.) packed with Separon SIG C_{18} reversed phase (7 μm) and (b) a stainless-steel column (80 × 8 mm I.D.) with Separon HEMA Bio-1000 DEAE weakly basic anion exchanger (7 μm) (both from Tessek, Czechoslovakia).

Chemicals

All chemicals used were of analytical-reagent grade, obtained from Lachema (Czechoslovakia), except for ethylenethiourea (ETU), which was obtained from Fluka (Switzerland).

The mobile phases were (A) 0.025 *M* aqueous phosphate buffer (pH 7.0), (B) 0.02 *M* aqueous acetate buffer (pH 7.3), (C) 0.04 *M* aqueous acetate buffer (pH 7.3) and (D) 0.02 *M* aqueous acetate buffer (pH 7.3)–methanol (85:15).

The flow-rate was 0.3 ml min^{-1}. The mobile phases were degassed in an ultrasonic bath and by passage of helium.

Stock solutions of ETU were prepared by dissolving an appropriate amount of ETU in a given mobile phase or in the sample matrix and were stored in a refrigerator. The stock solutions were diluted before measurement.

The test samples included Müller Thurgau white wine from Mikulov, Czechoslovakia, Pilsner Urquell lager beer and Florida peach and orange juice. The juice was filtered before injection. All the experiments were carried out at laboratory temperature (20 ± 2°C).

RESULTS AND DISCUSSION

In optimizing the experimental conditions for the separation of ETU from the other components of wine, beer and juice samples, the specific properties of amperometric detection on a copper electrode[27–29] must be considered. As the detection mechanism involves complex formation between the analyte and the copper (II) ions contained in the porous passive layer on the electrode, the pH of the mobile phase should not be lower than *ca.* 6; further, the composition of the buffer solution is important (phosphate or acetate buffers are preferable) and the content of any organic modifier should be low in order to attain a high detection sensitivity.

First, we tested the determination in a reversed-phase system that has been commonly employed for the purpose, using a C_{18} stationary phase. However, with the mobile phases A–D, which meet the requirements of amperometric detection on a copper electrode, the retention times of ETU are too short (2–3 min) and the separation from the matrix components is poor.

We therefore investigated the weakly basic anion exchanger Separon HEMA

Bio-1000 DEAE, based on a hydroxymethylmethacrylate matrix. The retention times of ETU were then around 14 min for mobile phases A–C. The detection was about twice as sensitive in the phosphate buffer than in the acetate buffer, but the determination of ETU in phosphate buffer was disturbed by substances present in the matrix that elute simultaneously with ETU. In subsequent work we therefore used the acetate buffer and studied the effect of an increased salt concentration (mobile phases B and C) and the presence of methanol (mobile phases B and D). An increase in the acetate concentration from 0.02 to 0.04 *M* caused no change in the retention time of ETU; addition of methanol led to a decrease in the retention time of ETU (7 min), but the separation efficiency simultaneously decreased. In agreement with our previous results[28], the detector response decreased with increasing flow-rate of the mobile phase (by *ca.* 20% for an increase from 0.3 to 0.5 ml min^{-1}). All the subsequent measurements were therefore carried out in the purely aqueous 0.02 *M* acetate mobile phase of pH 7.3 at a flow-rate of 0.3 ml min^{-1}. Under these conditions, ETU can be determined directly in wine, beer and soft drinks.

The UV absorption maximum of ETU lies at 224 nm. Most substances present in the test samples also absorb radiation around this wavelength, and hence the determination of ETU is impossible without prepurification of the sample. On the other hand, amperometric detection with a copper electrode is highly selective and permits the direct injection of the samples into the chromatographic system.

Determinations of ETU in wine and beer are illustrated in Fig. 1. A further advantage of this detection method is that the parent diethylendithiocarbamate fungicides (*e.g.*, Mancozeb) do not give detector response.

The parameters of the calibration dependence for ETU were obtained. The detection is very sensitive and the limit of detection, defined as the signal equal to twice the peak-to-peak noise value, is 0.4 ng of ETU in the injected volume of 20 μl. The linear dynamic range extends over three orders of magnitude of ETU concentration, from 0.4 to 1500 ng, with a correlation coefficient of 0.9996. The above limit of

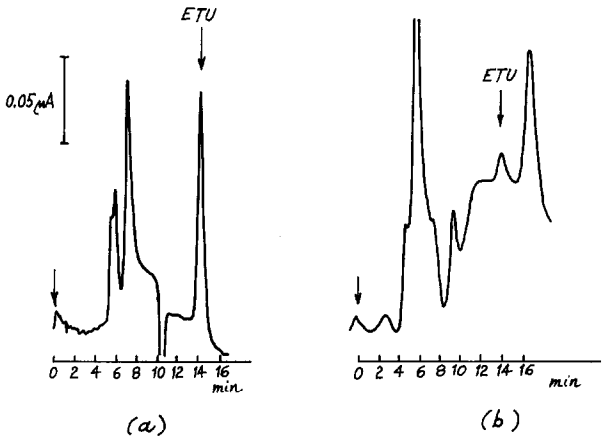

Fig. 1. Chromatograms of (a) wine and (b) beer spiked with ETU. Amount of ETU added: (a) 0.85 μg/ml; (b) 0.10 μg/ml. The Separon HEMA Bio-1000 DEAE column was used with mobile phase B at a flow-rate of 0.3 ml/min.

TABLE I

RESULTS OF THE DETERMINATION OF THE ETU IN WINE AND BEER

Results are means of seven replicate determinations.

Matrix	*ETU concentration*		*Relative standard deviation*
	Added (μg/ml)	*Found (μg/ml)*	*(%)*
Wine	0.10	0.09 ± 0.005	5.5
	0.70	0.69 ± 0.02	2.9
Beer	0.10	0.095 ± 0.005	5.2
	0.70	0.695 ± 0.002	2.9

detection corresponds to 20 ng/ml of the sample, and can be further decreased by injecting larger volumes. With injection of a 200-μl volume, the limit of detection decreases to *ca.* 3 ng/ml (of course, the injection of a ten times greater sample volume leads to a greater dispersion, so that the plate number of 47 800 per metre for a 20-μl sample decreases to 8640 per metre for a 200-μl sample).

The test samples of beer, wine and juice did not contain measurable amount of ETU, so we added measured amounts of ETU to them and determined the precision of the determination from repeated analyses of the spiked samples. The results are given in Table I. It can be seen that the method is applicable to the direct determination of low ETU concentrations in wine, beer and soft drinks without sample pretreatment.

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