

## Appendix 10.3 continued: Technical Procedure

Method 480/0

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## 1 INTRODUCTION

Dimethoate and Omethoate are insecticides which are used against sucking and chewing insects. The analytical method presented here determines these residues in water. This method was developed at BASF Aktiengesellschaft, Agricultural Center Limburgerhof, Germany.

## 2 PRINCIPLE OF THE METHOD

500 g of the water sample is extracted using solid phase extraction (activated charcoal). Dimethoate and Omethoate are eluted from the sorbent using a mixture of Dichloromethane and Methanol (80 + 20, v + v). The solvent is evaporated and the residue is redissolved in acetone.

The final chromatographic analysis of Dimethoate and Omethoate is performed by GC/MS.

## 3 TEST AND REFERENCE SUBSTANCES

3.1 Test substances  
(used for fortifications)

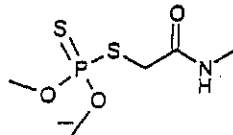
## 3.1.1 Dimethoate

Substance identification: Reg. No. 16236, Internal LIMS Code DIMETO

Common name: Dimethoate

Chemical name: O,O-dimethyl S-(methylaminocarbonylmethyl)-  
phosphorodithioate

Structural formula:



Empirical formula:  $C_3H_{12}NO_3PS_2$

Molecular weight: 229.26

Purity: 99.8 %, homogeneous

Lot. No.: 00831-164; PCP03878, Reanalysis: PCP05792  
supplied by BASF, APD/FC, Li 444

Stability: expected to be stable at least for 4 years at approx. -18°C

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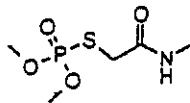
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## 3.1.2 Omethoate

Substance identification: Reg.No. 021370

Chemical name: O,O-dimethyl S-(methylcarbamoylmethyl)-  
phosphorothioate

Structural formula:

Empirical formula:  $C_3H_{12}NO_4$  PS

Molecular weight: 213.19

Purity: 98.3 %, homogeneous

Lot. No.: 80610; (Riedel-de Haën, date of analysis: Mar 25/1998)  
supplied by BASF, APD/FC, Li 444Stability: expected to be stable at least until Sep 02/2003 when stored at  
room temperature3.2 Reference substances  
(used for calibration)

Same substances as test substances, see 3.1

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## 4 MATERIALS AND INSTRUMENTS

## 4.1 Equipment for Extraction and Sample Clean-up

Note: The materials, chemicals, and the equipment specified below were used for method development. They are specified as examples only and may be substituted by supplies with similar specifications. If the use of supplies other than stated is intended, applicability to this method must be confirmed prior to method validation and/or routine analysis.

Equipment	Size, Description	Manufacturer/ Supplier	Catalog Number
Balance	PM 4800 delta range, d = 0.1 g / 0.01 g	Mettler (Germany)	
Balance	AT 261 delta range, d = 0.1 mg / 0.01 mg	Mettler (Germany)	
Separatory funnels	1000 mL (for water extraction see attachment 1)		
Peristaltic pump	IPN-16	Ismatec (Switzerland)	
SPE column dryer (N <sub>2</sub> )	see attachment 2		
N-EVAP with water bath	Vapostat TCS	Barkey (Germany)	
Vacuum rotary evaporator	Rotavapor R-124	Buechi (Switzerland)	
Vacuum controller	24	Vacuubrand (Germany)	
Vacuum pump	MZ 2C	Vacuubrand	
Water bath	B-481	Buechi (Switzerland)	
SPE extraction system		Baker (USA)	7018-00
SPE columns	3 mL	Baker (USA)	7121-03
Transfer pipets	1 ml	Gilson Abimed (Germany)	
Pipet tips	50 - 1000 µL	Brand (Germany)	7023 20
Erlenmeyer flasks	500 mL		
Glass funnels	d = 100 mm		
Tapered flasks	10 ml		
Vials	2 ml		
Volumetric flasks	25 mL		
Pipets (Volumetric)	1, 2, 2.5, 5 mL		
Pipet	5 mL		
Syringes	500 µL	Hamilton (Switzerland)	
GC vials	0.7 mL, N8-07 brown	Machery & Nagel (Germany)	70212.1
Vial caps		Machery & Nagel (Germany)	70252.1
Glass stoppers	various sizes		
Glass rod	d = 2 mm		

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## 4.2 Reagents

## 4.2.1 Chemicals

Note: Equivalent chemicals from other suppliers may be substituted but all chemicals used must be at least of "analytical grade" or must meet equivalent specifications.

Chemical	Grade	Manufacturer/ Supplier	Catalog Number
Activated charcoal	p. a.	Merck (Germany)	1.02186
Acetone	High purity	Merck (Germany)	1.00012
Dichloromethane (DCM)	High Purity	Merck (Germany)	1.06054
Methanol	High Purity	Merck (Germany)	1.06011
Water (in this method referred to as ddH <sub>2</sub> O)	Milli-Q-grade or equivalent	prepared with Millipore apparatus, Milli-Q plus 185, Millipore (France)	

## 4.2.2 Elution Mixture

Elution mixture: DCM + methanol, 80 + 20 (v + v)

## 4.3 Standard Solutions

## 4.3.1 Standard Solution Storage and Stability

Standard solutions are kept refrigerated. A study concerning the storage stability of standard solutions found no decomposition of solutions made in acetone when stored in the refrigerator for 17 days. This stability study was performed during method validation and is documented in the raw data.

BASF recommends that stock solutions (0.5 mg/ml acetone) be prepared freshly every two weeks. Dilutions of stock solutions should be kept refrigerated for no longer than two weeks.

Note: Use amber bottles with Teflon®-lined screw caps as storage containers for standard solutions. Suggested standard concentrations are listed below. A different concentration scheme may be used and additional standards may be prepared as needed.

## 4.3.2 Standard Solutions for Fortifications and Calibration

Note: The concentrations listed below are examples. Other concentration schemes may be used and different or additional standard concentrations may be used if required.

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Individual stock solutions of the test substances dimethoate and omethoate are prepared in acetone.

Stock solution 1: 500 µg/mL dimethoate in acetone

Stock solution 2: 500 µg/mL omethoate in acetone

Appropriate aliquots of both stock solutions are mixed with acetone to obtain working solutions used for fortifications and calibration.

Concentrations of standard working solutions prepared for fortification: 50, 5, 0.5, 0.05 µg/mL

Concentrations of standard working solutions prepared for calibration: 50, 5, 1, 0.7, 0.4, 0.2, 0.1, 0.05 µg/mL

Calibration is performed with standards covering a range from 0.05 µg/mL to 0.7 or 1.0 µg/mL.

#### 4.4 GC/MS Instrumentation and Chromatography Conditions

##### 4.4.1 GC/MS Instrumentation

GC/MS system: Hewlett Packard GC 6890 equipped with MSD 5973

Autosampler: Combi PAL, CTC Analytics

Software for data acquisition: Windows HP ChemStation

Software for data evaluation and calibration:  
HP ChemServer

##### 4.4.2 Chromatography conditions

GC-column: 30 m DB 1701, 0.25 mm i.d., 0.25 µm film, J & W, Folsom, CA, USA

Injector: PTV, program: 50 °C, 0.1 min, 700 °C/min, 250 °C, XX min  
Siltek™-Deactivated Baffled Glass Liner, Cat# 21704-214.10, Restek Corp., Bellefonte, USA

Injection volume: 1 µL

Column temperature: 60°C, 1 min, 30°C/min, 230°C, 5°C/min, 240°C, 40°C/min, 280°C, 2 min

Carrier gas: Helium, 2 mL/min

Transfer line: 280°C

Ionization mode: EI

MS quadrupole: 150°C

MS ion source: 230°C

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## 4.4.3 GC/MS Instrumentation Testing

Appropriate standard solutions should be injected into the GC/MS prior to each analytical series to verify the peak retention times of the reference substances and to demonstrate the stability and sensitivity of the system.

## 4.4.4 Mode of Detection

The analytes are detected in SIM. For quantification and confirmation the following m/z ratios are used.

Dimethoate: Quantifier: m/z = 125, Qualifier: m/z = 93, 87

Omethoate: Quantifier: m/z = 156, Qualifier: m/z = 110, 109

In case of interferences on one mass, quantification can be done with qualifiers or other mass fragments provided that peak identity is confirmed.

## 5 ANALYTICAL PROCEDURE

## 5.1 Sample Preparation and Storage

Store water samples in clean amber glass bottles in a refrigerator at ca. 4 °C.

## 5.2 Spiking of Samples for Recovery Experiments

500 g of untreated water samples is weighed into 500 mL Erlenmeyer flasks. 0.5 mL of the appropriate spiking solutions with analyte concentrations of 0, 0.05, 0.5 and 5.0 µg/mL are added to the samples. The correlation between the concentration of the spiking solution and the resulting final analyte concentration in the sample is shown below:

Sample Weight	Concentration of Spiking Solution	Volume of Spiking Solution	Level of Fortification
500 g	0.00 µg/mL	0.5 mL	0.00 µg/kg
500 g	0.05 µg/mL	0.5 mL	0.05 µg/kg *
500 g	0.50 µg/mL	0.5 mL	0.5 µg/kg
500 g	5.0 µg/mL	0.5 mL	5.0 µg/kg

\* proposed limit of quantitation of the method

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## 5.3 Extraction of the Sample Material

## 5.3.1 Preparation and conditioning of the SPE Columns

The empty SPE column is placed on the BAKER SPE extraction system and filled with 400 mg activated charcoal. 2.5 mL DCM is added into the column and the charcoal is suspended in the solvent by means of a thin glass rod. DCM is then removed from the charcoal applying a vacuum of about 95 kPa. To further condition the charcoal additional 2.5 mL DCM, 2 x 2.5 mL methanol and 2 x 2.5 mL ddH<sub>2</sub>O are sucked through the column.

**Note:** Make sure that the charcoal is always slightly covered with solvent. Don't let the charcoal go dry!

During this procedure the pressure is slightly lowered to about 85 kPa. After conditioning of the column it is filled with 2 mL ddH<sub>2</sub>O and moved to the water extraction apparatus (see attachment 1).

## 5.3.2 Water Extraction

500 g of water sample aliquots ( $S_w$ ) are weighed into 500-mL Erlenmeyer flasks. The samples are quantitatively transferred into the separatory funnels of the water extraction apparatus. The Erlenmeyer flasks are rinsed with a few mL's of ddH<sub>2</sub>O for quantitative transfer. The separatory funnels are connected with the preconditioned SPE columns. The whole water samples are then sucked through the SPE columns by means of a peristaltic pump. The column extraction takes a few hours and could preferably be done over night.

## 5.4 SPE Column Drying

After extraction is completed the SPE column is moved back to the SPE extraction system and filled with ddH<sub>2</sub>O. Vacuum is applied for about 15 min to remove the water (vacuum approx. 40 kPa). Then the column is dried with N<sub>2</sub> at 40°C for approx. 1 h as shown in attachment 2.

## 5.5 Elution of Dimethoate and Omethoate

The dried SPE column is again mounted on the SPE extraction system with a 10 mL tapered flask underneath. The analytes are eluted with 3 x 2.5 mL (DCM + Methanol, 80 + 20, v + v) applying a vacuum of about 85 kPa.

**Note:** It is recommended to suspend the charcoal in the solvent by slightly stirring with a thin glass rod after the first aliquot of solvent has been added.

The combined eluate is reduced to a volume of about 0.3 mL (vacuum rotary evaporator) at a temperature of 30°C. The concentrated extract is quantitatively transferred into a 2 mL vial. The tapered flask is rinsed with 3 x 0.3 mL acetone. The rinsing solution is added to the 2 mL vial. The combined extract is then evaporated to dryness using a gentle stream of N<sub>2</sub> at

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30°C (N-EVAP apparatus). The residue is then dissolved in acetone. To meet the LOQ the final volume  $V_{END}$  is 0.25 mL. Depending on the expected concentration the final volume is adjusted in an appropriate way. From this solution 1  $\mu$ L ( $V_i$ ) is injected into the GC/MS for quantitation.

## 5.6 GC/MS measurement

## 5.6.1 General aspects

Note: Due to the polarity of the analytes it is highly recommended to use new silanized glass liners in the injection port of the GC instrument.

## 5.6.2 Calibration Procedures

The injection volume is 1  $\mu$ L. Reference standards with concentrations listed in 4.3.2 are injected to generate the calibration curves. In the analytical queue standards and samples are injected alternately to show the stability of the GC/MS system during the whole series.

Calculation of results is based on peak area measurements using a calibration curve. The standard curve is obtained by direct injection of the mixed analytes into the GC/MS in the range of 0.05  $\mu$ g/mL to 0.7 or 1.0  $\mu$ g/mL. In a given injection run, the same volume is used for all samples and standards.

The calibration curves are obtained by plotting peak area versus the amount of the analytes. The linear least squares working curve in the form  $y = bx + c$  is used for the construction of the calibration curve.

Establish the stability of the detection response and retention time by injecting several standards with different concentrations. For each injection set, the set should begin and end with standard injections, and each standard level should be injected at least in duplicate.

## 5.6.3 Limit of Quantitation and Limit of Detection

The limit of quantitation is defined as the lowest fortification level successfully tested. For water, the limit of quantitation is 0.05  $\mu$ g/kg (ppb) for both dimethoate and omethoate. The limit of detection for both analytes is 0.05 ng. It is here defined as the absolute amount of analyte injected into the GC/MS instrument using the lowest standard of the calibration curve.



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## 6 CALCULATION OF RESULTS

## 6.1 Principle

Calculation of results is based on peak area measurements. The residues of diemthoate and omethoate are calculated from the calibration curve and the equations are shown in section 6.2.

## 6.2 Calculation of Residues

The residue (R) in the water sample in  $\mu\text{g}/\text{kg}$  is calculated as shown in the following equation I:

$$R = \frac{V_{\text{END}} \times C_{\text{B}}}{V_{\text{I}} \times S_{\text{M}}}$$

- R = Residue in the water sample [ $\mu\text{g}/\text{kg}$ ]  
 $V_{\text{END}}$  = End volume of the extract after all dilution steps [ml]  
 $C_{\text{B}}$  = Amount of analyte in the injection volume as read from the calibration curve [ng]  
 $V_{\text{I}}$  = Volume injected into GC/MS [ $\mu\text{l}$ ]  
 $S_{\text{M}}$  = Weight of the sample extracted [kg]

If residue data are to be corrected for loss of analyte during sample extraction and clean-up procedures the residue (R) has to be corrected with the results of the procedural recoveries as shown in the following equation II:

$$R_{\text{RC}} = R \times R_{\text{FE}}$$

- $R_{\text{RC}}$  = Residue concentration of the analyte in the sample corrected with the procedural recovery of the analyte in fortification experiments [ $\mu\text{g}/\text{kg}$  water]  
 $R_{\text{FE}}$  = Procedural recovery of the analyte as determined from fortification experiments performed in parallel to the sample analysis

$$= \frac{100 \% \text{ (level of fortification)}}{\% \text{ recovery}}$$

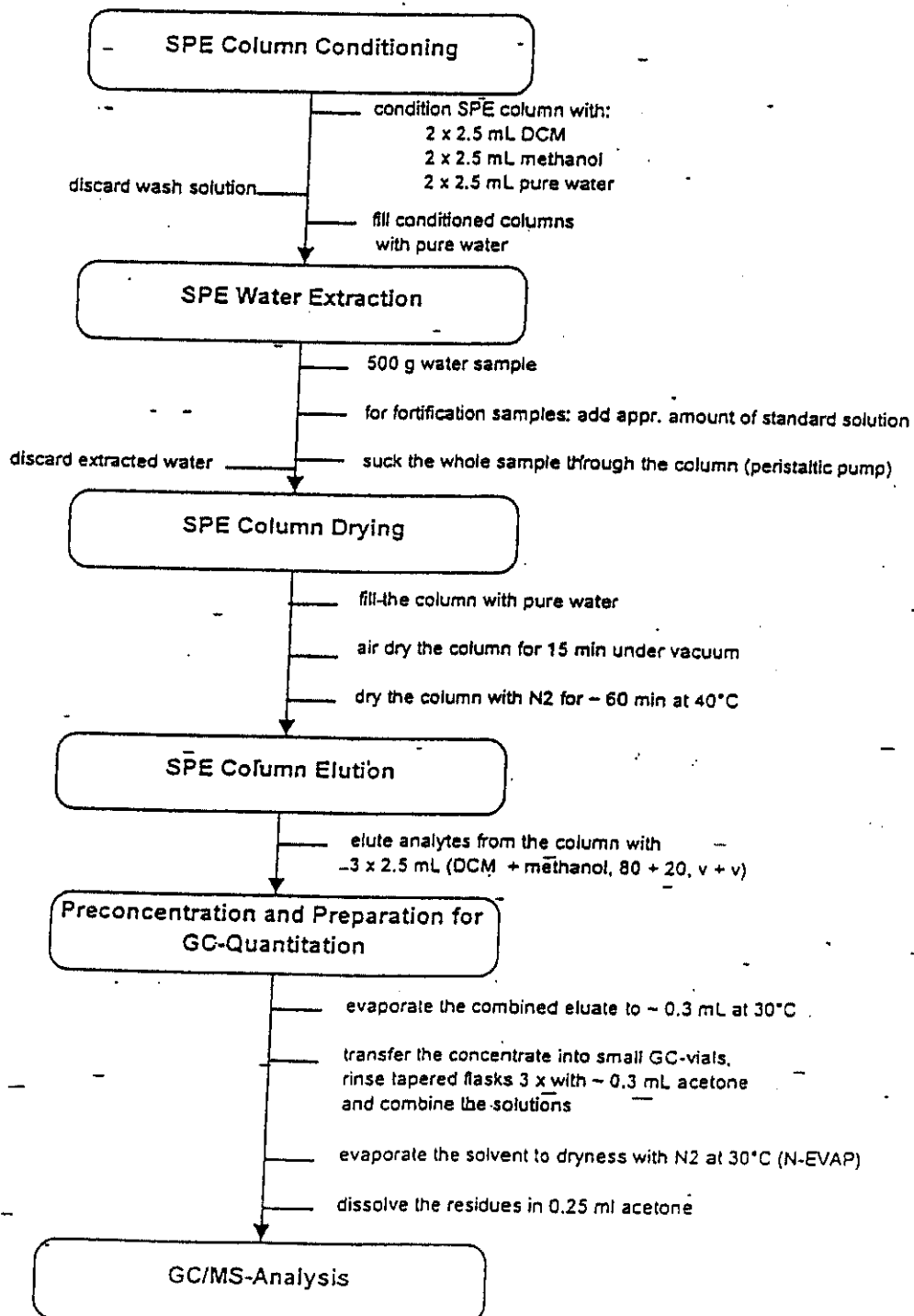
Note: For routine analysis requirements residue data should not be corrected for procedural recoveries. Results of fortification experiments should be listed individually.

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## 7 FLOW CHART OF METHOD 480/0



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## 8 METHOD MANAGEMENT AND TIME REQUIREMENT

The analysis of one series of samples (= 6 unknown samples, 2 fortified samples for recovery experiments, 1 blank sample) requires 16 working hours. This time includes the calculation of the results, the preparation of the equipment as well as the reporting of all raw data under GLP.

## 9 PROBLEMS, SAFETY AND HEALTH CONSIDERATIONS

Both Dimethoate and Omethoate are toxic. Dimethoate has possible risk to harm the unborn child and is possibly mutagen. (See Material Safety Data Sheets, e.g. from Sigma Aldrich, St. Louis, USA, Product No 45449 and 45600). All procedures should be performed under a well-ventilated hood. Personal protective equipment (gloves, lab coats) should be worn while performing this method. Heed all label statements and precautions.

## 10 CONFIRMATORY TECHNIQUE

A further analytical procedure as confirmatory technique is not necessary, because GC/MS is a very specific method. If unexpected residues are found in the SIM-mode, then one may record the full scan spectrum or use further characteristic mass fragments for confirmation of the compound.

Although the EU guideline requires three mass fragments higher than  $m/z = 100$ , two lower fragments have been chosen for dimethoate due to their higher intensity. The spectrum clearly indicates that the compound can be confirmed also with  $m/z = 143$  and  $m/z = 229$ .

However, in addition to the method described above another chromatographic column with different selectivity might be used. This is demonstrated in attachments 3 - 5. Results are presented which were obtained during method development stage using a DB 17 column. Since both dimethoate and omethoate elute at different retention times, this will give additional reliability. Tap water spiked at a level of  $0.05 \mu\text{g}/\text{kg}$  was analyzed in duplicate.

The results demonstrate that this alternative can also be used for confirmatory purposes.

## 11 RECOVERIES

Recovery data will be provided in the validation part of the analytical method 480/0. The study code of the validation is 58222.

### 11.1 Limit of Determination, Blank Values

The limit of determination (quantitation) was defined by the lowest fortification level successfully tested =  $0.05 \mu\text{g}/\text{kg}$ . The tested untreated blank water samples showed no interfering peaks (or less than 1/3 of the  $0.05 \mu\text{g}/\text{kg}$  fortification level) at the retention time of the analytes.