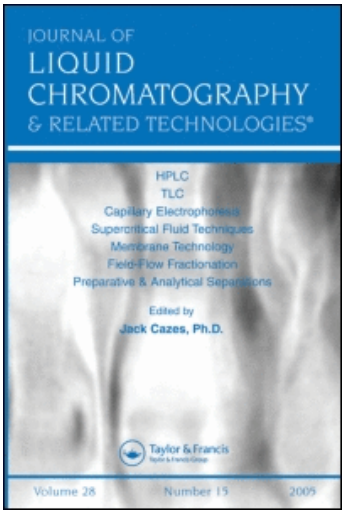

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ETHYLENETHIOUREA IN FOOD

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ABSTRACT

An HPLC procedure was developed for the determination of ethylenethiourea (ETU) in peels of potatoes, tomatoes and apples after application of mancozeb suspension (ethylenebisdithiocarbamate fungicide). The analytical procedure consisted of a single extraction step with methanol and ETU was eluated from SEPARON CN column. Mobile phase was 4 % methanol in chloroform cyclohexane (1:1). The level of ethylenthiourea after application of mancozeb decreased slowly, with a half time six days in each case.

INTRODUCTION

The widespread application of ethylenebisdithiocarbamate fungicides has led to considerable interest in their degradation. The fungicides employed are generally toxic and they can present some hazards to public health. There-

fore, legal requirements of many contries are increasing, making it necessary to determine fungicide residues at very low levels.

The major degradation product is ethylenethiourea (ETU). ETU has been shown to be goiterogenic /1/, carcinogenic /2/ and teratogenic /3/.

The methods of analysis of ETU in foodstuffs and other substrates have been reviewed /4/. The method, which has been adopted as a AOAC official method, is gas-liquid chromatography of the S-butyl derivate of ETU with flame photometric detection /5/. However, the derivatization is time-consuming, and some workers have found that the derivate is unstable /4/. HPLC with photometric /6/ or electrochemical /7/ detection provides an alternative which avoids the necessity for producing a volatile derivate.

The determination and persistance of ETU has been estimated by a number of other workers in cucumbers /8/, soybeans /9/, corn /10/, beans /11/, tomato plants /10-13/, kale /14/, wheat /8/, pears /15/, lettuces /14, 16/.

The present study has used and HPLC method to monitor of ETU levels after application of mancozeb to potatoes, tomatoes and apples and draw up profiles of the changes under controlled conditions in laboratory. Mancozeb is the coordination product of zinc ion and manganous ethylenebisdithiocarbamates.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was a Knauer equipped with a 10 µl loop injector a variable wavelenght UV detector and high pressure pump. The samples were separated using chromatographic column (Separon CN 0.32 x 15 cm, 5 µm

particle size) and were purchased from Tessek Prag. All solventes were twice distilled and were of analytical reagent grade (Lachema Brno, Czechoslovakia).

Chemicals

Standards of ETU was obtained from the Research Institute of Agrochemical Technology (Bratislava, Czechoslovakia). The identity and purity of ETU was confirmed by elemental analysis and mass spectrometry.

Procedures

Aqueous suspension of mancozeb (0.20 g containing 0.18 mg ETU in 20 ml) was sprayed onto individual potatoes, tomatoes and apples using a air-powered spray so that they were fully wetted. The sprayed foodstuff was kept in the laboratory at 20 ^{o}C.

A representative samples after storrage were peeled (the thickness of the peels was approximately 1 mm). The peels were chopped into pieces 1 cm x 1 cm and 30 g sample were weighed into 250 ml glass flask of homogenizer. To the sample, 70 ml methanol was added and the mixture was homogenized for 4 minutes at high speed. The mixture was then filtered under vacuum, using a Buchner funnel. The extract was transfered to a 250 ml round-bottom flask, the organic solvent was removed on a vacuum rotatory evaporator. The residue was dissolved with 1 ml chloroform - methanol (1 : 1) and centrifugated. The clear solution was injected into the chromatographic column.

The determination of ETU has been carried out by UV detector at 240 nm. The quantitative evaluation was made on the basis of the regression analysis where the dependence between the areas of peaks of ETU standard and the quantity was determined. The linearity range was between 0.5 to 10 ppm of ETU.

RESULTS AND DISCUSSION

In the extraction studies of the peels have been problems with losses of ETU during the extraction and the evaporation of methanol. It was shown that ETU in extract material can be unstable /17/. This effect is caused by enzymes released from the plant cells /18/. A widely used method has been the extraction of the macerated peels with methanol. Another solvents such as chloroform give many coextractives /19/ and lower extraction yield /16/.

In order to estimate the recovery of the ETU extraction with methanol the various addition of ETU was added (1 and 10 mg/kg) to the peels which were not sprayed with mancozeb suspension. Immediate extraction of the peels with methanol was used in studies. ETU recovery is shown in Table I. The average recovery trough any amounts (from 1 to 10 mg ETU/1 kg) was always higher than 80 %. The absolute recoveries could not be determined, because there is no method to estimate the in situ level of ETU in the samples.

The stability of ETU in methanol extract was also studied. The level of ETU in extracts of macerated peels dropped by 55 % (apples), 40 % (potatoes) and 45 % (tomatoes) in 24 h. It indicates that samples should be worked-up rapidly after extraction. Similar results were published in literature /16/. No loss of ETU was observed during the evaporation of methanol on a rotatory evaporator as long as the temperature was below 30 ^{o}C.

Care must be taken to optimize HPLC conditions for retention time of ETU sensitivity and selectivity of the separation process. The determination of ETU can be carried out on a CN column with a mobile phase 4 % methanol in chloroform - cyclohexane (1 : 1). In this system the interference of coextractives with ETU peak was not observed. In figures 1 - 3 representative chromatograms of methanol extracts as well as treated and untreated samples analysed by HPLC are shown.

TABLE 1

Recoveries (%) of ETU methanol extraction from samples

Sample	Standard addition of ETU mg/kg	Yield of extraction %
potatoe peels	10	97.1 ± 2.1
	1	84.0 ± 3.8
apple peels	10	95.4 ± 1.9
	1	83.1 ± 4.0
tomatoe peels	10	92.0 ± 2.2
	1	80.6 ± 3.8

Yield of extraction are the mean of three replicate experiments.

In order to estimate the ETU stability on the external surface of plants, four ETU analyses were done : immendiately after spraying and after two, four and seven days of spraying. ETU can be continuosly generated by the degradation of the fungicides. It was proved that the level of ETU in field-grown lettuces sprayed with maneb descreased rapidly over seven days and was mainly attributable to ETU present initially in the fungicide /14/.

Newsome et al. found that the ETU levels following the application of maneb to bean or tomato plants in the field declined over 6-9 days /13/. A study was therefore carried out in which a dose of mancozeb suspension was applied to the surface of apples, potatoes and tomatoes and then monitored at different interval. Fig. 4 illustrates the dependence of ETU level on the time after spraying. The initial levels corresponded to the ETU amounts present in the applied mancozeb.

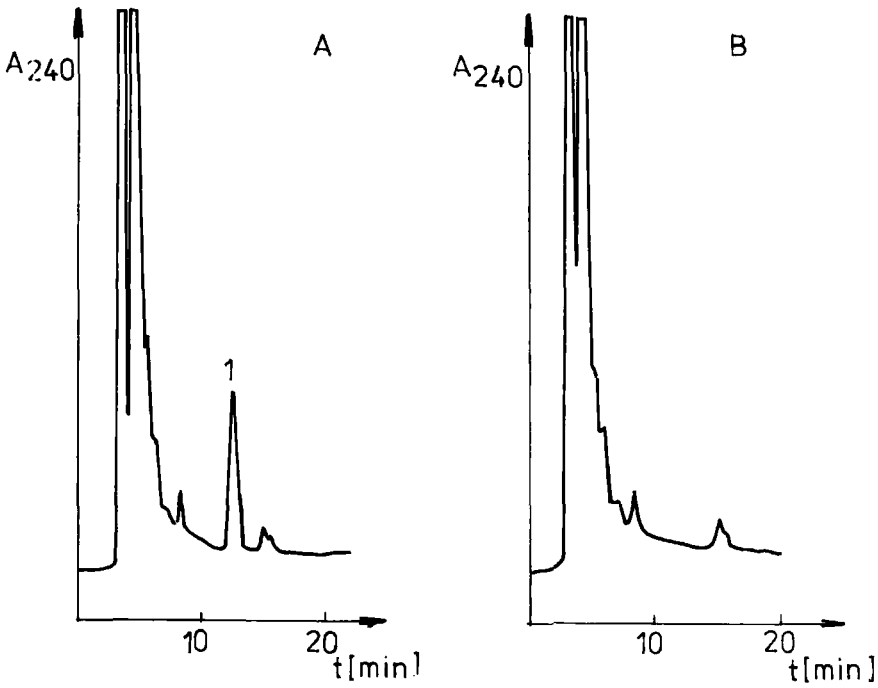

FIGURE 1

Chromatograms of methanol extracts of the potatoe peels

A. treated sample after 2 days

B. untreated sample

Conditions : column Separon CN; mobile phase 4 % methanol in chloroform - cyclohexane (1:1); flow rate 0.5 ml/min; Peak : 1 = ETU

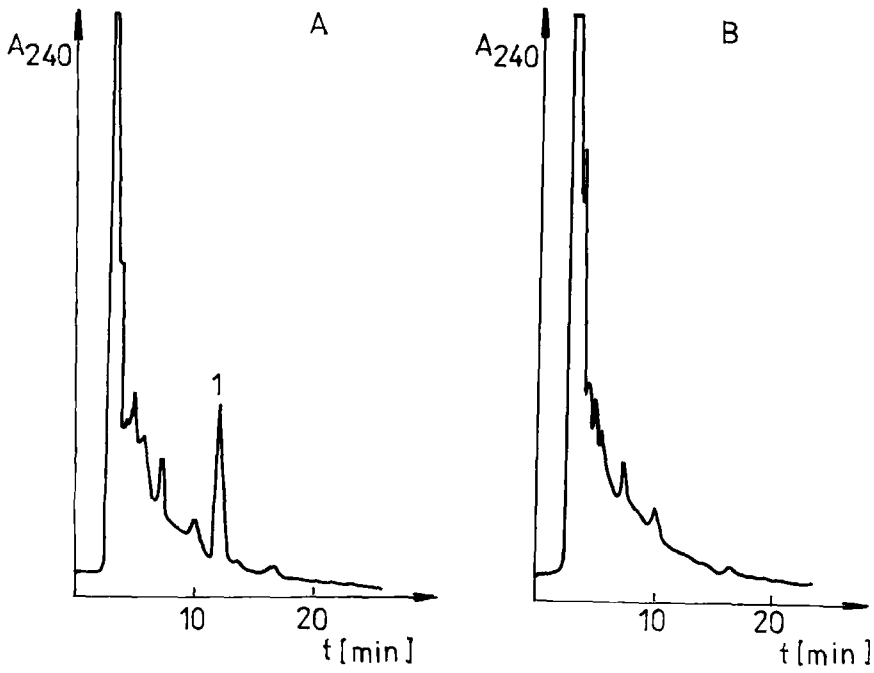

FIGURE 2

Chromatograms of methanol extracts of the apple peels

A. treated sample after 2 days

B. untreated sample

Conditions : column Separon CN; mobile phase 4 % methanol in chloroform - cyclohexane (1:1); flow rate 0.5 ml/min; Peak : 1 = ETU

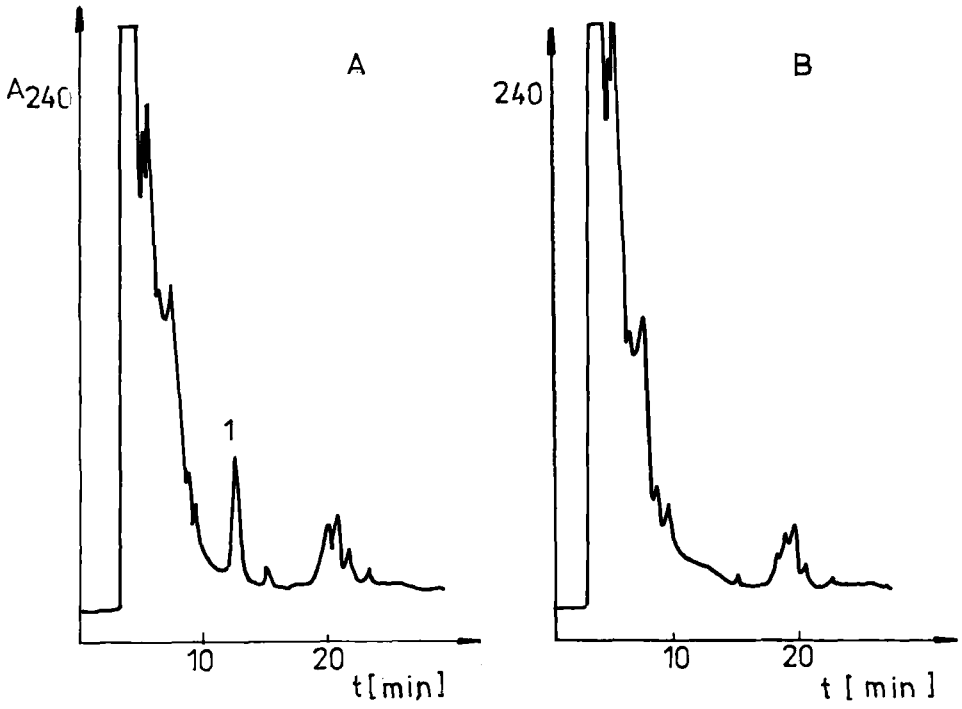

FIGURE 3

Chromatograms of methanol extracts of the tomatoe peels

A. treated sample after 2 days

B. untreated sample

Conditions : column Separon CN; mobile phase 4 % methanol in chloroform - cyclohexane (1:1); flow rate 0.5 ml/min;
Peak : 1 = ETU

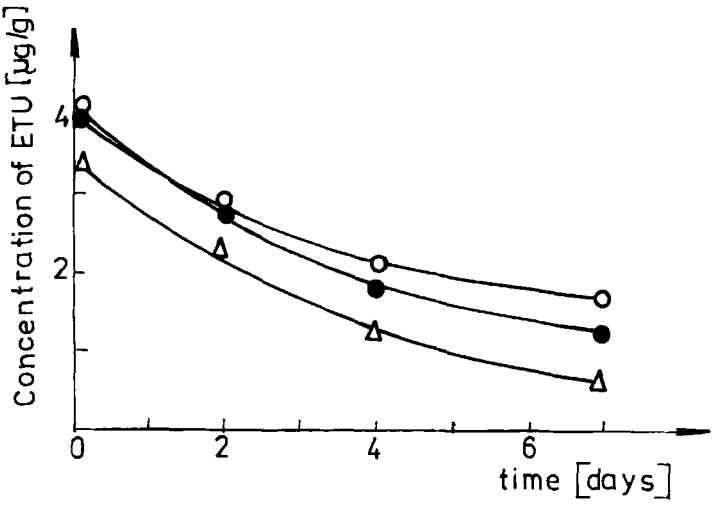

FIGURE 4

The dependence of mean level of ETU in peels on the time after spraying with mancozeb suspension.
Values obtained are the mean of three replicate experiments.

○ potatoe peels
● apple peels
△ tomatoe peels

The level of ETU decreased slowly, with a half-life of about six days in each case. It can be supposed that ETU was degraded faster than the formation of additional ETU from mancozeb. This loss of ETU was similar to the result reported for the application of mancozeb to lettuces /16/.

Results of this study have shown that HPLC can be used for trace analysis of ETU in peels of food after spraying with mancozeb suspension. The detection limit was 0.1 ppm.

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