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Note

High-performance liquid chromatographic determination of dibutyldithiocarbamate degradation products in cucumbers and soil

E. BRANDŠTETEROVÁ*, J. LEHOTAY and J. GARAJ

Department of Analytical Chemistry, Slovak Technical University, Radlinského 9, 812 37 Bratislava (Czechoslovakia)

and

P. A. LECLERCQ

Laboratory of Instrumental Analysis, Department of Chemical Engineering, Eindhoven University of Technology, Eindhoven (The Netherlands)

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Metal dialkyldithiocarbamates are used as active fungicides in agriculture (ferbam, ziram) and also as additives and antioxidants in rubber and plastic materials. Therefore, direct monitoring of their purities and their residues in the foods and agricultural products is necessary.

High-performance liquid chromatography (HPLC) of metal complexes of N-disubstituted dithiocarbamic acids (dimethyl and dibutyl derivatives) was described in previous papers^{1–4}. The results confirmed that all these complexes are unstable and decompose rapidly.

The effects of UV radiation, temperature and time on the degradation of iron(III) dibutyldithiocarbamate were studied⁵ and the use of HPLC and mass spectrometry (MS) for the identification and determination of this complex and the products of its transformation was discussed. Adsorption and reversed-phase chromatography were investigated.

Ethylenethiourea (ETU) was determined as the main degradation product of ethylene bis dithiocarbamate in some fungicides by HPLC⁶. The identification of some mixed-ligand dithiocarbamate complexes has been described^{7,8} and the rate constants for ligand-exchange reactions evaluated⁹.

Dithiocarbamate complexes are known to have toxicological and mutagenic effects and their degradation products have been found to be more biologically effective than the original chelates^{10–12}. The conversions of dimethyldithiocarbamates into tetramethylthiuram disulphide (TMTD) and its mechanism for ferbam [iron(III) dimethyldithiocarbamate] and ziram [zinc(II) dimethyldithiocarbamate] have been illustrated¹³.

Some papers have discussed in detail toxicological aspects of dithiocarbamates (DTCs) and related compounds^{14,15}. The short-term toxicity of 26 DTCs was

determined in tests with various organisms. It was found that dialkyl-DTCs were generally more toxic than ethylene bis-DTCs. The application of heavy metal DTCs is questionable as these compounds are generally more toxic than the metals.

Liquid-liquid extraction of some dialkyl-DTCs has been studied with various solvent combinations in order to establish reagent interferences in the final determination¹⁶.

Liquid chromatography has been applied to the identification and determination of some DTCs in vulcanized rubber products. After conversion of zinc DTCs into the corresponding cobalt(III) DTCs, the latter were separated by a reversed-phase method with methanol-water using UV detection¹⁷. HPLC with amperometric detection on a copper electrode has been used for the determination of ethylene-thiourea in beverages without sample pretreatment; the limit of detection was 3 ng/ml¹⁸.

The aim of this work was to use the optimum separation conditions for the simultaneous HPLC separation of iron(III) dibutyldithiocarbamate [Fe(III) DB DTC] and its degradation products in some agricultural samples grown on plastic foil containing dithiocarbamates as antioxidants. It is known that such foil is degraded during the growth period under the influence of UV radiation, temperature and oxidizing agents, and the degradation products can penetrate into the soil or crop grown in contact with the foil. For these reasons, it is necessary to monitor the residues of dithiocarbamates and their degradation products.

EXPERIMENTAL

Apparatus

All experiments were carried out on Packard Model 8200 HPLC system with a UV detector (254 nm). Stainless-steel columns (250 × 2.2 mm I.D.) packed with LiChrosorb Si 60 (particle diameter 5 μm) and LiChrosorb RP-18 (5 μm) were applied. Chloroform-*n*-heptane (20:80) and water-isopropanol (25:75) were used as mobile phases.

Some gas chromatographic-mass spectrometric (GC-MS) measurements were performed using a Finnigan 4000 instrument. Helium was used as the carried gas. A glass "falling needle" device was used as an injector. All experiments were carried out under the following GC conditions: inlet pressure 2.0 bar (absolute), injection temperature 240°C, column temperature 230°C and ion-source temperature 250°C.

The mass spectrometer was operated in the electron-impact (EI) mode under the following conditions: electron energy 70 eV, electron current 0.25 mA, multiplier voltage 1.70 kV, mass range 40-550 and scan speed one spectrum per second.

A Model 1102 elemental analyser (Carlo Erba, Milan, Italy) was used to establish the purities of the standards.

Chemicals

Iron(III) dibutyldithiocarbamate [Fe(III) DB DTC] and tetrabutylhiuram disulphide (TBTD) were synthesized by literature procedures^{11,19}. A standard Fe(III) DB DTC was obtained as an antioxidant used in the plastics industry (VUAPL, Research Institute of Plastic Materials, Nitra, Czechoslovakia). The identities and purities of the standards were confirmed by elemental analysis and mass spectrometry.

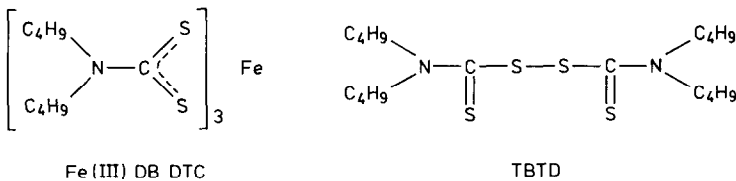
All organic solvents were of analytical-reagent grade (Lachema, Brno, Czechoslovakia) and were dried over magnesium perchlorate and redistilled.

Procedures

Chloroform was applied for the extractions of real samples (cucumbers and soil). Amounts of 500 g of the soil (cucumbers) were extracted gradually at room temperature with $3 \times 300 \text{ cm}^3$ of chloroform in the discontinuous mode. Each extraction was performed for 10 min. After evaporation of the solvent, dissolution of the residues in the mobile phase (1 cm^3) and filtration through glass-fibre filters (Tessek, Prague, Czechoslovakia), the extracts were injected onto the chromatographic column in volumes of $10 \mu\text{l}$.

RESULTS AND DISCUSSION

The optimum separation conditions for the simultaneous HPLC separation of Fe(III) DB DTC and TBTD have been published⁵. Reversed-phase HPLC with a LiChrosorb RP-18 column and isopropanol-water as the mobile phase was recommended.



Determination of Fe(III) DB DTC and TBTD in cucumbers

Samples of cucumbers grown on foil containing Fe(III) DB DTC as an antioxidant were extracted according to the procedure described. Four different cucumbers extracts were analysed: (1) cucumbers grown without using the foil; (2) cucumbers grown on foil containing 0.6% of Fe(III) DB DTC; (3) cucumbers grown on foil containing 0.3% of Fe(III) DB DTC in combination with another antioxidant; and (4) cucumbers grown on foil without Fe(III) DB DTC.

HPLC separation of cucumber extracts fortified with Fe(III) DB DTC and TBTD is shown in Fig. 1 and HPLC traces for cucumbers extracts of samples 2 and 4 are illustrated in Fig. 2a and b. The extraction recovery of Fe(III) DB DTC was 89% for an Fe(III) DB DTC concentration in cucumbers of 0.05 mg/kg. The standard deviation ($n=5$) was 2% and linearity achieved at the spiking levels of 0.02–0.1 mg/kg. The limit of detection of Fe(III) DB DTC in cucumber extracts was 0.020 mg/kg. For TBTD the recovery from cucumbers was 90% and the limit of detection of TBTD in extracts was 0.018 mg/kg.

From the chromatogram in Fig. 2a, it is obvious that this extract did not contain residues of Fe(III) DB DTC. However, very low concentrations of TBTD were determined in cucumber samples 2 and 3 (0.036 and 0.022 mg/kg, respectively).

The presence of TBTD in the extracts was verified by GC-MS. The results confirm that dibutylthiocarbamates are not stable and after several months they could be decomposed completely to their degradation products (e.g., TBTD)⁵.

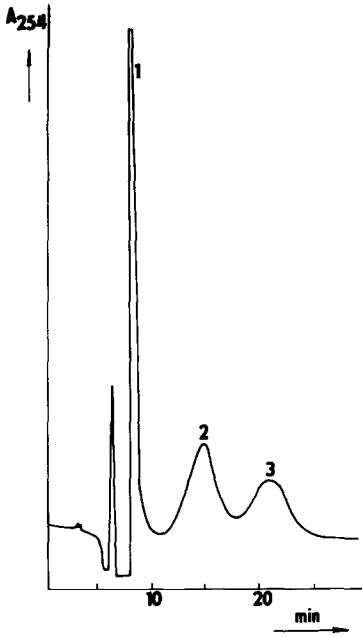


Fig. 1. HPLC separation of cucumber extract fortified with TBTD and Fe(III) DB DTC (0.1 mg/kg). Column, LiChrosorb RP-18, 5 μm (250 \times 2.2 mm I.D.); mobile phase, water-isopropanol (25:75); flow-rate, 0.2 cm^3/min ; injection volume, 10 μl ; attenuation 32. Peaks: 1 = inert; 2 = TBTD; 3 = Fe(III) DB DTC. resolution $R_{2,3} = 1.28$.

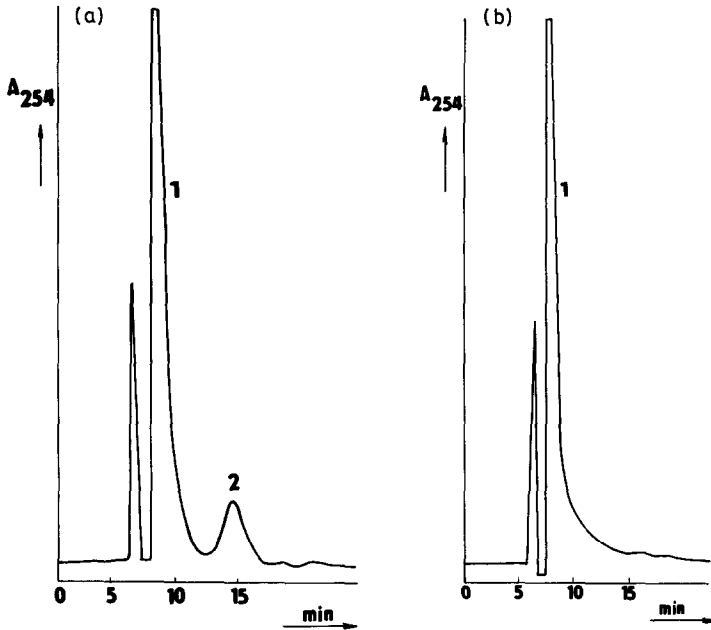


Fig. 2. HPLC traces of cucumber extracts, samples (a) 2 and (b) 4. Separation conditions as in Fig. 1; attenuation 16. Peaks: 1 = inert; 2 = TBTD.

Determination of Fe(III) DB DTC and TBTD in soil

Extracts of soils from cucumber growing (samples 1–4) were analysed in the same way as the cucumbers samples. After filtration the extracts were injected onto the chromatographic column. The separation conditions were the same as for HPLC analysis of cucumber extracts. HPLC separations of the soil extracts (samples 2 and 4) are illustrated in Fig. 3a and b. Both Fe(III) DB DTC and TBTD were identified in soil sample 2 [0.022 mg/kg Fe(III) DB DTC and 0.026 mg/kg and TBTD in sample 3 (0.018 mg/kg). Th results obtained were verified by GC–MS. A gas chromatogram of the soil extract (sample 2) is shown in Fig. 4.

It was discussed previously⁵ that TBTD had been decomposed to tetrabutylthiuram monosulphide (TBTM) during GC separation at temperatures above 473 K. For this reason, the peak of TBTM was identified in Fig. 4 in site of the fact that TBTM had not been present in the soil extract. At the temperature of GC separation (503 K) it is certain that TBTD present in the extract would also have been converted into TBTM. Mass spectra of TBTD and TBTM from the soil extract are illustrated in Fig. 5a and b.

The identification of TBTD and TBTM was confirmed at low voltage at 12–16 eV. The recovery of Fe(III) DB DTC in the soil (fortified at 0.05 mg/kg) was 91% and that of TBTD 92%. The detection limits for Fe(III) DB DTC and TBTD were 0.016 mg/kg and 0.014 mg/kg in soil, respectively.

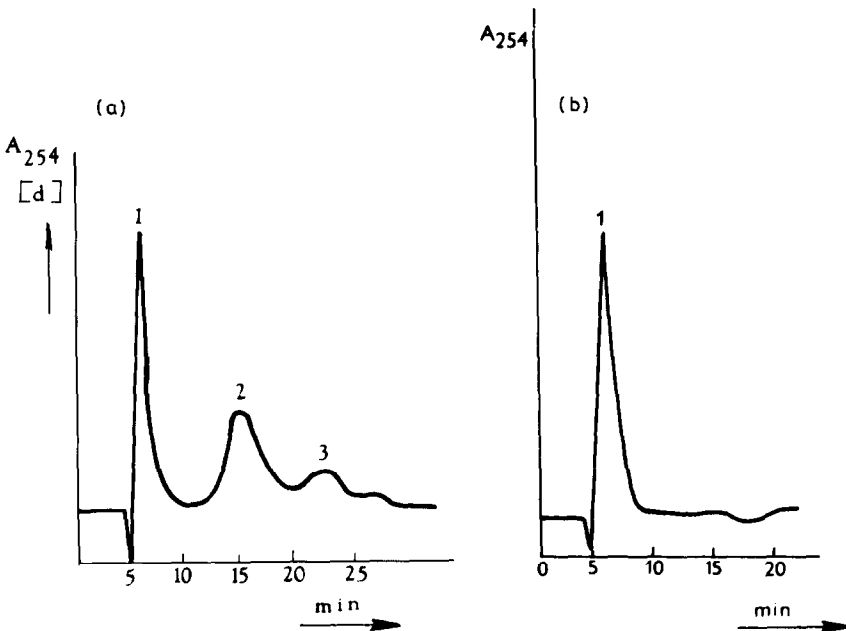


Fig. 3. HPLC traces of soil extracts, samples (a) 2 and (b) 4. Separation conditions as in Fig. 1; attenuation 4. Peaks: 1 = inert; 2 = TBTD; 3 = Fe(III) DB DTC.

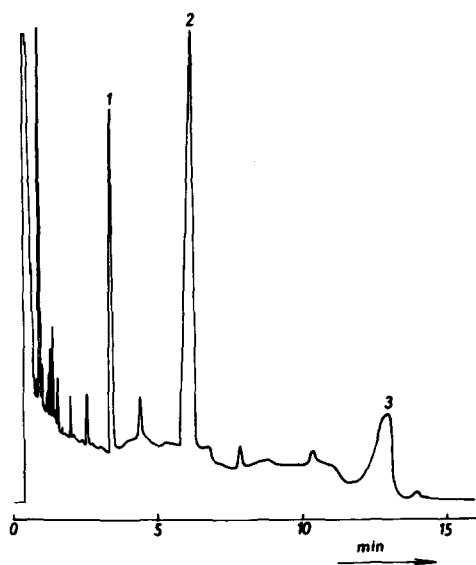


Fig. 4. Gas chromatogram of soil extract, sample 2. Column, SE-30 (30 m \times 0.4 mm I.D.); pressure (helium), 200 kPa; temperature, 503 K. Peaks: 1 = unknown; 2 = TBTM; 3 = TBTD.

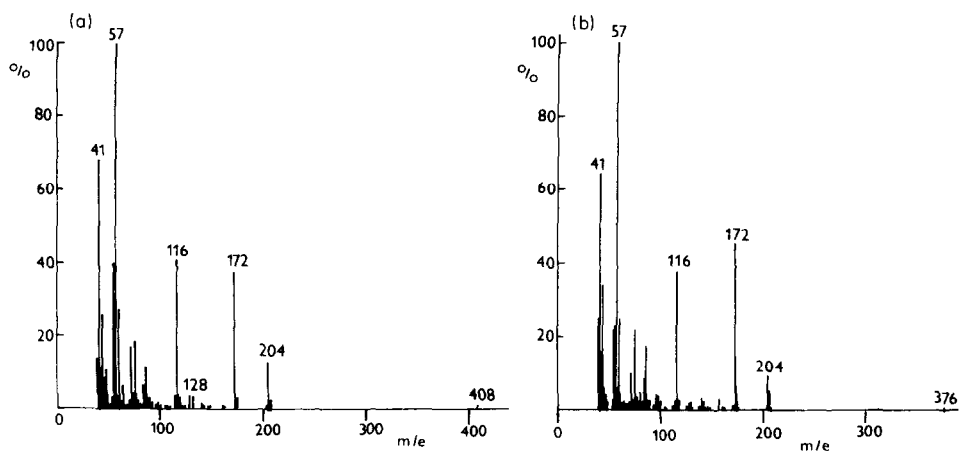


Fig. 5. Mass spectra of (a) TBTM and (b) TBTM.

The proposed method has already been accepted by VU APL Nitra (a producer of plastic foils used in agriculture) and has been applied for the control analysis of DTC antioxidants in rubber and plastic products.

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