INDEPENDENT LABORATORY VALIDATION OF "ANALYTICAL METHOD 00877 FOR THE DETERMINATION OF TOTAL RESIDUES OF DELTAMETHRIN (AE F032640) IN/ON SOIL AND SEDIMENT BY HPLC-MS/MS WITH MODIFICATION SIGNED 07/15/13"

Tim Vincent

1.0 SUMMARY

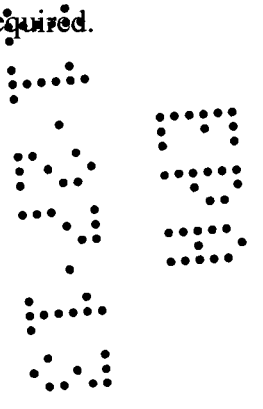
To satisfy US regulatory ILV requirements, a residue analytical method must be validated at an independent laboratory prior to its submission to the appropriate regulatory authority. The purpose of this study is to conduct an independent laboratory validation of the residue analytical method for deltamethrin (cis-deltamethrin and its isomers trans-deltamethrin and alpha-R-deltamethrin), in soil by LC/MS/MS as described in Bayer Method 00877, with method modification dated 07/15/13, as written (References 3 and 4). This study was designed to fulfill the requirements of the U.S. EPA guidelines found in OCSPP 850.6100 and OPPTS 860.1340(c)(6) (References 1 and 2).

The study was conducted by ABC Laboratories, Inc. of Columbia, Missouri, according to the protocol for Sponsor Study No. MEDAL039, entitled "Independent Laboratory Validation of "Analytical Method 00877 for the Determination of Total Residues of Deltamethrin (AE F032640) in/on Soil and Sediment by HPLC-MS/MS with Modification signed 07/15/13.""

The method under evaluation has a stated Limit of Quantitation (LOQ) of 0.10 µg/kg for all three isomers in soil. In this study, the method was validated on a composite sample of soil at the LOQ and 10 × LOQ.

The first method validation trial conducted on soil was successful for deltamethrin (cis-deltamethrin and its isomers trans-deltamethrin and alpha-R-deltamethrin) with no modifications to the method. However, standard and fortification solutions were adjusted and/or diluted proportionally to accommodate available glassware and test material, as allowed by the method. The 15 ng/L (the method estimated LOD level) standard was not used due to the signal to noise being under the three-to-one ratio, and the 30 and 40 ng/L standards were added in order to attain bracketing of the fortified samples.

No communication, other than clarification of solutions, chromatography issues, and recovery updates between the Sponsor Representative and Study Director, was required.



A single analyst completed sample sets consisting of 33 samples in the course of approximately one-and-a-half typical workday (13 hours) for one set. To analyze for all isomers, three extractions per set would be completed. LC-MS/MS analysis would be performed overnight and into the next day, for each set, for approximately 15 hours.

2.0 INTRODUCTION

To satisfy US regulatory ILV requirements, a residue analytical method must be validated at an independent laboratory prior to its submission to the appropriate regulatory authority. This study was conducted to fulfill those requirements.

The residue analytical method described in Bayer Analytical Method 00877, entitled "Analytical Method 00877 for the Determination of total Residues of Deltamethrin (AE F032640) in/on Soil and Sediment by HPLC-MS/MS" (Appendix 1), with method modification dated 07/15/13 (Appendix 2), is applicable for the quantitation of cis-deltamethrin, trans-deltamethrin, and alpha-R-deltamethrin in soil. In this study, the analytical method was validated on a representative matrix for which the method was designed: a sample of soil.

Soil samples of 20 g are extracted in a microwave extractor with 40 mL of a mixture of acetonitrile/ammonium acetate 10 mMol/L in water (900/100, v/v). After extraction, portions of the samples are centrifuged to remove fine particles of the soil. Identification

and quantitation of the test item is done by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode. Possible matrix effects of deltamethrin are eliminated by using an isotopically labeled internal standard solution. This solution is added to the sample solutions after extraction. The limit of quantitation (LOQ) for all isomers was 0.10 µg/kg. The limit of detection (LOD) in the method was estimated to be 0.03 µg/kg, but as the equivalent standard (15 ng/L) was not used due to performance issues, the lowest attainable standards (30 ng/L) was used to determine the LOD as 0.06 µg/kg for this study.

Except for the minor modifications discussed in Section 3.6 of this report, these methods were performed as written. No communication, other than clarification of solutions, chromatography issues, and recovery updates between the Sponsor Representative and Study Director, was required.

3.0 MATERIALS AND METHODS

3.1 Test Substances

The reference analytical standards (test substances) used for this study were:

cis-deltamethrin:

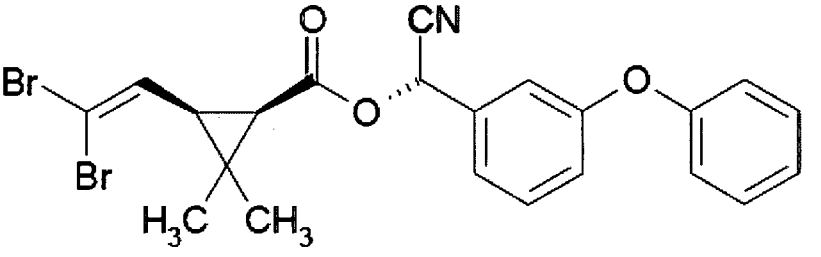
Chemical Name:

IUPAC: (S)–α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS: [1R-[1α(S*),3α]]-cyano(3-phenoxyphenyl)methyl3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

CAS No.: 52918-63-5

Chemical Structure:



Molecular Weight:	505.2 g/mol
Source:	Bayer CropScience
Purity:	99.4%
Lot no.:	0902200301
Receipt date:	04 Sep 13
Expiration date:	18 Nov 15
Storage:	5°C

trans-deltamethrin:

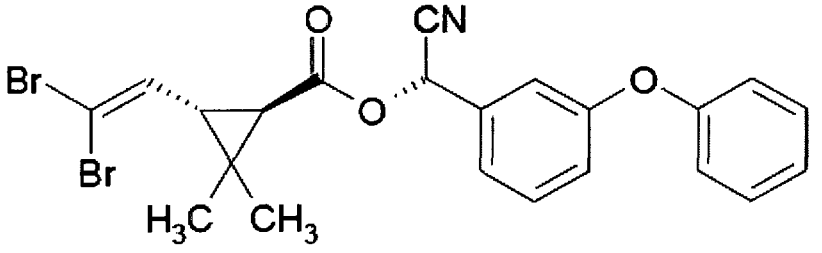
Chemical Name:

IUPAC: (S)–α-cyano-3-phenoxybenzyl (1R,3S)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS: [1R-[1α(S*),3β]]-cyano(3-phenoxyphenyl)methyl3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

CAS No.: 64363-96-8

Chemical Structure:



Molecular Weight: 505.2 g/mol
Source: Bayer CropScience
Purity: 95.1%
Lot no.: 0106200406
Receipt date: 04 Sep 13
Expiration date: 19 Feb 15
Storage: -20°C

alpha-R-deltamethrin:

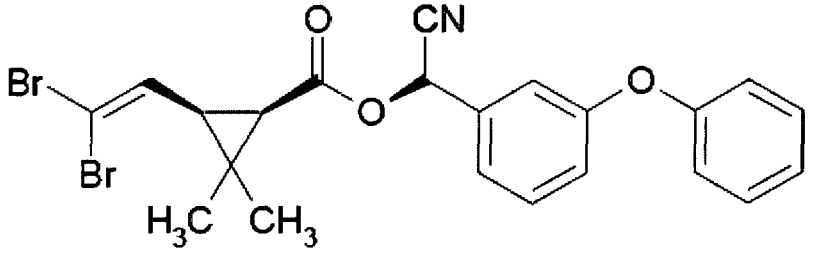
Chemical Name:

IUPAC: (R)–α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS: [1R-[1α(R*),3α]]-cyano(3-phenoxyphenyl)methyl3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

CAS No.: 55700-99-7

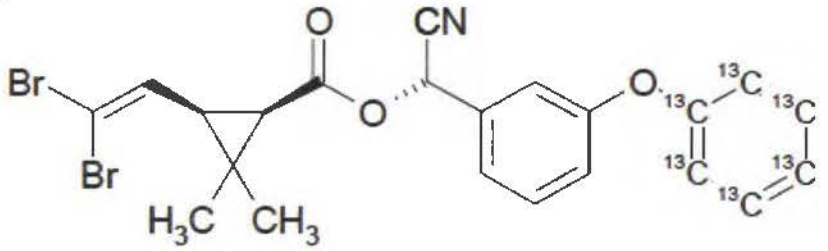
Chemical Structure:



Molecular Weight: 505.2 g/mol
Source: Bayer CropScience
Purity: 90.1%
Lot no.: 0522200602
Receipt date: 04 Sep 13
Expiration date: 01 Mar 18
Storage: -20°C

cis-deltamethrin-^{13}C6 Internal standard:

Chemical Name:
IUPAC: (S)–cyano(3-phenoxyphenyl)methyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS No.: Not available
Chemical Structure:



Molecular Weight:	511.14 g/mol
Source:	Bayer CropScience
Purity:	100%
Lot no.:	BECH 1068-2-1
Receipt date:	04 Sep 13
Expiration date:	09 Mar 16
Storage:	-20°C

The cis-deltamethrin, trans-deltamethrin, and alpha-R-deltamethrin standards, as well as the cis-deltamethrin-^{13}C6 internal standard, were supplied by Bayer CropScience. Information pertaining to the characterization and stability of the test substances is archived by Bayer CropScience. The Certificates of Analysis are included in Appendix 3.

3.2 Test Systems

In this study, the analytical method was validated on the following matrix: samples of soil. This matrix was chosen as representative of the matrices for which the method was designed – soil and sediment.

Control samples of soil used in the study were provided by the ABC Laboratories, Inc. from a site in Iowa. All soil samples were sieved (No. 10, 2.00 mm mesh sieve) and homogenized by ABC Laboratories, Inc. prior to use in this study. The soil samples were placed into limited-access refrigerated storage (GXP 437) at a temperature range of 5 ± 5°C after being homogenized at ABC Laboratories, Inc. The samples remained in refrigerated storage until removed for subsampling and analysis. The soil was processed following ABC SOPs.

The soil specimen was GLP characterized by AGVISE Laboratories; details of the characterization results are presented in Appendix 4.

The samples were assigned unique identification by the laboratory. Additional designations such as "control" and "fortified control," as appropriate, were also assigned by the laboratory.

3.3 Equipment

Equipment used is the same as that specified in the analytical method, except as noted below:

Balances:	Mettler Toledo, Model XP205DR, for weighing solid standards (Mettler Instrument Corp.) Mettler Toledo, Model BB2440, for weighing ammonium acetate (Mettler Instrument Corp.) Mettler Toledo, Model MS3002S/03, for weighing soil samples (Mettler Instrument Corp.)
Centrifuge:	Model 5430R (Eppendorf)
Graduated cylinders:	glass, various sizes, class B
HPLC/MS System:	AB Sciex Mass Spectrometer API 4000 LC/MS/MS system with a Leap Autosampler HTC PAL, Agilent 1100 Bin. Pump, Agilent 1100 Degasser, Agilent 1100 Col. Comp., and Valco Switching Valve. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software. HPLC column: Zorbax Eclipse XDB-C8, 5µm, 2.1 x 150mm HPLC vials: Target Snap Cap Wide opening, clear glass, 2 mL (National Scientific) HPLC vial caps: Target National Scientific Kim-Snap closure caps with PTFE/Silicone septa (National Scientific) HPLC vials: Target DP Screw Thread Vial, amber glass, 12 x 32 mm, Flat base, 2 mL (National Scientific) HPLC vial caps: Target DP RoboCap, polypropylene with PTFE/Silicone/PTFE septum (National Scientific)
Microwave:	MARS Microwave Accelerated Reaction System (CEM)
Tachometer	Model 02-401-3 (Fisher Scientific)
Timer:	Mobile Timer (Control Company)
Pipets:	class A glass, graduated, serological; various sizes

Pipets, adjustable:	Gilson pipets: Microman, 10-100 µL, 50-250 µL (Gilson Scientific)
	Hamilton syringes 100 and 250 µL (Hamilton Company)
Volumetric flasks:	Glass, various sizes, class A

3.4 Reagents and Standards

Reagents and standards used were of equivalent grade as that specified in the analytical method.

3.5 Principles of the Analytical Method

The residue analytical method described in Bayer Analytical Method 00877, entitled "Analytical Method 00877 for the Determination of total Residues of Deltamethrin (AE F032640) in/on Soil and Sediment by HPLC-MS/MS" (Appendix 1), with method modification dated 07/15/13 (Appendix 2), is applicable for the quantitation of cis-deltamethrin, trans-deltamethrin, and alpha-R-deltamethrin in soil. The following is a summary of that method:

Soil and sediment samples of 20 g are extracted in a microwave extractor with 40 mL of a mixture of acetonitrile/ammonium acetate 10 mMol/L in water (900/100, v/v). After extraction, portions of the samples are centrifuged to remove fine particles of the soil. Identification and quantitation of the test item is done by high performance liquid chromatography using MS/MS detection in the Multiple Reaction Monitoring mode. Possible matrix effects of deltamethrin are eliminated by using an isotopically labeled internal standard solution. This solution is added to the sample solutions after extraction.

3.6 Modifications, Interpretations, and Critical Steps

There were no modifications to the method, but some changes expressly allowed within the method were utilized during this study. The analytical method was run exactly as written except as follows:

Section 7 Standard Solutions and Section 9.1 Fortification. Standard and fortification solutions were adjusted and/or diluted proportionally to accommodate available glassware and test material.

Section 7 Standard Solutions: The 15 ng/L (the method estimated LOD level) standard was not used due to the signal to noise being under the three-to-one ratio, and the 30 and 40 ng/mL standards were added in order to attain bracketing of the fortified samples.

Section 9.2 Extraction: The microwave power was set at 32% of the 800 W unit (256 W), to at least meet the 250 W prescribed in method.

The method-listed retention time of 8.75 minutes (see modification dated 07/15/13 in Appendix 2) was not obtained, but retention times do vary with specific instruments and conditions.

The method also included a modification dated 07/15/13 (Appendix 2), which is considered a part of the method for the purposes of this study.

3.7 Instrumentation

The quantitative analysis of cis-deltamethrin, trans-deltamethrin, and alpha-R-deltamethrin was performed using Agilent 1100 HPLC units coupled to an AB Sciex Mass Spectrometer API 4000 LC/MS/MS system. The system parameters are shown in the tables below for each of the isomers. Peak area was used for quantitation.

HPLC Conditions (cis-deltamethrin):

System:	AB Sciex Mass Spectrometer API 4000 LC/MS/MS system with a Leap Autosampler HTC PAL, Agilent 1100 Bin. Pump, Agilent 1100 Degasser, Agilent 1100 Col. Comp., and Valco Switching Valve. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.
Column:	Zorbax Eclipse XDB-C8, 5µm, 2.1 x 150mm
Column Temperature:	60 °C
Injection Volume:	60 µL
Autosampler Temperature:	Not Available
Flow Rate:	0.3 mL/minute
Mobile Phase:	A: Water with Ammonium Acetate 10mM/Methanol (9/1) B: Methanol with Ammonium Acetate 10mM

Mobile Phase Conditions:

Time	%A	%B	Flow (mL/min)
0.00	20.0	80.0	0.3
4.00	20.0	80.0	0.3
4.01	5.0	95.0	0.3
10.00	5.0	95.0	0.3
10.10	20.0	80.0	0.3
14.00	20.0	80.0	0.3

Retention Times:	cis-deltamethrin	~3.6 minutes
	cis-deltamethrin-^{13}C6 (Internal standard)	~3.6 minutes
Total Run Time:	~14 minutes	

HPLC Conditions (trans-deltamethrin):

System:	AB Sciex Mass Spectrometer API 4000 LC/MS/MS system with a Leap Autosampler HTC PAL, Agilent 1100 Bin. Pump, Agilent 1100 Degasser, Agilent 1100 Col. Comp., and Valco Switching Valve. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.
Column:	Zorbax Eclipse XDB-C8, 5µm, 2.1 x 150mm
Column Temperature:	60 °C
Injection Volume:	60 µL
Autosampler Temperature:	Not Available
Flow Rate:	0.3 mL/minute
Mobile Phase:	A: Water with Ammonium Acetate 10mM/Methanol (9/1) B: Methanol with Ammonium Acetate 10mM

Mobile Phase Conditions:

Time	%A	%B	Flow (mL/min)
0.00	20.0	80.0	0.3
4.00	20.0	80.0	0.3
4.01	5.0	95.0	0.3
10.00	5.0	95.0	0.3
10.10	20.0	80.0	0.3
14.00	20.0	80.0	0.3

Retention Times:	trans-deltamethrin	~3.6 minutes
	cis-deltamethrin-^{13}C6 (Internal standard)	~3.6 minutes
Total Run Time:	~14 minutes	

HPLC Conditions (alpha-R-deltamethrin):

System:	AB Sciex Mass Spectrometer API 4000 LC/MS/MS system with a Leap Autosampler HTC PAL, Agilent 1100 Bin. Pump, Agilent 1100 Degasser, Agilent 1100 Col. Comp., and Valco Switching Valve. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.
Column:	Zorbax Eclipse XDB-C8, 5µm, 2.1 x 150mm
Column Temperature:	60 °C
Injection Volume:	60 µL
Autosampler Temperature:	Not Available
Flow Rate:	0.3 mL/minute
Mobile Phase:	A: Water with Ammonium Acetate 10mM/Methanol (9/1) B: Methanol with Ammonium Acetate 10mM

Mobile Phase Conditions:

Time	%A	%B	Flow (mL/min)
0.00	20.0	80.0	0.3
4.00	20.0	80.0	0.3
4.01	5.0	95.0	0.3
10.00	5.0	95.0	0.3
10.10	20.0	80.0	0.3
14.00	20.0	80.0	0.3

Retention Times:	alpha-R-deltamethrin	~3.6 minutes
	cis-deltamethrin-^{13}C6 (Internal standard)	~3.6 minutes
Total Run Time:	~14 minutes	

The detection method utilized was LC-MS/MS employing electrospray (TIS) interface in the positive mode on a triple quadrupole instrument. The instrument was tuned by infusing the analytes into a TIS (turbo ion spray) source, then creating a tune file to maximize the response of each analyte using the TIS source. The acquisition method was adjusted to maximize the response of the fragment ions detected. The ion transitions for each analyte are shown in the table below:

MS Conditions:

System: Applied BioSystems/MDS Sciex API 4000 LC/MS/MS system

Analytes Monitored	Ions Monitored (AMU)	Declustering Potential (volts)	Collision Energy (volts)	Dwell Time (milli-seconds)	EP (volts)	CXP (volts)	Acquisition Timing (minutes)
cis-deltamethrin	523 → 280.9	55	22	1000	10	20	0-14
trans-deltamethrin	523 → 280.9	55	22	1000	10	20	0-14
alpha-R-deltamethrin	523 → 280.9	55	20	1000	10	20	0-14
cis-deltamethrin-^{13}C6 (Internal standard)	529 → 280.9	55	24	100	10	18	0-14

Additional detector settings are shown in the table below:

Parameter	**Setting**			
	cis-deltamethrin	trans-deltamethrin	alpha-R-deltamethrin	cis-deltamethrin-^{13}C6 (Internal standard)
Acquisition Mode:	MRM	MRM	MRM	MRM
Ionization Mode:	positive (+)	positive (+)	positive (+)	positive (+)
Source Temp.:	500 °C	500 °C	500 °C	500 °C
Nebulizer (GS1):	60	60	60	60
Auxiliary Gas (GS2):	60	60	60	60
Curtain Gas:	50	50	50	50
CAD Gas:	6	6	6	6
Ion Spray Voltage:	5500	5500	5500	5500

The instrument was operated in the MS/MS (MRM) positive ion mode for quantitative analysis. Single transition chromatograms for each analyte were integrated and the peak areas used for quantitation. Quantitation was performed using a single transition for each analyte.

For each analytical run, a nine-point standard curve was prepared by injecting constant volumes of standard solutions of each isomer. All injections also contained an internal standard, cis-deltamethrin-^{13}C6, and the ratio of the analyte peak to the internal standard peak area was used to determine the residue.

3.8 Calculations

Calculations were performed as directed by the method. A validated software application was used to create a standard curve based on linear regression. Linear regression was monitored to support the response linearity of the mass spectrometer detector. The regression functions were used to calculate a best fit line (from a set of standard concentrations in ng/L versus peak response intensity ratio (i.e. target analyte isomer response divided by internal standard (IS) response) to demonstrate that a linear

relationship exists between analyte concentration and peak response, and that a response factor approach to calculation was appropriate.

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	intensity ratio (analyte peak response/IS peak response)
x	=	ng/L found for peak of interest
m	=	slope
b	=	y-intercept

Equations for Deltamethrin Isomers (cis-, trans-, & alpha-R-)

The calculations for µg/kg (parts per billion, or ppb) found and percent recovery (for fortified samples) were:

The amount of analyte (in µg/kg) found in the sample was calculated according to the following equation:

$$\mu g/kg\ \text{Found} = \frac{\text{ng/L Found} \times \text{FV (mL)}}{\text{SW (g)} \times 1000}$$

where:

ng/L Found	=	(Intensity Ratio – b)/m Intensity Ratio = Analyte Peak Area/Internal Standard Peak Area Analyte Peak Area = peak area response of the analyte in sample extract; Internal Standard Peak Area = peak area response of the internal standard in sample extract
FV	=	Final Volume. The mL volume of the final sample extract submitted to HPLC (typically 40.0 mL)
SW	=	Sample Weight. Grams of sample extracted (20.0 g for soil)
1000	=	1000 mL/L

Also written as µg/kg Found = (ng/L × DF)/1000, where:

DF	=	Dilution Factor. Final Volume (FV)/Sample Weight (SW)

Percent recovery of fortified samples (procedural fortifications) was determined using the following equation:

$$\%\ \text{Recovery} = \frac{\text{ppb Found in Fortified Sample}}{\text{ppb Added}} \times 100$$

Example Calculations for Deltamethrin Isomers (cis-, trans-, & alpha-R-)

Deltamethrin isomers were calculated for soil as follows:

80406-002, cis-deltamethrin, soil, Set #1, **Fortified Control @ 0.10 µg/kg:**

Sample peak response = 3119

IS peak response = 143712

IS ratio = 3119/143712 = 0.0217

m = 0.0004844957

b = -0.00160996

ng/L Found = (0.0217 + 0.00160996)/0.0004844957 = 48.1 ng/L

$$\mu g/kg\ Found = \frac{48.1\ ng/L \times 40.0\ mL}{20.0\ g \times 1000\ mL/L} = 0.0962\ \mu g/kg$$

$$\%\ Recovery = \frac{0.0962\ ppb}{0.10\ ppb} \times 100 = 96\%$$

7.0 REFERENCES

1. United States Environmental Protection Agency (U.S. EPA). January 2012. Office of Chemical Safety and Pollution Prevention (OCSPP). Ecological Effects Test Guidelines, OCSPP 850.6100: Environmental Chemistry Methods and Associated Independent Laboratory Validation (Formerly OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods). EPA 712-C-001, Washington, D.C.
2. United States Environmental Protection Agency (U.S. EPA). August 1996. Office of Chemical Safety and Pollution Prevention (OCSPP). Residue Chemistry Test Guidelines OPPTS (OCSPP) 860.1340, "Residue Analytical Method", EPA 712-C-96-174, Washington, D.C.
3. Brumhard, B.; Analytical Method 00877 for the Determination of total Residues of Deltamethrin (AE F032640) in/on Soil and Sediment by HPLC-MS/MS, Bayer CropScience AG Report: MR-081/04, Amended 31 March 2009, Bayer CropScience AG, Germany.
4. Method Modification signed for: Analytical Method 00877 for the Determination of Total Residues of Deltamethrin (AE F032640) in/on Soil and Sediment by HPLC-MS/MS, Bayer CropScience Study Number: MEDAL039, 13 July 2013, Bayer CropScience, Research Triangle Park, North Carolina.

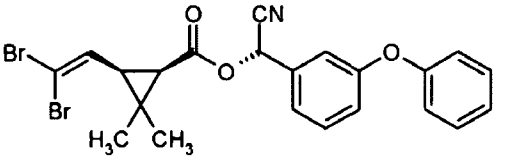
APPENDIX 1 BAYER CROPSCIENCEAG REPORT MR-081/04, ENTITLED "ANALYTICAL METHOD 00877 FOR THE DETERMINATION OF TOTAL RESIDUES OF DELTAMETHRIN (AE F032640) IN/ON SOIL AND SEDIMENT BY HPLC-MS/MS" (CONTINUED)

2 Introduction

The objective of the study was to validate a new residue analytical method for the determination of the total residue of deltamethrin in soil and sediment by means of HPLC-MS/MS. The total residue is defined as the sum of cis-deltamethrin (AE F032640), α-R-deltamethrin (AE F108569) and trans-deltamethrin (AE F0035073).
It is known that cis-deltamethrin can be transformed by chemical or biological processes to its diasteromeres α-R-deltamethrin and trans-deltamethrin. For ecotoxicological risk assessment purposes α-R- and trans-deltamethrin are assumed to have the same biological/pesticidal activity as cis-deltamethrin. This method is able to quantify cis-deltamethrin as well as its isomers trans-deltamethrin and alpha-R-deltamethrin. The method was optimised and validated using cis-deltamethrin.
The method was validated in accordance to the Guidance Document on Residue Analytical Methods (Ref. 1), the Commission Directive 96/46/EC (Ref. 3) and BBA Guideline for Residue Analytical Methods (Ref. 2).

3 Test and Reference Items

cis-deltamethrin:



Common name:	Deltamethrin
Code name:	cis-deltamethrin
Chemical code:	AE F032640
Chemical name (IUPAC):	(S)–α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
Chemical name (CA):	[1R-[1α(S*),3α]]-cyano(3-phenoxyphenyl)methyl3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate
CAS No.:	52918-63-5
Molecular formula:	$C_{22}H_{19}Br_2NO_3$
Molecular weight:	505.2 g/mol
Solubility in water:	<5 µg/L (20 °C)
Vapour Pressure:	1.24 x 10^{-8} Pa (25 °C)
Octanol-water partition coefficient log Pow:	4.6 (25 °C)
Hydrolytic stability:	pH 5 & 7: $t_{1/2}$ >30 days (25 °C) pH 9: $t_{1/2}$ 2.5 days (25 °C)

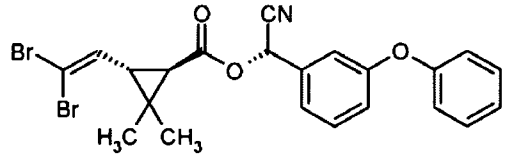
APPENDIX 1 BAYER CROPSCIENCEAG REPORT MR-081/04, ENTITLED "ANALYTICAL METHOD 00877 FOR THE DETERMINATION OF TOTAL RESIDUES OF DELTAMETHRIN (AE F032640) IN/ON SOIL AND SEDIMENT BY HPLC-MS/MS" (CONTINUED)

Laboratory Project ID: P 601030028 Bayer CropScienceAG Report: **MR-081/04**
Deltamethrin Method Soil

Reference standard:

Certificate of analysis:	AZ 10090
Batch ID:	97B0276B3
Purity:	99.6%
Expiry date:	July 2007
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

trans-deltamethrin:

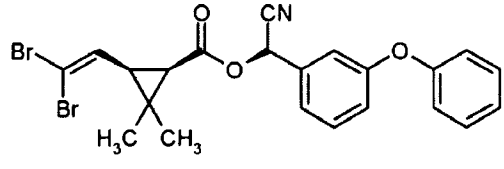


Code name:	trans-deltamethrin
Chemical code:	AE 0035073
Chemical name (IUPAC):	(S)–α-cyano-3-phenoxybenzyl (1R,3S)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
Chemical name (CA):	[1R-[1α(S*),3β]]-cyano(3-phenoxyphenyl)methyl3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate
CAS No.:	64363-96-8
Molecular formula:	$C_{22}H_{19}Br_2NO_3$
Molecular weight:	505.2 g/mol

Reference standard:

Certificate of analysis:	AZ 09021
Batch ID:	5E0551
Purity:	94.0%
Expiry date:	March 2005
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

alpha-R-deltamethrin:



Code name:	alpha-R-deltamethrin
Chemical code:	AE F108569
Chemical name (IUPAC):	(R)–α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

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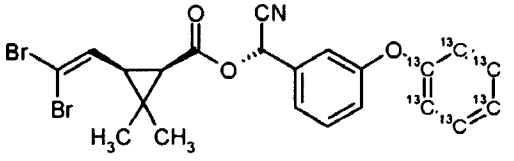
Laboratory Project ID: P 601030028 Bayer CropScienceAG Report: **MR-081/04**
Deltamethrin Method Soil

Chemical name (CA):	[1R-[1α(R*),3α]]-cyano(3-phenoxyphenyl)methyl3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate
CAS No.:	55700-99-7
Molecular formula:	$C_{22}H_{19}Br_2NO_3$
Molecular weight:	505.2 g/mol

Reference standard:

Certificate of analysis:	AZ 11194
Batch ID:	SEL/1322
Purity:	93.1%
Expiry date:	Nov. 2004
Origin:	Bayer CropScience GmbH, PT – Analytics Frankfurt, D-65926 Frankfurt am Main, Germany

cis-deltamethrin-^{13}C6:
cis-deltamethrin-^{13}C6 is used as internal standard.



Code name:	[phenoxy-^{13}C6]deltamethrin
Chemical name (IUPAC):	(S)–cyano(3-phenoxyphenyl)methyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
Empirical formula:	$C_{22}H_{19}Br_2NO_3$
Molecular weight:	511.14 g/mol

Reference standard:

Batch ID:	BECH 1068-1-1
Chemical purity:	>99%
Isotope enrichment:	total ^{13}C: 99.6%
Expiry date:	not given
Origin:	Bayer CropScience AG, PT – Isotope Chemistry, D-42096 Wuppertal, Germany

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4 Test System

The method was validated using the two German soils *Höfchen* and *Laacher Hof* and the German sediment *Nesten*. Two different soils and the sediment were used in order to assess a possible influence of different soil properties. The soil and sediment samples were classified according to DIN and/or USDA specifications. Soil characteristics are summarised in Table 1.
Complete soil and sediment parameterisation is reported in the Appendix (Table 7 to Table 9).

Table 1: Soil Types

Designation	Soil type (Texture)	Organic matter [%]
Höfchen	silt loam (USDA)	1.6
Laacher Hof	sandy loam (USDA)	2.1
Sediment	silt loam (USDA)	7.2

5 Instruments

Microwave Extractor:	MLS-Ethos MWS Vertriebs GmbH 88299 Leutkirch, Germany
Balance:	PC 4400, PM 4800 and AT 261 Mettler Instruments GmbH 35387 Giessen, Germany
Ultrasonic bath:	Transsonic 890/H Heinrich Faust 51145 Cologne, Germany
Liquid chromatograph:	HP 1100 Column Compartment G1316A HP 1100 Binary Pump G1312A HP 1100 Isocratic Pump G1310A HP 1100 Degasser G1322A Agilent 40880 Ratingen, Germany
Autosampler:	HTC PAL System CTC Analytics AG 4222 Zwingen, Switzerland
Column:	Purospher STAR RP-18e Li ChroCard Size: 55 x 4 mm Cat. No.: 1.50231 Merck Eurolab GmbH 64293 Darmstadt, Germany

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Mass spectrometer:	API 4000 with turbo-ionspray interface mass selective detector (MS/MS) Perkin Elmer Sciex Instruments 64331 Weiterstadt, Germany

6 Reagents and Equipment

Magnetic stirring bar:	plain (large, e.g. 35 x 8 mm [length x i.d.]) or "dumb-bell" type (e.g. 35 x 8 mm [length x i.d.], diameter of end disk is 20 mm, from COWIE Technology, parts no. 1.1335)
Acetonitrile:	for HPLC, super gradient grade Riedel de Haen, No. 34998 30926 Seelze, Germany
Methanol:	for HPLC Promochem GmbH 46469 Wesel, Germany
Toluene	for analysis Merck, No.1.08325.2500 64271 Darmstadt, Germany
Ammonium acetate	extra pure Merck, No.1.01115.1000 64271 Darmstadt, Germany
Water:	purified in a Milli-Q unit Milli-Pore GmbH 65731 Eschborn, Germany
Solvent I:	acetonitrile/ammonium acetate 10 mMol/L in water(900/100; v/v)

Volumetric flasks, pipettes and other instruments commonly used in the laboratory.

7 Standard Solutions

The following Sections 7.1 to Section 7.3 describe general working procedures for the preparation of standard solutions. Therefore, given names, weights and volumes do not correspond exactly to the used names, weights, volumes and concentrations documented in the raw data.

7.1 Standard Stock Solutions

(STMDEL) 500 mg/L stock solution of cis-deltamethrin:
Weigh approximately 10.0 mg* cis-deltamethrin into a 20-mL volumetric flask. Dilute to volume with toluene.

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(STM13C) 1000 mg/L stock solution of [phenoxy-^{13}C6]deltamethrin:
Weigh approximately 10.0 mg* [phenoxy-^{13}C6]deltamethrin into a 10-mL volumetric flask. Dilute to volume with toluene.

*: 0.01 mg characterises the precision of the balance, not the precision of the weighed reference substance

Before further use, the standard stock solution has to be ultrasonicated for about one minute to achieve complete dissolution of the test substance.

7.2 Working Standard Solutions

The working standard solutions are used for the preparation of the calibration standard solutions and for sample fortification.

(1 LSGDEL) 1000 µg/L solution of cis-deltamethrin:
Pipette 0.2 mL of (STMDEL) into a 100-mL volumetric flask and dilute to volume with Solvent I.

(2 LSG13C) 1000 µg/L solution of [phenoxy-^{13}C6]deltamethrin:
Pipette 0.1 mL of (STM13C) into a 100-mL volumetric flask and dilute to volume with Solvent I.

(3 LSGDEL) 100 µg/L solution of cis-deltamethrin:
Pipette 10 mL of (1LSGDEL) into a 100-mL volumetric flask and dilute to volume with Solvent I.

(4 LSG13C) 2 µg/L solution of [phenoxy-^{13}C6]deltamethrin:
Pipette 0.5 mL of (2LSG13C) into a 250-mL volumetric flask and dilute to volume with Solvent I.

(5 LSGDEL) 10 µg/L solution of cis-deltamethrin:
Pipette 1 mL of (1LSGDEL) into a 100-mL volumetric flask and dilute to volume with Solvent I.

7.3 Solvent Standard Solutions

(1 MIXDEL) mixed solution of 5 µg/L cis-deltamethrin and 2 µg/L of [phenoxy-^{13}C6]-deltamethrin:
Pipette 0.25 mL of (1 LSGDEL) and 0.1 ml of (2LSG13C) into a 50-mL volumetric flask and dilute to volume with Solvent I.

(2 MIXDEL) mixed solution of 2.5 µg/L cis-deltamethrin and 2 µg/L of [phenoxy-^{13}C6]-deltamethrin:
Pipette 0.125 mL of (1 LSGDEL) and 0.1 ml of (2LSG13C) into a 50-mL volumetric flask and dilute to volume with Solvent I.

(3 MIXDEL) mixed solution of 0.5 µg/L cis-deltamethrin and 2 µg/L of [phenoxy-^{13}C6]-deltamethrin:
Pipette 0.25 mL of (3 LSGDEL) and 0.1 ml of (2LSG13C) into a 50-mL volumetric flask and dilute to volume with Solvent I.

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(4 MIXDEL) mixed solution of 0.2 µg/L cis-deltamethrin and 2 µg/L of [phenoxy-^{13}C6]-deltamethrin:
Pipette 0.1 mL of (3 LSGDEL) and 0.1 ml of (2LSG13C) into a 50-mL volumetric flask and dilute to volume with Solvent I.

(5 MIXDEL) mixed solution of 0.1 µg/L cis-deltamethrin and 2 µg/L of [phenoxy-^{13}C6]-deltamethrin:
Pipette 0.05 mL of (3 LSGDEL) and 0.1 ml of (2LSG13C) into a 50-mL volumetric flask and dilute to volume with Solvent I.

(6 MIXDEL) mixed solution of 0.08 µg/L cis-deltamethrin and 2 µg/L of [phenoxy-^{13}C6]deltamethrin:
Pipette 0.4 mL of (5 LSGDEL) and 0.1 ml of (2LSG13C) into a 50-mL volumetric flask and dilute to volume with Solvent I.

(7 MIXDEL) mixed solution of 0.05 µg/L cis-deltamethrin and 2 µg/L of [phenoxy-^{13}C6]deltamethrin:
Pipette 0.25 mL of (5 LSGDEL) and 0.1 ml of (2LSG13C) into a 50-mL volumetric flask and dilute to volume with Solvent I.

(8 MIXDEL) mixed solution of 0.015 µg/L cis-deltamethrin and 2 µg/L of [phenoxy-^{13}C6]deltamethrin:
Pipette 0.075 mL of (5 LSGDEL) and 0.1 ml of (2LSG13C) into a 50-mL volumetric flask and dilute to volume with Solvent I.

All standard solutions need to be stored in a refrigerator.

8 Safety Precautions

The German guidelines for laboratories issued by the Trade Co-operative Association (e.g. Bulletin M006) or comparable guidelines in other countries must be considered when working according to this method.
The following solvents and chemicals are used which are classified according to the Hazardous Substances Regulations:

cis-deltamethrin	toxic T and irritant Xi (R-phrases 21, 23/25, 36/38)
alpha-R-deltamethrin	toxic T *)
trans-deltamethrin	toxic T *)
[phenoxy-^{13}C6]-deltamethrin	toxic T *)
Ammonium acetate	
Acetonitrile	harmful Xn, highly flammable F
Toluene	harmful Xn, highly flammable F
Methanol	toxic T, highly flammable F

*) A classification is not yet available. Due to this fact the compound has to be treated as very toxic substance.

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This classification is based on German guidelines and has to be adapted according to the respective national guidelines in case the method is used outside of Germany. While working with these substances, the relevant safety regulations are to be considered (see R- and S-rules).

9 Performance of Analysis

Within this method validation it was demonstrated that cis-deltamethrin and its isomers trans-deltamethrin and alpha-R-deltamethirn show the same selectivity for MS/MS detection (see Figure 2 to Figure 6). This will allow to minimize time for chromatographic separation and to elute the isomer mixture from the separation column as a single peak. Since the current method was validated using MS/MS parameters which are optimised for cis-deltamethrin these settings may not be optimum for quantitation of the isomers alpha-R-deltamethrin and trans-deltamethrin. This has to be considered when quantifying deltamethrin residues in unknown samples. The current method validation was performed using control material fortified with cis-deltamethrin only.

9.1 Fortification

The method was validated by analysing control samples and control samples fortified with cis-deltamethrin at and above the limit of quantitation.
Sample fortification was done by adding a certain amount of a fortification standard solution to 20 g of soil or sediment. After a waiting time of one hour between fortification and begining of the extraction, to allow for the standard to be soaked into the soil, the samples were extracted according to the procedure described in section 9.2.

The following fortification levels were analysed:

0.1 µg/kg:	addition of 200 µL of (5 LSGDEL) to 20 g of soil or sediment
1 µg/kg:	addition of 200 µL of (3 LSGDEL) to 20 g of soil or sediment

The preparation of the fortification standards is described in section 7.2.

9.2 Extraction

1. Weigh 20 g of the soil or sediment sample into a 100-mL beaker containing a magnetic stirrring bar. (Large stirring bar necessary to ensure soil/solvent mixture is completely mixed during extraction.)
2. Add 40 mL of a mixture of acetonitrile/ammonium acetate 10 mMol/L in water (9/1 v/v).
3. Place ten beakers with soil/sediment and solvent mixture into the microwave extractor.
4. Switch on the magnetic stirrer.

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5. Extract for three minutes at 250 W.
6. After extraction, add 80 µL of internal standard solution (2 LSG13C) into the sample and stir for one minute.
7. Transfer about 1.5 mL of the extract into a centrifuge tube.
8. Centrifuge for 3 minutes at >12000 g to remove fine particles of the soil.
9. Transfer a portion of the sample solution into an HPLC vial.
10. Inject an aliquot of 50 µL into the HPLC-MS/MS system.

A flow diagram of the analytical procedure is given in Figure 1.

9.3 Liquid Chromatographic Conditions

Column:	LiChroCART 55-4 Purospher STAR RP-18e, 3 µm, length 5.5 cm, i.d. 4.0 mm
Injection volume:	50 µL
Oven temperature:	40 °C
Mobile phases:	A: water with ammonium acetate 10 mMol/L / methanol (9/1 v/v) B: methanol with ammonium acetate 10 mMol/L
Run time:	4 min
Flow rate (column):	1.0 mL/min
Flow rate (interface):	1.0 mL/min

Isocratic Pump (for flushing the interface):
Mobile phase: water with ammonium acetate 10 mMol/L / methanol (1/1 v/v)

Retention time:

cis-deltamethrin (AE F032640):	approx.1.2 min
alpha-R-deltamethrin (AE F108569) :	approx.1.2 min
trans-deltamethrin (AE F0035073) :	approx.1.2 min
[phenoxy-^{13}C6]deltamethrin:	approx.1.2 min

Table 2: HPLC Time Table

Time [min]	Setting
0.10	switching eluent stream to waste
0.50	switching eluent stream to interface
3.00	switching eluent stream to waste

Remark: For the time, the eluent stream of the binary pump is switched to the interface, the eluent stream of the isocratic pump is switched to waste and vice versa.

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9.4 Mass Spectrometry - Principle of Measurement

Substances introduced into the mass spectrometer are ionised using a turbo-ionspray interface. Sample ions are accelerated by an adequate voltage regulation and separated by mass in the first quadrupole (Q1). The most abundant ions (the protonated and deprotonated ions) of the analyte (parent ions) are impulsed with nitrogen in the collision cell (Q2). Fragments of these ions (daughter ions) are separated by mass in the third quadrupole (Q3) and detected. The mass spectrometric parameters for the analytes and the selected ions are listed in Table 3.

9.5 Mass Spectrometric Parameters

The reported parameters are examples for an optimum adjustment of the mass spectrometer for cis-deltamethrin. With these parameters, the results in sections 11 to 15 were obtained. From time to time these parameters have to be checked and adjusted if necessary.

Table 3: MS/MS Operating Parameters

	cis-deltamethrin	[phenoxy-^{13}C6]deltamethrin
Q1 Mass [amu]	523 *	529 *
Q3 Mass [amu]	281	281
Dwell [msec]	500	500
Ionisation Mode	ESP+	ESP+
IS [V]	5500	5500
EP [V]	10	10
DP [V]	51	51
CE [V]	21	21
CXP [V]	12	12
Turbo Gas	50	50
Curtain Gas	50	50
Nebuliser Gas	50	50
Collision Gas [L/min]	0.92	0.92
Turbo gas Temp. [°C]	500	500

* masses m/z 523 and m/z 529 correspond to $[M+2+NH_4]^+$

IS: Ion Spray Voltage EP: Entrance Potential DP: Declustering Potential
CE: Collision Energy CXP: Collision Cell Exit Potential
ESP+: positive ion mode, i.e. production of positive ions

The mass spectra of the parent and the product ions of cis-deltamethrin, trans-deltamethrin, alpha-R-deltamethrin and [phenoxy-^{13}C6]deltamethrin are presented in the Appendix (Figure 2 to Figure 6).

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10 Calculations

For calculation of the concentrations, multi-point calibration curves were used. These curves were calculated automatically after each sequence run with the Perkin-Elmer quantitation software *Analyst (vers. 1.3)* using linear regression. Further calculations were performed using the software *EXCEL 97 (Office 97®).*
Matrix effects for cis-deltamethrin are eliminated by using an internal standard solution of the isotopically labelled test item ([phenoxy-^{13}C6]deltamethrin). This solution will be added to the sample solutions after extraction.

The linear equation for calibration is expressed as:

$$y = \text{Intercept} + \text{Slope} \cdot x$$

y = Area, x = Concentration

When an internal standard is used:

$$y = \frac{\text{Area}_{\text{Standard}}}{\text{Area}_{\text{Internal Standard}}} = \text{Int.Ratio} \quad \text{and} \quad x = \frac{\text{Conc}_{\text{Standard}}}{\text{Conc}_{\text{IS}}} = \text{Conc}_{\text{ratio}}$$

Int.Ratio = Intensity ratio
$Conc_{Standard}$ = Concentration of standard solution [µg/L]
$Conc_{IS}$ = Concentration of internal standard solution [µg/L]
$Conc_{ratio}$ = Concentration ratio

If the concentration of the isotopically labeled internal standard is the same in all solutions that are injected into the HPLC instrument, it has not to be taken into consideration. However, the concentration of the internal standard solution should be in the range of the concentration of the sample solutions.

By means of the linear equation, the content of deltamethrin in dry soil can be calculated as follows:

$$\text{Dilution}_{\text{Factor}} = \frac{\text{Volume}_{\text{final}}}{\text{Weight}}$$

$$\text{Conc}_{\text{Analyte}} = \frac{\text{Int.Ratio} - \text{Intercept}}{\text{Slope}}, \quad \text{Int.Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

$$\text{Conc}_{\text{Soil Wet}} = \text{Conc}_{\text{Analyte}} \cdot \text{Dilution}_{\text{Factor}}$$

$$\text{Conc}_{\text{Soil Dry}} = \text{Conc}_{\text{Soil Wet}} \cdot \frac{100\%}{100\% - \text{Moisture}}$$

$Volume_{final}$ = Volume of the extraction solution added [L]
Weight = Weight of the soil sample [kg]
$Conc_{Analyte}$ = Concentration of the analyte in the sample solution [µg/L]
$Conc_{Soil\ Wet}$ = Concentration of the analyte in wet soil [µg/kg]
$Conc_{Soil\ Dry}$ = Concentration of the analyte in dry soil [µg/kg]
Moisture = Moisture (water content) of the sample [%]
$Area_{Analyte}$: mean area of the analyte in the sample solution
$Area_{Internal\ Standard}$: mean area of the internal standard in the sample solution

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The recovery is calculated according to the following equation:

$$\text{Recovery} = \frac{\text{Conc}_{\text{Soil Wet}} \cdot 100\%}{\text{Conc}_{\text{Soil Spiked}}}$$

$\text{Conc}_{\text{Soil Spiked}}$ = Concentration of the reference item spiked [µg/kg]

Example calculation for recovery of cis-deltamethrin in soil Höfchen, 1.0 µg/kg (recovery 7, file: 040601a9_us.wiff, sample ID: 49)

$$\text{Dilution}_{\text{Factor}} = \frac{0.04\,L}{0.02\,kg} = 2.0\,L/kg \qquad \text{Dilution}_{\text{Factor}} = \frac{\text{Volume}_{\text{final}}}{\text{Weight}}$$

$$\text{Conc}_{\text{Analyte}} = \frac{0{,}27338 - 0.00367069}{0.513769} = 0{,}52496\,\mu g/L \qquad \text{Conc}_{\text{Analyte}} = \frac{\text{Int.Ratio} - \text{Intercept}}{\text{Slope}}$$

$$\text{Conc}_{\text{Soil Wet}} = 0{,}52496\,\mu g/L \cdot 2.0\,L/kg = 1{,}0499\,\mu g/kg \qquad \text{Conc}_{\text{Soil Wet}} = \text{Conc}_{\text{Analyte}} \cdot \text{Dilution}_{\text{Factor}}$$

$$\text{Recovery} = \frac{1{,}0499\,\frac{\mu g}{kg} \cdot 100\%}{1.0\,\frac{\mu g}{kg}} = 105\% \qquad \text{Recovery} = \frac{\text{Conc}_{\text{Soil Wet}} \cdot 100\%}{\text{Conc}_{\text{Soil Spiked}}}$$

Remark: Calculations were performed using the computer software *Analyst (vers. 1.3)* and *EXCEL 97*. The results given are rounded values. Thus, rounding deviations may occur if recalculations are made using the rounded figures.

11 Detector Linearity

Standard solutions containing cis-deltamethrin, were measured in a concentration range of about 0.015 to 5 µg/L corresponding to a concentration in soil of 0.03 - 10 µg/kg. In this concentration range, the mass spectrometric detector showed linear correlation between concentration and peak area ratio (area ratio = analyte area / internal standard area) (Table 4, 1/x weighted linear regression).
The graphical presentation of the linearity curve is included in the Appendix (Figure 7).

Table 4: Correlation Between Concentration and Area Ratio

Compound	Concentration range [µg/L]	Correlation coefficient r
cis-deltamethrin	0.015 – 5	0.9999

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12 Control Samples

The analytical results for all control samples from soil Höfchen and soil Laacher Hof were below 0.15 µg/kg. Figure 10 and Figure 11 show representative chromatograms of the analyte ions of control soils Höfchen and Laacher Hof.
The control samples of the sediment system showed peaks equivalent to approx. 30% of the LOQ (see Figure 12). Therefore, the measured concentrations in the recovery samples with sediment were corrected for the background concentration in the corresponding control samples.

13 Limit of Quantitation and Limit of Detection

The limit of quantitation of the method is 0.1 µg/kg for cis-deltamethrin.

The limit of detection of the method is 0.03 µg/kg for cis-deltamethrin.

Remarks: The limit of quantitation (LOQ) is defined as the lowest value of a compound in a real sample, which is still quantifiable by the analytical method. In this particular case, the LOQ is defined by the lowest fortification level. The limit of detection (LOD) is defined as the lowest value of a compound showing a signal, which significantly differs from the blank values. In this particular case, the LOD was set to 1/3 of the LOQ (LOD = 0.03 µg/kg). It could be shown with triplicate measurements that the chromatographic peaks at the LOD were greater than the signal equivalent to three times the background noise.

14 Determination of the Recovery Rates

Samples from soil *Höfchen*, soil *Laacher Hof* and *sediment* were fortified with cis-deltamethrin. For method validation, a total of 30 recovery experiments were conducted. Each sample was injected twice into the HPLC instrument. Fortification levels as well as recoveries and relative standard deviations (RSD) are presented in Table 5. Fortification levels are expressed as the amount of compound in wet soil and wet sediment, respectively. Figure 13 to Figure 18 show representative chromatograms of the analysis of control samples fortified with 0.1 µg/kg and 1 µg/kg.

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17 References

Ref. 1 EC Guidance Document on Residue Analytical Methods, SANCO/825/00 rev.7 of March 17, 2004

Ref. 2 BBA Guideline: Residue Analytical Methods for Post-Registration Control Purposes of July 21, 1998

Ref. 3 Commission Directive 96/46/EC amending Council Directive 91/414/EEC of July 16, 1996

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18 Appendices

Figure 1: Flow Diagram of the Extraction Procedure