2.0 INTRODUCTION

Background:

Famoxadone (DPX-JE874) is a fungicide used for various crops. The structure of famoxadone and other relevant information on the chemical are given in Figure 1.

Objective:

To provide two analytical methods for the determination of famoxadone (DPX-JE874) in water: One method is based on solid phase extraction (SPE), the other method on liquid / liquid extraction. Both methods are validated for three types of water: drinking water, ground water and surface water. The validation ranges were 0.050 µg/L (LOQ, limit of quantitation) to 0.25 µg/L for drinking water and ground water and 0.25 µg/L (LOQ) to 1.25 µg/L for surface water. Limits of detection (LOD) were not studies but estimated to be 0.008 µg/L (SPE) or 0.016 µg/L (liquid/liquid extraction) for drinking water and ground water, and 0.040 µg/L (SPE and liquid/liquid extraction) for surface water.

Data requirements:

To support requirements by the EEC Directive 91/414/EEC, Annex II 4.2.3.

Method principles for the solid phase extraction (SPE) method:

Water is sampled through pre-conditioned 1 g C_{18} SPE cartridges (1 L drinking water or ground water, 0.2 L surface water). The cartridges are dried and then eluted with 3 * 2 mL acetonitrile. The eluates are concentrated to dryness and the residue is redissolved in toluene (e.g. 0.2 to 1.0 mL) for GC/ECD analysis on a DB 1 capillary column (15 m * 0.32 mm * 0.25 µm).

Method principles for the liquid / liquid extraction method:

Water (0.5 L) is extracted three times with 50 mL dichloromethane using an Ultra Turrax homogenizer at ≈ 13500 rpm. The combined dichloromethane extracts are dried over sodium sulfate and concentrated to dryness. The residue is redissolved in toluene (e.g. 0.2 to 2.5 mL) for GC/ECD analysis on a DB 1 capillary column (15 m * 0.32 mm * 0.25 µm).

For method validation water samples were fortified and then extracted by SPE or liquid / liquid partition. Control water samples were used to demonstrate the absence of famoxadone signals in untreated samples. Furthermore, method validation demonstrated limits of determination / quantitation (LOQ) and the range of applicability.

GC/MS was examined and demonstrated as confirmatory method.

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following description before making substitutions. Substitutions should only be made if equivalency / suitability has been verified with acceptable control and fortification recovery data.

3.1 Standard

Analytical reference standard:

Famoxadone (DPX-JE874) provided by the Sponsor with a Certificate of Analysis: IN # JE874-92, purity: 99.6 %. For detailed information see Figure 1.

3.2 Solvents for Stock, Fortification and Chromatographic Standard Preparation

Solvent used for stock standard preparation:

Methanol (HPLC grade, part no. 3041) (Promochem, Wesel, Germany).

Solvent used for fortification standard preparation:

Acetonitrile (HPLC grade, part no. 2856) (Promochem). DO NOT SUBSTITUTE!

Solvent used for chromatographic standard preparation:

Toluene (residue grade, part no. 8092) (Promochem).

3.3 Equipment and Reagents for Solid Phase Extraction (SPE)

Miscellaneous equipment:

Balance: Sartorius RC 210D (Sartorius, Göttingen, Germany).

SPE processing stations (T.J. Baker, Deventer, Netherlands or Macherey-Nagel, Düren, Germany) equipped with stop cocks for flow control and vacuum gauge for vacuum control

Water suction pumps.

Sample concentration device with water bath and nitrogen supply or rotary evaporators R-114 and RE-111 (Büchi, Flawil, Switzerland).

Ultrasonic bath: Transonic 460 (Elma, Singen, Germany).

Typical glassware and laboratory equipment.

Sampling cartridges:

Mega Bond Elut™, Bonded Phase C_{18}, size 6 mL, part no. 1225-6001 (Varian, Darmstadt, Germany).

Solvents:

Acetone (residue grade, part no. 0018), acetonitrile (HPLC grade, part no. 2856),

toluene (residue grad, part no. 8092) (Promochem, Wesel, Germany).
Technical acetone (purity < 99 %, part no. 00585) for rinsing of rotary evaporators (Fluka, Neu-Ulm, Germany).
Ampuva water (part no. 1080181, Fresenius, Bad Homburg, Germany).

3.4 Equipment and Reagents for Liquid / Liquid Extraction

Miscellaneous equipment:

Balance: Sartorius RC 210D (Sartorius, Göttingen, Germany).
Ultra Turrax IKA T25 (Janke & Kunkel, Staufen, Germany).
Rotary evaporators R-114 and RE-111 (Büchi, Flawil, Switzerland).
Ultrasonic bath: Transonic 460 (Elma, Singen, Germany).
Typical glassware and laboratory equipment.

Reagents:

Sodium sulfate anhydrous (purity: > 99 %, part no. 0313, T.J. Baker, Deventer, Netherlands).

Solvents:

Dichloromethane (residue grade, part no. 3023), toluene (residue grade, part no. 8092) (Promochem, Wesel, Germany).
Technical acetone for rinsing of rotary evaporators (purity: > 99 %, part no. 2856, Fluka, Neu-Ulm, Germany).
Ampuva water (part no. 1080181, Fresenius, Bad Homburg, Germany).

3.5 GC/ECD System and Evaluation

GC/ECD system:

Varian (Darmstadt, Germany) GC system equipped with:
Varain 8200 Autosampler, Varian 3400 GC with split / splitless injector, Varian ECD, J & W Scientific DB 1 capillary column (15 m, 0.32 mm i.d., 0.25 µm film, J & W Scientific, Folsom, CA, USA).

Evaluation:

PC-based Varian Star chromatographic software, Microsoft Excel.

3.6 Safety and Health

Each analyst must be aquainted with the potential hazards of the reagents, products and solvents used in the method before working in the laboratory. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used

4.0 METHODS

4.1 Principles of the Analytical Methods

4.1.1 Solid Phase Extraction (SPE) Method

Water (1 L drinking water or ground water, 0.2 L surface water) is sampled through 1 g C_{18} SPE cartridges which are preconditioned with acetone, acetonitrile and water. The cartridges are dried and then eluted with 3 * 2 mL acetonitrile. The eluates are concentrated to dryness and the residue is redissolved in toluene (e.g. 0.2 to 1.0 mL) for GC/ECD analysis (DB 1: 15 m * 0.32 mm * 0.25 µm).

A schematic presentation of the method is given in Figure 2.

4.1.2 Liquid / Liquid Extraction Method

Water (0.5 L all types) is extracted three times with 50 mL dichloromethane using an Ultra Turrax homogenizer at ≈ 13500 rpm. The combined dichloromethane extracts are dried over sodium sulfate and then concentrated to dryness. The residue is redissolved in toluene (e.g. 0.2 to 2.5 mL) for GC/ECD analysis (DB 1: 15 m * 0.32 mm * 0.25 µm).

A schematic presentation of the method is given in Figure 3.

4.2 Analytical Procedure

4.2.1 Glassware and Equipment Cleaning Procedure

All reusable glassware should be rinsed with solvent, washed with hot tap water, non-phosphate detergent, rinsed with deionized water (may be performed in laboratory dish washer) and dried fully before use

The Ultra Turrax homogenizers are rinsed between sample extractions in a beaker filled with an appropriate solvent (e.g. dichloromethane) to prevent carry over.

Rotary evaporators are rinsed between sample evaporations with acetone to prevent carry over.

Care should be taken to avoid working with high levels of the analyte being monitored in the same laboratory where samples are being extracted and analyzed.

4.2.2 Stock Standard Solution Preparation (Example) and Stability

A stock standard solution of the reference standard was prepared at 1.00 mg/mL in-methanol: Weigh 10.00 ± 0.10 mg of famoxadone (IN # JE874-92, purity: 99.6 %) into a 10 mL volumetric flask. Dissolve completely in 10.0 mL methanol.

Store refrigerated, stable for at least one month.

4.2.3 Fortification Standard Solution Preparation (Example) and Stability

A 25 µg/mL intermediate solution in acetonitrile was prepared by volumetric dilution:
Dilute 250 µL of stock standard solution in 10.0 mL acetonitrile.
A 250 ng/mL fortification standard solution in acetonitrile was prepared by volumetric dilution:
Dilute 500 µL of intermediate solution in 50.0 mL acetonitrile.

Store refrigerated, stable for at least two weeks.

4.2.4 Chromatographic Standard Solution Preparation (Examples) and Stability

Chromatographic standard solutions were prepared at 25 to 500 pg/µL in toluene by volumetric dilution as exemplified below:
50 ng/µL: 500 µL of stock standard solution (1.00 mg/mL) in 10 mL (intermediate solution).
500 pg/µL: 200 µL of intermediate solution (50 ng/µL) in 20 mL.
300 pg/µL: 150 µL of intermediate solution (50 ng/µL) in 25 mL.
250 pg/µL: 100 µL of intermediate solution (50 ng/µL) in 20 mL.
100 pg/µL: 50 µL of intermediate solution (50 ng/µL) in 25 mL.
50 pg/µL: 25 µL of intermediate solution (50 ng/µL) in 25 mL.
25 pg/µL: 1000 µL of chromatographic solution (500 pg/µL) in 20 mL.
Store refrigerated, stable for at least one month.

4.2.5 Source, Storage and Characterization of Samples

Drinking water: Tap water from the local drinking water supply collected at PTRL Europe, Helmholtzstr. 22, D-89081 Ulm, Germany. See Appendix A1 for representative analysis data provided by the City Water Supplier (column Uni Ulm).
Ground water: Obtained from the well "Fassung 4" (Donauried near D-89129 Langenau, Germany) of the State Water Supplier "Landeswasserversorgung Stuttgart". See Appendix A1 for representative analysis data provided by the supplier.
Surface water: Danube water (sampling point Donau 7 of the State Water Supplier "Landeswasserversorgung Stuttgart" sampled near D-89340 Leipheim, Germany). See Appendix A1 for representative analysis data provided by the supplier.

Drinking water was sampled from the tap as needed. Ground and surface water were sampled once and stored refrigerated in brown glass bottles.

4.2.6 Solid Phase Extraction (SPE) Method

4.2.6.1 Preparation of Samples

Decant ground and surface water for reduction of particles that may clog the frit of the SPE cartridge.

Measure water sample into beaker or bottle: 1 L of drinking water or ground water and 0.2 L of surface water.

4.2.6.2 Sample Fortification Procedure

Fortify untreated water samples with fortification standard (250 ng famoxadone per mL) as exemplified below:

Drinking water and ground water (1 L):	LOQ:	200 µL	=>	0.050 µg/L famoxadone.
	5 * LOQ:	1000 µL	=>	0.25 µg/L famoxadone.
Surface water (0.2 L):	LOQ:	200 µL	=>	0.25 µg/L famoxadone.
	5 * LOQ:	1000 µL	=>	1.25 µg/L famoxadone.

Different volumes may be chosen for modified sample volumes.

4.2.6.3 Analyte Extraction Procedure

1. Place SPE cartridges (1 g Varian Bond Elute C_{18}) on SPE station and pre-condition with 2 cartridge fillings of solvent in the following order: acetone, acetonitrile and bidistilled water.
CAUTION: CARTRIDGE MUST NOT RUN DRY AFTER ADDITION OF FIRST PORTION OF ACETONITRILE!
2. Fill cartridge with bidistilled water.
3. Connect cartridge to water suction pump and immerse the open part of the cartridge in the water sample.
CAUTION: AVOID AIR BUBBLES IN THE CARTRIDGE!
DO NOT ALLOW AIR TO BE SUCKED THROUGH THE CARTRIDGE!
DO NOT IMMERSE TUBING INTO THE WATER SAMPLE!
4. Sample water through the pre-conditioned SPE cartridge (flow rate: ≈ 10 - 15 mL/min).
5. Transfer cartridge onto SPE station and dry the cartridge with air by application of vacuum for ≈ 0.5 hours.

4.2.6.4 Analyte Elution Procedure

1. Place 10 mL vials under the cartridge in the SPE station to collect the eluate.
2. Add 2 mL acetonitrile to the cartridge.
3. Draw ≈ 1 mL solvent into the cartridge to wet the adsorbent.
4. Close stop cock and wait for 1 min.

5. Open stop cock and wait until all solvent has penetrated into the adsorbent.
6. Apply vacuum to remove remaining solvent.
7. Repeat elution twice as above with 2 mL acetonitrile and collect eluats in the 10 mL vial.

4.2.6.5 Concentration of Eluate and Adjustment of Final Volume

1. Concentrate acetonitrile extract to dryness by application of a slight stream of nitrogen (water bath temperature: ≈ 35 °C).
2. Add toluene to adjust final volume as follows:

Blank controls, LOQ samples or samples in which low residues (drinking water and ground water: ≤ 0.1 µg/L, surface water: ≤ 0.5 µg/L) are expected: 0.2 mL.

5 * LOQ samples or samples in which high residues (drinking water and ground water: > 0.1 µg/L, surface water: > 0.5 µg/L) are expected: 1.0 mL.

3. Redissolve analyte by ultra sonication (≈ 1 min).
4. GC/ECD analysis on a DB1 capillary column with split / splitless injection.

It is recommended to analyze final extracts as soon as possible. Final extracts are stable for at least 3 days at room temperature. If storage is necessary store refrigerated (stability > 5 days). Dilution or concentration of final extract may become necessary if the concentration of the sample extract exceeds the established calibration range.

4.2.7 Liquid / Liquid Extraction Method

4.2.7.1 Preparation of Samples

Decant ground and surface water for reduction of particles that may cause problems in phase separation.

Measure 0.5 L of water sample into beaker.

4.2.7.2 Sample Fortification Procedure

Fortify untreated water samples with fortification standard (250 ng famoxadone per mL) as exemplified below:

Drinking water and ground water (0.5 L):	LOQ:	100 µL	=>	0.050 µg/L famoxadone.
	5 * LOQ:	500 µL	=>	0.25 µg/L famoxadone.
Surface water (0.5 L):	LOQ:	500 µL	=>	0.25 µg/L famoxadone.
	5 * LOQ:	2500 µL	=>	1.25 µg/L famoxadone.

Different volumes may be chosen for modified sample volumes.

4.2.7.3 Analyte Extraction Procedure

1. Add 50 mL dichloromethane to water sample.
2. Extract with Ultra Turrax homogenizer at ≈ 13500 rpm for 3 min.

3. Transfer extraction mixture into separatory funnel (e.g. 500 mL).
4. Collect lower dichloromethane phase in a 500 ml erlenmeyer flask with 10 g of sodium sulfate anhydrous (for drying of extract).
5. Transfer upper aqueous phase back into beaker.
6. Repeat extraction twice with 50 mL portions of dichloromethane and combine all dichloromethane phases in the erlenmeyer flask.
7. Wait at least 15 min with occasional shaking for drying of combined dichloromethane extracts.

4.2.7.4 Concentration of Extract and Adjustment of Final Volume

1. Decant dichloromethane extract into 500 mL round bottomed flask.
2. Wash sodium sulfate twice with 10 mL dichloromethane and add rinses to 500 mL round bottomed flask.
3. Concentrate extract to < 5 mL (water bath temperature: ≈ 35 °C).
4. Transfer concentrate into 10 to 15 mL centrifuge vial.
5. Rinse round bottomed flask with 2 * ≈ 2 mL dichloromethane and add rinsates to centrifuge vial.
6. Concentrate extract to dryness (water bath temperature: ≈ 35 °C).
7. Add toluene to adjust final volume as follows:

Drinking water and ground water:

Blank controls, LOQ samples or samples in which unknown residues are expected: 0.2 mL.
5 * LOQ samples or samples in which high residues (> 0.2 µg/L) are expected: 0.5 mL.

Surface water:

Blank controls, LOQ samples or samples in which unknown residues are expected: 0.5 mL.
5 * LOQ samples or samples in which high residues (> 0.5 µg/L) are expected: 2.5 mL.

8. Redissolve analyte by ultra sonication (≈ 1 min).
9. GC/ECD analysis on a DB1 capillary column with split / splitless injection.

It is recommended to analyze final extracts as soon as possible. Final extracts are stable for at least 3 days at room temperature. If storage is necessary store refrigerated (stability > 5 days). Dilution or concentration of final extract may become necessary if the concentration of the sample extract exceeds the established calibration range.

4.3 Instrumentation

4.3.1 Description

See section 3.5 "GC/ECD System and Evaluation" for detailed description of the gas chromatographic system used in this study.
Use a capillary GC instrument with split/splitless injector and electron capture detector (GC/ECD).

The confirmatory method uses a capillary GC/MS ion trap system with temperature programmable SPI injector. For detailed description of GC/MS system see Appendix A2

4.3.2 Operating Conditions

Establish chromatographic conditions for GC/ECD analysis such as (exemplified):

Injection:	1 µL split / splitless injection with autosampler. Splitless time: 0.5 min. Injector temperature: 290 °C.
GC capillary column:	J&W Scientific DB 1 (15 m, 0.32 mm i.d., 0.25 µm film).
Carrier gas:	Helium at 10 psi.
Oven program:	90 °C, 0.5 min; 30 °C/min to 220 °C; 5 °C/min to 240 °C; 30 °C/min to 300 °C; 300 °C, 1.0 min.
Retention time:	Famoxadone (DPX-JE874): ≈ 8.6 - 8.9 min (≈ 239 °C).
Detection:	Electron capture detector (ECD), Argon / CH_4 (90:10) make-up gas. Detector temperature 300 °C.

4.3.3 Calibration Procedure

Prepare at least four chromatographic standards for calibration, intended to bracket the levels of famoxadone (DPX-JE874) in the sample extracts.

REMARK:
It is advised to run chromatographic standards first to demonstrate reproducibility of injection, separation and to establish the calibration curve.

Once the calibration curve is established, chromatographic standards should be interspersed with sample extracts and evaluated as verifications. In the case that verifications indicate increased or decreased response, use verifications to establish new calibration curve.

With the GC/ECD system used in this study linear calibration functions from 25 to 500 pg/µL resulted in acceptable regression coefficients (Figures 4 and 5) and recovery results.

4.3.4 Sample Analysis

Before analyzing blanks or low level extracts, verify contamination or memory effect of syringe and injector by solvent injections. See Figures 6 to 11 for chromatograms of sample extracts.

4.4 Calculations

4.4.1 Methods

The famoxadone (DPX-JE874) signals are integrated to give peak areas which are evaluated with the correct calibration function (e.g. linear) to yield a final extract concentration C_{end} reported in pg/µL (calculated by chromatographic software).

Concentration C_{end} (pg/µL = ng/mL; calculate mean value if more than one injection was performed; usually fortified samples were injected twice) is multiplied with the final volume V_{end} (mL) to obtain the total amount of famoxadone (DPX-JE874) in the water sample (µg).

The total amount is divided by the total water sample volume Vol (L) to obtain the concentration of famoxadone in water found (C_{water} in µg/L).

$$C_{water}\ (found) = \frac{(C_{end}) * V_{end}}{1000 * Vol\ (sample)} \quad [\mu g/L]$$

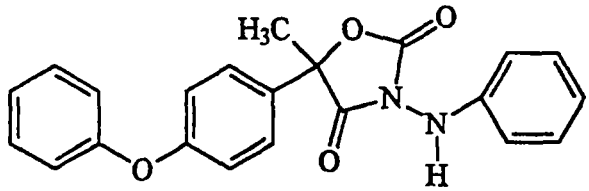
Recoveries for fortified water samples are calculated as follows:

$$Recovery = \frac{C_{water}\ (found)}{C_{water}\ (spiked)} * 100 \quad [\%]$$

Calculations in the Excel evaluation tables were performed with full precision and results were rounded to 2 significant digits.

Figure 1

Chemical structure of famoxadone (DPX-JE874) and related information.



IUPAC name: 3-anilino-5-methyl-5-(4-phenoxyphenyl)-2,4-oxazolidinedione.

CA name: 5-methyl-5-(4-phenoxyphenyl)-3-(phenylamino)-2,4-oxazolidinedione.

CAS RN.: [131807-57-3].

Standard obtained from the Sponsor with the following information:

IN # JE874-92, Ref. # E72194-122.

Purity: 99.6 %.

Expiration date: January 21, 1998.

Figure 2

Schematic presentation of the solid phase extraction (SPE) method.

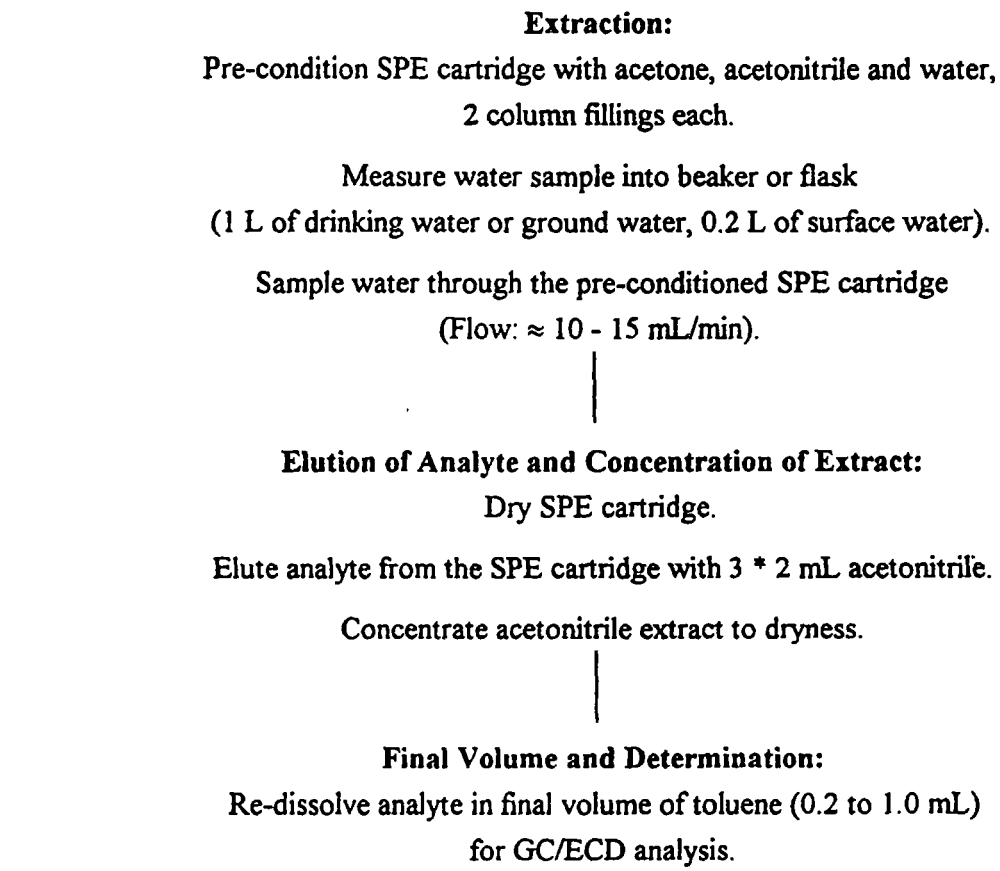
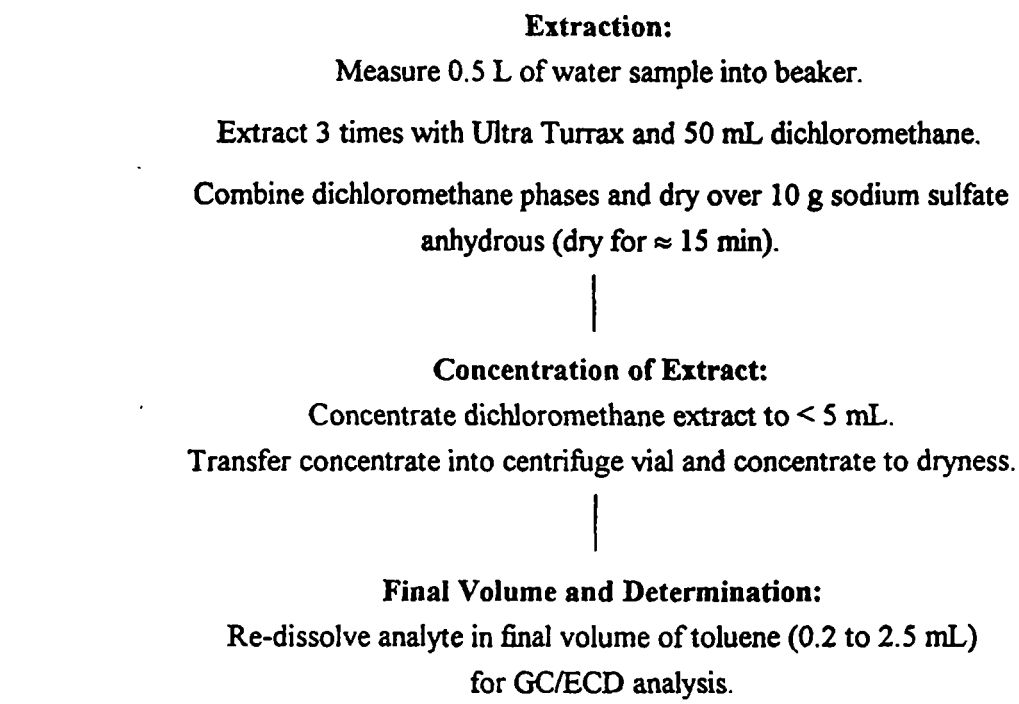


Figure 3

Schematic presentation of the liquid / liquid extraction method.



Appendix A 2

Description of confirmatory GC/MS and MS/MS methods with representative mass spectra and chromatograms.

GC/MS system:

A Varian GC/MS system equipped with a Varian 8100 autosampler, a Varian 3400 GC with a temperature programmed SPE injector, a Varian Saturn 3 Iontrap MS (EI ionization, MS/MS option) and Compaq Data System was used.

GC/MS and GC/MS/MS methods:

Injection: 1 µL splitless injection using autosampler and temperature programmed SPI injector (120 °C, 0.10 min, 180 °C/min to 260 °C, 1 min).

Column: BPX-5 (25 m, 0.32 mm i.d., 0.25 µm film, SGE, Weiterstadt, Germany).

Oven program: 90 °C for 1 min; 30 °C/min to 240 °C; 10 °C/min to 300 °C; 300 °C for 2 min.

Full scan MS detection method:

Mass range: 70 to 400 m/e. The sum of the major fragment ions 330/224/196 m/e is used for detection and quantitative evaluation . Representative spectra and chromatograms of chromatographic standards and water sample extracts are given on the following pages.

MS/MS detection method:

EI-MS/MS with resonant collision-induced dissociation (CID) of the 330 m/e parent ion. The 193 m/e daughter ion is used for evaluation.
Mass range: 180 to 200 m/e.
Resonant excitation: 20 msec with an amplitude of 1.7 V.
Parent fragment ion: 330 m/e, isolation window 3 m/e.
Excitation storage level: 130 m/e.
Representative chromatograms of a chromatographic standard and water sample extracts are given on the following pages.

With the detection method 2 (EI-MS/MS) the 330 m/e fragment ion is isolated in the ion trap and then exposed to an additional resonant excitation voltage. The 330 m/e fragment ion dissociates to the specific 193 m/e daughter ion. This procedure results in improved selectivity and better sensitivity for the monitored 193 m/e daughter ions