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Note

Analysis of ethylenethiourea in beer by high-performance liquid chromatography

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Ethylenebis(dithiocarbamates) including Maneb, Zineb and Mancozeb form an important class of fungicides for controlling crop diseases including those of hops. Ethylenethiourea (ETU) can be produced as a degradation product of these fungicides and in view of its toxicity there is a need for analytical methods to check residues which may arise in treated crops. Current methods include thin-layer chromatography (TLC)[1] and gas–liquid chromatography (GLC)[1,2,3] but these require derivatization and also extensive clean-up procedures particularly for samples of plant origin. High-performance liquid chromatography (HPLC) has considerable potential in trace organic analysis of biological samples and has very recently been used in the ETU analysis of rat plasma[4] and urine[5]. This note illustrates the use of HPLC in the analysis of ETU in beer and also the application of column-switching to resolve the analyte from coeluting matrix components.

EXPERIMENTAL

High-performance liquid chromatography

Two independent isocratic HPLC systems linked by a 4-way switching valve were used for analysis. System 1 comprised a Waters 6000A solvent delivery pump linked in series to a Rheodyne loop injector (20-μl loop), two Spherisorb CN 5 μm columns (25 cm × 5 mm I.D.), a Rheodyne 4-way switching valve and a waste reservoir. System 2 comprised a second Waters 6000A pump linked in series to the 4-way switching valve, a Spherisorb NH_2 5-μm column (25 cm × 5 mm I.D.) and a Pye LC3 UV detector operating at 240 nm. A mobile phase of hexane–ethanol (2:1), flow-rate 1.0 ml/min, was used for both systems.

The beer extract (20 μl) was injected onto the first Spherisorb CN column of system 1 and the eluent passed to waste via the switching valve. After 15.0 min the eluent from system 1 was switched to the Spherisorb NH_2 column of system 2 for 1 min and then switched back to waste again. Under these conditions ETU eluted from the Spherisorb NH_2 column 25.3 min after the initial injection.

Beer extraction procedure

Ethanol (1 ml) was added to 5.0 g beer and the sample quantitatively transfer-

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red to the top of a sintered glass column (25 × 2.5 cm I.D.) containing 50 g anhydrous sodium sulphate. After absorption of the sample (*ca.* 1 min) the column was eluted with 3 × 20 ml dichloromethane–methanol (99:1) and the extract concentrated to 2 ml on a water bath at 60°C using a Kuderna-Danish concentrator. The concentrate was diluted with 8 ml dichloromethane and passed through a silica gel Sep-Pak and the eluent discarded. ETU was eluted from the Sep-Pak with 10 ml dichloromethane–methanol (98:2) and the eluent concentrated to 300 μl at 60°C.

RESULTS AND DISCUSSION

A typical HPLC chromatogram of a beer extract using two 5-μm bonded-cyano columns linked in series to a UV detector operating at 240 nm is shown in Fig. 1. Under these conditions a peak corresponding to 200 μg/kg ETU eluted after 15.6 min. However, reanalysis of the sample at 250 nm showed the absorbance ratio of this peak (250 nm:240 nm = 1:0.5) to be quite dissimilar to that of standard ETU (250 nm:240 nm = 1:1.3) indicating the presence of coeluting beer components. When the fraction containing this peak was switched to a bonded-amino column (as described in the Experimental section) no peaks eluting with the same overall retention time as ETU (25.3 min) were observed subject to a detection limit of 10 μg/kg. The column switching HPLC chromatogram of a different beer sample at 240 nm is shown in Fig. 2. The three major components were well resolved by the bonded-amino column with

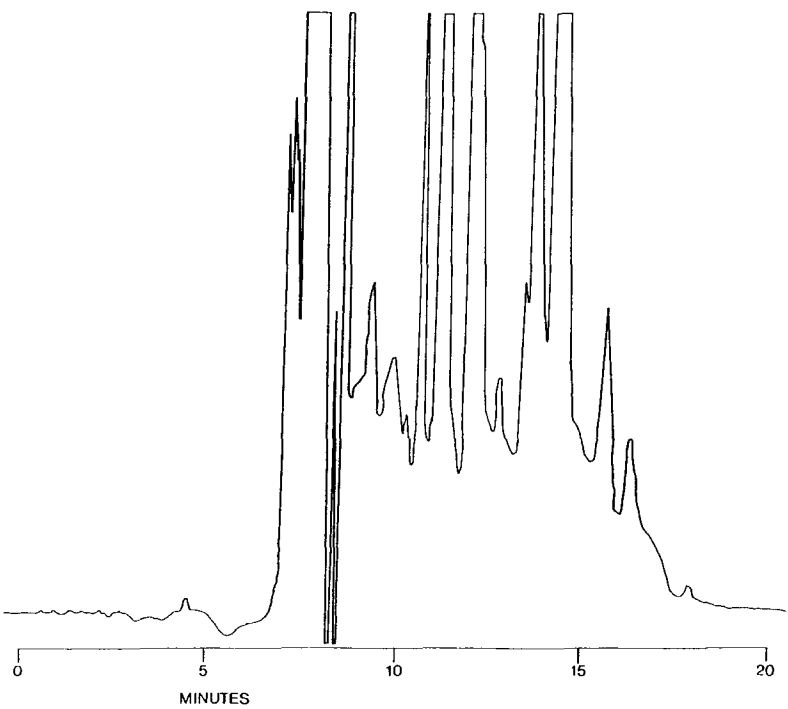

Fig. 1. Chromatogram of one-dimensional HPLC–UV analysis of typical beer extract; two 5-μm CN columns in series; detection at 240 nm, 0.08 a.u.f.s. (retention time of standard ETU 15.6 min).

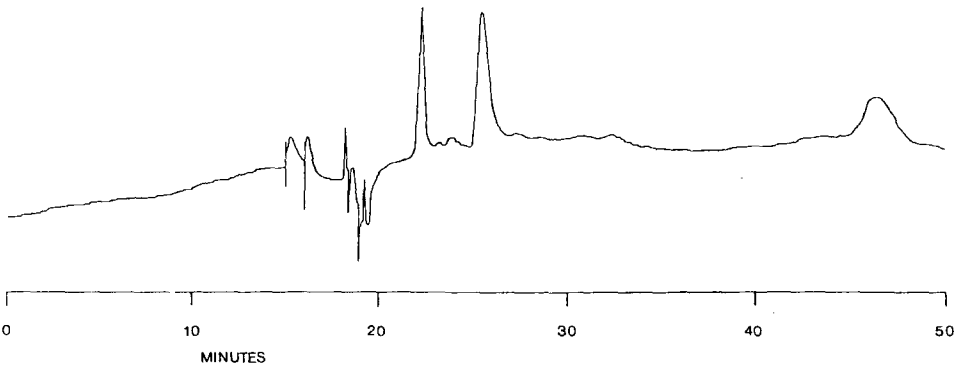

Fig. 2. Chromatogram of column switching HPLC–UV analysis of beer extract; two 5-μm CN columns and one 5-μm NH_2 column; detection at 240 nm, 0.01 a.u.f.s. (retention time of standard ETU 25.3 min).

the peak at 25.3 min corresponding to 370 μg/kg ETU. Reanalysis of the sample at 250 nm showed this peak to have the same absorbance ratio as standard ETU.

Using the extraction procedure described in the experimental section the recovery of ETU added to beer at 20 μg/kg and 600 μg/kg averaged 62.4% (standard deviation 4.9, 5 determinations) and 75% (standard deviation 1.9, 5 determinations) respectively with a detection limit of 10 μg/kg.

Conventional one-dimensional HPLC has been found to give spuriously high results in the analysis of ETU in beer due to the presence of coeluting matrix components. However, the more powerful resolving ability of column switching HPLC using polar-bonded columns of differing selectivities has proved highly effective in separating ETU from these coeluting materials. Additionally the procedure of monitoring the eluent at two different wavelengths has been found to be a valuable confirmatory technique.

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