1. INTRODUCTION

Background and Objective:

The objective of this study was to develop and to validate an analytical method for the separate determination of the cis- and the trans-isomers of d-phenothrin in surface water. The target limit of quantification (LOQ) is 0.1 µg/L (ng/mL), expressed as total d-phenothrin (TG: technical grade). d-Phenothrin contains ⩾95% (1R)- isomers, ⩾75% trans- isomer, and thus ⩽ 25 % cis-isomer.

d-Phenothrin (TG) is analysed in water after liquid/liquid (L/L) extraction by electron impact (EI) GC-MS/MS, separating cis- and trans-isomers into 2 peaks, which are subsequently evaluated separately, monitoring three MS/MS mass transitions for quantitation/confirmation for each isomer.

Calibrations were to be prepared separately for the cis- and the trans-isomer analytical standards. Calibrations extended over a range covering nominal concentrations of the analytes between ⩽ 25 % of LOQ and ⩾ 120 % of 10xLOQ.

2. EXPERIMENTAL

2.1 Test System

The analytical method was validated with surface water.

Surface water from the river Danube sampled in Ulm was used. The pH was 8.16, the total water hardness was 17.9°d (Deutsche Härtegrade, approximately 3.20 mmol/L).
The surface water was characterized by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP, see APPENDIX 1):

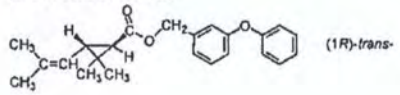
DOC (diluted organic carbon, EN 1484: 1997):	1.7 mg/L
Silt content (EN 872 Whatman GF 6):	3.1 mg/L
Electric conductivity (at 25 °C):	584 µS/cm

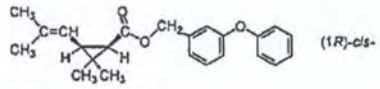
The surface water was kept in the dark at ambient temperature after collection and prior to extraction.

2.2 Analytical Test and Reference Items

Analytical standards of d-phenothrin (TG: technical grade), of trans- and of cis-phenothrin were provided by the Sponsor (see APPENDIX 2 for Certificates of Analysis) and used as test / reference items. The analytical standards were stored refrigerated when not use.

d-Phenothrin





d-Phenothrin contains 97.0% (1*R*)- isomers.

The ratio of trans / cis- isomer is 80.31 / 19.69 (communicated by the Sponsor).

IUPAC name: 3-phenoxybenzyl (1*R*)-*cis-trans*-chrysanthemate

Molecular formula: $C_{23}H_{26}O_3$ Molecular mass: 350.5 g/mol

cis-d-Phenothrin: (1*R*)-*cis*- isomer

CAS No.: *[51186-88-0]*

trans-d-Phenothrin: (1*R*)-*trans*- isomer

CAS No.: *[26046-85-5]*

2.3 Analytical Method

2.3.1 Apparatus

2.3.1.1 Laboratory Equipment

Mettler-Toledo XP205DR analytical balance for analytical standard.

Vortex mixer REAX top, Heidolph.

Rotary evaporator: Laborota 4002, Heidolph: and Büchi Rotavapor R 210 V850, Büchi.

Typical glassware and laboratory equipment.

All the glassware was cleaned in a laboratory dishwasher and air-dried before use.

2.3.1.2 GC-MS/MS System

Thermo Trace 1310 GC equipped with Split/Splitless injector, TriPlus RSH Autosampler and TSQ 8000 triple-quadrupole mass spectrometer.

Autosampler Injection: 2 µL splitless.

Carrier gas: Helium 1.5 mL/min constant flow.

Splitless injection:

Temperature : 225 °C

Splitless time: 2 min

Capillary Column:

Optima 5-MS Accent (Macherey-Nagel): 30 m length, 0.25 mm inner diameter, 0.25 µm film thickness.

Oven Temperature Program:

95°C for 0.75 min, then with 15 °C/min to 250 °C, finally with 10 °C/min to 275°C, 7 min hold.

Mass spectrometric conditions:

Temperature:	275°C
Ion polarity:	positive
Ion source:	Equipped with EI ion volume
Transitions monitored:	183 m/z -> 168 m/z (0.02 sec, collision energy: 10) 183 m/z -> 165 m/z (0.02 sec, collision energy: 10) 183 m/z -> 153 m/z (0.02 sec, collision energy: 10)

Figure 1 shows the full scan and product ion spectra for d-phenothrin. The quantitative determination was carried out by external calibration using calibration solutions in solvent. Calibration functions ranging from 1.0 to 150 ng/mL for cis-phenothrin and from 10 to 1000 ng/mL for trans-phenothrin were used to evaluate the extracts (exemplified in Figure 2). Representative GC-MS/MS ion chromatograms of calibration solutions in solvent and for extracts of fortified and control specimens are presented in Figure 4 to Figure 6.

2.3.2 Solvents, Chemicals and Miscellaneous

Acetone, Promochem, for Pesticide Residue Analysis.
Hexane, Promochem, for Pesticide Residue Analysis.
Toluene, Promochem, for Pesticide Residue Analysis.
Silanized glass wool, Supelco; anhydrous sodium sulfate, p.a., Merck.

2.3.3 Preparation of Standard Solutions

Stock solutions of d-phenothrin, cis-phenothrin and d-trans-phenothrin were prepared in toluene as follows e.g.:

Substance name	Weight [mg]	Dissolve in [mL]	Obtain [mg/mL]
d-phenothrin (purity: 97%)	10.45	10	1.0
cis-d-phenothrin (purity: 100%)	10.09	10	1.0
trans-d-phenothrin (purity: 95%)	10.43	10	1.0

Fortification solutions of d-phenothrin with concentrations of 2.0 and 0.20 µg/mL were prepared by accurate dilution of the stock solution in acetone.

Calibration solutions for cis-phenothrin and trans-phenothrin were prepared by volumetric dilution in toluene to obtain concentrations of 1.0, 2.0, 4.0, 10, 50 and 150 ng/mL (for cis-phenothrin) and 10, 20, 40, 500 and 1000 ng/mL (for trans-phenothrin).

For preparation of matrix matched standards final extracts of residue-free control specimen (processed concurrently with the fortified samples) were used. Aliquots of the final extracts were fortified with cis-phenothrin or trans-phenothrin using the calibration solutions in solvent resulting in concentrations of 10 ng/mL (cis-phenothrin) and 40 ng/mL (trans-phenothrin).

All standard solutions were stored refrigerated in amber glass bottles when not in use.

2.3.4 Stability of Standard Solutions and Extracts

The recoveries in the fortified samples are within the acceptable range of 70-120 %, thus stability is sufficiently proven (SANCO/825/00 rev. 8.1).

2.3.5 Effects of Matrix on Analytes Responses

No significant effects of matrix (enhancement or suppression) on GC-MS/MS responses were observed (see Table 2).

2.3.6 Residue Analysis

1. Measure 200 mL of water into a 0.25-L separation funnel.
2. For recovery controls, add at this stage:
   100 µL of a 0.20 µg/mL phenothrin fortification solution to establish a concentration of 0.10 µg/L in water as LOQ fortification.
   100 µL of a 2.0 µg/mL phenothrin fortification solution to establish a concentration of 1.0 µg/L in water as 10xLOQ fortification.
3. Extract the water sample 2-times, each time with 20 mL of hexane for 30 seconds by manual shaking. Finally, extract the water sample with 10 mL of hexane. Combine all hexane extracts through a funnel plugged with silanized glass wool, topped with ~10 g of sodium sulphate anhydrous, in a 100 mL pear shaped flask. Then wash the sodium sulphate with hexane (10 mL) collecting it into the pear shaped flask.
4. Rotary evaporate the combined, dried extract over a water bath at ~40 °C to reduce the volume of hexane to near dryness and remove the remaining solvent under a nitrogen stream.
5. Take up the residue with 0.50 mL of toluene, mix well and transfer the extracts into a glass vial for analysis by GC/MS.

2.4 Calculations

Results derived from GC-MS/MS and calculations are shown in detail in Table 1.

The following equation was used to calculate the individual residues R in µg/L:

$C_{Water} = C_{End} \times (V_{End} / V_W) / 1000\ ng/\mu g = C_{End} \times M$

with :

C_{Water}: Analyte concentration in surface water in µg/L.

C_{End}: Final concentration of analyte in specimen extract, in ng/mL.

V_W: 0.200 L (200 mL) of surface water used for extraction.

V_{End}: Volume of final extract: 0.50 mL

Recoveries (Rec.) were calculated for the fortified specimens as follows:

Rec. = $(C_{Water} / C_{fortified}) \times 100\ \%$

The calculation is exemplified with the surface water specimen P3046-34 (see Table 1) fortified at 0.10 µg/L (LOQ). The final extract was examined by GC-MS/MS in run file P3066TSQ8-017 (Figure 5) to give a final concentration for the cis-isomer C_{End} of 6.96 ng/mL for 183 m/z -> 168 m/z. The respective trans-isomer in specimen P3046-34 (see Table 1) gave a final concentration C_{End} of 36.4 ng/mL, for 183 m/z -> 168 m/z.

Thus:

For cis-phenothrin :

C_{Water} = $C_{End} \times (V_{End} / V_W) / 1000\ ng/\mu g$

= $C_{End} \times M$

= 6.96 ng/mL x (0.5 mL / 0.200 L) / 1000 ng/µg

= 6.96 ng/mL x 0.0025 (mLxµg)/(ng/L)

= 0.0174 µg/L

For trans-phenothrin similarly:

C_{Water}= 0.0909 µg/L

Total d-phenothrin concentration $C_{\Sigma cis+tr}$ found in water:

$C_{\Sigma cis+tr}$ = 0.0174 µg/L + 0.0909 µg/L

= 0.108 µg/L

Rec. = $[(C_{Water\,(cis)} + C_{Water\,(trans)}) / C_{fortified}] \times 100\ \%$

= [(0.0174 µg/L + 0.0909 µg/L) / 0.10 µg/L] x 100 % = 108 %

Calculations were performed with full precision. Thus discrepancies may arise when recalculated.