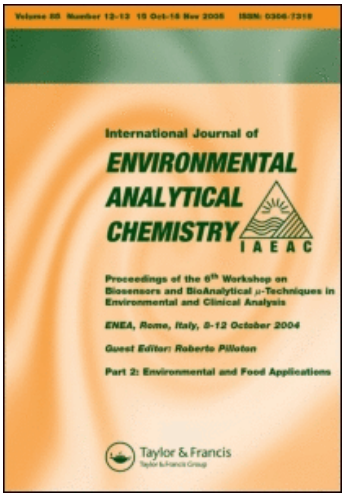

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DETERMINATION OF DITHIOCARBAMATES IN RESIDUES BY LIQUID CHROMATOGRAPHY WITH SELECTIVE PRECOLUMN OR REACTION-DETECTION SYSTEMS

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Two liquid chromatographic methods have been developed for the selective determination of dithiocarbamate fungicides in residues. Thiram was detected at 435 nm as copper(II) dimethyldithiocarbamate after post-column complexation with Cu(II). The reaction takes place in a solid-state reactor which is packed with metallic copper. Due to the high detection wavelength possibly interfering compounds do not disturb the determination of thiram. In combination with on-line trace enrichment on C18-bonded silica detection limits in the low-ppb range can be obtained. Examples for the determination of thiram in soil and on apples and lettuce are shown.

For ethylenebisdithiocarbamates such as maneb or zineb, a selective preconcentration system was developed. Nabam which is formed during extraction with EDTA is preconcentrated as an ion-pair on a C_{18}-precolumn which is loaded on-line with cetrimide. Interfering apolar compounds are removed by adsorption on a C18 clean-up precolumn which is inserted in front of the preoncentration precolumn. With this set-up maneb and zineb could be determined in soil extracts without off-line clean-up steps at concentrations of 100 ppb.

INTRODUCTION

A major proportion of the world fungicide market is represented by dithiocarbamates (DTCs) and their derivatives, the so-called thiuram disulphides.[1] Compounds such as the thiuram disulphide thiram, the ethylenebisdithiocarbamates maneb and zineb, and the dimethyldithiocarbamates ferbam and ziram (Figure 1) are widely used as fungicides. Other uses of DTCs are as accelerators in rubber vulcanization and of disulfiram as a drug against alcohol abuse.

DTCs can decompose in different ways, but the main route in the environment is via hydrolysis.[2] The actual mechanism is strongly dependent on the pH. Study of the behavior of DTCs in environmental systems is difficult because the compounds often are rather unstable and many degradation products can be formed. One of the most important decomposition products is ethylenethiourea because this compound is mutagenic and teratogenic. A complicating factor is the ability of DTCs to form strong complexes with a wide range of metal ions.[3,4] Moreover, residue analysis is complex because selective analytical methods for the

*Deceased.

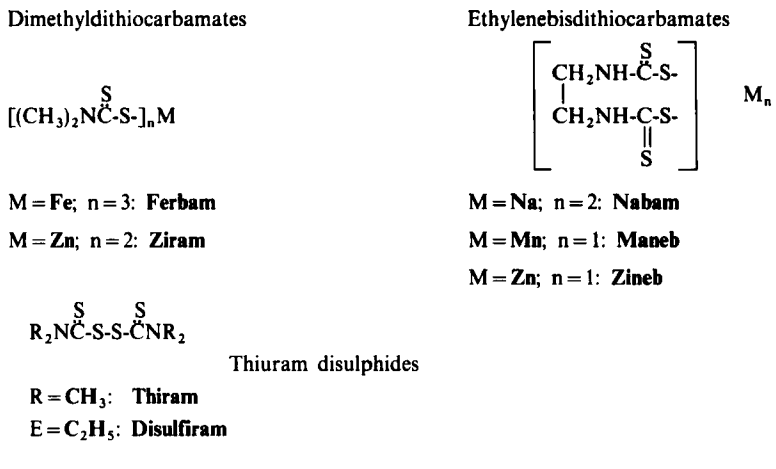

Figure 1 Structures of important dithiocarbamate fungicides.

determination of DTCs and their degradation products are not used in practice. Most routine methods are based on the rapid hydrolysis of DTCs, which produces carbon disulphide. Carbon disulphide can react with copper(II) and diethanolamine to form copper diethanolaminedithiocarbamate which can be determined photometrically at 435 nm (Keppel method).[5] A sensitive determination of carbon disulphide is also possible by means of head-space gas chromatography.[6] The main disadvantage of these methods is that they do not discriminate between different DTCs and many of their degradation products. Only the total amount of all compounds that form carbon disulphide is determined.

In recent years, several liquid chromatographic (LC) methods for the determination of DTCs have been developed.[7–11] However, these methods also are rather non-selective because UV detection is employed. Therefore, an elaborate clean-up procedure is required for the analysis of complex samples. Recently, we reported a selective reaction detection system for thiram.[12] The detection is based on the post-column complexation of thiram in a solid-state reactor packed with finely divided metallic copper to form a coloured complex with an absorption maximum at 435 nm. The LC method was combined with a preconcentration and clean-up step on a C_{18}-bonded silica precolumn for the sub-ppb determination of thiram in surface water. In the present paper, the same method has now been applied to the trace-level determination of thiram in residue samples. Besides, a selective precolumn system has been developed for the LC determination of the ethylenebisdithiocarbamates maneb and zineb which are of great environmental concern.[13]

EXPERIMENTAL

Chemicals

Thiram was purchased from Fluka (Buchs, Switzerland). Technical-grade maneb,

zineb and nabam were obtained as gifts from the Governmental Food Inspection Service (Alkmaar, The Netherlands). Cetrimide (cetyltrimethylammonium bromide), sodium borohydride and EDTA were supplied by Baker (Deventer, The Netherlands). All other chemicals were of analytical-reagent grade (Baker).

LC System

Thiram The LC system (Figure 2a) for the determination of thiram consisted of a Gilson 302 pump (Gilson, Villiers-le-Bel, France) and a 200 × 2.1 mm I.D. stainless-steel column packed with 5 μm Hypersil ODS (Shandon Southern, Cheshire, U.K.). The preconcentration pump was an Altex 110A pump (Altex, Berkeley, CA, U.S.A.) used at a flow-rate of 1.0 ml/min. Preconcentration was carried out using a laboratory-made[14] 4.0 × 2.1 mm I.D. precolumn which was hand-packed with a slurry of 5 μm LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.) in methanol, using a syringe. For the post-column reactor the same type of (pre)columns with a length of 2.0 or 4.0 mm and 2.1 mm I.D. were used. The metallic copper was prepared by the reaction of copper(II) chloride and sodium borohydride.[12] A Perkin–Elmer LC 55 UV/Vis detector (Perkin-Elmer, Norwalk, CT, U.S.A.) was used. Acetonitrile, 10 mM aqueous acetate buffer (pH 5.2) (70:30, v/v), was used as the LC mobile phase at a flow-rate of 0.3 ml/min.

Ethylenebisdithiocarbamates The scheme of the LC system is shown in Figure 2b. The system consisted of a Gilson 302 pump and a 200 × 3.2 mm I.D. stainless-steel column packed with 5 μm LiChrosorb RP-18 (Merck). The preconcentration pump was a Kontron LC 114 pump (Kontron, Zürich, Switzerland) used at a flow-rate of 1.0 ml/min. The clean-up column was a 10 × 2.1 mm I.D. Chrompack (Middelburg, The Netherlands) precolumn which was hand-packed with a slurry of 10 μm styrene-divinylbenzene copolymer PRP-1 (Hamilton, Bonaduz, Switzerland) in methanol. The cetrimide column was a 70 × 4.2 mm I.D. stainless-steel column which was hand-packed with solid cetrimide. Preconcentration was carried out using a Chrompack precolumn of 10 × 4.2 mm I.D. which was hand-packed with a slurry of 5 μm LiChrosorb RP-18 in methanol. A Pye Unicam PU 4025 UV/Vis detector (Pye Unicam, Cambridge, U.K.) was used. Methanol, 10 mM aqueous acetate buffer (pH 6.2) (90:10, v/v) containing 5 mM cetrimide, was used as the LC mobile phase at a flow-rate of 0.7 ml/min.

Pretreatment of Residues

Thiram Fifty grams of the residue sample are mixed with 50 g of anhydrous sodium sulphate and homogenized in a mortar. Fifty grams of this mixture are extracted three times with 60 ml dichloromethane for about 20 min in an ultrasonic bath and filtered over a paper filter. The combined organic phases are concentrated in a rotavapor at about 40 °C to about 10 ml; next 10 ml methanol are added. Subsequently, the mixture is evaporated to 5 ml which are transferred to a calibrated vessel and evaporated under a gentle stream of nitrogen at room temperature to 1.0 ml. Finally, 9.0 ml of an aqueous solution containing 1 mM

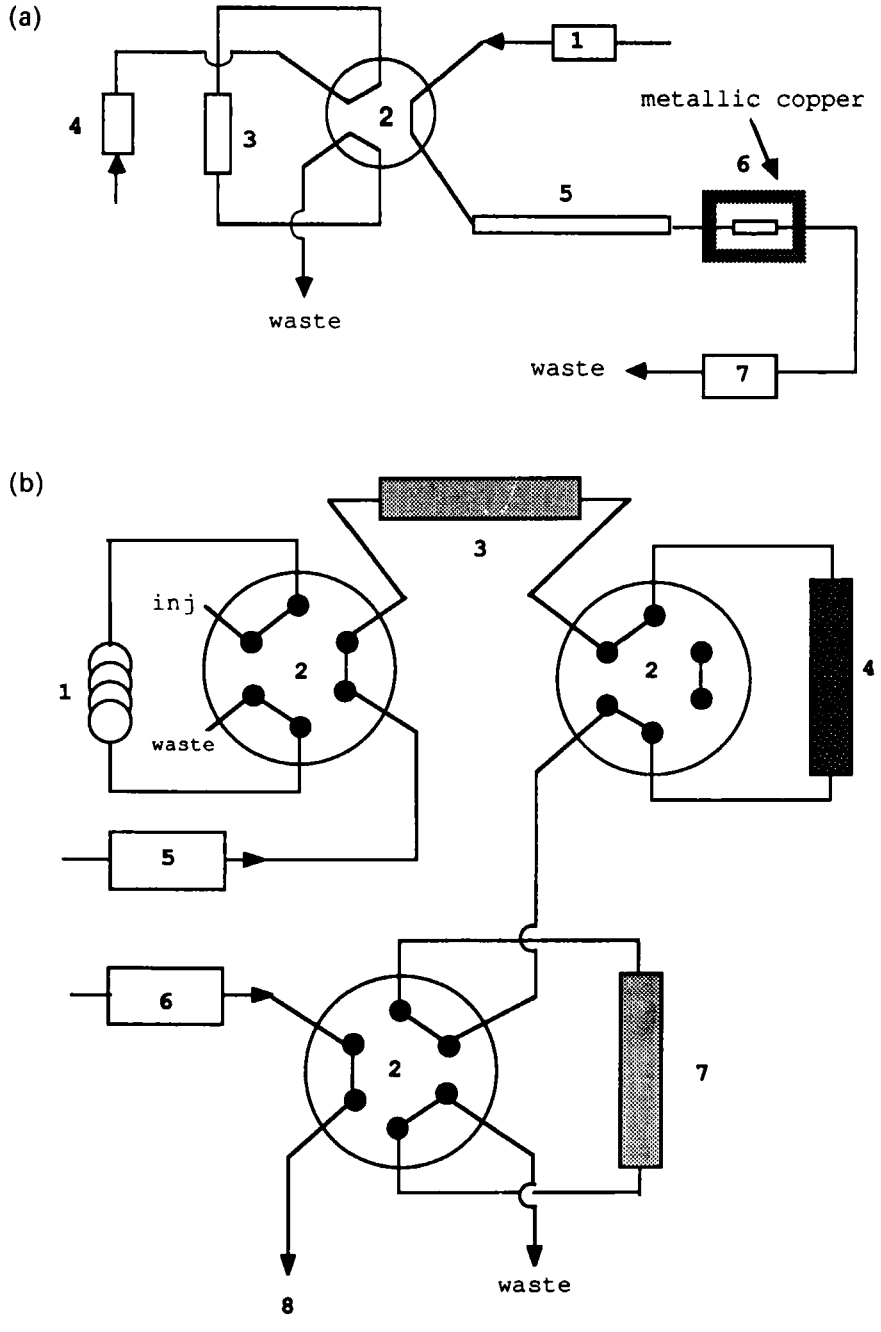

Figure 2 Schematic diagrams of the apparatus. (a) System for thiram: 1 = LC pump; 2 = valve; 3 = preconcentration precolumn; 4 = pump; 5 = analytical column; 6 = copper reactor; 7 = detector. (b) System for maneb and zineb: 1 = injection loop; 2 = valve; 3 = cleanup precolumn; 4 = cetrimide column; 5 = pump; 6 = LC pump; 7 = preconcentration precolumn; 8 = to analytical column and detector. For further details, see Experimental section.

EDTA and 1 mM potassium citrate are added and the mixture is filtered. An aliquot is pumped or injected via a loop onto the precolumn. After preconcentration and flushing with a 1 mM EDTA/citrate solution, thiram is desorbed to the analytical column with the LC eluent.

Ethylenebisdithiocarbamates Twenty-five grams of the sample are extracted with 50 ml of an aqueous solution of 0.1 M EDTA (pH 9.0) for about 45 min in an ultrasonic bath and filtered. The extract is diluted with a 1 M phosphate buffer (pH 7.0) in a ratio which is dependent on the concentration of the analytes in the sample and filtered through a 0.2 μm membrane filter.

Before introduction of the sample, the preconcentration column is loaded with cetrimide by flushing of the precolumn system (cetrimide column switched on-line) with 5 ml of a 10 mM aqueous acetate buffer (pH 6.2) containing 5 mM sodium nitrate. Subsequently, the cetrimide column is switched off-line and the preconcentration column is washed with 3 ml of the acetate buffer/sodium nitrate solution. The sample is then injected via a 1.0 ml loop and pumped via the cleanup precolumn through the preconcentration column. After flushing the system with 3 ml of the acetate buffer/sodium nitrate solution, nabam is desorbed to the analytical column with the LC eluent.

RESULTS AND DISCUSSION

Determination of Thiram

LC system The LC system used for the determination of thiram and, especially, the post-column complexation on a solid-state copper reactor were described in ref. 12. The conversion of the colourless thiram into a coloured copper(II) dithiocarbamate complex (λ_{max} = 435 nm) effects a considerable increase of the selectivity, while the sensitivity is about the same as with the UV detection of thiram itself at 254 nm. As an example, the preconcentration of up to 20 ml surface water on a C_{18} precolumn was shown to give very clean chromatograms. Owing to the selectivity of the detection, an analysis time of only about 5 min was required. More than 200 injections of solutions containing nanogram amounts of thiram could be made with a 2.0 × 2.1 mm I.D. reactor that contains about 8 mg of copper.

Sample pretreatment Thiram is very apolar and, therefore, extraction from residue samples is only possible with non-aqueous solvents. First, methanol was tested because after dilution with water this solvent can be directly injected onto a reversed-phase LC system. However, with this solvent many interfering compounds were also extracted. Subsequently, dichloromethane and hexane were tested and dichloromethane was found to be more selective. For 50 g of a dried and homogenized sample, 3-fold extraction with 60 ml dichloromethane is necessary in order to obtain a recovery of about 98%.

After filtration the combined organic phases are evaporated. Dichloromethane is

not miscible with water and, therefore, it has to be removed completely prior to LC analysis. However, if dichloromethane was fully evaporated, a considerable loss of thiram was observed. This problem has been solved by evaporation of the 180 ml to 10 ml and, subsequently, the addition of 10 ml of methanol. Dichloromethane can be fully removed by a further evaporation step down to 1 ml (methanol). The sample can now be mixed with water in order to permit preconcentration of a large volume on a hydrophobic stationary phase. For pure water the breakthrough volume of thiram on a 4.0×2.1 mm I.D. precolumn packed with C_{18}-bonded silica is more than 50 ml,[12] but the breakthrough volume decreases to about 6 ml for water-methanol (90:10). That is, after dilution of 1 ml of methanol with 9 ml of water, about 50% of the sample can be loaded onto the precolumn. In order to prevent complexation of thiram with metal ions an aqueous EDTA/citrate solution is used instead of pure water for dilution of the sample. A 1:1 mixture of 1 mM EDTA and 1 mM potassium citrate is able to prevent the reaction of thiram with Cu(II) (cf. ref. 12). No significant loss of thiram was observed if a solution containing 35 ppm of thiram and 0.1 mM copper(II) sulphate was stored for 16 h, while without the EDTA/citrate mixture a 50% decrease in the thiram concentration was found after 20 min.

The stability of DTCs in soil also needs attention. Preliminary experiments have shown a rapid decrease (about 40% in one day) of the thiram concentration (500 ppb) in spiked soil. The addition of an EDTA/citrate mixture had no positive influence on the stability of thiram in soil.

Determination of Ethylenebisdithiocarbamates

LC system Initially the system developed for the determination of thiram was applied to the determination of ethylenebisdithiocarbamates. Maneb and zineb are virtually insoluble in water and almost all organic solvents. Therefore, a direct LC determination of these compounds seemed impossible. However, they can easily be converted into nabam via a ligand-exchange reaction, i.e. by the addition of an aqueous EDTA solution. Reversed-phase LC of nabam can be carried out on a C_{18}-bonded silica column with a mobile phase containing cetrimide as an ion-pair reagent. In a system with 10 mM cetrimide, the presence of 90% methanol is necessary to obtain a retention time of below 10 min. The consequence of the conversion of maneb and zineb into nabam is that only the sum of these three compounds can be determined. This is no particular drawback because the use of nabam has been decreased drastically during the last years and the toxicity and environmental behavior of maneb and zineb are very similar.[13] The post-column copper reactor was found to be unsuitable for nabam because the copper complex formed is very apolar and precipitates in the reactor itself. Therefore, one has to be content with non-selective UV detection at 280 nm and sufficient selectivity will have to be found in another way. An elegant solution to this problem is presented hereunder.

In its anionic form nabam can be preconcentrated on a precolumn loaded with cetrimide. On such a precolumn only anions will be retained by the ion-pair mechanism. Unfortunately, however, cetrimide is loaded on C_{18}-bonded silica as

the carrier material and this stationary phase also exhibits high retention towards many apolar, and possibly interfering compounds. These compounds can be removed by inserting another precolumn packed with C_{18}-bonded silica or the still more apolar styrene-divinylbenzene copolymer PRP-1 in front of the precolumn loaded with cetrimide (clean-up precolumn, see Figure 2b). In order to load the preconcentration column with cetrimide, a column packed with solid cetrimide was inserted between the two precolumns; one may expect that enough cetrimide will dissolve in water to obtain sufficient loading of the C_{18} precolumn. However, if during the preconcentration step samples were pumped through the cetrimide column, recoveries were low and distinct memory effects were observed. The low recovery found must at least partly be attributed to metal impurities because addition of a chelating agent such as EDTA to the sample increased the recovery.

In order to overcome the problem of memory effects, the procedure was modified and the preconcentration column was loaded with cetrimide *before* injection of the sample. This was carried out by flushing the precolumn system for about 3 min with an acetate buffer/sodium nitrate solution. Subsequently, the cetrimide column was switched off-line and the sample was injected or pumped into the precolumn system. The cetrimide column can be relatively long—and thus be used for a large number of analyses—because the column is not part of the analytical chromatographic system and does not contribute to band broadening. The 70×4.2 mm I.D. column used was replaced daily. Because the preconcentration column is flushed with acetate buffer/sodium nitrate after loading with cetrimide, metal impurities are removed and do not interfere in the determination of nabam.

Preconcentration of at least 50 ml was found to be possible on a 10×4.2 mm I.D. preconcentration precolumn without breakthrough. The high selectivity of an apolar clean-up precolumn combined with a precolumn loaded with an ion-pair reagent for preconcentration of anions will be demonstrated below.

Sample pretreatment Maneb and zineb can efficiently be extracted from soil samples with an aqueous EDTA-containing solution. The selectivity of such an extraction which is based on a ligand-exchange reaction is very high, because most other apolar compounds will not be extracted. For the extraction of 25 g soil spiked with about 1 ppm maneb or zineb with 50 ml 0.1 M EDTA (pH 9.0) recoveries of over 98% were found. At lower pH values the recovery was less. Since a solution of pH 9 will cause deterioration of chemically bonded silica materials, the sample was diluted with a phosphate buffer of pH 7. This solution can directly be used for preconcentration on a C_{18}-bonded silica precolumn loaded with cetrimide (see above).

Applications

The chromatograms in Figure 3 demonstrate the selectivity and sensitivity of the analytical system which has been developed for the determination of thiram residues in soil. Figure 3a shows a chromatogram of a blank soil with UV detection at 254 nm. The broad, tailing band in the early part of the chromato-

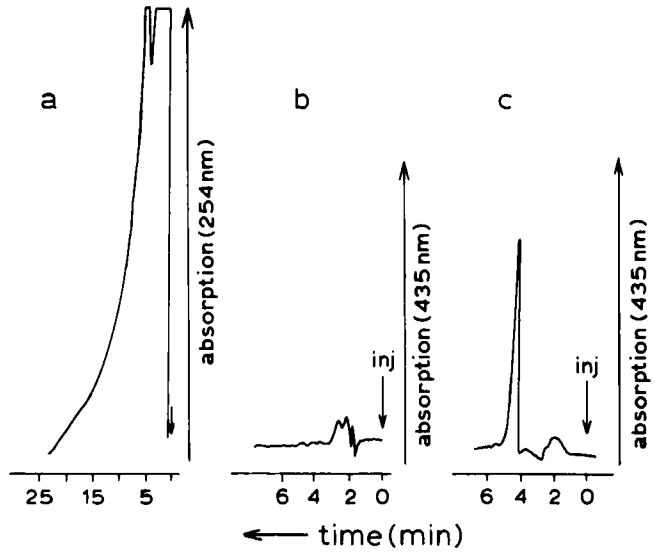

Figure 3 Determination of thiram in soil. LC conditions: analytical column 200 × 2.1 mm I.D. packed with 5 μm Hypersil ODS; precolumn, 4.0 × 2.1 mm I.D. packed with 5 μm LiChrosorb RP-18; copper reactor, 4.0 × 2.1 mm I.D. (only b and c); mobile phase, acetonitrile-aqueous acetate buffer (10 mM, pH 5.2) (70:30); flow-rate, 0.3 ml/min; detection wavelength, 254 nm (a) and 435 nm (b and c). Preconcentration of 5 ml of a blank soil extract (a and b) and of a soil extract spiked with 400 ppb of thiram (c). For sample pretreatment, see Experimental section.

gram makes detection of thiram at this non-selective wavelength impossible. Figs. 3b and c, representing blank soil and soil spiked with 400 ppb thiram, respectively, demonstrate selectivity gained by using the post-column copper reactor with detection at 435 nm. The recovery for thiram in soil spiked with 500 ppb was 98% with a detection limit of 10 ppb.

The above procedure is also suitable for the determination of thiram on lettuce and apple, as is demonstrated in Figures 4 and 5 (the different retention times of thiram are caused by different mobile phase compositions). The large band in the early part of both chromatograms must probably be attributed to chlorophylls and related compounds which are coextracted after the disintegration of the plant cells. The band is significantly smaller for apple extracts than for lettuce extracts; with the latter material, the detection limit is therefore higher by a factor of ten. The large interferences in the case of lettuce extracts can possibly be reduced by employing "softer" extraction methods than ultrasonic treatment. With the present method detection limits of 5–10 ppb for apples and 50–100 ppb for lettuce can be obtained.

As in the case of thiram the determination of ethylenebisdithiocarbamates in soil was only possible when the selectivity of the analytical system was drastically increased. Direct preconcentration of the analytes from soil extgracts on a cetrimide-loaded C_{18} precolumn resulted in chromatogram similar to the one shown in Figure 3a. The insertion of a C_{18} or a PRP-1 cleanup column before the preconcentration column affected the removal of most interfering apolar com-

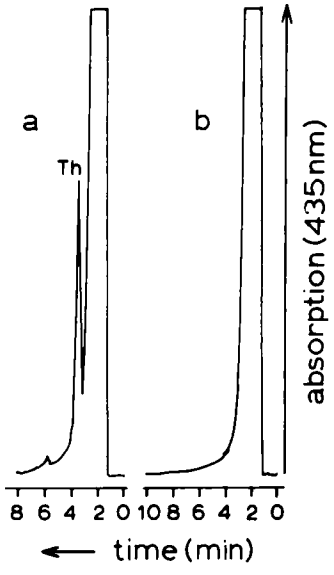

Figure 4 Determination of thiram on lettuce. (a) Preconcentration of 1 ml of an extract of lettuce spiked with 350 ppb thiram and (b) of a blank extract of lettuce; for LC conditions, see Figure 3 except for LC mobile phase, acetonitrile-aqueous acetate buffer (10 mM, pH 5.2) (75:25); attenuation, 0.02 a.u.f.s. For sample pretreatment, see Experimental section.

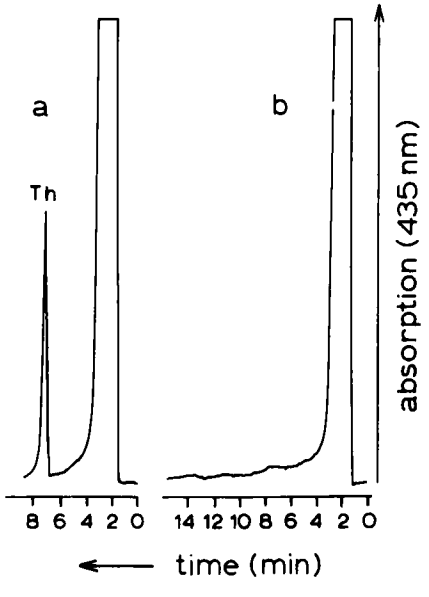

Figure 5 Determination of thiram on apples. (a) Preconcentration of 3 ml of an extract of apple spiked with 50 ppb thiram and (b) or a blank extract of apple peels; for LC conditions, see Figure 3 except for LC mobile phase, acetonitrile-aqueous acetate buffer (10 mM, pH 5.2) (65:35); attenuation, 0.01 a.u.f.s. For sample pretreatment, see Experimental section.

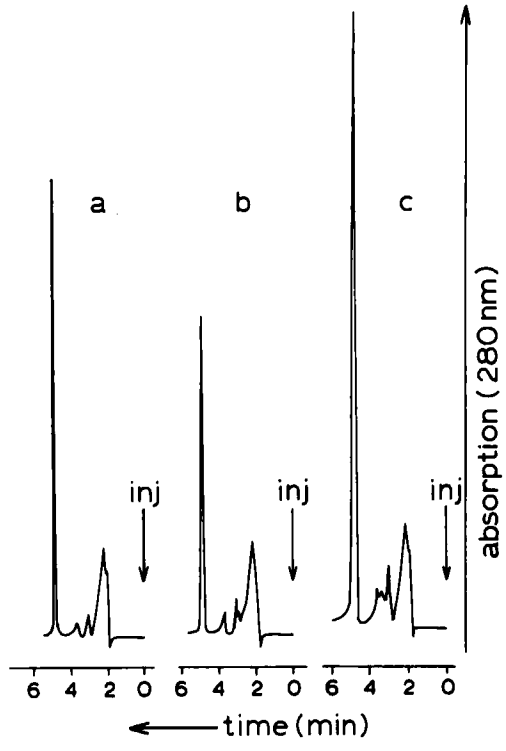

Figure 6 Determination of ethylenebisdithiocarbamates in soil. LC conditions: analytical column, 200 × 3.2 mm I.D. packed with 5 μm LiChrosorb RP-18; clean-up precolumn; 10 × 2.1 mm I.D. packed with PRP-1; cetrimide column; 70 × 4.2 mm I.D. packed with cetrimide; preconcentration precolumn, 10 × 4.2 mm I.D. packed with 5 μm LiChrosorb RP-18 and loaded for each analysis with cetrimide by flushing the whole system for 3 min with an aqueous acetate buffer (10 mM, pH 6.2) containing 5 mM sodium nitrate; mobile phase, methanol-aqueous acetate buffer (10 mM, pH 6.2) (90:10) containing 5 mM cetrimide; flow-rate, 0.7 ml/min; detection wavelength, 280 nm. Preconcentration of 1 ml of a standard solution of 1.8 ppm of zineb (a), of 1 ml of a standard solution of 1.3 ppm of maneb (b) and of a soil extract spiked with 2.6 ppm of nabam (c). For sample pretreatment, see Experimental section.

pounds. Besides, most of the interfering anions which pass through the cleanup column are eluted early in the chromatogram due to the high methanol content of the LC mobile phase. Figures 6a and b represent chromatograms of maneb and zineb standards. Figure 6c shows the chromatogram of a soil extract spiked with 2.6 ppm nabam. The selectivity of the preconcentration system allows the LC analysis to be performed within about 20 min with a detection limit of 100 ppb.

The present method was used to determine ethylenebisdithiocarbamate residues in several soil samples taken two weeks after pesticide application. The concentrations found were between 5 and 50 ppm. This is in the same range as was found by headspace GC analysis using the carbon disulphide method.

CONCLUSION

Two relatively simple LC methods have been developed for the trace-level

determination of several DTCs in various types of environmental samples. The selectivity of the novel procedures is much higher than that of methods based on hydrolysis with subsequent determination of the carbon disulphide formed, because in the latter case only the sum of the dithiocarbamates and many possible degradation products is determined.

The new methods nicely illustrate that sample handling and detection in chromatographic methods are closely related, because both can improve the sensitivity and selectivity of the total analytical procedure. The on-line post-column complexation seems useful for many other chelate-forming analytes with favourable detection characteristics of the resulting complexes. The selective precolumn system based on ion-pair formation looks attractive for the determination of both acidic and basic compounds in complex matrices.

Further research in the field of the trace-level determination of DTCs and related compounds will be directed at the elaboration of selective methods for dimethyldithiocarbamates such as ferbam and ziram and degradation products of DTCs. This is highly important because the study of the environmental behaviour and toxicity of DTCs and their degradation products is strongly dependent on the availability of reliable analytical methods.

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