

ABSTRACT / SUMMARY**Independent laboratory Validation (ILV) of an Analytical Method for the Determination of the cis- and trans Isomers of d-Phenothrin in Soil by GC/MS****Objective:**

The objective of this study was to independently validate an analytical method¹ for separate determination of the cis- and the trans-isomers of d-phenothrin in soil. The target limit of quantification (LOQ) for the ILV is ≤ 0.01 mg/kg (10 ng/g), expressed as total d-phenothrin (TG: technical grade).

Method Principle:

d-Phenothrin (TG: technical grade) was extracted twice from 20-g soil samples with methanol by shaking and filtration, followed by L/L extraction (adding 10 % aqueous NaCl) with dichloromethane and glass column chromatography using 15-g of activated Florisil. Finally the analyte(s) were determined by GC/MS(SIM), separating cis- and trans-isomers into 2 peaks which were evaluated separately. The original method from 1993 monitored only two fragment ions (123 and 183 m/z) and used solely the 183 m/z ion for quantitative evaluation.

During the present ILV study, GC/MS(SIM) was used monitoring and evaluating three fragment ions (123, 183 m/z and 184 m/z). Additionally GC/MS/MS was employed and evaluated three MS/MS mass transitions (168, 165 m/z and 153 m/z) which are formed from an intensive 183 m/z fragment ion isolated in the 1st quadrupole of the MS/MS instrument.

Independent Laboratory Validation Results:

For complete method validation, soil samples were fortified (5 replicates per fortification level) with d-phenothrin (TG: contains according to information obtained by the sponsor 80.3 % trans-isomer and 19.7 % cis-isomer) at 0.010 mg/kg (LOQ) and at 0.10 mg/kg (10xLOQ), always expressed as total d-phenothrin.

Additional two soil samples were kept untreated as blank controls and one sample was processed as reagent blank. Signal / interference in all blank control sample extracts were not detected and thus below 20 % of the LOQ (< 0.002 mg/kg).

The average recoveries of each isomer and of total d-phenothrin (sum of cis- and trans-isomers) were for all three selected fragment ions and all three parent-daughter ion transitions monitored within the acceptable range of 70 % to 110 % with relative standard deviations (RSD) of ≤ 20 %.

The summary of the method validation results is given below:

¹ Jacobson, B., F. Kaiser, 17-Aug-93. Dissipation of Sumithrin Applied to Bare Ground, California Location. ABC Laboratories Report ID #40310. Sumitomo ID ER-31-0020. Pages 25-28.

1. INTRODUCTION

Background and Objective:

The objective of this study is to independently validate an analytical method² for separate determination of the cis- and the trans-isomers of d-phenothrin in soil. The target limit of quantification (LOQ) for the ILV is ≤ 0.01 mg/kg (10 ng/g), expressed as total d-phenothrin (TG: technical grade).

2. EXPERIMENTAL

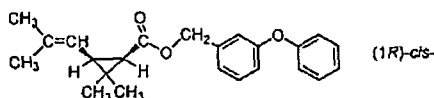
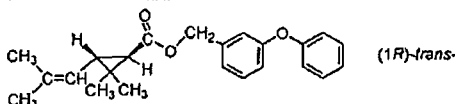
2.1 Test System

The analytical method was validated with one soil type, see APPENDIX 1 for soil characteristics.

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% (1R)- isomers.

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

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

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

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

CAS No.: [51186-88-0]

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

CAS No.: [26046-85-5]

² Jacobson, B., F. Kaiser, 17-Aug-93. Dissipation of Sumithrin Applied to Bare Ground, California Location. ABC Laboratories Report ID #40310. Sumitomo ID ER-31-0020. Pages 25-28.

2.3 Analytical Method

2.3.1 Apparatus

2.3.1.1 Laboratory Equipment

Mettler-Toledo XP205DR analytical balance for analytical standards.

Sartorius ED 2202S-CW laboratory balance for samples and reagents.

Vortex mixer REAX top, Heidolph. Horizontal shaker HS501D, IKA

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

Drying oven UFB 500, Memmert. Sartorius MA150Q drying balances.

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 μ L 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 μ m 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.

Electron impact (EI) mass spectrometric conditions:

Source temperature: 275°C

Ion polarity: positive

Ion source: Equipped with EI ion volume

Ions monitored in SIM: 183 m/z (0.02 sec), 123 m/z (0.02 sec), 184 m/z (0.02 sec)

Ions transitions in MS/MS: 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 full scan electron impact (EI) and MS/MS product ion spectra for d-phenothrin.

The quantitative determinations were carried out by external calibration using calibration solutions in solvent. Calibration functions ranging from 4.0 to 1000 ng/mL for cis-phenothrin

and from 40 to 2500 ng/mL for trans-phenothrin were used to evaluate the extracts (exemplified in Figure 2 to Figure 5).

Representative GC-MS(SIM) ion chromatograms of calibration solutions in solvent and for extracts of fortified and control specimens are presented in Figure 6 to Figure 8.

Representative GC-MS/MS ion chromatograms of calibration solutions in solvent and for extracts of fortified and control specimens are presented in Figure 9 to Figure 11.

2.3.2 Solvents, Chemicals and Miscellaneous

Acetone, Promochem, for Pesticide Residue Analysis.

Dichloromethane, Promochem, for Pesticide Residue Analysis.

Ethyl acetate, Promochem, for Pesticide Residue Analysis.

Hexane, Promochem, for Pesticide Residue Analysis.

Toluene, Promochem, for Pesticide Residue Analysis.

Methanol, analytical grade, Fisher Scientific.

Millipore water, PTRL Europe supply.

Silanized glass wool, Supelco. Glass fibre filter, Macherey-Nagel.

Sodium chloride, Fluka. Anhydrous sodium sulfate, Merk. All analytical grade.

Florisil, Fluka.

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	10.136	1.0
cis-d-phenothrin (purity: 100%)	10.09	10.090	1.0
trans-d-phenothrin (purity: 95%)	10.43	10.126	1.0

Fortification solutions of d-phenothrin with concentrations of 100, 20 and 2.0 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 4.0, 10, 25, 50, 100, 150, 250, 1000 and (intermediate) 10,000 ng/mL (for cis-phenothrin) and 40, 100, 250, 500, 1000, 1500, 2500 and (intermediate) 10,000 and 100,000 ng/mL (for trans-phenothrin).

For preparation of matrix matched standards a final soil extract of residue-free control soil (processed concurrently with the fortified samples) was used. Aliquots of the final extracts were fortified with cis-phenothrin or trans-phenothrin using calibration solutions in solvent resulting in concentrations of 25 ng/mL (cis-phenothrin) and 250 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 < 20 %) on GC-MS responses were observed (see Table 3).

2.3.6 Residue Analysis following Procedures as given in 1993 Report, Pages 26-27

For the ILV the procedures as given in the original 1993 report pages 26-27 were followed as given below:

Extraction

Sumithrin was extracted from soil using methanol. Twenty grams of soil were weighed into a 250-mL polypropylene bottle. Control samples were fortified at this point by application of the appropriate spiking solution directly onto the soil. Forty milliliters of methanol were added to the sample, the jar was capped and placed on a reciprocating shaker for 10 minutes. The sample was then filtered under vacuum through glass-fiber filter paper contained in a Büchner funnel. The liquid portion was collected in a 500-mL separatory funnel. The residue was re-extracted with an additional 40 mL of methanol, filtered, and the extracts combined in the separatory funnel. The sample container and Büchner funnel were rinsed with approximately 30 mL of methanol and the rinse combined with the sample extract. The extract was partitioned using 80 mL of a 10% sodium chloride solution and 40 mL of methylene chloride. The methylene chloride was passed through a bed of sodium sulfate to remove water and collected in a 250-mL

flat bottom flask. The partitioning was done again using 40 mL of methylene chloride, drained through the sodium sulfate, and collected in the flat bottom flask. The methylene chloride was concentrated to dryness under vacuum with a rotary evaporator and a water bath was held at approximately 30 °C.

Additional cleanup of the sample extract was performed using 15 g of activated Florisil contained in a glass chromatographic column. The residue in the flat bottom flask was dissolved in 3 mL of a mixed solvent (hexane/ethyl acetate, 20:1, v/v). This was quantitatively transferred to the Florisil column using three additional 3-mL washes of the mixed solvent. The sample solution and rinses were allowed to percolate through the column until the solvent reached the top of the packing. The eluate was discarded. The Sumithrin was eluted from the column using 45 mL of the mixed solvent. The first 5 mL were discarded. The remaining 40 mL were collected in a flat bottom flask, concentrated to 1-2 mL using rotary evaporation and then transferred to a glass screw-capped culture tube using hexane. The solution was taken to dryness using N₂. The residue was dissolved in a known volume of toluene and quantitated using gas chromatography (GC) and a mass selective detector (MSD).

Remarks:

1. Soil weight W was 20 g dry soil.
2. For recovery controls, add prior to extraction:
100 µL of a 2.0 µg/mL d-phenothrin fortification solution to establish a concentration of

- 10 $\mu\text{g}/\text{kg}$ (ng/g , 0.010 mg/kg) in soil as LOQ fortification.
 100 μL of a 20 $\mu\text{g}/\text{mL}$ d-phenothrin fortification solution to establish a concentration of 100 $\mu\text{g}/\text{kg}$ (ng/g , 0.10 mg/kg) as 10xLOQ fortification.
3. Florisil was activated in a drying oven at 130 °C over night.
 4. The final volume V_{End} was adjusted to 1.0 mL of toluene for GC/MS analysis.

2.4 Calculations

Results derived from GC-MS(SIM) and calculations are shown in detail in Table 1. Results derived from GC-MS/MS and calculations are shown in detail in Table 2.

The following equation was used to calculate the individual isomer residues R in ng/g :

$$R = C_{\text{End}} \times (V_{\text{End}} / W) = C_{\text{End}} \times M$$

with:

- R: Analyte concentration in soil in ng/g ($\mu\text{g}/\text{kg}$).
 C_{End} : Final concentration of analyte in soil extract, in ng/mL .
 W: Weight of soil extracted: 20 g
 V_{End} : Volume of final extract: 1.0 mL
 M: Multiplier
 $R_{\Sigma\text{cis+tr}}$: Sum of both isomers

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

$$\text{Rec.} = (R_{\Sigma\text{cis+tr}} / R_{\text{fortified}}) \times 100 \%$$

The calculation is exemplified with the soil sample P3047-37 (see Table 1) fortified at LOQ with 10 ng/g (0.010 mg/kg) of d-phenothrin TG. The final extract was examined by GC-MS(SIM) in run file P3067TSQ8-065 (Figure 8) to give for the cis-isomer in the 183 m/z ion chromatogram evaluated by external calibration with standards in solvent a final concentration C_{End} of 40.0 ng/mL . The trans-isomer gave a concentration C_{End} of 168 ng/mL .

Thus for the cis-isomer:

$$\begin{aligned} R &= C_{\text{End}} \times (V_{\text{End}} / W) \\ &= 40.0 \text{ ng/mL} \times (1.0 \text{ mL} / 20 \text{ g}) \\ &= 40.0 \text{ ng/mL} \times 0.050 \text{ mL/g} \\ &= 2.00 \text{ ng/g} \end{aligned}$$

And for the trans-phenothrin similarly:

$$\begin{aligned} R &= 8.38 \text{ ng/g} \\ R_{\Sigma\text{cis+tr}} &= 2.00 \text{ ng/g} + 8.38 \text{ ng/g} = 10.4 \text{ ng/g} \end{aligned}$$

And further:

$$\text{Rec.} = 10.4 \text{ ng/g} / 10.0 \text{ ng/g} \times 100 \% = 104 \%$$

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