

Rapid and Simple Method for Determination of Thiram in Fruits and Vegetables with High-Performance Liquid Chromatography with Ultraviolet Detection

Susanne B. Ekroth,* Birgit Ohlin, and Bengt-Göran Österdahl

Chemistry Division 1, National Food Administration, Box 622, SE-751 26 Uppsala, Sweden

A rapid and sensitive HPLC-UV method was developed for determination of the dithiocarbamate thiram in fruit and vegetables. The method was based on extraction with a mixture of ethyl acetate and cyclohexane in an ultrasonic bath for 15 min. The analysis was done on an Apex II diol column in a straight (normal) phase HPLC system with UV detection. The average recoveries for spiked samples, 0.1–1.3 mg/kg, of apple, cucumber, lettuce, nectarine, plum, pear, and grapes were in the range of 73–86% with a relative standard deviation of 3.1–18%. Lower recoveries, 40–66%, were obtained for tomatoes, green pepper, raspberries, and strawberries, which might be explained by an enzymatic degradation of thiram. Limit of quantification was found to be 0.1–0.2 mg/kg for all tested matrices.

Keywords: *Thiram; HPLC-UV; fruits; vegetables; degradation*

INTRODUCTION

The fungicide thiram is widely used in the world on a large variety of crops such as apples, pears, strawberries, tomatoes, grapes, peaches, and lettuces. The methods in use today, although promising, are either time-consuming (Baumann et al., 1991), involving many steps in sample treatment and different reagents, or limited to one matrix, for example, apple (Baumann et al., 1991), lettuce (Meloni et al., 1984), strawberry, and maize (Brandsteterová et al., 1986). It is of high priority to find a suitable method for routine control that will be applicable to both acidic and neutral products. Because the juices from fruits and vegetables rapidly decompose thiram (Vuik et al., 1992; Ministry of Welfare, Health and Cultural Affairs, 1988), it is important to chop the sample as coarsely and quickly as possible before one proceeds with extraction. Thiram is then likely to be found only on the surface of the crop. The maximum residue limit (MRL) for the group of dithiocarbamates is the sum of dimethyl-, ethylenebis- and propylenebisdithiocarbamate, calculated and expressed as CS₂ (Table 1). It is hoped that soon new MRLs for thiram will be available, presumably at lower levels. The European Union has MRLs for thiram of 3.8 mg/kg for strawberries and grapes and 3.0 mg/kg for other fruits and vegetables.

The aim of this work was to develop a simple analytical method for thiram. The second aim was to improve and simplify current methods and validate the new version for as many matrices as possible because the yield is very dependent upon the choice of crop. It is then to be used as a routine method in the Swedish monitoring program. The method was finally tested on sprayed green pepper from Almeria, Spain.

* Author to whom correspondence should be addressed (telephone +46 18 17 56 46; fax + 46 18 10 58 48; e-mail suek@slv.se).

Table 1. MRL According to SLV FS 1996:16 (Sweden), Where 1 mg of CS₂ = 1.58 mg of Thiram/kg

matrix	MRL as CS ₂	MRL as thiram
apple	1	1.6
cucumber	0.5	0.8
strawberry	2	3.2
lettuce	5	7.9
nectarine	2	3.2
plum	1	1.6
pear	1	1.6
tomato	1	1.6
grape	2	3.2
green pepper	2	3.2
raspberry	1	1.6

EXPERIMENTAL PROCEDURES

Chemicals used included thiram (Dr. Ehrenstorfer, 99.9%), ethyl acetate (pesticide grade), cyclohexane (HPLC grade), methanol (gradient grade), 2-propanol (HPLC grade), ammonia (reagent grade), and sodium sulfate, anhydrous (gradient grade).

Standard. A stock solution of thiram with a concentration of ~400 µg/mL was made in a mixture of ethyl acetate and cyclohexane (1 + 3, v/v). The stock solution is stable for at least 4 or 5 months when stored at 4 °C.

Apparatus. A liquid chromatograph for gradient programming with three channels, a Hewlett-Packard 1090, equipped with a diode array detector (DAD), was used. An ultrasonic bath, type Bandelin Sonorex, RK 100, was also used.

Chromatographic Conditions. An Apex II diol column (Jones Chromatography), 3 µm, 150 × 4.6 mm, was used. The mobile phase was (A) cyclohexane, (B) cyclohexane/2-propanol/methanol/1 drop (10 µL) of 25% ammonia per 250 mL of solution (80 + 15 + 5 + 0.01, v/v/v/v), and (C) 2-propanol.

The gradient program started with 95% A and 5% B and changed to 54% A, 20% B, and 26% C after 18 min. The injection volume was 15 µL. The retention time of thiram was 10 min, and the total time was 40 min with a flow rate of 0.65 mL/min. The UV detection was performed at 225, 240, 283, and 300 nm.

Sample Preparation. Two kinds of samples were distinguished: small units such as strawberries, raspberries, and

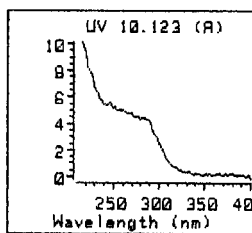
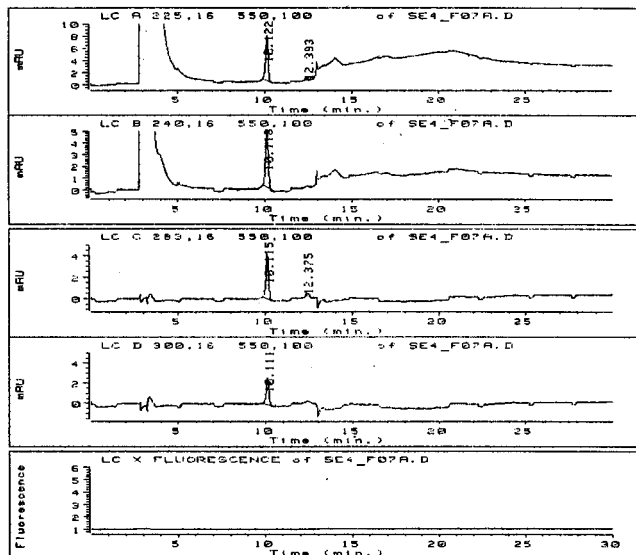


Figure 1. Chromatogram of thiram with four wavelengths and spectra in standard solution (2 µg/mL).

grapes and bigger units such as apples, plums, peppers, cucumbers, tomatoes, and lettuce. It is very important to cut the crop as little and as representatively as possible. At least five units were cut into four equal parts. Crops such as lettuce were cut into smaller pieces so that all of the sample was covered by the extraction liquid.

Extraction Procedure. For smaller units 300 g was analyzed and for larger units 175–200 g. The sample was weighed into a 1 L glass flask with a screw cap and extracted with 300 mL of spiked ethyl acetate/cyclohexane (1 + 3, v/v) for 15 min in an ultrasonic bath. The sample was filtrated through 20 g of sodium sulfate, and 10 g of sodium sulfate was added to the filtrate. After 3 min, 50.0 mL (small units) or 85.0 mL (large units) of the extract was decanted and concentrated to ~2 mL on Rotavapor with a water bath set to 37 °C. The volume was adjusted to 20.0 mL (small units) or 10.0 mL (large units) with ethyl acetate/cyclohexane (1 + 3, v/v). The extract was filtrated through a syringe with a Teflon filter (0.45 µm). An aliquot of 15 µL was then injected into the column.

RESULTS AND DISCUSSION

One problem was how to perform an extraction applicable to all products. Two different sample pre-treatments were done, but the extraction procedure was the same for both. It is very important that when chopping is necessary, a representative part is taken.

Because thiram does not penetrate the exterior, the extraction was done on the surface, without homogenizing. Normally, the samples are spiked prior to the extraction, but because the juices from fruits and vegetables rapidly decompose thiram, recoveries were calculated by spiking the extraction solvent.

The chromatograms, seen in Figure 1 show the selectivity of thiram when four different wavelengths are used combined with retention time and spectra. A

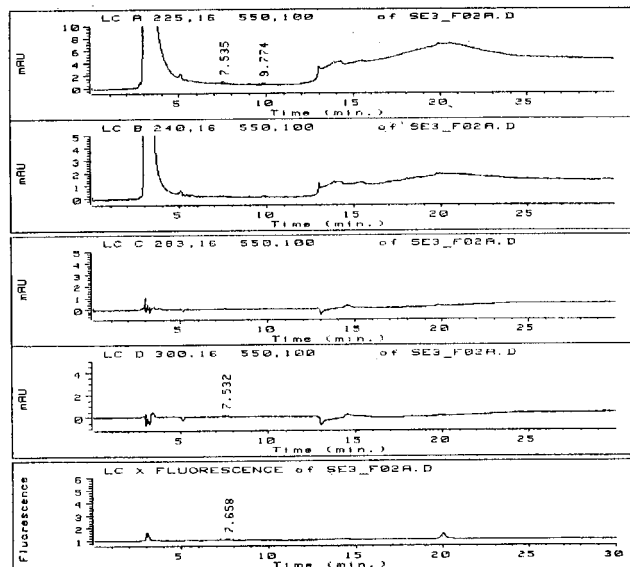


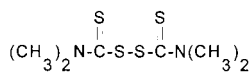
Figure 2. Blank sample of cucumber extract (5 g of sample/mL).

Table 2. Recovery of Thiram from Fortified Fruits and Vegetables

matrix	fortifn level (mg/kg)	no. of determinations	recovery (%)	mean ± RSD (%)	LOQ (mg/kg)
apple	0.1	4	87, 82, 75, 91	84 ± 8	0.1
	0.2	4	94, 92, 94, 87	92 ± 4	0.1
	0.4–0.6	4	36, 103, 111, 81	83 ± 41	0.1
	1.0	4	84, 80, 71, 78	78 ± 7	0.1
	0.7–1.3	4	79, 94, 96, 95	91 ± 9	0.1
cucumber	0.2	4	91, 82, 87, 84	86 ± 5	0.2
	0.3	4	73, 73, 73, 69	72 ± 3	0.2
	0.7	2	87, 83	85 ± 3	0.2
strawberry	1.0	4	86, 85, 88, 87	87 ± 1	0.2
	0.2	4	54, 56, 50, 52	53 ± 5	0.2
lettuce	1.0	4	80, 76, 88, 76	80 ± 7	0.2
	0.2	4	72, 69, 78, 73	73 ± 5	0.2
nectarine	1.0	4	86, 84, 84, 82	84 ± 2	0.2
plum	0.2	4	66, 77, 74, 74	73 ± 6	0.2
pear	0.2	4	77, 87, 80, 75	80 ± 7	0.2
tomato	0.2	4	81, 83, 107, 70	85 ± 18	0.2
grape	0.2	4	52, 47, 51, 78	57 ± 25	0.2
green pepper	0.2	3	87, 87, 82	85 ± 3	0.2
raspberry	0.2	3	56, 58, 59	58 ± 3	0.2
	0.2	1	40	40	0.2

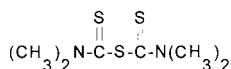
fifth chromatogram at the bottom shows fluorescence, which thiram does not have. The chromatograms also illustrate how efficient this rapid method is without a cleanup step (see blank sample in Figure 2).

As seen in Table 2, despite spiking at low levels, this work has given high recoveries (80–86%) with an acceptable relative standard deviation (RSD; 3.1–18%). Some crops show lower recovery (40–78%), but still with an acceptable standard deviation (2.5–25%) for most matrices. The low recoveries of thiram are likely to be explained by an enzymatic degradation when the crop is cut into pieces. In most cases, low recoveries depend on the type of crop, for example, strawberries, raspberries, tomatoes, and green peppers. To remove this source of error, it is important not only to chop coarsely but also to limit the time in sample treatment and extraction procedure to as short as possible. This method gives a high sensitivity, and the limit of quantification (LOQ) was found to be 0.1–0.2 mg/kg on all tested matrices, which is significantly lower than the



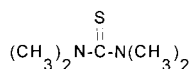
Thiram: tetramethylthiuram disulfide (TMTD)

[137-26-8]



Tetramethylthiuram monosulfide (TMTM)

[97-74-5]



Tetramethylthiourea (TMTU)

[2782-91-4]

Figure 3. Structures and CAS Registry Numbers (provided by the authors) of thiram and its two possible degradation products.

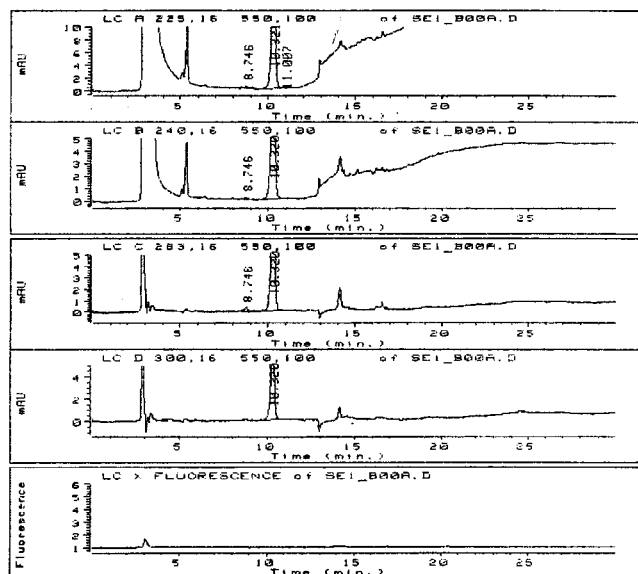


Figure 4. Treated Spanish green pepper (5.4 g of sample/mL).

MRL (see Table 1). LOQ is based on recoveries and RSD (see Table 2).

It would be interesting to make further investigation to determine if the loss of yield can be explained by the formation of the two degradation products, tetramethylthiuram monosulfide (TMTM) and tetramethylthiourea (TMTU), of thiram (TMTD). Both can be detected in more concentrated standard solutions ($>40 \mu\text{g/mL}$) at a rate of ~ 0.2 – 3.4% of the signal of thiram. The structures of thiram and its two possible degradation products (Brandšteterová et al., 1986) are shown in Figure 3.

The optimal time for extraction of thiram residues in treated (grown and sprayed with thiram in a greenhouse, exclusively for this research) green pepper (Table 3) was determined to 15 min. The values are based on

Table 3. Extraction Time versus Concentration in Treated Green Pepper^a

time (min)	concn (mg of thiram/kg)	time (min)	concn (mg of thiram/kg)
5	0.7	45	0.8
15	1.0	90	0.6
30	0.9		

^a Green peppers treated in a greenhouse were used to optimize the time for extraction in an ultrasound bath.

a single analysis because of the restricted access of incurred samples of green pepper. It is very important that the stalk of the green pepper is properly removed before sample treatment. An analysis of only one stalk showed a concentration of 23 mg of thiram/kg compared to 1.0 mg of thiram/kg in the green pepper fruit (Figure 4).

The HPLC gradient in this method has been adapted to suit the multiresidue method used in the Swedish pesticide monitoring program.

Linearity Curve. Linearity curves were constructed by plotting standard peak areas versus concentrations both in ethyl acetate/cyclohexane (1 + 3, v/v) and in spiked matrix. Good linearity was found in the regular standard solution at 0.3–39 $\mu\text{g/mL}$ and in matrix at 0.1–1.3 mg/kg. The equation of the curve in standard solution at 225 nm was $y = 52x - 21$ with $r^2 = 0.9995$ and in matrix at 225 nm, $y = 224x - 1$ with $r^2 = 0.9958$.

Summary. This method is unique because it covers a number of matrices. It is rapid and simple, and the measurement is done on thiram directly instead of the group of dithiocarbamates.

ACKNOWLEDGMENT

We thank Prof. Antonio Valverde, University of Almeria, Spain, for growing and sending the sprayed vegetables.

LITERATURE CITED

- Baumann, R. A.; Dijkman, E.; Hogendoorn, E. A.; van Zoonen, P. Comparison of two RPLC-methods for the residue determination of thiram in apple. *Med. Fac. Landbouww. Rijksuniv. Gent*. **1991**, 56/3a, 941–948.
- Brandšteterová, E.; Lehotay, J.; Liška, O.; Garaj, J. High-performance liquid chromatographic determination of dimethyldithiocarbamate residues in some agricultural products. *J. Chromatogr.* **1986**, 354, 375–381.
- Meloni, M.; Pirisi, F. M.; Cabras, P.; Cabitza, F. Residues of Fungicides on Greenhouse Lettuce. *J. Agric. Food Chem.* **1984**, 32, 183–185.
- Ministry of Welfare, Health and Cultural Affairs. Dithiocarbamates. *Analytical Methods for Residues of Pesticides*, 5th ed.; Rijswijk, The Netherlands, 1988; Part II, Special Methods.
- Vuik, J.; van Dinter R.; de Vos, R. H. Improved sample pretreatment of the carbon disulfide evolution method for the determination of dithiocarbamate residues in lettuce. *J. Agric. Food Chem.* **1992**, 40, 4.

Received for review August 14, 1998. Accepted September 10, 1998.

JF9809100