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Determination of Methomyl and Methomyl-Oxime in Fruit Crops and Water by HPLC

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Bestimmung von Methomyl und Methomyl-oxim in Obst und Wasser mit Hilfe der HPLC

Zusammenfassung. Es wird eine hochleistungs-flüssigkeits-chromatographische Methode zur quantitativen Bestimmung der Methomylrückstände in Obst und Wasser beschrieben, mit der noch Rückstandsmengen von 1 ng/g (ppb) erfaßbar sind. Das Verfahren besteht aus einem Extraktionsschritt, Aufreinigungsschritt, der Trennung der Rückstände auf einer Umkehrphase (RP-C_{18}) und der UV-Detektion. Die Methode ermöglicht die gleichzeitige Bestimmung von Methomyl und seinem Hydrolyseprodukt (Methomyl-oxim) durch die Anwendung des UV-Detektors allein oder durch zusätzliche elektrochemische Detektion. Durch diese Schaltanordnung wurden Konzentrationen von 1 ng/ml Methomyl und 100 pg/ml (ppt) Methomyl-oxim in Wasser bestimmt. Die Methode gestattet im Bereich von 0,01 – 1 µg/g eine Wiederfindungsrate von 101,4 ± 6% in Obst (Äpfel) und 93,7 ± 4% in Wasser.

Summary. A simple and specific quantitative HPLC method for the analysis of methomyl residues in fruit crops and water is described. The method is based on simple extraction steps, high-performance liquid chromatographic separation using a reversed phase column (RP-C_{18}) and UV detection. With this analytical system, methomyl residues at 1 ng/g level can be detected. This approach allows also the simultaneous determination of methomyl and its degradation product methomyl-oxime. This is possible either by using the UV detector at 233 nm or by using both the UV- and the electrochemical detectors in series. Using this combined detection system, it was possible to determine methomyl at the 1 ng/ml level and methomyl-oxime at the 100 pg/ml level in water. Recovery rate in the range of 0.01 – 1 µg/g is 101.4 ± 6% in fruit crops (apples) and 93.7 ± 4% in water.

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Key words: Best. von Methomyl, Methomyl-oxim in Obst und Wasser; Chromatographie, HPLC; elektrochem. Detektor

Introduction

The use of N-methylcarbamate insecticides in agriculture increases rapidly, replacing the more environmentally stable aromatic-halogen insecticides. Carbamate insecticides have a broad spectrum of activity, effectivity and, generally, have a low mammalian toxicity.

Methomyl, S-methyl-N-[(methylcarbamoyl)-oxy]-thioacetimidate, is applicable to a wide range of crops and controls a broad spectrum of insects when used as a foliar spray.

Different methods have been described for the separation and determination of methomyl in vegetable crops [1 – 3, 6 – 8], soil [2, 5, 7], water [5], tissues [7] and food [9].

The modes of separation and detection include thin-layer chromatography (TLC) [8, 9], gas chromatography (GC) using the alkali-flame ionization detector [1], gas liquid chromatography (GLC) after the hydrolysis of methomyl to the corresponding oxime (methyl-N-hydroxythioacetimidate) [7], colorimetry after hydrolysis [2] and high-performance liquid chromatography (HPLC) [3 – 6].

The method of Ogata et al. [1] has the disadvantage that after each sample injection intervals of 30 – 40 min are necessary to retain the original detector sensitivity. Other methods are either too time-consuming for routine work [2, 3, 5, 7] or not sensitive enough for residue analysis [2, 6] or give a low recovery [6].

The simple and specific method described below is based on the modification of the extraction method of Pease and Kirkland [7], separation on a reversed phase

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high-performance liquid chromatographic column and the detection using UV- and electrochemical detectors.

We determined as much as 1 ng/ml (ppb) methomyl in water and 10 ng/g in apple and as much as 5 ng/g methomyl-oxime in both water and apple by the combination of a reversed phase column (RP-C_{18}) with UV detection (233 nm). With this HPLC system using the electrochemical detector (1.30 V), we could determine as much as 100 pg/ml (ppt) methomyl-oxime in water.

Experimental

Reagents

a) n-Hexane, ethyl acetate, acetonitril and methylene chloride all p.a. quality were purchased from Merck-Darmstadt.

b) Bidistilled water used in this method was purified once more prior to analysis on a RP-C_8 column (∅ 40 – 63 µm).

c) The mobile phase was made by adding 200 ml of acetonitril to 800 ml of purified water. For the electrochemical detection, 1.6 g of $LiClO_4$ was dissolved in 1 L of eluent. The eluent was degassed daily and prior to use in a sonic bath.

d) Methomyl standard solution: 10 mg of methomyl was dissolved in 100 ml of methanol (100 µg/ml) and stored in a refrigerator. Working standards were prepared by suitable dilution with the eluent.

e) Methomyl-oxime solution: 10 mg of oxime was dissolved in 100 ml of methanol (100 µg/ml) and stored in a refrigerator. Working standards were prepared by suitable dilution with the eluent.

Standard Curve for Methomyl

The average reproducibility of injection quantities from 3 – 200 ng (constant injected volumes of 10 µl) was 3.8 % relative standard deviation for methomyl. The calibration curve is linear up to 300 – 400 ng and passes through the origin. Detection limits were approximately 1 – 2 ng per injection taken as a signal/noise ratio (Fig. 1 a).

Standard Curve for Methomyl-Oxime

The average reproducibility of injection quantities from 5 – 500 ng (constant injected volumes of 10 µl) was 3.25 % relative standard deviation with the UV detector and 4.1 % relative standard deviation with the electrochemical detector. Calibration curves are linear up to 800 – 1,000 ng and pass through the origin. Detection limits were approximately 2 ng with the UV detector (Fig. 1 b) and 100 pg with the electrochemical detector (Fig. 1 d, e).

General Apparatus

a) Homogenizer jar model MX 32 (Braun AG, Frankfurt).

b) Ultra-Turrax homogenizer model 18/10 (Janke & Kunkel).

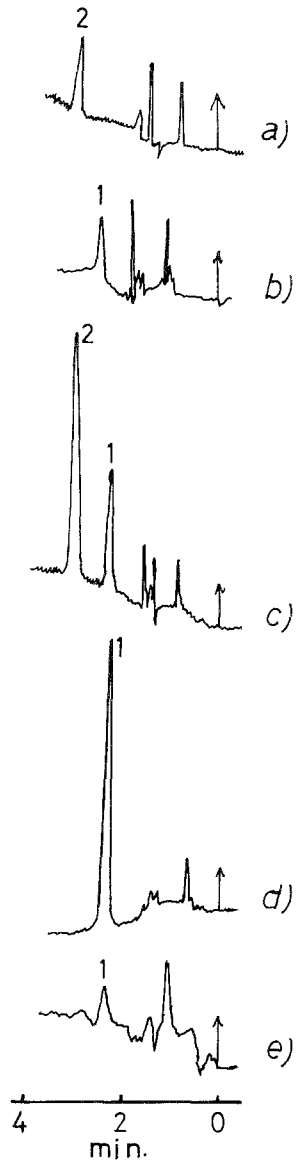

Fig. 1a–e. Chromatogramms of standards: (*1*) methomyl-oxime; (*2*) methomyl. **a** 3 ng of methomyl, UV-0.005 AUFS. **b** 5 ng of methomyl-oxime, UV-0.005 AUFS. **c** Methomyl and methomyl-oxime each 10 ng, UV-0.005 AUFS. **d** 5 ng of oxime-electrochem. detector 8 nA/cm. **e** 200 pg of oxime-electrochem. detector 5 nA/cm

c) Ultra sonic bath model RK 102 (Badelin electronic, Berlin).

d) Centrifuge model UJ 1 S (Christ, Osterode/Harz).

e) Vacuum rotary evaporator model SB (Büchi, Switzerland).

f) Chromatographic tubes: 250 × 4.6 mm id VA-steel column (Knauer, Berlin).

HPLC-Apparatus

The High-Performance Liquid Chromatograph consisted of the following parts:

a) Two-piston high-pressure pump model 52.00 (Knauer, Berlin).

b) Injection system model 71.20 (Rheodyn, California) with a 20 µl loop.

Table 1
Percent recovery of methomyl from vegetable crops (apple) control: < 5 ng/g (ppb)

Concentration [µg/g]	Extracted apple [g]	Added [ng]	Found [ng] $\bar{x} \pm s$ ($n = 6$)	% Recovery $\bar{x} \pm s$ ($n = 6$)
0.01	40	400	442 ± 43	110.5 ± 10.7
0.05	20	1.000	1.026 ± 67	102.6 ± 6.7
0.20	20	4.000	4.002 ± 390	100.1 ± 9.8
0.50	20	10.000	10.205 ± 220	102.1 ± 2.2
1.00	20	20.000	18.340 ± 231	91.7 ± 1.2

Table 2
Percent recovery of methomyl from water control: < 5 ng/g

Concentration [µg/ml]	Extracted water [ml]	Added [ng]	Found [ng] $\bar{x} \pm s$ ($n = 6$)	% Recovery $\bar{x} \pm s$ ($n = 6$)
0.01	40	400	404 ± 44.2	100.9 ± 11.6
0.05	20	1.000	900 ± 190	90.0 ± 1.9
0.20	20	4.000	3.712 ± 108	92.8 ± 2.7
0.50	20	10.000	9.460 ± 156.8	94.6 ± 1.6
1.00	20	20.000	18.040 ± 588	90.2 ± 2.9

c) Self-packed reversed-phase (RP-C18) column (high viscosity method).

d) Variable wavelength UV-detector model 87.00 (Knauer, Berlin) equipped with 10 µl flow cell ($d = 10$ mm).

e) Electrochemical detector model E 611 (Metrohm, Herisau, Switzerland) in conjunction with a three-electrode flow cell (1.3 µl) model EA 1096. The cell system has two glassy carbon electrodes as the working and auxiliary electrodes and Ag/AgCl (3 M KCl) as the reference electrode.

f) 100 mV Compensation recorder model 41.00 (Knauer, Berlin).

g) 10 µl Syringe model 101.00.40 (Knauer, Berlin).

HPLC Operating Parameters

a) Column:	RP-C18
b) Mobile phase:	acetonitril/water 20 : 80 (v/v)
c) Flow rate:	2 ml/min
d) Pressure:	110 bar
e) Electrolyte:	0.01 M $LiClO_4$
f) UV-detector:	233 nm; 0.02 AUFS
g) Electrochem.-detector:	+1.30 Volt; 5 – 120 nA/cm
i) Recorder:	100 mV

Determination

Ten microlitre samples were injected onto the HPLC column using the chromatographic apparatus and parameters as described. Residue peaks were tentatively identified on the basis of retention times [methomyl 2.9 ± 0.1 min ($n = 58$); methomyl-oxime 2.3 ± 0.1 min ($n = 28$)]. Residue amounts were determined by comparing peak height to peak height obtained from known amount of methomyl. Reference solution was chromatographed immediately after sample.

Extraction and Clean-up

Homogenize 2 – 3 apples in the homogenizer jar. Weigh 20 g of this homogenized sample into a 100-ml centrifuge bottle, add 50 ml of ethyl acetate and blend with Ultra-Turrax homogenizer for 2 min. Centrifuge at 3,000 r.p.m. for 10 min and carefully decant the extract into 250-ml beaker. Repeat blending and centrifugation two times more, using each time 50 ml of ethyl acetate. Filter the combined extracts through a filter paper loaded with 10 g of celite into a 500 ml round-bottomed flask. Filter paper and beaker were washed with 50 ml of ethyl acetate. Add 50 ml of bidistilled water and evaporate the ethyl acetate using the vacuum rotary evaporator at 30° C and 20 mbar vacuum. Filter the water through a small funnel with glass wool plug into a 100 ml volumetric flask, wash the round-bottomed flask and the funnel with bidistilled water and dilute to the mark. Shake well, withdraw a 20.0 ml aliquot into a 100-ml separatory funnel and acidify with 1 ml of 1 N H_2SO_4. Shake three times each with 25 ml of n-hexane, discard the hexane layer. Extract with 25 ml of methylene chloride for 2 min, withdraw the methylene chloride layer into 100 ml beaker containing 10 g of Na_2SO_4. Repeat the extraction two times more. Filter the combined extracts into a 100 ml round-bottomed flask, wash the beaker and filter with 20 ml of CH_2Cl_2. Evaporate at 30° C and 20 mbar. Dissolve the residue in 200 µl eluent.

Determination of Recoveries

Vegetable Crops (Apples). Six samples, each 20 g of a representative sample were spiked with known amounts of methomyl (0, 0.4[1], 1, 4, 10, 20 µg). The spiked samples were then treated as described under extraction and clean-up. Ten microlitres of the residue solution were injected onto the column. Six replicate determinations were carried out. Results are shown in Table 1.

Water Samples. Known amounts of methomyl (0, 0.4[1], 1, 4, 10, 20 µg) were added to six separatory funnels containing each 20[1]-ml of local lake water. The spiked samples were extracted as described under extraction and clean-up. Six replicate determinations were carried out. The results are shown in Table 2.

1 For the concentration of 0.01 µg/g or ml, 0.4 µg of methomyl was added to 40 g (ml) of sample

Table 3
Degradation of methomyl on apple

Days after the last spraying	0	7	14	28	35
Residue [ng/g] $\bar{x} \pm s$ ($n = 4$)	450 ± 20	110 ± 5	47 ± 8	52 ± 0	35 ± 7
Control [ng/g]	< 5	< 5	< 5	9 ± 2	19 ± 4

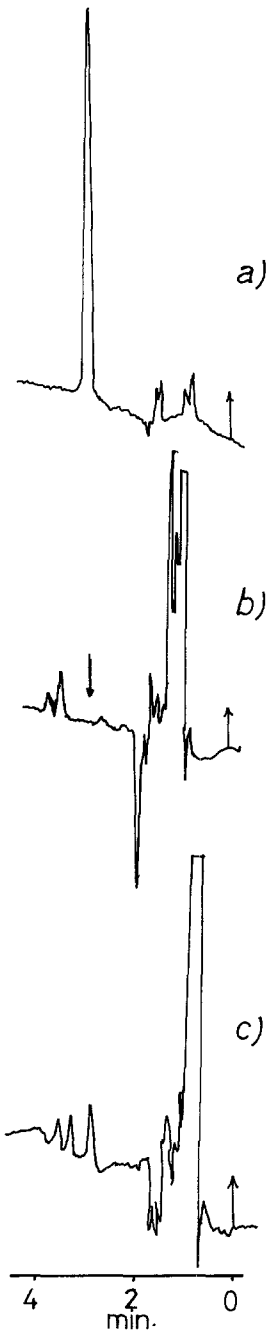

Fig. 2a—c. Chromatogramms of **a** methomyl standard, 100 ng, **b** untreated apple sample, **c** untreated apple sample fortified with 0.01 µg/g of methomyl 0.02 AUFS

Degradation of Methomyl on Apples

Apple samples used in this degradation study were obtained from an agricultural experiment station and had been sprayed with 0.15% of insecticide (25% of methomyl). Apple samples were gathered at five different intervals from the last spraying. These samples and their parallel controls were analysed as described under extraction and cleanup. Results are shown in Table 3.

Results and Discussion

Representative chromatogramms in Fig. 2 illustrate the results of the analysis of methomyl residues from 20 g of apple samples.

Figure 2b and c show untreated sample and untreated sample fortified with 0.01 µg/g of methomyl, respectively. Blank extracts showed no interfering peaks with similar retention times of methomyl.

The approach described has been used successfuly to determine methomyl in water at the 1 ng/ml (ppb) level.

Recoveries of methomyl from apple samples were 101.4 ± 6.0% and from water, 93.7 ± 4.0% at the 0.01 – 1 µg/g or ml concentration range (Table 1 and 2).

The procedure presented allows the simultaneous determination of methomyl and its degradation product methomyl-oxime (Fig. 1c). Using the UV-detector at 233 nm, we were able to determine as much as 2 ng of oxime. Using the electrochemical detector at +1.30 V, we could determine as little as 100 pg of oxime (Fig. 1e). These results show that the electrochemical detection mode of oxime is 20 times more sensitive than the UV-mode.

The advantages of the method presented are:

a) No need for derivatization or hydrolysis.

b) Short retention times for methomyl and methomyl-oxime (2.9 and 2.3 min, respectively).

c) The possibility of monitoring both substances simultaneously at 233 nm.

d) Combining both UV- and electrochemical detectors in series, it is possible to determine methomyl at the 1 ppb level (UV) and methomyl-oxime at the 100 pg/ml (ppt) level (electrochem.).

References

1. Ogata, J. N., Yanagihara, K. H., Hylin, J. W., Bevenue, A.: J. Chromatog. **157**, 401–403 (1978)
2. Rangaswamy, J. R., Prakash, S. R., Vijayashankar, Y. N.: J. Assoc. Offic. Anal. Chemists **60**, 1093–1096 (1977)
3. Krause, T.: J. Assoc. Offic. Anal. Chemists **63**, 1114–1124 (1980)
4. Sparacino, C. M., Hines, J. W.: J. Chrom. Sci. **14**, 549–566 (1976)
5. Frei, R. W., Lawrance, J. F., Hope, J., Cassidy, R. M.: J. Chrom. Sci. **12**, 40–44 (1974)
6. Thean, J. E., Fong, W. G., Lorenz, D. R., Stephens, T. L.: J. Assoc. Offic. Anal. Chemists **61**, 15–17 (1978)
7. Pease, H. L., Kirkland, J. J.: J. Agr. Food. Chem. **16**, 554–557 (1968)
8. Mendoza, C. E., McCleod, H. A., Shields, J. B., Phillips, W. E. J.: Pesticide Sci. **5**, 231–237 (1974)
9. McCleod, H. A., Mendoza, C. E., McCully, K. A.: Pesticide Sci. **6**, 11–16 (1975)

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