

Analytical method for pyrethrins in soil and sediment

Reports: ECM: EPA MRID No. 50475403. DeVellis, S.R. 2017. Validation of an Environmental Chemistry Method for the Determination of Pyrethrins in Soil and Sediment. Smithers Viscient Study No.: 14118.6101. Report prepared by Smithers Viscient, Wareham, Massachusetts; sponsored and submitted by Pyrethrin Steering Committee/Joint Venture (PJV) c/o Consumer Specialty Products Association, Inc., Washington, D.C.; 63 pages. Final report issued June 8, 2017.

ILV: EPA MRID No. 50475401. Jutson, J.I. 2017. Independent Laboratory Validation of: Validation of an Environmental Chemistry Method for the Determination of Pyrethrins in Groundwater, Surface Water, Soil and Sediment. Concord Biosciences Study No.: 036079 and Document No.: 036079-1. Report prepared by Concord Biosciences, LLC, Concord, Ohio; sponsored and submitted by Pyrethrin Steering Committee/Joint Venture (PJV) c/o Consumer Specialty Products Association, Inc., Washington, D.C.; 154 pages. Final report issued December 15, 2017.

Document No.: MRIDs 50475403 & 50475401

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with the USEPA FIFRA (40 CFR Part 160) and OECD Good Laboratory Practices (GLP; p. 3 of MRID 50475403). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of the authenticity of the report was included with the QA statement.

ILV: The study was conducted in accordance with the USEPA FIFRA GLP (p. 3 of MRID 50475401). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A certification of the authenticity of the report was not included.

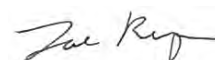
Classification: This analytical method is classified as unacceptable. An updated ECM was not submitted incorporating the fact that the source of the acetonitrile was critical. The specificity of the method was not supported by ILV representative chromatograms. Linearity was not satisfactory for most of the ILV and ECM analyses. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method.

PC Code: 069001

EFED Final Reviewers:

Zoe Ruge, Physical Scientist

Signature:



Date:

9/27/2018

Mohammed Ruhman, Ph.D.,
Senior Scientist

Signature:



Date:

9/27/2018

**CDM/CSS-
Dynamac JV**

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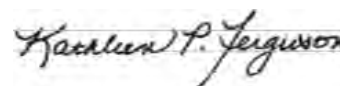


Date:

4/26/18

Reviewers:Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature:



Date:

4/26/18

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

This analytical method, Smithers Viscient Study No. 14118.6101, is designed for the quantitative determination of Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) at the LOQ of 8.00 µg/kg and of Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II) at the LOQ of 6.54 µg/kg in water using LC/MS/MS. Analytes were identified using one ion transition; a confirmation method is usually not required when LC/MS or GC/MS is used as the primary method for quantifying residues. The acceptability of the LOQs cannot be determined because the lowest toxicological level of concern in soil is currently unknown. At this time, LOD/LOQ should be within the values specified in the terrestrial field study guidance.¹ The ECM and ILV used one characterized sediment and one characterized soil matrices; matrices differed between the ECM and ILV. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method since terrestrial field dissipation studies were not referenced. The ILV validated the ECM in the first trial for pyrethrins in soil with insignificant modifications of the analytical instrumentation and parameters, as well as the use of Fisher acetonitrile; this was identified through previous ILV experience as a critical component of the method. The ECM did not contain a statement that the use of Fisher-brand acetonitrile was critical; an Updated ECM should be submitted specifying the source of the acetonitrile as Fisher as critical. All ILV and ECM data was satisfactory regarding accuracy and precision for all analytes; however, all ILV data regarding linearity and specificity was unsatisfactory for all analytes.

¹ URL: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/nafta-guidance-document-conducting-terrestrial-field>

Table 1. Analytical Method Summary.

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Pyrethrin I	50475403	50475401	Unacceptable	Soil/ Sediment ^{2,3}	8/6/17	Pyrethrin Steering Committee/ Joint Venture (PJV) c/o Consumer Specialty Products Association Inc.	LC/MS/ MS	8.00 µg/kg
Pyrethrin II								6.54 µg/kg

1 Pyrethrins = Pyrethrin Concentrate (BAS 383 HB I), which consists of six different esters grouped as such: Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) and Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II). Pyrethrin Concentrate (% purities) = 29.46% Pyrethrin I, 24.02% Pyrethrin II, 53.48% Total Pyrethrins.

2 In the ECM, natural freshwater sand sediment (Smithers Viscient Batch No. 102915-M-1; 88% sand 10% silt 2% clay; pH 5.4 in 1:1 matrix:water, 3.3% organic carbon) obtained from Glen Charlie Pond, Wareham, Massachusetts, and sand soil (Smithers Viscient Batch No. 012616A; 94% sand 6% silt 0% clay; pH 6.9 I 1:1 matrix:water, 0.70% organic carbon) obtained from Sunny Nook Farms, Rochester, Massachusetts, were used (USDA soil textural classification). Soil/sediment characterization was performed by Agvise Laboratories, Northwood, North Dakota. Sand soil was labelled sandy loam soil.

3 In the ILV, and sediment (Golden Lake 0-5 cm; 93% sand 7% silt 0% clay; pH 8.1 in 1:1 soil:water; 2.7% organic matter) obtained from Golden Lake, North Dakota, and loamy sand soil (DU#2 0-6"; 79% sand 12% silt 9% clay; pH 6.6 in 1:1 soil:water; 2.2% organic matter) obtained from Grand Forks, North Dakota, were used. Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

Samples (5.00 g, dry weight) were fortified as necessary with 0.04 mL or 0.40 mL of the fortification solution pyrethrins (0.100 mg/L:0.0818 mg/L or 10.0 mg/L:8.18 mg/L of Pyrethrin I:Pyrethrin II) in acetonitrile (pp. 13-15 of MRID 50475403). The soil/sediment samples were extracted twice with 20.0 mL of acetonitrile. The samples were shaken for 20 minutes on a shaker table at 150 rpm then centrifuged at 3000 rpm for 10 minutes and transferred to a 50-mL volumetric flask. The volume of the combined extracts was brought to 50 mL with acetonitrile. The samples were further diluted 1:80 for LOQ and 1:267 for 10×LOQ with acetonitrile:water (50:50, v:v) before analysis via LC/MS/MS.

Samples were analyzed by Agilent 1200 HPLC system coupled with an MDS Sciex 4000 QTRAP® MS (Version 1.6.2; Agilent Poroshell 120 EC-C8 column, 3.0 mm x 50 mm, 2.7 µm column; column temperature 25°C) using a mobile phase gradient of (A) 0.1% formic acid in water (B) 0.1% formic acid in acetonitrile [percent A:B at 0.01-0.50 min. 98.0:2.0, 2.00 min. 30.0:70.0, 5.00-6.00 min. 2.0:98.0, 6.10-7.00 min. 98.0:2.0] with MS/MS detection and Multiple Reaction Monitoring (MRM; TEM 550°C; pp. 11, 15-16 of MRID 50475403). Ion source was positive ESI for all analytes. One ion transition was monitored for each analyte: m/z 329.0→161.30 for pyrethrin I, m/z 331.40→163.20 for jasmolin I, m/z 317.40→149.30 for cinerin I, m/z 373.40→161.10 for pyrethrin II, m/z 375.30→163.20 for jasmolin II, and m/z 361.30→149.00 for cinerin II. Approximate

retention times were reported as 4.2 minutes for pyrethrin I, 4.5 minutes for jasmolin I, 4.2 minutes for cinerin I, 3.6 minutes for pyrethrin II, 3.8 minutes for jasmolin II, and 3.6 minutes for cinerin II for soil and sediment. Injection volume was 100 μ L.

In the ILV, the ECM was performed as written, except for insignificant modifications of the analytical instrumentation and parameters (pp. 17-18, 24; Table A-9, p. 36 of MRID 50475401). The ILV reported that Shimadzu Nexera UPLC system (System X) coupled with an AB Sciex 4000 MS was used (TEM 500°C). All monitored ion transitions were the same as those of the ECM. Retention times were not reported. A critical component of the method was elucidated by the ILV through the failure of the LOQ fortification in the first trial of the water method validation (ILV performed soil/sediment and water method validations in the same study report – water validations were performed before soil/sediment validations; p. 18; Appendix E, pp. 127-130). The Study Monitor informed the ILV that Fisher acetonitrile must be used for the sample processing since problems have occurred when other sources of acetonitrile have been used for pyrethrins. After switching from Sigma-Aldrich to Fisher brand acetonitrile, the ILV successfully validated the ECM for both water and soil/sediment methods.

In the ECM and ILV, the method Limits of Quantification (LOQs) in water were 8.00 μ g/kg for Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) and 6.54 μ g/kg for Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II; pp. 18-20, 22-24 of MRID 50475403; pp. 6, 25 of MRID 50475401). In the ECM and ILV, the method Limits of Detection (LOD) were 1.21 μ g/kg in soil and 1.03 μ g/kg in sediment for Pyrethrin I and 1.46 μ g/kg in soil and 0.963 μ g/kg in sediment for Pyrethrin II.

II. Recovery Findings

ECM (MRID 50475403): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) at 8.00 μ g/kg (LOQ) and 80.0 μ g/kg (10 \times LOQ) and for analysis of Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II) at 6.54 μ g/kg (LOQ) and 65.4 μ g/kg (10 \times LOQ) in soil and sediment matrices (Tables 1-4, pp. 27-30). Analytes were identified using one ion transition; a confirmation method is usually not required when LC/MS or GC/MS is used as the primary method for quantifying residues. Natural freshwater sand sediment (Smithers Viscient Batch No. 102915-M-1; 88% sand 10% silt 2% clay; pH 5.4 in 1:1 matrix:water, 3.3% organic carbon) obtained from Glen Charlie Pond, Wareham, Massachusetts, and sand soil (Smithers Viscient Batch No. 012616A; 94% sand 6% silt 0% clay; pH 6.9 I 1:1 matrix:water, 0.70% organic carbon) obtained from Sunny Nook Farms, Rochester, Massachusetts, were used (USDA soil textural classification; pp. 12-13). Soil/sediment characterization was performed by Agvise Laboratories, Northwood, North Dakota. Sand soil was labelled sandy loam soil.

ILV (MRID 50475401): Mean recoveries and RSDs were within guideline requirements for analysis of Pyrethrin I at 8.00 μ g/kg (LOQ) and 80.0 μ g/kg (10 \times LOQ) and for analysis of Pyrethrin II at 6.54 μ g/kg (LOQ) and 65.4 μ g/kg (10 \times LOQ) in soil and sediment matrices (Tables 3-4, pp. 22-23; Tables A-5 to A-8, pp. 32-35). LC/MS/MS Analytes were identified using one ion transition. Sand sediment (Golden Lake 0-5 cm; 93% sand 7% silt 0% clay; pH 8.1 in 1:1 soil:water; 2.7% organic matter) obtained from Golden Lake, North Dakota, and loamy sand soil (DU#2 0-6"; 79% sand 12% silt 9% clay; pH 6.6 in 1:1 soil:water; 2.2% organic matter) obtained from Grand Forks, North Dakota, were used (p. 14; Appendix A, pp. 93-94). Soil characterization was performed by

Agvise Laboratories, Northwood, North Dakota. The ILV validated the ECM in the first trial for pyrethrins in soil with insignificant modifications of the analytical instrumentation and parameters, as well as the use of only Fisher-brand acetonitrile (pp. 17-18, 24-25; Appendix E, pp. 127-130). ILV performed soil/sediment and water method validations in the same study report – water validations were performed before soil/sediment validations. After the failure of the LOQ fortification in the first trial of the water method validation, the Study Monitor informed the ILV that Fisher acetonitrile must be used for the sample processing since problems have occurred when other sources of acetonitrile have been used for pyrethrins. After switching from Sigma-Aldrich to Fisher brand acetonitrile, the ILV successfully validated the ECM for both the water and soil/sediment methods. The ECM did not contain a statement that the use of Fisher-brand acetonitrile was critical; an Updated ECM should be submitted specifying the source of the acetonitrile as Fisher as critical.

Table 2. Initial Validation Method Recoveries for Pyrethrins as Pyrethrin I and Pyrethrin II in Soil and Sediment.^{1,2,3}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Natural Freshwater Sand Sediment						
Pyrethrin I	8.00 (LOQ)	7	77.9-88.4	82.3	4.09	4.96
	80.0	5	84.3-101	90.1	6.67	7.40
Pyrethrin II	6.54 (LOQ)	7	85.6-95.2	80.3	5.88	7.32
	65.4	5	81.6-94.0	91.3	5.02	5.50
Sand Soil						
Pyrethrin I	8.00 (LOQ)	7	73.4-89.7	91.3	3.83	4.20
	80.0	5	85.5-97.9	88.4	4.80	5.43
Pyrethrin II	6.54 (LOQ)	7	76.3-97.2	89.7	7.11	7.92
	65.4	5	89.3-97.5	92.0	3.34	3.63

Data (uncorrected recovery results, pp. 18-19) were obtained from Tables 1-4, pp. 27-30 of MRID 50475403.

1 Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) and Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II).

2 One ion transition was monitored for each analyte: m/z 329.0→161.30 for pyrethrin I, m/z 331.40→163.20 for jasmolin I, m/z 317.40→149.30 for cinerin I, m/z 373.40→161.10 for pyrethrin II, m/z 375.30→163.20 for jasmolin II, and m/z 361.30→149.00 for cinerin II.

3 Natural freshwater sand sediment (Smithers Viscient Batch No. 102915-M-1; 88% sand 10% silt 2% clay; pH 5.4 in 1:1 matrix:water, 3.3% organic carbon) obtained from Glen Charlie Pond, Wareham, Massachusetts, and sand soil (Smithers Viscient Batch No. 012616A; 94% sand 6% silt 0% clay; pH 6.9 I 1:1 matrix:water, 0.70% organic carbon) obtained from Sunny Nook Farms, Rochester, Massachusetts, were used (USDA soil textural classification; pp. 12-13). Soil/sediment characterization was performed by Agvise Laboratories, Northwood, North Dakota. Sand soil was labelled sandy loam soil.

Table 3. Independent Validation Method Recoveries for Pyrethrins as Pyrethrin I and Pyrethrin II in Soil and Sediment.^{1,2,3}

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sand Sediment						
Pyrethrin I	8.00 (LOQ)	5	83.7-103	95.8	9.3	9.7
	80.0	5	77.0-93.9	85.8	6.8	7.9
Pyrethrin II	6.54 (LOQ)	5	75.2-99.8	90.1	9.1	10.0
	65.4	5	69.8-89.4	79.8	7.9	10.0
Loamy Sand Soil						
Pyrethrin I	8.00 (LOQ)	5	71.5-89.8	83.7	7.4	8.8
	80.0	5	89.4-103	96.9	4.9	5.0
Pyrethrin II	6.54 (LOQ)	5	81.2-85.5	82.7	1.7	2.0
	65.4	5	93.4-103	98.2	3.8	3.9

Data (uncorrected recovery results, Tables A-5 to A-8, pp. 32-35) were obtained from Tables 3-4, pp. 22-23 and Tables A-5 to A-8, pp. 32-35 of MRID 50475401 and DER Attachment 2.

1 Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) and Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II).

2 One ion transition was monitored for each analyte: m/z 329.0→161.30 for pyrethrin I, m/z 331.40→163.20 for jasmolin I, m/z 317.40→149.30 for cinerin I, m/z 373.40→161.10 for pyrethrin II, m/z 375.30→163.20 for jasmolin II, and m/z 361.30→149.00 for cinerin II.

3 Sand sediment (Golden Lake 0-5 cm; 93% sand 7% silt 0% clay; pH 8.1 in 1:1 soil:water; 2.7% organic matter) obtained from Golden Lake, North Dakota, and loamy sand soil (DU#2 0-6"; 79% sand 12% silt 9% clay; pH 6.6 in 1:1 soil:water; 2.2% organic matter) obtained from Grand Forks, North Dakota, were used (p. 14; Appendix A, pp. 93-94). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

III. Method Characteristics

In the ECM and ILV, the method LOQs in water were 8.00 µg/kg for Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) and 6.54 µg/kg for Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II; pp. 18-20, 22-24 of MRID 50475403; pp. 6, 25 of MRID 50475401). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. In the ECM, it was noted that background noise should not exceed 20% of the LOQ. In the ECM and ILV, the method LODs were 1.21 µg/kg in soil and 1.03 µg/kg in sediment for Pyrethrin I and 1.46 µg/kg in soil and 0.963 µg/kg in sediment for Pyrethrin II. In the ECM, the LOD was calculated as the standard deviation at the LOQ multiplied by $t_{0.99}$, where $t_{0.99}$ equalled 3.143 for n-1 degrees of freedom where n = 7. The LOD was not justified in the ILV.

Table 4. Method Characteristics for Pyrethrins in Soil and Sediment.

Analyte ¹		Pyrethrin I		Pyrethrin II		
Limit of Quantitation (LOQ)	ECM	8.00 µg/kg		6.54 µg/kg		
	ILV					
Limit of Detection (LOD)	ECM	Nominal	Not reported			
		Calculated	1.21 µg/kg (soil) 1.03 µg/kg (sediment)		1.46 µg/kg (soil) 0.963 µg/kg (sediment)	
	ILV ²	1.21 µg/kg (soil) 1.03 µg/kg (sediment)		1.46 µg/kg (soil) 0.963 µg/kg (sediment)		
Linearity (calibration curve r ² and concentration range)	ECM	Soil	r ² = 0.99562		r ² = 0.99128	
		Sediment	r ² = 0.99642		r ² = 0.99394	
	ILV	Soil	r ² = 0.9948		r ² = 0.9939	
		Sediment	r ² = 0.9915		r ² = 0.9904	
	Range	0.05-0.50 µg/L		0.0409-0.409 µg/L		
Repeatable	ECM ³	Yes at LOQ and 10×LOQ in one sediment and one soil matrices (characterized).				
	ILV ^{4,5}					
Reproducible		Yes at LOQ and 10×LOQ				
Specific	ECM	Soil	Yes, matrix interferences were < 20% of the LOQ (based on peak area) for each of the three components. Baseline noise interference at the LOQ was significant in chromatograms of pyrethrin I/II and jasmolin I/II.			
		Sediment	Yes, matrix interferences were < 30% of the LOQ (based on peak area) for each of the three components. Baseline noise interference at the LOQ was significant in chromatograms of cinerin I/II and jasmolin I/II.			
	ILV	Soil	No , baseline noise interference at the LOQ was significant in chromatograms of cinerin I/II and very significant (height <i>ca.</i> 50% of LOQ peak height) in chromatograms of jasmolin I/II. Matrix interferences were < 30% of the LOQ (based on peak area) for each of the three components.			
		Sediment	No , baseline noise interference at the LOQ was very significant (height <i>ca.</i> 40-70% of LOQ peak height) in chromatograms of cinerin I/II, and jasmolin I/II was barely distinguishable from the baseline noise at the LOQ. ⁶ Matrix interferences were < 6% of the LOQ (based on peak area) for each of the three components.			

Data were obtained from pp. 18-20, 22-24 (LOQ/LOD); Tables 1-4, pp. 27-30 (recovery results); Figures 1-20, pp. 31-50 (chromatograms); Figures 21-24, pp. 51-54 (calibration curves) of MRID 50475403; pp. 6, 25 (LOQ/LOD); Tables 3-4, pp. 22-23 and Tables A-5 to A-8, pp. 32-35 (recovery results); Figures 5-8, pp. 42-45 (calibration curves); Figures 29-48, pp. 66-85 (chromatograms) of MRID 50475401. Analytes were identified using one ion transition; a confirmation method is usually not required when LC/MS or GC/MS is used as the primary method for quantifying residues.

Red values indicate discrepancies with meeting guideline requirements.

1 Pyrethrins = Pyrethrin Concentrate (BAS 383 HB I), which consists of six different esters grouped as such: Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) and Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II). Pyrethrin Concentrate (% purities) = 29.46% Pyrethrin I, 24.02% Pyrethrin II, 53.48% Total Pyrethrins.

2 ILV LODs were reported from the ECM.

3 In the ECM, natural freshwater sand sediment (Smithers Viscient Batch No. 102915-M-1; 88% sand 10% silt 2% clay; pH 5.4 in 1:1 matrix:water, 3.3% organic carbon) obtained from Glen Charlie Pond, Wareham, Massachusetts, and sand soil (Smithers Viscient Batch No. 012616A; 94% sand 6% silt 0% clay; pH 6.9 I 1:1 matrix:water, 0.70% organic carbon) obtained from Sunny Nook Farms, Rochester, Massachusetts, were used (USDA soil textural classification; pp. 12-13 of MRID 50475403). Soil/sediment characterization was performed by Agvise Laboratories, Northwood, North Dakota. Sand soil was labelled sandy loam soil.

4 In the ILV, sand sediment (Golden Lake 0-5 cm; 93% sand 7% silt 0% clay; pH 8.1 in 1:1 soil:water; 2.7% organic matter) obtained from Golden Lake, North Dakota, and loamy sand soil (DU#2 0-6"; 79% sand 12% silt 9% clay; pH 6.6 in 1:1 soil:water; 2.2% organic matter) obtained from Grand Forks, North Dakota, were used (p. 14; Appendix A,

pp. 93-94 of MRID 50475401). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota.

- 5 The ILV validated the ECM in the first trial for pyrethrins in soil with insignificant modifications of the analytical instrumentation and parameters, as well as the use of only Fisher-brand acetonitrile (pp. 17-18, 24-25; Appendix E, pp. 127-130 of MRID 50475401). ILV performed soil/sediment and water method validations in the same study report – water validations were performed before soil/sediment validations. After the failure of the LOQ fortification in the first trial of the water method validation, the Study Monitor informed the ILV that Fisher acetonitrile must be used for the sample processing since problems have occurred when other sources of acetonitrile have been used for pyrethrins. After switching from Sigma-Aldrich to Fisher brand acetonitrile, the ILV successfully validated the ECM for both the water and soil/sediment methods. The ECM did not contain a statement that the use of Fisher-brand acetonitrile was critical; an Updated ECM should be submitted specifying the source of the acetonitrile as Fisher as critical.

6 Based on Figure 42, p. 79 and Figure 47, p. 84 of MRID 50475401.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. An updated ECM was not submitted incorporating the fact that the source of the acetonitrile was critical. ILV performed soil/sediment and water method validations in the same study report – water validations were performed before soil/sediment validations. After the failure of the LOQ fortification in the first trial of the water method, the ILV presumed that matrix effects caused suppression of the analyte signal. After some communication with the Study Monitor and testing, the ILV determined that matrix effects were not the cause. The Study Monitor informed the ILV that Fisher acetonitrile must be used for the sample processing since problems have occurred when other sources of acetonitrile have been used for pyrethrins. The ILV communicated that Sigma-Aldrich acetonitrile had been used in previous trials. After switching from Sigma-Aldrich to Fisher brand acetonitrile, the ILV successfully validated the ECM for both the water (second trial) and soil/sediment (first trial) methods. The ECM did not contain a statement that the use of Fisher-brand acetonitrile was critical; an Updated ECM should be submitted specifying the source of the acetonitrile as Fisher as critical. The use of the Fisher brand acetonitrile by the ILV was necessary for the successful validation of the ECM method.
2. The specificity of the method was not supported by ILV representative chromatograms (Figures 29-48, pp. 66-85 of MRID 50475401). Although matrix interferences were $< 30\%$ of the LOQ (based on peak area) for each of the three components in both the soil and sediment matrices, the height of baseline noise interference at the LOQ ranged from *ca.* 30-90% of the LOQ peak height for cinerin I/II and jasmolin I/II. This level of baseline noise interference at the LOQ interfered with peak integration confidence, especially when the analyte peak was barely distinguishable from the baseline noise. The reviewer noted that the extraction procedure was fairly simple; the reviewer did not know if the use of additional SPE clean-up processes had been attempted in order to increase resolution of the analyte signals.
3. ILV linearity was not satisfactory for any analyses: Pyrethrin I in soil ($r^2 = 0.9948$) and sediment ($r^2 = 0.9915$); and Pyrethrin II in soil ($r^2 = 0.9939$) and sediment ($r^2 = 0.9904$; Figures 5-8, pp. 42-45 of MRID 50475401; DER Attachment 2).

ECM linearity was not satisfactory for Pyrethrin II in soil ($r^2 = 0.99128$) and sediment ($r^2 = 0.99394$; Figures 21-24, pp. 51-54 of MRID 50475403).

Linearity is satisfactory when $r^2 \geq 0.995$.

4. The communications between the ILV Study Director and the CSPA Study Monitor were documented (p. 26; Appendix E, pp. 127-130 of MRID 50475401). Communications involved trial successes and failures, as well as procedure clarification (specifically the dichloromethane extraction procedure and not allowing the extract to be reduced to dryness). The reviewer noted that the CSPA Study Monitor involved the Smithers Study Director after the ILV Study Director asked him to check with the method developer, and this communication was forwarded to the ILV Study Director. However, the reviewer did not deem this interaction to be collusion since the Smithers Study Director only clarified and re-emphasized points of the method. The Smithers Study Director did not direct supplementary work or modifications to the ILV.

The reviewer noted that the solution to the ILV LOQ performance data problem came from the Study Monitor talking to another chemist familiar with analyzing Pyrethrins (Appendix E, p. 129 of MRID 50475401). The reviewer would have liked this chemist to have been identified.

5. Method LODs were not reported by the ECM or ILV; calculated LODs were provided. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 18-20, 22-24 of MRID 50475403; pp. 6, 25 of MRID 50475401). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. In the ECM, it was noted that background noise should not exceed 20% of the LOQ. In the ECM, the LOD was calculated as the standard deviation at the LOQ multiplied by $t_{0.99}$, where $t_{0.99}$ equalled 3.143 for $n-1$ degrees of freedom where $n = 7$. The LOD was not justified in the ILV. Detection limits should not be based on arbitrary values.
6. The reviewer noted the following significant typographical error in the ECM: LODs were not reported correctly in the method summary tables on pages 23-24 (pp. 23-24 of MRID 50475403). These LODs were not placed correctly according to p. 20 of MRID 50475403 (ECM) and p. 6 of MRID 50475401 (ILV); the reviewer reported the LODs based on these values.
7. It was reported for the ILV that one sample set of 13 samples required *ca.* 8 hours including calculation of results (p. 25 of MRID 50475401).

V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method
Detection Limit-Revision 1.11, pp. 317-319.

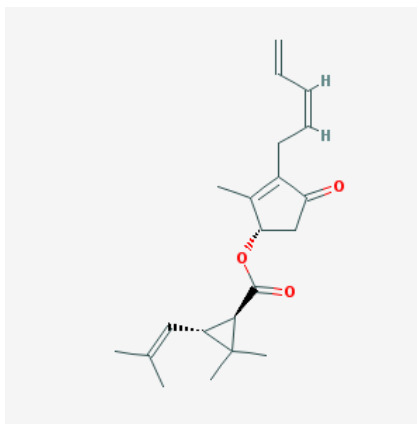
DER Attachment 1: Chemical Names and Structures.**Pyrethrin I**

IUPAC Name: [(1S)-2-methyl-4-oxo-3-[(2Z)-penta-2,4-dienyl]cyclopent-2-en-1-yl] (1R,3R)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate

CAS Name: Not reported

CAS Number: 121-21-1

SMILES String: CC1=C(C(=O)C[C@@H]1OC(=O)[C@@H]2[C@H](C2(C)C)C=C(C)C)C/C=C\C=C

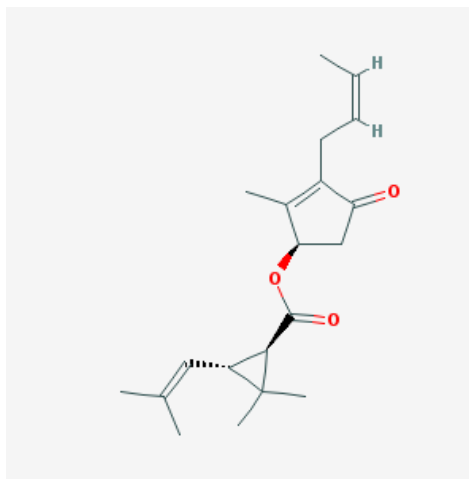
**Cinerin I**

IUPAC Name: [(1R)-3-[(Z)-but-2-enyl]-2-methyl-4-oxocyclopent-2-en-1-yl] (1R,3R)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate

CAS Name: Not reported

CAS Number: 25402-06-6

SMILES String: C/C=C\CC1=C([C@@H](CC1=O)OC(=O)[C@@H]2[C@H](C2(C)C)C=C(C)C)C



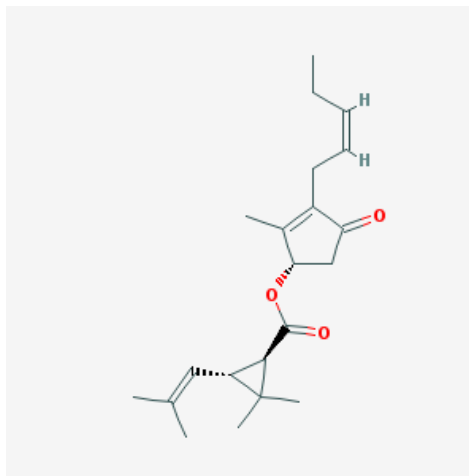
Jasmolin I

IUPAC Name: [(1S)-2-methyl-4-oxo-3-[(Z)-pent-2-enyl]cyclopent-2-en-1-yl] (1R,3R)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate

CAS Name: Not reported

CAS Number: 4466-14-2

SMILES String: CC/C=C\CC1=C([C@H](CC1=O)OC(=O)[C@@H]2[C@H](C2(C)C)C=C(C)C)C

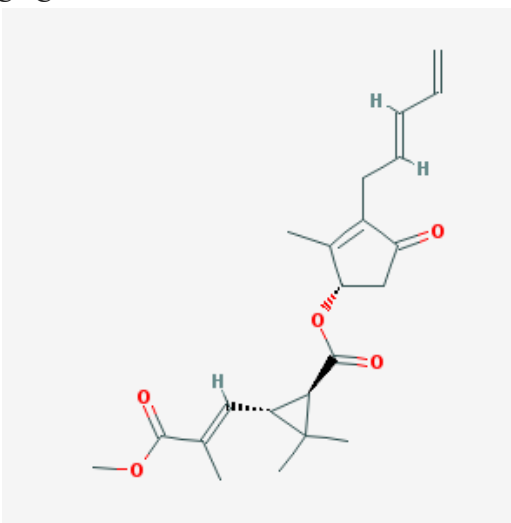
**Pyrethin II**

IUPAC Name: [(1S)-2-methyl-4-oxo-3-[(2E)-penta-2,4-dienyl]cyclopent-2-en-1-yl] (1R,3R)-3-[(E)-3-methoxy-2-methyl-3-oxoprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate

CAS Name: Not reported

CAS Number: Not reported

SMILES String: CC1=C(C(=O)C[C@@H]1OC(=O)[C@@H]2[C@H](C2(C)C)/C=C(\C)/C(=O)OC)C/C=C/C=C



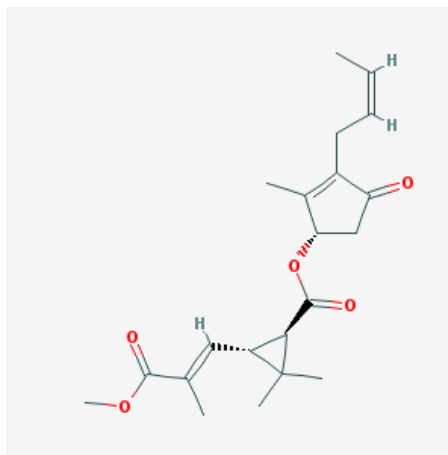
Cinerin II

IUPAC Name: [(1S)-3-[(Z)-but-2-enyl]-2-methyl-4-oxocyclopent-2-en-1-yl] (1R,3R)-3-[(E)-3-methoxy-2-methyl-3-oxoprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate

CAS Name: Not reported

CAS Number: 121-20-0

SMILES String: C/C=C\CC1=C([C@H](CC1=O)OC(=O)[C@@H]2[C@H](C2(C)C)/C=C(\C)/C(=O)OC)C

**Jasmolin II**

IUPAC Name: [(1S)-2-methyl-4-oxo-3-[(Z)-pent-2-enyl]cyclopent-2-en-1-yl] (1R,3R)-3-[(E)-3-methoxy-2-methyl-3-oxoprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate

CAS Name: Not reported

CAS Number: 1172-63-0

SMILES String: CC/C=C\CC1=C([C@H](CC1=O)OC(=O)[C@@H]2[C@H](C2(C)C)/C=C(\C)/C(=O)OC)C

