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Microwave-Assisted Extraction (MAE)–Acid Hydrolysis of Dithiocarbamates for Trace Analysis in Tobacco and Peaches

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A simple and rapid method is presented for the analysis of residues of ethylenebis(dithiocarbamate) (maneb, zineb, and mancozeb) and *N*,*N*-dimethyldithiocarbamate (thiram and ziram) fungicides in dry tobacco leaves and peaches. Residues are extracted and hydrolyzed to CS_2 in a single step by use of microwave energy in a closed-vessel system while the evolved CS_2 trapped in a layer of iso-octane overlaying the reaction mixture is taken for gas chromatographic−flame photometric analysis. This combined extraction−hydrolysis step is carried out in 10 and 15 min for sets of 12 samples of tobacco and peach matrices, respectively. Total sample preparation time for GC analysis is 40 min. The limits of detection (LOD) are 0.005 mg/kg for thiram and ziram on peaches and 0.1 mg/kg for maneb, zineb, and mancozeb on tobacco. The respective LOD and limit of quantification (LOQ) levels in CS_2 equivalents are 0.003 and 0.006 mg/kg on peaches and 0.04 and 0.2 mg/kg on tobacco, respectively. Recoveries in the 0.01−60 mg/kg fortification range are 80−100% with respective relative standard deviations $<$20%. The method was used for the analysis of $>$3000 commercial tobacco samples including also different marketed cigarette brands.

KEYWORDS: Dithiocarbamates; microwave-assisted extraction (MAE); tobacco; peaches; trace analysis; gas chromatography

INTRODUCTION

Dithiocarbamates (DTCs) and especially *N*,*N*-dimethyldithiocarbamates (DMDTCs) and ethylenebis(dithiocarbamate)s (EBDCs) are very important protective nonsystemic fungicides of broad-spectrum activity registered for the control of fungal and bacterial pathogens on a large variety of crops such as tomatoes, grapes, apples, peaches, tobacco, lettuce, and ornamentals. In Greece, the most commonly used DTCs on peaches are ziram and thiram (DMDTCs), and on tobacco the EBDCs maneb, zineb, and mancozeb are used.

The analysis of intact DTCs is complex due to their insolubility in most solvents, their ability to form strong complexes with a variety of metal ions, and their instability, which is affected by oxygen, moisture, temperature, pH, and even components of plant juice (*1*−*3*).

Because most of DTCs have been in use in agriculture for more than 60 years, a variety of methods have been developed for the analysis of their residues in different substrates; these methods have been recently reviewed (*4*). Many of these methods are based on acid hydrolysis of DTCs in the presence of stannous chloride, as proposed by Keppel (*5*), and analysis of the evolved CS_2, H_2S, or amines is carried out by different techniques. Many modifications of the original method have been also reported with regard to sample pretreatment, conditions of acid hydrolysis, and trapping and analysis of released CS_2, with the aim of reducing the time and complications of Keppel's method. In some recent methods sample preparation is carried out in closed vials in heated water baths or ovens and CS_2 is analyzed by headspace GC (*6*−*9*); alternatively CS_2 trapped into an organic solvent is analyzed either by GC (*10*) or by flow injection and colorimetric quantification of CS_2 as a copper complex (*11*). However, most of these methods are still time-consuming, include steps that are not entirely controlled, and are not appropriate for all substrates, as is the case with tobacco matrices. In the method described here sample preparation is carried out in less than one-tenth of the time required by existing methods and all operations are carried out under strictly controlled conditions by use of a microwave-assisted digestion/extraction oven.

Microwave ovens commonly used for sample digestion in inorganic elemental analysis have become popular in recent years, and applications of microwave-assisted extraction (MAE) systems, especially in the area of environmental analysis, are increasing rapidly (*12*); however, the main body of applications is devoted so far to the extraction of persistent hydrocarbons from marine sediments, soils, and solid wastes (*13*). Applications of MAE for the extraction of a few chemical groups of pesticides from soil (*14*−*19*) and plant matrices (*20*, *21*) have been also reported. Stability problems under MAE conditions have been already demonstrated for some organophosphorus pesticides (*22*), and certainly the same will be true for many other chemical

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Table 1. Operation Program of MAE

	tobacco	peaches
magnetron power (%)	80	80
max temp (°C)	100	100
max pressure (psi)	90	90
time indispensable to reach the above conditions (min)	7	6.5
time the system remains at above conditions (min)	10	15
no. of vessels	12	12
vol of solvents inside each vessel (mL)	4	40
wt of samples (g)	2	10

groups of pesticides known to be thermally labile. However, for certain cases, as is the case of the DTCs, decomposition under controlled conditions is favored, and thus the MAE-combined hydrolysis step of DTCs was exploited.

The purpose of this study was to investigate the feasibility of using microwave energy for the efficient extraction and simultaneous hydrolysis of weathered residues of DTCs present on/in plant matrices, especially dry tobacco leaves and peaches.

EXPERIMENTAL PROCEDURES

Reagents. Hydrochloric acid, carbon disulfide, iso-octane, sodium hydroxide, and ethylenediaminetetraacetic acid disodium salt (EDTA), all of pro-analysis grade, were purchased from Riedel-de Haen (Seelze-Hannover, Germany). Acetone and pyridine were purchased from Merck (Darmstadt, Germany) and anhydrous stannous chloride ($SnCl_2$) from Sigma (St. Louis, MO).

Analytical Standards. Pesticide analytical standard materials of zineb, mancozeb (purity = 80%), and ziram (purity = 97%) were purchased from Promochem (Augsburg, Germany); maneb and thiram (purity = 95%) were obtained from Chem Service (West Chester, PA).

Solutions. Stannous chloride was used as a solution of 1.5% $SnCl_2$ in 5 N hydrochloric acid. Carbon disulfide solutions were made in iso-octane. Pesticide standard solutions were made as follows: Solutions of maneb and mancozeb, due to their low water solubility, were made in 0.2 M EDTA solution with the pH adjusted to 8 to reduce the rate of hydrolysis; zineb was dissolved in pyridine, and solutions of thiram and ziram were made in acetone. For each pesticide a stock solution of 20 mg/L was made (solution A), which was serially diluted to produce solutions B (2 mg/L) and C (0.2 mg/L). Solutions A−C are the working standard solutions used for the recovery experiments and the construction of analyte calibration curves. The working standard solutions were used freshly made.

Apparatuses and Respective Operational Parameters. The MSP 1000 laboratory microwave system (CEM, Matthews, NC) equipped with a 12-vessel carousel was used for the microwave-assisted single extraction−hydrolysis step. PTFE-lined extraction vessels were used, and during operation both temperature and pressure were monitored in a single vessel; a sensor monitoring the solvent leaks in the interior of the microwave oven was also in use. The operational parameters of the MAE−hydrolysis apparatus are shown in **Table 1**.

Gas chromatography was carried out on a Tremetrics model 9001 (Finnigan Corp./Tremetrics Inc., Austin, TX) gas chromatograph equipped with a flame photometric detector (FPD), operated in the sulfur mode, an on-column injector, and an autosampler, model AS-9000 (Finnigan Mat). Chromatography was carried out on Poraplot Q (i.d. = 0.53 mm, length = 10 m) megabore column obtained from Chrompack BV (Middelburg, The Netherlands). The analytical column was connected to a 1.5 m precolumn of deactivated silica. The carrier gas was helium (99.999% purity) at a constant velocity of 47 cm/s, and the detector gases were set at 150 and 100 mL/min for air and hydrogen, respectively. The injector and detector temperatures were set at 120 and 200 °C, respectively. The column oven was programmed from 40 °C (hold for 1 min at 40 °C) to 100 °C at 7 °C/min (hold for 1 min at 100 °C) and at 30 °C/min to 150 °C (hold for 2 min at 150 °C). Six-microliterμL injections were made. The data were acquired and processed by the LabQuest software (SSI, State College, PA).

Sample Preparation. *Tobacco.* Dry tobacco leaves were coarsely cut by use of a paper shredder. After appropriate mixing by hand to produce a homogeneous bulk sample, subsamples of 2 ± 0.1 g were transferred into the extraction vessels; 10 mL of iso-octane was added in each vessel followed by 30 mL of freshly prepared 1.5% stannous chloride made in 5 N HCl. Each vessel was closed gastight, right after the addition of the hydrolysis solution, and was shaken vigorously by hand for 30 s; sets of 12 vessels were microwave-digested according to the operational program shown in **Table 1**.

Peaches. Peach fruits were homogenized by use of a food processor, and subsamples of 10 ± 0.1 g were transferred into the microwave vessels. These samples were handled as above and processed according to the respective operational conditions shown in **Table 1**.

The vessels removed from the microwave oven were allowed to cool at 38−40 °C (below the boiling point of CS_2) by use of an ice bath. During cooling, the vessels were periodically shaken by hand for 15 s to assist the partitioning of carbon disulfide (CS_2) into the iso-octane layer. After cooling, 1-mL aliquots from the upper iso-octane phase of each vessel were pipetted into the autosampler screw-cap-sealed vials taken for GC analysis.

Preparation of Standard Calibration Curves. In this method, calibration was carried out by hydrolyzing analytical standards instead of using carbon disulfide standard solutions. For each compound, the linearity of the method was evaluated by preparing calibration standards (three replicates) in a wide range of concentrations.

Tobacco. Suitable aliquots of working standard solutions A and B (maneb, zineb, or mancozeb) were pipetted, in triplicate, into separate clean vessels giving rise to a series of solutions containing 0.5, 1.0, 2.0, 4.0, 8.0, 10.0, 20.0, 30.0, 60.0, 80.0, 100.0, and 130.0 μg. These standards were processed as the tobacco samples. A 12-point calibration curve was necessary for the analysis of tobacco due to the wide range of DTC residue levels (0.5−60 mg/kg) usually present in field tobacco samples.

Peaches. For the preparation of calibration curves of thiram and ziram, aliquots from the respective working standard solutions A, B, and C were also transferred, in triplicate, into separate clean vessels giving rise to a series of solutions containing 0.05, 0.1, 0.5, 1, 5, and 10 μg. The rest of the procedure was the same as the one followed for the tobacco samples.

All standard solutions should always be processed by MAE separately from samples.

Qualitative analysis was based on the retention time (7.2 min) of carbon disulfide by injecting carbon disulfide solutions made in iso-octane.

Stability of Analytical Standard Stock Solutions. To determine the stability of the analytical standard solutions, solutions were stored under refrigerated conditions (4−8 °C) for a period of one week and aliquots were analyzed daily. On each day, calibration curves from freshly prepared standard solutions were made.

Preparation of Fortified Samples for the Recovery Studies. *Tobacco.* Recovery studies were carried out at 0.5, 1, 5, 15, 30, and 60 mg/kg fortification levels for maneb and zineb, respectively, and at 0.5, 1, 5, 15, and 45 mg/kg for mancozeb by spiking 2 g of untreated tobacco subsamples with the appropriate volume of solutions A and B. After spiking, samples were handled and processed as described above.

The intra-assay precision (repeatability) was assessed by analyzing on the same day three replicated samples at each fortification level. The inter-assay precision (reproducibility) of the method was checked by analyzing the same commercial samples (unprocessed desiccated tobacco and blend tobacco from marketed cigarette brands) at intervals of 2 weeks; during this period the tobacco samples were stored at ambient temperature.

Peaches. Uncontaminated and freshly blended peach subsamples of 10 g each transferred into the microwave extraction vessels were fortified at 0.01, 0.1, 0.5, and 1 mg/kg level by addition of the appropriate volume from the working standard solution A, B, or C. The following handling and processing of samples was the same as described above for the tobacco samples. Repeatability was assessed

by analyzing on the same day five replicated samples fortified at the same level. For the assessment of the reproducibility of the method, recovery data derived from the analysis of fortified samples prepared at weekly intervals were compared.

Determination of the Detection and Quantification Limits. The limit of detection (LOD, mg/kg) was determined as the lowest concentration giving a response of 3 times the standard deviation of the baseline noise defined from the analysis of three control (untreated) samples. The limit of quantification (LOQ, mg/kg) was determined as the lowest concentration of a given pesticide giving a response that could be quantified with a relative standard deviation (RSD) of <20%.

RESULTS AND DISCUSSION

General Considerations. A series of preliminary experiments were conducted in selecting the optimum operation conditions of the MAE−hydrolysis step (**Table 1**). Many experimental variables such as temperature and duration of the extraction−hydrolysis step, weight of sample, and chemical composition and volume of the extraction−hydrolysis medium needed to be optimized. For this optimization process the factorial design approach has been adopted in some recently reported MAE-based methods (*19*, *21*, *23*, *24*). However, in our case the combined extraction−hydrolysis of DTCs in closed vessels incubated in conventional ovens or sonication baths was in routine use for many years, and thus what was really needed was to adjust and/or modify the previously selected conditions for the MAE operation.

Among the parameters included in **Table 1**, the magnetron power was set arbitrarily at 80% and the 90 psi maximum pressure was selected to avoid rupturing of the safety membranes (maximum resistance = 100 psi) of the MAE vessels during operation, whereas the 100 °C maximum temperature was a consequence of the 90 psi maximum pressure cutoff. When tobacco samples were processed, the internal pressure of the MAE vessels increased rapidly, and in <6 min the limit of 90 psi was reached; this was not the case when standard solutions or peach samples were processed. In the latter case, the maximum pressure attained in the MAE vessels was ~30 psi. Therefore, upper limit set points of 100 °C and 90 psi were selected, and the MAE extraction conditions were limited by whichever set point was reached first.

The sample weight, 2 g for tobacco and 10 g for peaches, was chosen on the basis of preliminary experiments and considering mainly technical problems such as adequacy of sample dispersion, mixing, and moistening, especially for tobacco, in 100-mL cavity vessels. The composition of the extraction−hydrolysis medium (1.5% $SnCl_2$ in 5 N HCl) is the same as the one previously used in closed vessel hydrolysis systems of DTCs incubated in conventional ovens, and this has approximately the same composition as the one used in methods published in the literature (*5*, *6*, *9*, *10*). However, preliminary experiments were carried out with the $SnCl_2$ content ranging from 1 to 5% in 1−10 N HCl solutions to confirm the suitability of this medium under MAE conditions. Also, the addition of L-cysteine, reported to facilitate the extraction of dithiocarbamic anions from plant tissues (*25*), was evaluated with no remarkable changes in the accuracy (improvement of percent recoveries) or the reproducibility of the method when applied to tobacco samples (data are not shown).

The volume of the extraction−hydrolysis medium and the duration of MAE processing, when all other parameters are set as selected above, were the main parameters extensively studied and optimized. Dry tobacco (coarsely cut or ground) floats on the surface of the digestion mixture, forming a layer in the interface of the lower aqueous and the upper iso-octane phase.

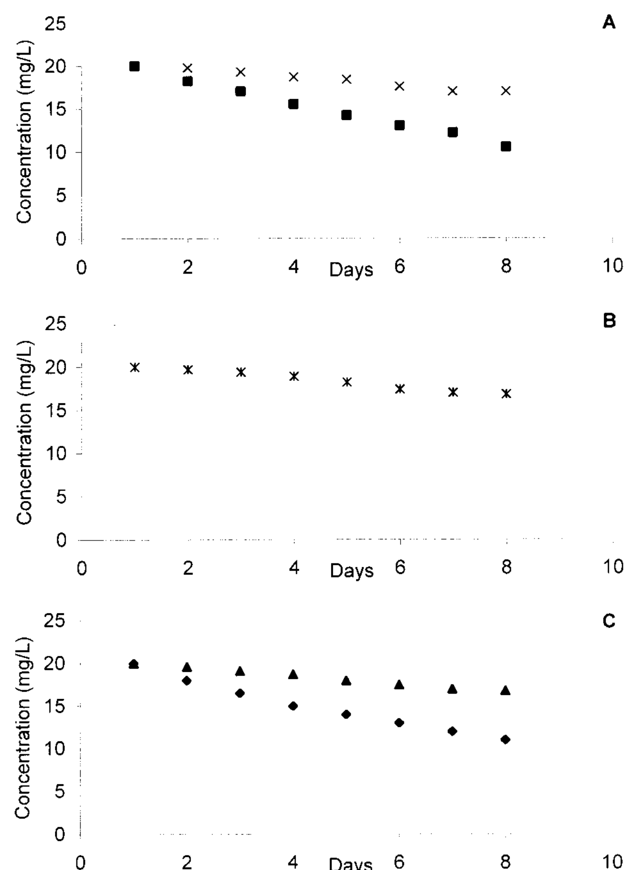

Figure 1. Stability of working standard solutions when stored under refrigerated conditions: (A) mancozeb (■) and ziram (×); (B) thiram (∗); (C) maneb (◆) and zineb (▲).

Thus, the absence of precautions to assist the rapid moistening of plant tissues will result in incomplete and variable extraction−hydrolysis of DTCs. Therefore, the vigorous manual shaking of closed vessels before they are placed onto the MAE carousel is recommended. However, additional measures were also necessary. In preliminary experiments, tobacco tissues transferred into the extraction vessels were moistened first with 10 mL of water and allowed to equilibrate for 10 min before the addition of the processing solvents. This measure improved the accuracy of the method; however, the reproducibility was still a major drawback. The problem was highly diminished when the volume of the extraction−hydrolysis medium was set at 30 mL (data are not shown). It is suspected that with such a volume a slight swirling is created, which assists in a more efficient moistening and full submergence of plant tissues into the aqueous phase; however, the plant material is still floating in the upper zone of the aqueous phase. At lower or higher volumes of the aqueous phase, recoveries were generally decreased. This problem is expected to be alleviated by use in each vessel of a sample stirring option, which is now available in recent models of MAE ovens.

Iso-octane was selected as the CS_2 trapping solvent because it is not miscible with water and has a low dielectric constant, and thus the evolved CS_2 is trapped in a colder solvent and it does not interfere with the chromatographic analysis of CS_2. Hexane and heptane were also evaluated, but both solvents interfered with the chromatographic analysis of CS_2 (data not shown). A minimum volume of 10 mL of the iso-octane phase was found to be necessary to quantitatively trap the evolved CS_2 and, on the other hand, overlay effectively the tobacco tissues; otherwise, electrical sparks are observed, which slowly

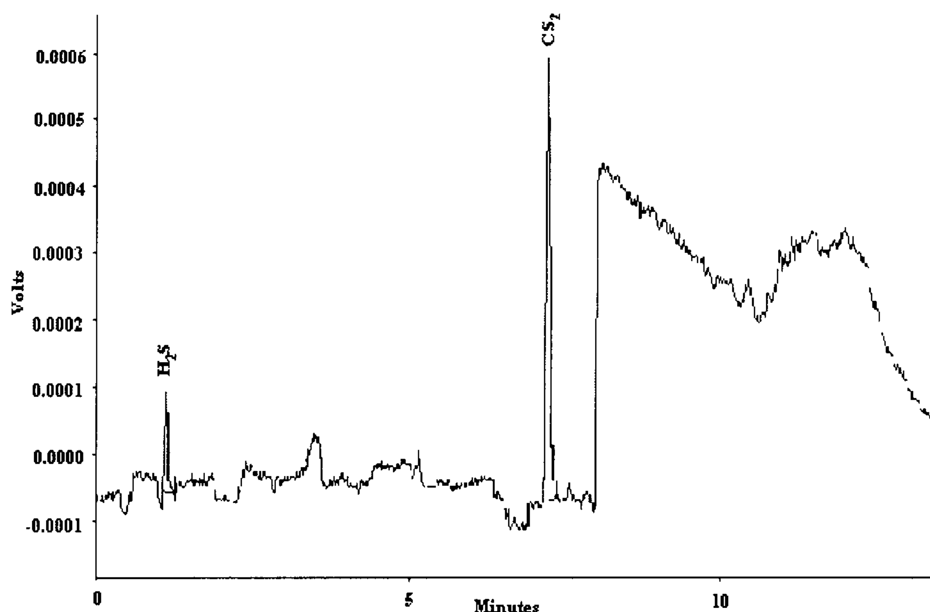

Figure 2. Sample chromatogram from the analysis of a tobacco sample fortified with mancozeb at 0.5 mg/kg. Conditions of analysis are those given under Experimental Procedures. Carbon disulfide is eluted with a retention time of 7.2 min and hydrogen sulfide at 1.1 min.

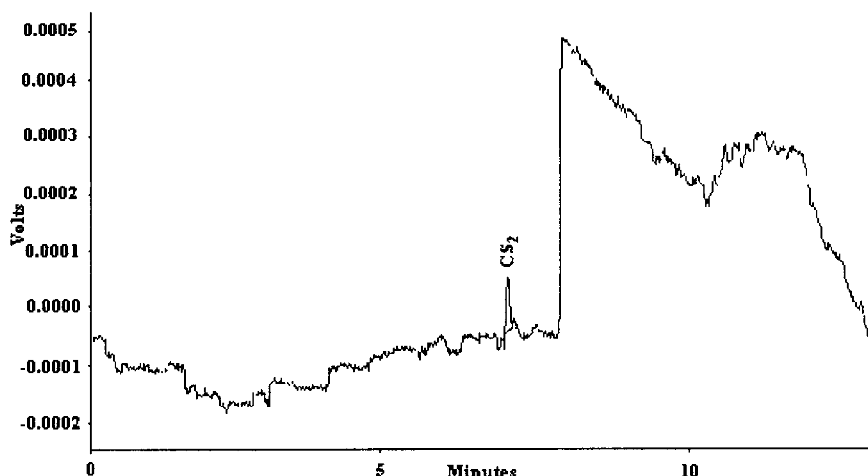

Figure 3. Sample chromatogram from the analysis of a peach sample fortified with thiram at 0.005 mg/kg (LOD level). Conditions of analysis are those given under Experimental Procedures. Carbon disulfide is eluted with a retention time of 7.2 min.

deteriorate the extraction vessels. Higher volumes of iso-octane up to 30 mL did not affect the performance of the method when both tobacco and peach samples were processed.

Stability of Sought Analytes in Solution. It is well-known that DTCs are not stable in solution (*1*), and thus the stability of stock and working standard solutions was evaluated. The data of this exercise are shown in **Figure 1**. From the storage stability test (4−8 °C) it was concluded that mancozeb and maneb (**Figure 1A,C**) decomposed at a rate of 8% per day, and only 50−55% of the initial concentration was recovered after 1 week of storage. On the other hand, solutions of ziram, thiram, and zineb (**Figure 1A−C**) were more stable, and 85% of the initial concentrations was recovered after 1 week of storage. Nevertheless, it is recommended that for all tested compounds fresh stock and working standard solutions be made daily for the daily update of calibration curves and recovery studies.

Method Validation and Performance. Sample chromatograms from the analysis of tobacco and peach samples are shown in **Figures 2** and **3**, respectively. The tobacco sample was fortified with mancozeb at the 0.5 mg/kg level and the peach sample with thiram at the 0.005 mg/kg level, the latter being the LOD level for the analysis of DTCs in peach tissues. In some chromatograms of tobacco samples hydrogen sulfide was also present, eluting with a retention time of 1.1 min (**Figure 2**). In both chromatograms shown in **Figures 2** and **3** there is an abrupt increase of signal response at 8.8 min and a concomitant rise of the chromatographic baseline, which coincides with the elution of iso-octane. Chromatographic conditions were selected as such to increase the time lapse between elution of CS_2 and iso-octane and avoid any distorting effect of iso-octane elution on the symmetry of the CS_2 peak.

There were no matrix peaks in the chromatograms shown in **Figures 2** and **3** to interfere with the analysis of CS_2. Furthermore, the retention time of CS_2 was relatively constant at 7.2 ± 0.3 min. Even so, cleaning the column at slightly elevated temperatures after each run was found to be imperative to maintain the chromatographic efficiency of the GC analytical column and extend its effective life span.

Calibration curves of the sought analytes are shown in **Figure 4** for the ethylenebis(dithiocarbamate)s and in **Figure 5** for the *N*,*N*-dimehyldithiocarbamates. Calibration curves for all tested compounds and at all concentration ranges were better described

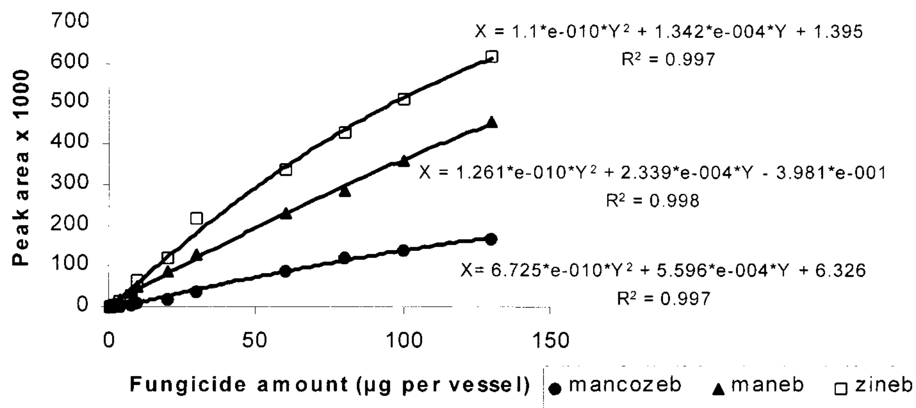

Figure 4. Calibration curves of maneb, zineb, and mancozeb.

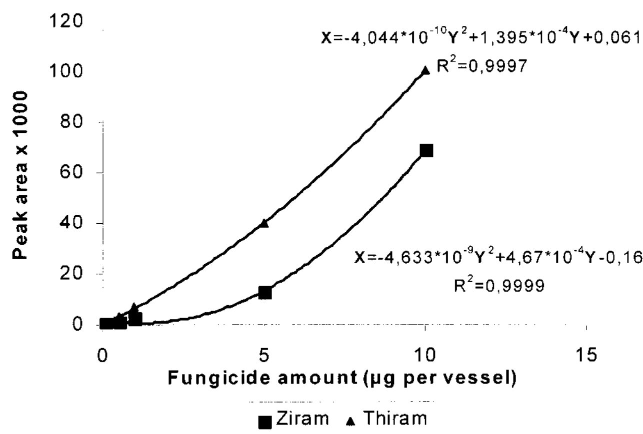

Figure 5. Calibration curves of thiram and ziram.

by quadratic equations with correlation coefficients >0.99. Good linear correlation coefficients ($r^2 = 0.999$) between concentrations (X) and peak areas (Y) were also found at narrower concentration ranges. The slopes of the calibration curves of the tested compounds differ from each other, and this is obviously due to the variable amount of CS_2 equivalents present per unit weight of each compound.

For tobacco, the percent mean recovery values ± relative standard deviations (RSDs) of DTCs in the fortification range from 0.5 to 60 mg/kg are shown in **Table 2**. These recovery values are in the range of 81−100% for all of the compounds and for most fortification levels. In a few cases mean recovery values >100% were found, especially for the lower fortification levels, due to the presence of DTCs in most available tobacco samples used for the recovery studies. During routine application of the proposed method for the analysis of DTCs in tobacco samples, the daily validation of the method at only three fortification levels (0.5, 15, and 30 mg/kg) is more than adequate to ensure the quality and accuracy of the derived analytical data.

The recovery data for peaches are shown in **Table 3**. Recoveries of both ziram and thiram were >89%. For peaches, the method was validated for a narrower fortification range (0.01−1 mg/kg) because this was the range of residues most commonly found in peaches.

The intraday (repeatability) precision of the method is satisfactory (RSDs < 20% for tobacco, **Table 2**, and <13% for peaches, **Table 3**, for all compounds at all fortification levels). The interday (reproducibility) precision is also acceptable [relative percent difference (RPD) <22% for tobacco and <18% for peaches, **Tables 2** and **3**].

Table 2. Recovery of the Four Dithiocarbamates Tested from Fortified Tobacco ($n = 3$)

compound	fortification level (mg/kg)	mean recovery (%) (RSD, %)
maneb	0.5	100 (20.0)
	1	95 (19.5)
	5	93 (15.0)
	15	103 (1.7)
	30	101 (0.4)
	60	102 (1.0)
zineb	0.5	142 (12.0)
	1	100 (20.0)
	5	87 (12.7)
	15	89 (11.8)
	30	92 (3.9)
	60	91 (3.0)
mancozeb	0.5	93 (12.4)
	1	100 (20.0)
	5	81 (5.2)
	15	85 (7.8)
	45	90 (0.6)
thiram	0.5	87 (13.3)
	1	113 (5.0)
	5	85 (5.9)
	15	88 (1.3)
	30	101 (3.8)
	60	106 (1.1)

Table 3. Results of Intraday (Repeatability) and Interday (Reproducibility) Assay of Precision at Four Levels of Concentrations Tested for Ziram and Thiram in Peaches

concn	first analysis recoveries ($n = 5$) av (RSD, %)		second analysis recoveries ($n = 5$) av (RSD, %)		reproducibility ($n = 2$) av (RPD, %)	
(mg/kg)	ziram	thiram	ziram	thiram	ziram	thiram
0.01	104 (5.3)	92 (11.9)	98 (8.5)	94 (12.2)	101 (11.9)	93 (4.3)
0.1	89 (8.8)	101 (3.7)	86 (5.3)	102 (4.3)	88 (5.5)	102 (1.6)
0.5	94 (8.7)	90 (11.2)	93 (8.2)	98 (5.1)	93 (2.8)	94 (17.9)
1	100 (1.5)	100 (2.2)	97 (2.2)	100 (1.8)	98 (4.1)	100 (0.8)

For tobacco samples the LOD and LOQ levels were set at 0.1 and 0.5 mg/kg, respectively, for all DTCs tested corresponding to 0.04 and 0.2 mg/kg of CS_2 equivalents. For peaches the LOD and LOQ levels of both ziram and thiram were set at 0.005 and 0.01 mg/kg, respectively, corresponding to 0.003 and 0.006 mg/kg CS_2 equivalents. These LOD and LOQ values represent an improvement compared to respective levels reported in other methods (0.16−1.2 mg/kg). Furthermore, the criteria and definitions of LOD and LOQ levels are not always clearly defined in the literature and, therefore, no direct comparisons can be made.

Table 4. Results of the Validation Procedure of the Analysis of Different Marketed Cigarette Brands and Field-Treated Unprocessed Tobacco Samples (Different Concentrations) Expressed as Carbon Disulfide

	repeatability ($n = 3$)		
code	first analysis, mean concn (mg/kg) ± SD	second analysis (a week later), mean concn (mg/kg) ± SD	reproducibility ($n = 2$) mean (RPD, %)
A	0.9 ± 0.1	0.8 ± 0.0	0.9 (11.8)
B	1.0 ± 0.1	1.0 ± 0.1	1.0 (0.0)
C	3.5 ± 1.0	4.1 ± 0.9	3.8 (15.8)
D	0.6 ± 0.3	0.7 ± 0.1	0.7 (15.4)
E	0.8 ± 0.2	0.9 ± 0.1	0.9 (11.8)
F	0.8 ± 0.1	0.9 ± 0.1	0.9 (11.8)
G	0.5 ± 0.1	0.5 ± 0.1	0.5 (0.0)
H	0.9 ± 0.0	0.9 ± 0.0	0.9 (0.0)
I	0.2 ± 0.0	0.1 ± 0.1	0.2 (66.7)
J	0.6 ± 0.3	0.6 ± 0.1	0.6 (0.0)
K	3.3 ± 0.7	3.5 ± 0.2	3.4 (5.9)
L	45.7 ± 3.5	42.9 ± 3.2	44.3 (6.3)

For peaches, the repeatability, reproducibility, and sensitivity of the method are good enough to ensure a reliable determination of both ziram and thiram at levels 100-fold lower than the respective maximum residue levels (MRLs) set at 3 mg/kg. For tobacco, there are no internationally established MRLs. Nevertheless, nowadays pesticide residues are extensively monitored in tobacco, and there is an increasing demand for accurate, reproducible, and sensitive methods for their analysis.

The main drawback of this method is its lack of specificity to distinguish DTCs from one another. This is a common characteristic of all methods based on initial hydrolysis of DTCs and quantification of hydrolysis products (*2*, *5*−*11*).

The proposed method has been in use in our laboratory for the past 5 years, and during this period >3000 tobacco samples field-treated with mancozeb including also tobacco from commercial cigarette brands were analyzed. Sample data from this work are shown in **Table 4**. The residue levels found in these samples ranged from 0.2 to 3.6 mg of CS_2/kg. However, occasionally residue levels >45 mg of CS_2/kg were also found. Residues of DTCs are very stable on the surface of tobacco leaves, and this is also evident from the data shown in **Table 4**; the residue levels in this set of samples remained almost unchanged during a week of sample storage at ambient temperature, and the small changes found are within the experimental error of the analytical method. However, DTC residue levels dissipate rapidly during the subsequent tobacco fermentation and other processing because residues of DTCs >1 mg of CS_2/kg were never found in commercial cigarette brands.

DTCs were also found to be relatively stable on the surface of peaches. Peaches sprayed with DTCs at the early stage of development (in late April) contained at harvest residue levels ranging from 0.1 to 0.01 mg of CS_2/kg (based on the whole fruit weight).

It is concluded that the presented method is well validated for the analysis of DTCs in tobacco and peaches and that the method as it stands or slightly modified can be used for the analysis of DTCs in a variety of other crops.

SAFETY PRECAUTIONS

Due to the handling of strong acid solutions in this method and the fact that some of the DTCs are suspected carcinogens, all operations should be performed in a well-ventilated fume hood with the analyst wearing protective gloves.

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