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APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE TRACE ANALYSIS OF SOME FUNGICIDES

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SUMMARY

A method has been developed for the determination of trace concentrations of iron(III) dimethyldithiocarbamate (ferbam) and its three degradation products (thiram or TMTD, tetramethylthiuram disulphide; TMTM, tetramethylthiuram monosulphide; and TMTU, tetramethylthiourea) in model mixtures and real samples. Quantitative analysis using adsorption and reversed-phase chromatography enables reproducible results to be achieved of in the high-performance liquid chromatographic separation of these compounds in a very short time. All results were verified by mass spectrometry. The limit of detection for thiram in soil was 0.005 mg/kg, which corresponds with the criteria of the FAO (Codex Alimentus) concerning methods for the determination of dithiocarbamate residues in food.

INTRODUCTION

The fact that many of the dialkyldithiocarbamates used in agriculture as fungicides are not stable has led to interest in the synthesis of some of the degradation products and to study the degradation process of dithiocarbamates (DTCs)[1–5]. Some workers studied the reactions of thiuram disulphides and monosulphides as the oxidation degradation products of DTCs[6,7]. Very interesting results have been obtained[8,9] on the heat degradation of thiram (TMTD; tetramethylthiuram disulphide).

Many of the derivatives and oxidation degradation products are inhibitors of enzymatic processes[10–12]. Dithiocarbamates and their degradation products are of clinical importance. Many workers have studied the biological activity of DTCs and their influence on animal and human organisms[13–17], and confirmed that some degradation products of DTCs were more toxic in man than the original DTCs. Studies of the biological activity of TMTD and TMTM (tetramethylthiuram monosulphide) have been published[18,19]. The conversion of dimethyldithiocarbamates into TMTD and its mechanism for ferbam [iron(III) dimethyldithiocarbamate] and ziram (zinc dimethyldithiocarbamate) has been illustrated[20]. Very interesting results were pub-

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lished by the Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs[21]. This paper illustrated the maximal allowed residue concentrations of DTCs in various kinds of vegetables and fruit.

We have been interested in the study of the degradation process of dimethyldithiocarbamates (ferbam, thiram). In this paper, results are presented on the degradation of DTC fungicides by UV irradiation and with time and the trace HPLC analysis of DTC degradation. The main reason for our interest in this quantitative analysis of DTC degradation products was that the toxicity of TMTD is higher (LD_{50} 2.5 mg/kg) than that of ferbam (LD_{50} 12.5 mg/kg)[22]. The combination of HPLC and (MS) mass spectrometry will improve the identification and determination of ferbam and thiram degradation products in real samples (soil).

EXPERIMENTAL

Apparatus

A Spectra-Physics Model 3500 HPLC system with a UV detector was used for all HPLC separations. Stainless-steel columns (250 × 4.6 mm I.D.) packed with LiChrosorb Si-100 (particle diameter 5 μm) and LiChrosorb RP-18 (5 μm) were applied for the separation of model mixtures and real samples. Chloroform–cyclohexane and acetonitrile–methanol–water were used as the mobile phases.

All mass spectra were measured with a 902 S mass spectrometer (AEI, Manchester, U.K.) at 7–70 eV, 0.1 mA and 323–375°K.

For degradation by UV irradiation a UV-RI lamp (UVR, Chirana, Stará Turá, Czechoslovakia) was applied.

Chemicals

Ferbam[23], TMTD[24] and TMTM[25] were synthesized by literature procedures. TMTU (tetramethylthiourea) was prepared by the reaction of dimethylamine and thiophosgene in benzene. The identities and purities of these products 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

All products were decomposed by UV irradiation in the dry state and also in solutions (chloroform, methanol and water) as thin films on glass plates. The time intervals for UV degradation were 5, 10, 30, 60, 120 and 180 min.

For the extraction of the real samples (soil) the solvents applied were chloroform, methanol and water. A 500-g amount of the soil sample was carefully mixed w ith different amounts of thiram and extracted gradually with 3 × 300 cm^3 of chloroform, methanol and water. Each extraxtion was performed for about 10 min.

RESULTS AND DISCUSSION

The choice of the optimal separation conditions for the simultaneous separation of ferbam and its three degradation products has been published[26]. Adsorption chromatography on LiChrosorb Si-100 and reversed-phase chromatography using

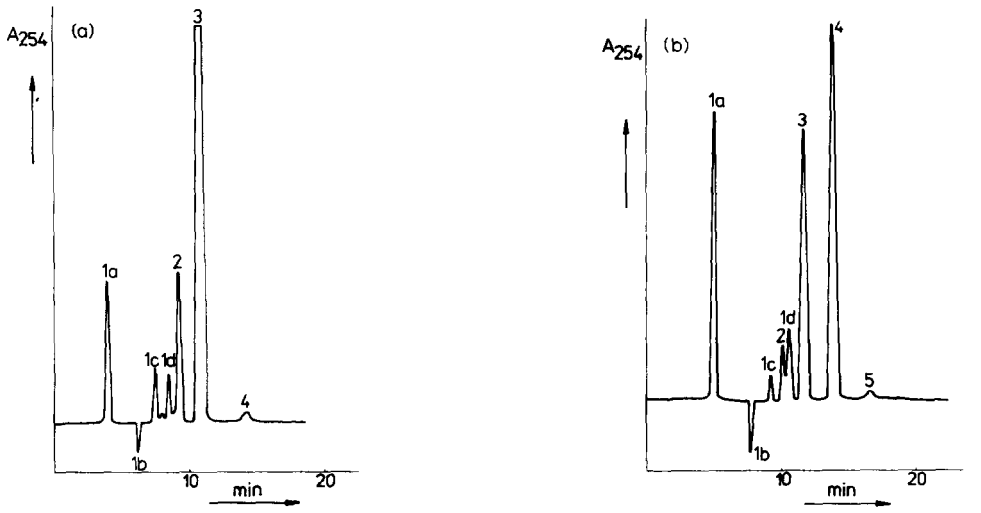

Fig. 1. Separation of ferbam (chloroform solutions) after UV irradiation. Column: LiChrosorb RP-18, 5 μm (250 × 4.6 mm I.D.). Mobile phase: acetonitrile–methanol–water (37:33:30). Flow-rate:0.37 cm^3/min. Pressure: 17.5 MPa. (a) Irradiation for 5 min. Peaks: 1 = inert; 2 = TMTM: 3 = TMTD; 4 = ferbam. (b) Irradiation for 180 min. Peaks: 1 = inert; 2 = TMTU: 3 = TMTM; 4 = TMTD; 5 = ferbam.

a LiChrosorb RP-18 column were recommended.

The influence of UV radiation, which is very important for all processes in real samples, was studied in a series of model experiments. Degradation was effected in solvents with different polarities (chloroform, methanol and water) at a concentration of 1 mg/cm^3. After degradation by UV irradiation the samples were injected into the column and the chromatograms were compared with standard chromatograms. Fig. 1 illustrates the chromatograms of chloroform solutions of ferbam after degradation. It is obvious that irradiation for only 5 min is sufficient for the decomposition of the

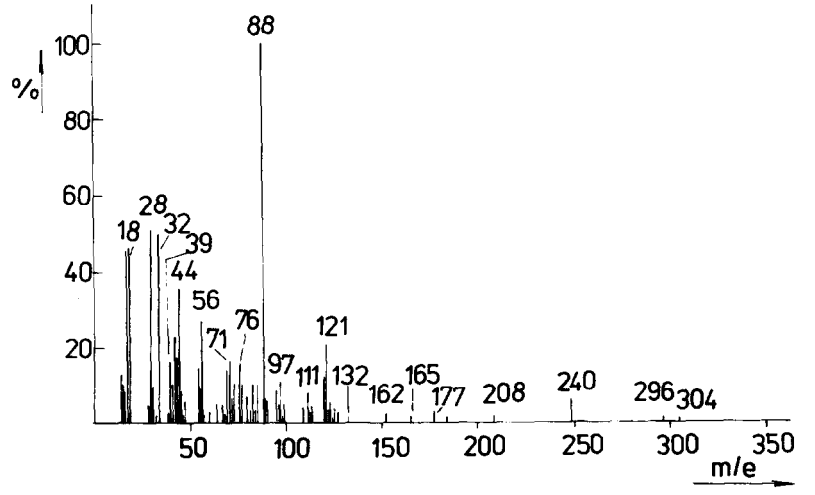

Fig. 2. Mass spectrum of ferbam (chloroform solution) after UV irradiation for 180 min.

TABLE I

UV DEGRADATION OF FERBAM (CHLOROFORM SOLUTIONS)

Time (min)	*TMTU (%)*	*TMTM (%)*	*TMTD (%)*	*Ferbam (%)*
5	–	4.05	94.27	1.67
10	0.71	19.57	78.26	1.46
30	1.29	15.41	82.09	1.21
60	3.12	31.03	64.81	1.04
120	3.19	16.70	80.11	0.89
180	5.11	38.21	55.85	0.83

metal complex into its degradation products, mainly TMTD. After 180 min the peak of TMTU is also clear. The identification of TMTU, TMTM and TMTD was confirmed by mass spectrometry (12–16 eV). The mass spectrum of ferbam in chloroform after UV degradation for 180 min is shown in Fig. 2.

The amounts of the degradation products in the whole series of ferbam chloroform solution after UV irradiation are shows in Table I. The chromatograms of aqueous ferbam solutions after UV irradiation are shown in Fig. 3. The results obtained confirm that in aqueous solutions ferbam is also decomposed mainly into TMTD and TMTM but after irradiation for 10 min it is also possible to identify TMTU. Fig. 4 shows the mass spectrum of aqueous solutions of ferbam after UV irradiation and Table II gives the amounts of ferbam and its degradation products after UV irradiation (aqueous solutions. The individual degradation products (standards) were also analysed by HPLC after UV degradation in order to investigate their stability. Fig. 5 shows the chromatograms of TMTD, TMTM and TMTU chloroform solutions after UV degradation.

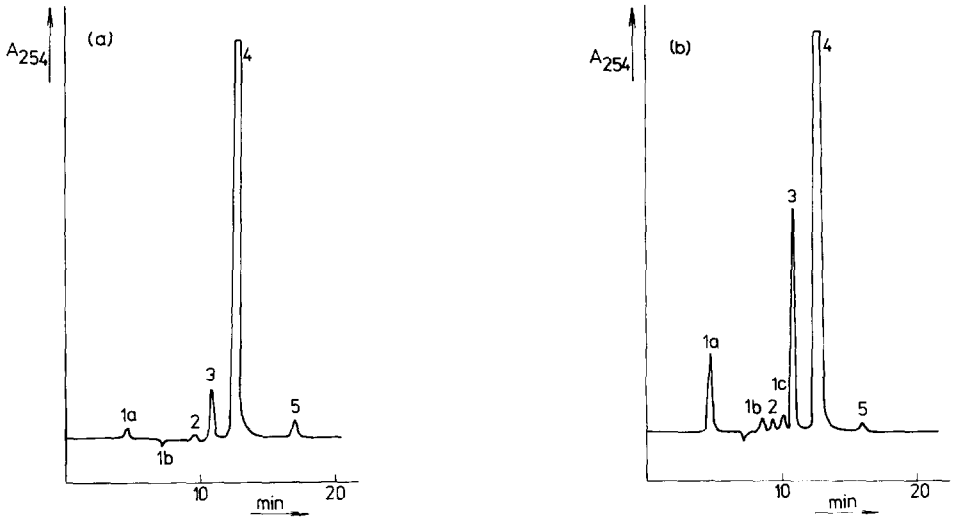

Fig. 3. Separation of ferbam (aqueous solutions) after UV irradiation. Conditions as in Fig. 1, except flow-rate, 0.33 cm^3/min; pressure, 15.9 MPa. (a) Irradiation for 10 min. Peaks: 1 = inert; 2 = TMTU; 3 = TMTM; 4 = TMTD; 5 = ferbam. (b) Irradiation for 180 min. Peaks as in (a).

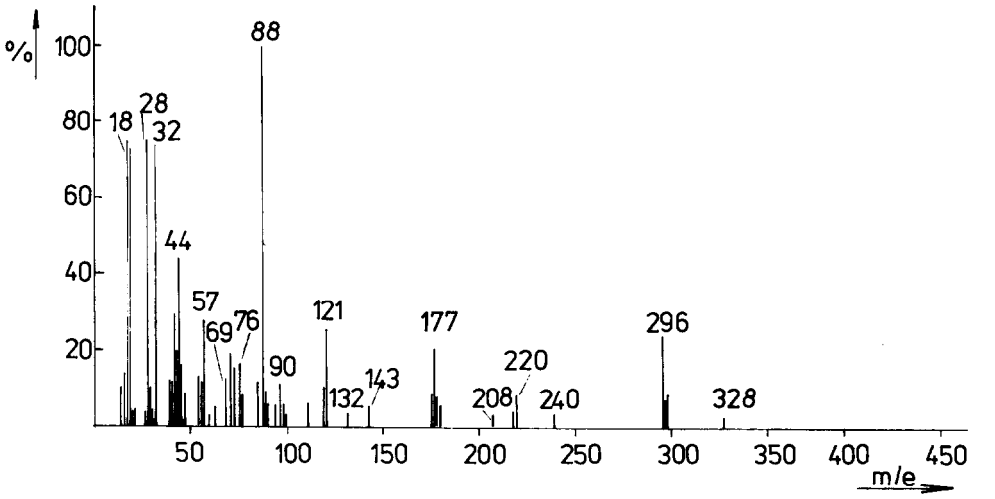

Fig. 4. Mass spectrum of ferbam (aqueous solution) after UV irradiation for 10 min.

On the basis of the results obtained we can observe the instability of two of the degradation products (TMTD and TMTM) after UV irradiation. TMTU is relatively stable in all solvents. The chromatograms shown indicate the interesting course of the reciprocal conversion of TMTD and TMTM. TMTM was transformed into TMTD after UV irradiation influence, whereas TMTM was formed after the degradation of TMTD.

We also investigated the degradation of ferbam with time and identified the degradation products as solutions in chloroform. The results are given in Table III. We found that ferbam was totally decomposed after about 35 days and TMTD was the main degradation product. The results were confirmed by mass spectrometry.

The chromatographic behaviour of DTC fungicides and thiram was used for the separation, identification and determination of very low concentrations of thiram in soil. This fungicide was added to the soil at the concentrations recommended for agriculture application:

Sample 1: soil without thiram;
Sample 2: soil with 3 g/kg of thiram;
Sample 3: soil with 1 g/kg of thiram;
Sample 4: soil with 1 g/kg of thiram, heated at 353°K;
Sample 5: soil with 1 g/kg of thiram, UV degradation for 120 min;
Sample 6: soil with 6 mg/kg thiram.

TABLE II

UV DEGRADATION OF FERBAM (AQUEOUS SOLUTIONS)

Time (min)	*TMTU (%)*	*TMTM (%)*	*TMTD (%)*	*Ferbam (%)*
10	0.77	5.88	91.65	1.70
60	4.67	18.44	75.61	1.27
180	1.22	20.50	77.55	0.73

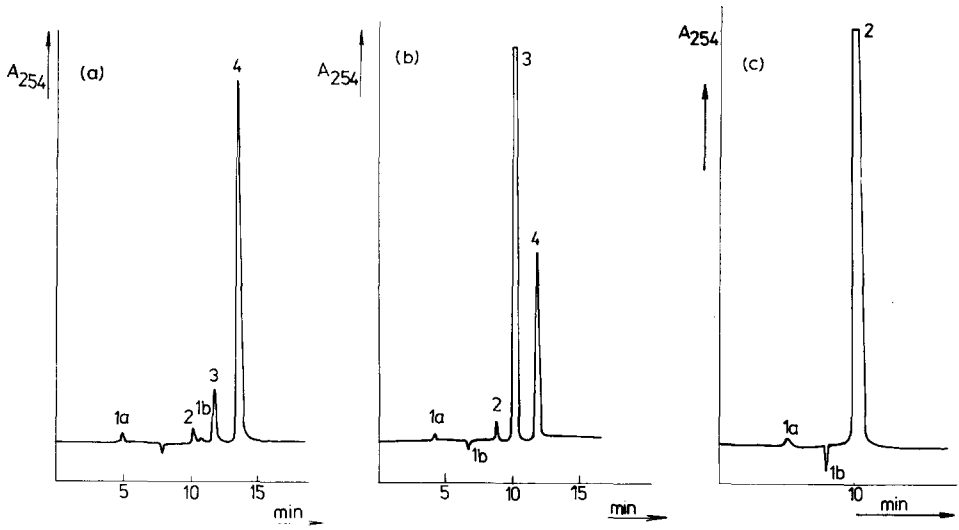

Fig. 5. Separation of (a) TMTD, (b) TMTM and (c) TMTU and their degradation products after UV irradiation for 120 min. Conditions as in Fig. 3. Peaks: 1 = inert; 2 = TMTU; 3 = TMTM; 4 = TMTD.

The soil with thiram was extracted with chloroform, methanol and water and, after evaporation of the extracts and dissolution of the residue in the mobile phase, the solutions were injected into the column (LiChrosorb Si-100). As the mobile phase, the optimal combination of chloroform–cyclohexane (70:30)[26] was chosen. In order to establish the influence of temperature and UV radiation on the soil with thiram, the soil samples were heated at 353°K and UV irradiated for 120 min.

The determination of thiram in all soil samples was effected using constant separation conditions. Calibration graphs for various concentration ranges were statistically evaluated by linear regression ($n = 5$). For injected amounts of 2–7.5 μg the equation of the straight line was $y = 1.67x + 0.11$, correlation coefficient $r_{x,y} = 0.9997$ and systematic error $S_{x,y} = 1.51\%$. For injected amounts of 0.25–0.75 μg the equation was $y = 1.77x + 0.04$, $r_{x,y} = 0.9995$ and $S_{x,y} = 1.71\%$. The limit of detection (the amount which gives a peak equivalent to three times the baseline noise) of thiram in the soil was 0.005 mg/kg.

TABLE III

DEGRADATION OF FERBAM (CHLOROFORM SOLUTIONS) WITH TIME

Time	*Ferbam (%)*
10 min	47.50
2 h	47.00
48 h	43.80
4 days	39.15
9 days	31.00
21 days	10.50
35 days	–

TABLE IV

EXTRACTION RECOVERY OF THIRAM IN SOIL

Soil sample	*Chloroform extract (mg)*	*Methanol extract (mg)*	*Aqueous extract (mg)*	*Extraction recovery (%)*
1	–	–	–	–
2	1450	10.375	0.017	97.36
3	470	0.570	–	94.11
4	462.5	0.855	–	92.67
5	460.3	0.811	–	92.02

The extraction recoveries are given in Table IV, and show the advantage of HPLC for the trace analysis of the DTC fungicides in natural samples.

CONCLUSION

HPLC provides the possibility of determining very low DTC residue concentrations in a very short time. The detection limit of thiram (0.005 mg/kg) corresponds with the FAO (Codex Alimentus) criteria for analytical methods for the determination of dithiocarbamates in food. The daily dose for man is 0–0.02 mg/kg/day for ferbam and 0–0.005 mg/kg/day for thiram.

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