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Note

High-performance liquid chromatographic determination of ethylenethiourea in Perozin and Dithane M-45

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Ethylenethiourea (ETU) is a degradation product of a widely used group of fungicides, the ethylenebisdithiocarbamates (EBDC). It has been detected in formulated materials[1–3] and as residues in certain food crops[4–7]. Toxicological investigations have demonstrated that at high concentrations ETU is goitrogenic[8,9] and teratogenic[10]. Because of the potential health hazard, sensitive and reliable methods are required for determining the residue on or in foods.

This study investigates the influence the extraction solvent on the determined amount of ETU and whether during extraction EBDC is converted into ETU. The fungicides examined were Dithane M-45 and Perozin. In Dithane M-45 the active ingredient is the co-ordination product of zinc ion and manganous ethylenebisdithiocarbamates, and in Perozin it is zinc ethylenebisdithiocarbamate.

EXPERIMENTAL

Perozin or Dithane M-45 (0.25 g; 1 year old) was repeatedly extracted for 5-min periods with 5 ml of water or 5 ml of methanol until the ETU concentration in the extract decreased below the limit of detection. The suspension of Perozin or Dithane M-45 was centrifuged and the clear solution was injected into a liquid chromatograph consisting of a high-pressure membrane pump equipped with a flow-through Bourdon tube manometer as damping device, an injection valve, a separation column and a UV detector. The absorbance of the column effluent was measured at 254 nm. The separation was carried out on 5-μm Separon AE and Separon SE columns (25 × 0.8 cm I.D.). The mobile phase was water–methanol (35:65). The amount of ETU was determined by linear least-squares fitting of the curves of the amount of ETU injected against peak area. The concentrations of the standard solutions were 0.05–1.1 mg of ETU in 10 ml of methanol, and the calibration graph was linear.

RESULTS AND DISCUSSION

One problem associated with investigations of ETU residues is whether EBDC is converted into ETU during workup of samples. A variability of results was noted for samples of the mentioned fungicides; this may have been caused by different

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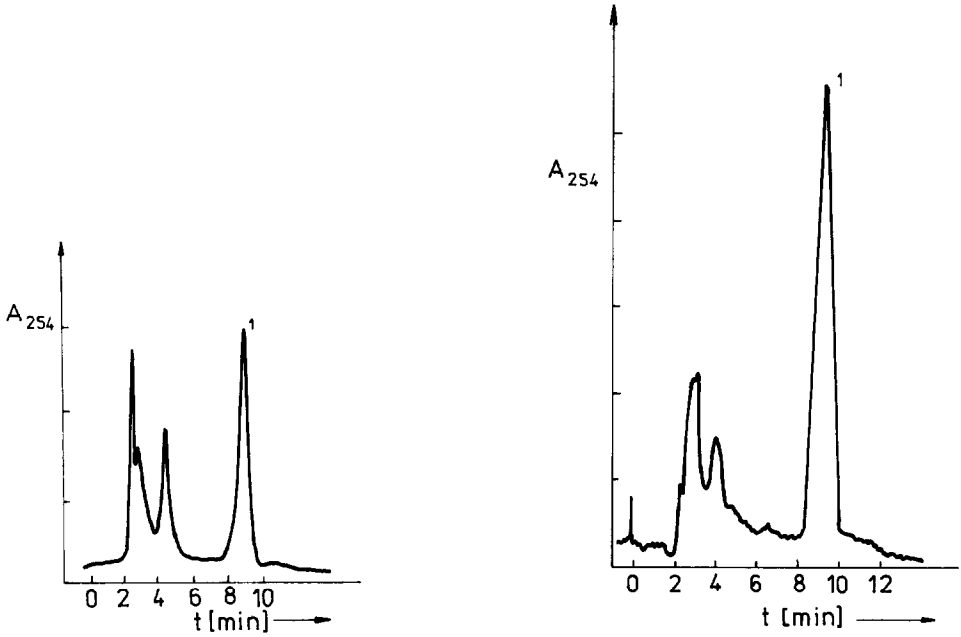

Fig. 1. Chromatogram of methanol extract of Perozin. Conditions: column, Separon AE; mobile phase, water–methanol (35:65); flow-rate, 1.6 ml/min. Peak: 1 = ETU.

Fig. 2. Chromatogram of water extract of Dithane M-45. Conditions: column, Separon AE; mobile phase, water–methanol (35:65); flow-rate, 1.6 ml/min. Peak: 1 = ETU.

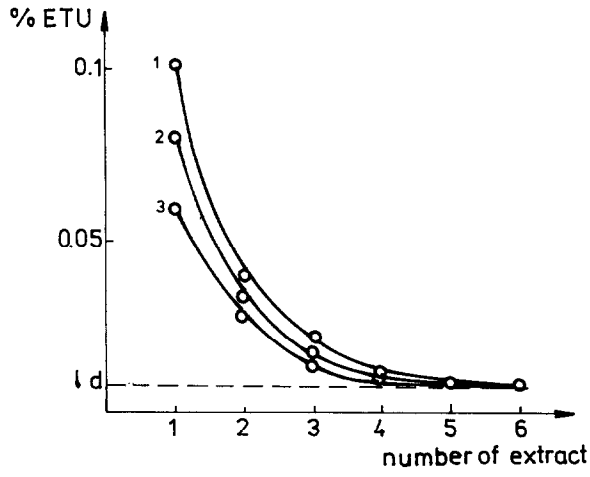

Fig. 3. Dependence of ETU concentration on the number of methanol extractions of Dithane M-45. (1) First extraction of the sample; (2) re-extraction of the same sample after three days; (3) re-extraction of the same sample after one day (l.d., limit of determination).

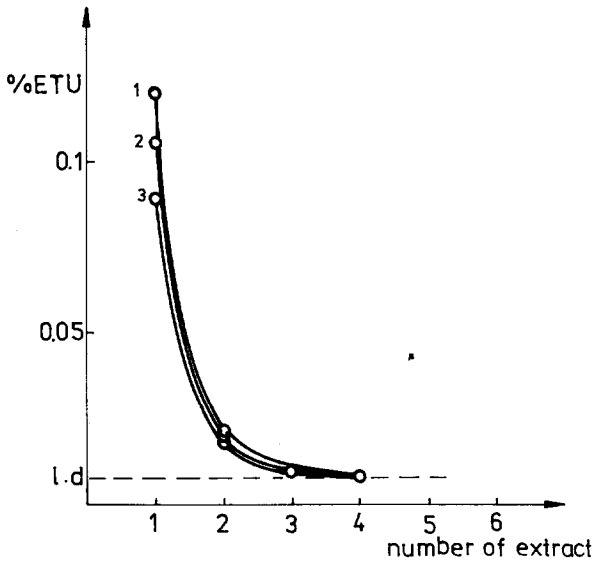

Fig. 4. Dependence of ETU concentration on the number of water extractions of Dithane M-45. (1) First extraction of the sample; (2) re-extraction of the same sample after three days; (3) re-extraction of the same sample after one day (l.d., limit of determination).

conditions of storage or by the use of different analytical techniques. Care must be taken to optimize the high-performance liquid chromatographic (HPLC) conditions for retention time, linearity over the concentration range, and sensitivity. Interferences from substrate background were eliminated by varying the HPLC conditions. (Two columns were used for the separation.)

Figs. 1 and 2 show the separation of some extracts on the Separon AE column.

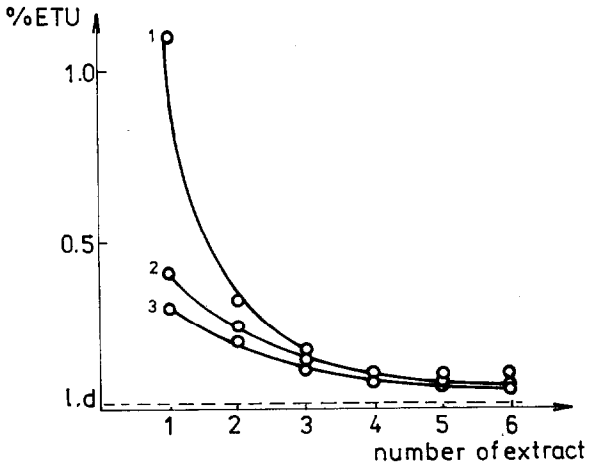

Fig. 5. Dependence of ETU concentration on the number of methanol extractions of Perozin. (1) First extraction of the sample; (2) re-extraction of the same sample after three days; (3) re-extraction of the same sample after one day (l.d., limit of determination).

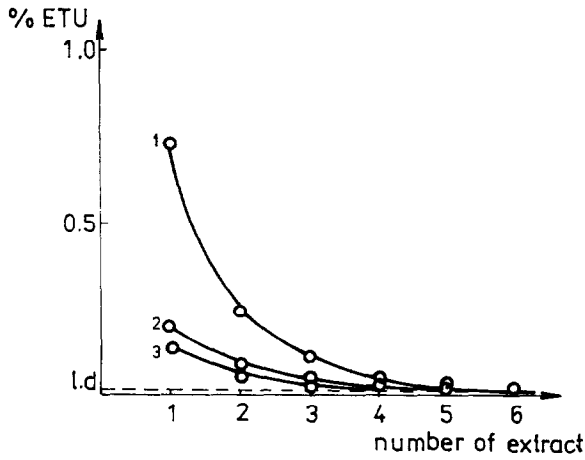

Fig. 6. Dependence of ETU concentration on the number of water extractions of Perozin. (1) First extraction of the sample; (2) re-extraction of the same sample after three days; (3) re-extraction of the same sample after one day (l.d., limit of determination).

A similar separation was achieved on the Separon SE column. Figs. 3–6 illustrate the dependence of the ETU concentration on the number of times that the fungicides were extracted with water or methanol. It can be seen that, after the first day, more ETU has been formed from EBDC. This may be due to the influence of the residue of the extraction solvent. Interesting results were obtained in the case of the extraction of Perozin with methanol. The concentration of ETU in the extraction solvent did not decrease below the limit of determination of the HPLC method, which indicates that further ETU can be formed during the extraction. Similar results were published by Van Damme *et al.*[11]. This is confirmed by comparing the determinations of ETU in methanol and water extracts (Table I). The concentration of ETU in methanol extracts was higher than the concentration in water extracts. So results must be adjusted for the amount of ETU that is formed during workup of samples. This amount can be estimated from the ETU concentration in the later extracts (Figs. 5 and 6).

These results show that formation of ETU from EBDC sometimes depends on

TABLE I

THE DETERMINATION OF ETU IN DITHANE M-45 AND PEROZIN

Fungicide	*Extraction solvent*	*ETU (%)*
Dithane M-45	Methanol	0.17 ± 0.02
	Water	0.13 ± 0.02
Perozin	Methanol	1.30 ± 0.07
	Methanol*	1.60 ± 0.07
	Water	1.21 ± 0.07

* No allowance made for the amount of ETU formed during extraction.

the extraction solvent, so the latter must be chosen very carefully. The limit of detection was 0.8 mg per litre of extract.

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