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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF DIMETHYLDITHIOCARBAMATE RESIDUES IN SOME AGRICULTURAL PRODUCTS

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SUMMARY

Dimethyldithiocarbamates are widely used in agriculture as active fungicides. The degradation of dimethyldithiocarbamates (ferbam, thiram) confirmed the fact that they are not stable and decompose very rapidly. The aim of this work was to apply the results obtained in high-performance liquid chromatographic quantitative analysis of residues of dithiocarbamate fungicides in some agricultural products (strawberries, maize, tobacco).

The developed method enables very simple control analysis of low concentrations of dimethyldithiocarbamate residues in very short time. All limits of detection correspond with the criteria of FAO (*Codex Alimentarius*).

INTRODUCTION

We have previously studied the degradation of dimethyldithiocarbamates (DTCs) and found that metal dimethyldithiocarbamates are not stable and decompose very quickly. Three degradation products were identified and the optimal conditions for the simultaneous high-performance liquid chromatographic (HPLC) separation of ferbam–iron(III) dimethyldithiocarbamate, thiram–tetramethyl thiuram disulphide and their possible degradation products in the minimum time and with maximum resolution were established. Adsorption and reversed-phase chromatography have been investigated[1,2].

Ferbam and thiram are widely used in agriculture as active fungicides[3–5] and the HPLC analysis of DTC fungicides using UV detection has been reported[6,7]. Extraction recoveries have been published for some agricultural products[8]. Maximum allowed residue concentrations of pesticides and fungicides for various kinds of vegetables and fruit have been given[9]. Other papers have described the determination of DTCs by HPLC using transition metal satls as 'ion-pair' reagents[10,11] and the HPLC of sodium and ammonium salts of DTCs using a chemically bonded stationary phase[12].

The aim of this work was to apply the results obtained using HPLC to the determination of residues of DTC complexes in some agricultural products.

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TABLE I

EXTRACTION RECOVERIES OF THIRAM FROM STAWBERRIES AND MAIZE

Column: Separon SIX, 5 μm (150 × 3.2 mm I.D.). Mobile phase: 45% chloroform in cyclohexane.

Sample	*Strawberries*				*Maize*			
	Chloroform extract (%)	*Methanol extract (%)*	*Extraction recovery (%)*	*Standard deviation (%)*	*Chloroform extract (%)*	*Methanol extract (%)*	*Extraction recovery (%)*	*Standard deviation (%)*
1	–	–	–	–	–	–	–	–
2	50.3	38.4	88.7	2.9	56.6	36.3	92.9	1.2
3	53.0	33.0	86.0	3.7	56.7	34.6	91.3	1.4

EXPERIMENTAL

Apparatus

All experiments were carried out on a Packard Model 8200 HPLC system with a UV detector (254 nm). For HPLC separations three glass columns (150 × 3.2 nm I.D., particle diameter 5 μm) were used, packed with Separon SIX, Separaton C_{18} or Separon NH_2 (Laboratorní přístroje, Prague, Czechoslovakia). Chloroform–cyclohexane and acetonitrile–methanol–water mixtures were used as the mobile phases.

Chemicals

All standards were synthetized by literature procedures[13–15] and their purities were verified by elemental analysis using a Model 1102 elemental analyser (Carlo Erba, Milan, Italy) and mass spectrometry using a 902S mass spectrometer (AEI, Manchester, U.K.).

Procedures

For the extraction of real samples (maize, strawberries and tobacco) the solvents applied were chloroform and methanol. A 100-g amount of the sample was extracted with three 100-cm^3 volumes of chloroform and methanol. Each extraction was performed for about 10 min. Natural samples were extracted in large series and all data given in Tables I and II are average values from five extractions.

RESULTS AND DISCUSSION

Determination of thiram in strawberries

Thiram (TMTD) is widely used as an active fungicide in strawberry growing (Hermal L-50, Wolfen, Thiuram 85, Benlate T 20). The thiram concentrations applied here conformed with the amounts allowed in agricultural applications.

For HPLC separations of thiram and its two possible degradation products (TMTM = tetramethylthiuram monosulphide and TMTU = tetramethylthiourea), a mixture of 45% chloroform in cyclohexane and a silica gel column (Separon SIX)

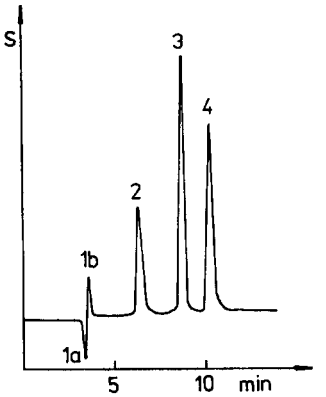

Fig. 1. Separation of TMTD, TMTM and TMTU (model mixture) on a silica gel column. Column: Separon SIX, 5 μm (150 × 3.2 mm I.D.). Mobile phase: 45% chloroform in cyclohexane. Flow-rate: 0.30 cm^3/min. Pressure: 5 MPa. Peaks: 1 = inert; 2 = TMTU; 3 = TMTM; 4 = TMTD.

were used. The optimal flow-rate of the mobile phase was 0.3 cm^3/min and the pressure was 5 MPa.

The $R_{j/j}$ values for the optimal separation conditions were $R_{TMTD/TMTM} = 2.25$ and $R_{TMTM/TMTU} = 1.52$. The optimal linear velocity was determined according to the H–u curve (0.71 mm/s).

The HPLC separation of TMTD, TMTM and TMTU on the Separon SIX is demonstrated in Fig. 1.

Three samples of strawberries were analysed. Thiram was added to strawberries at the concentration recommended for agricultural applications: sample 1, no thiram; sample 2, 0.03 g/kg; and sample 3, 0.01 g/kg. The samples were extracted and, after evaporation of the extracts and dissolution of the residues in the mobile phase, the solutions were injected into the chromatograph. The determination of thiram was effected using optimal separation conditions.

Calibration graphs were statistically evaluated by linear regression ($n = 5$). For injected amounts of 5 mm^3 the equation was $y = 0.9x + 0.07$, correlation coefficient $r_{xy} = 0.9995$ and $S_{xy} = 2.3\%$. The limit of detection (the amount that gives a peak equivalent to three times the baseline noise) of thiram in strawberries was 0.08 mg/kg. The extraction recoveries are given in Table I.

Determination of thiram in maize

Thiram has also been used for the protection of maize before seeding. Samples of maize were mixed with dry thiram and extracted as described above. Separon SIX and Separon NH_2 columns were used to establish the optimal separation system. Suitable separation conditions for the Separon SIX column were described above (Fig. 1). For the Separon NH_2 column, the following conditions were established: mobile phase, 20% chloroform in cyclohexane, $R_{12} = 1.35$, $R_{23} = 1.03$; linear velocity of the mobile phase, 1.27 mm/s; flow-rate, 0.54 cm^3/min; and pressure, 7 MPa.

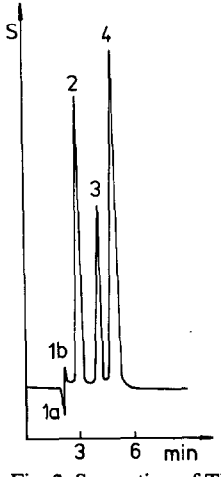

Fig. 2. Separation of TMTD, TMTM and TMTU (model mixture) on a column with chemically bonded stationary phase. Column: Separon NH_2, 5 μm (150 × 3.2 mm I.D.). Mobile phase: 20% chloroform in cyclohexane. Flow-rate: 0.54 cm^3/min. Pressure: 7 MPa. Peaks: 1 = inert; 2 = TMTU; 3 = TMTM; 4 = TMTD.

The separation of TMTD, TMTM and TMTU on a Separon NH_2 column is demonstrated in Fig. 2. Three maize samples were analysed: sample 1, no thiram; sample 2, 0.3 g/kg; and sample 3, 0.2 g/kg. The extraction recoveries are given in Table II.

Various mobile phases were investigated and the optimal separation conditions were chosen for the two columns used (silica gel and NH_2 phase). The numbers of theoretical plates (N_R), minimal separation times necessary for the separation of the last component (t_R), detection limits and optimal flow-rates of the mobile phases were as follows: silica gel column, $N_{R(TMTU)} = 5720$, $t_{R(TMTU)} = 10$ min, detection limit of thiram, 0.012 mg/kg maize and flow-rate, 0.30 cm^3/min; NH_2 phase column, $N_{R(TMTU)} = 2434$, $t_{R(TMTU)} = 6$ min, detection limit of thiram, 0.089 mg/kg maize and flow-rate, 0.54 cm^3/min.

It is obvious that the N_R values for adsorption chromatography were higher and the detection limit of thiram was considerably lower, so we adopted the silica gel column for the analysis of maize extracts. However, both columns were convenient for the successful separation and determination of DTCs and their degradation products in an acceptable time.

Determination of ferbam in tobacco

Ferbam is used as an active fungicide in tobacco growing. It was added to tobacco leaf samples as follows: sample 1, no ferbam; sample 2, 0.03 g/kg; and sample 3, 0.02 g/kg. The conditions used were as follows: column, Separon C_{18}; mobile phase, acetonitrile–methanol–water (40:35:25); flow-rate, 0.4 cm^3/min; pressure; 9.5 MPa. The limit of detection of ferbam in tobacco was 0.014 mg/kg.

The determination of ferbam was more complicated owing to its instability[1,2,16]. It was found that two of its degradation products (TMTD and TMTM) were present in all tobacco extracts. For these reasons, the calibration graphs were measured for three standards (ferbam, TMTD and TMTM). TMTU has not been identified in tobacco extracts.

The equations of the straight lines were as follows:

for ferbam:

$$y = 2.7x - 0.12$$
$$r_{x,y} = 0.9996$$

TABLE II

EXTRACTION RECOVERIES OF FERBAM, TMTD AND TMTM FROM TOBACCO

Column: Separon C_{18}, 5 μm (150 × 3.2 mm I.D.). Mobile phase: acetonitrile–methanol–water (40:35:25).

Sample	*Chloroform extract (%)*			*Methanol extract (%)*			*Extraction recovery (%)*	*Standard deviation (%)*
	Ferbam	*TMTD*	*TMTM*	*Ferbam*	*TMTD*	*TMTM*		
1	–	–	–	–	–	–	–	–
2	57.7	15.0	7.3	8.0	1.3	0.4	89.7	2.3
3	50.4	16.4	9.1	9.1	1.5	1.0	87.5	2.8

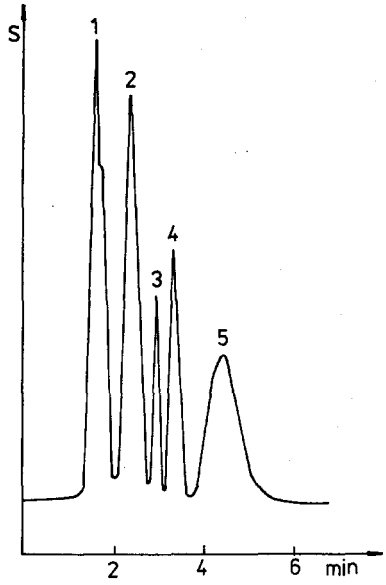

Fig. 3. Chromatogram of tobacco extract in chloforom. Column: Separon C_{18} 5 μm (150 × 3.2 mm; I.D.). Mobile phase: acetonitrile–methanol–water (40:35:25). Flow-rate: 0.42 cm^3/min. Pressure: 9.5 MPa. Peaks 1 and 2 = unidentified; 3 = TMTM; 4 = TMTD; 5 = ferbam.

for TMTD:

$$y = 2.4x - 0.23$$
$$r_{x,y} = 0.9994$$

for TMTM:

$$y = 1.9x - 0.35$$
$$r_{x,y} = 0.9996$$

Extraction recoveries for ferbam and its degradation products in tobacco are given in Table III and the chromatogram of a tobacco extract in chloroform is shown in Fig. 3. The detection limit of ferbam in tobacco was 0.032 mg/kg.

CONCLUSION

The method developed for the determination of dimethyldithiocarbamate fungicides in natural products permits very simple control analyses of low concentrations of DTC residues and their degradation products simultaneously with short separation times (6–10 min). All components were soluble in mobile phases containing chloroform. This fact reduced the detection limits of DTC fungicides published previously. All limits of detection correspond to the criteria of the FAO (*Codex Alimentarius*) concerning methods for the determination of DTC residues in food. The method has

already been accepted for the control analysis of DTC fungicides applied to some agriculatural products in Czechoslovakia.

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