

Analytical method for pyrethrins in water

Reports: ECM: EPA MRID No. 50475402. DeVellis, S.R. 2017. Validation of an Environmental Chemistry Method for the Determination of Pyrethrins in Groundwater and Surface Water. Smithers Viscient Study No.: 14118.6100. 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.; 65 pages. Final report issued June 9, 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 50475402 & 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 50475402). 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.

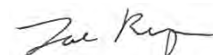
Classification: This analytical method is classified as unacceptable. An updated ECM was not submitted incorporating the fact that the source of the acetonitrile was critical. ILV performance data at the LOQs was unacceptable in both water matrices. ILV linearity was not satisfactory for Pyrethrin I in ground water. ECM linearity was not satisfactory for all analyses.

PC Code: 069001

EFED Final Reviewers:

Zoe Ruge, Physical Scientist

Signature:



Date:

9/27/18

Mohammed Ruhman, Ph.D.,
Senior Scientist

Signature:



Date:

9/27/18

**CDM/CSS-
Dynamac JV**

Lisa Muto, M.S.,
Environmental Scientist

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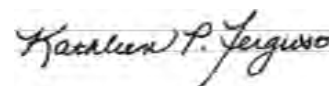


Date:

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. 14118.6100, is designed for the quantitative determination of Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) at the LOQ of 0.10 µg/L and of Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II) at the LOQ of 0.0818 µg/L 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 LOQs are less than the currently known lowest toxicological level of acute concern in water (0.76 µg/L), however they are greater than the currently known chronic levels of concern (0.04 µg/L). It is suggested that the registrant adjust the ECM so that the LOQ is lower than the currently known chronic value of 0.04 µg/L in the new submitted study. The ECM and ILV used one characterized surface water and one characterized ground water matrices; matrices differed between the ECM and ILV. The ILV validated the ECM in the second trial for pyrethrins in water with insignificant modifications of the analytical instrumentation and parameters; however, the failure of the first ILV trial was due to Sigma-Aldrich acetonitrile, not Fisher acetonitrile, being used. 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 ECM data was satisfactory regarding accuracy and precision for all analytes, but ILV performance data at the LOQs was unacceptable in both water matrices. All ECM and ILV data was satisfactory regarding specificity for all analytes; however, linearity was not satisfactory for some of the ECM and ILV analyses.

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	50475402	50475401	Unacceptable	Water ^{2,3}	9/6/17	Pyrethrin Steering Committee/J oint Venture (PJV) c/o Consumer Specialty Products Association Inc.	LC/MS/ MS	0.10 µg/L
Pyrethrin II								0.0818 µg/L

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, surface (river) water (pH 7.6, 85 mg equiv. CaCO₃/L, 0.53 mmhos/cm conductivity) obtained from Taunton River, Taunton, Massachusetts, and ground (well) water (pH 6.24, 84 mg equiv. CaCO₃/L, 20 mg/L alkalinity, 642 µS/cm conductivity) obtained from Rock Creek, Ohio, were used. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

3 In the ILV, surface (river) water (EFS-629; pH 8.3, 104 mg equiv. CaCO₃/L, 24 ppm total dissolved solids) obtained from Grand River, Madison, Ohio, and ground (well) water (EFS-625; pH 8.0, 242 mg equiv. CaCO₃/L, 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

I. Principle of the Method

Samples (40.0 mL) 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 of Pyrethrin I:Pyrethrin II) in acetonitrile (pp. 13-17 of MRID 50475402). The water samples were extracted twice with 5.00 mL of dichloromethane. The extraction procedure was not described. The dichloromethane layers were removed, placed in a 45-mL glass vials, and evaporated under a gentle stream of nitrogen set at 40°C to *ca.* 100 µL. The residue was reconstituted with 7.50 mL of acetonitrile via vortex-mixing for 30 seconds and sonication for five minutes. An aliquot of purified water (7.50 mL) was added in the same manner, bringing the final composition of the samples to acetonitrile:water (50:50, v:v). LOQ samples were analyzed via LC/MS/MS. For 10×LOQ fortifications, the samples were further diluted 1:10 with acetonitrile:water (50:50, v:v) before analysis via LC/MS/MS.

Samples were analyzed by an 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, 17-18 of MRID 50475402). 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 ground water and surface water. Injection volume was 100 µL.

In the ILV, the ECM was performed as written, except for insignificant modifications of the analytical instrumentation and parameters (pp. 15-16, 24; Table A-9, p. 36 of MRID 50475401). Details for the dichloromethane extraction procedure were provided. 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 (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.

In the ECM and ILV, the method Limits of Quantification (LOQs) in water were 0.10 µg/L for Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) and 0.0818 µg/L for Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II; pp. 19-22, 25-26 of MRID 50475402; pp. 6, 25 of MRID 50475401). In the ECM and ILV, the method Limits of Detection (LOD) were 0.0132 µg/L in ground water and 0.0138 µg/L in surface water for Pyrethrin I and 0.0181 µg/L in ground water and 0.00986 µg/L in surface water for Pyrethrin II.

II. Recovery Findings

ECM (MRID 50475402): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) at 0.10 µg/L (LOQ) and 1.0 µg/L (10×LOQ) and for analysis of Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II) at 0.0818 µg/L (LOQ) and 0.818 µg/L (10×LOQ) in water matrices (Tables 1-4, pp. 29-32). 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. Surface (river) water (pH 7.6, 85 mg equiv. CaCO₃/L, 0.53 mmhos/cm conductivity) obtained from Taunton River, Taunton, Massachusetts, and ground (well) water (pH 6.24, 84 mg equiv. CaCO₃/L, 20 mg/L alkalinity, 642 µS/cm conductivity) obtained from Rock Creek, Ohio, were used (p. 12). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50475401): Mean recoveries and RSDs were within guideline requirements for analysis of Pyrethrin I at 1.0 µg/L (10×LOQ) and for analysis of Pyrethrin II at 0.818 µg/L (10×LOQ) in water matrices (Tables 1-2, pp. 20-21; Tables A-1 to A-4, pp. 29-31; DER Attachment 2). Analysis of Pyrethrin I at 0.10 µg/L (LOQ) and Pyrethrin II at 0.0818 µg/L (LOQ) were unacceptable in both water matrices: Pyrethrin I (ground water, mean 67.8% and RSD 34.6%; surface water, RSD 44%) and Pyrethrin II (ground water, RSD 28.1%; surface water RSD 35%). LOQ recovery statistics were reviewer-calculated based on all reported recovery values (n = 6 or 7). The study author only used five values for statistics. Recovery values which were deemed outliers were omitted, and the samples were replaced with additional samples. LC/MS/MS Analytes were identified using one ion transition. Surface (river) water (EFS-629; pH 8.3, 104 mg equiv. CaCO₃/L, 24 ppm total dissolved solids) obtained from Grand River, Madison, Ohio, and ground (well) water (EFS-625; pH 8.0, 242 mg equiv. CaCO₃/L, 348 ppm total dissolved solids) obtained from Rock

Creek, Ohio, were used (p. 14; Appendix A, pp. 91-92). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The ILV validated the ECM in the second trial for pyrethrins in water with insignificant modifications of the analytical instrumentation and parameters; however, through the failure of the LOQ fortification in the first trial, the ILV learned that Fisher acetonitrile must be used for the sample processing since problems have occurred when other sources of acetonitrile have been used for pyrethrins (pp. 15-16, 18, 24-25; Appendix E, pp. 127-130). After switching from Sigma-Aldrich to Fisher brand acetonitrile, the ILV successfully validated the ECM. 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 Water.^{1,2,3}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground (Well) Water						
Pyrethrin I	0.10 (LOQ)	7	86.4-98.6	91.8	4.21	4.59
	1.0	5	92.3-94.1	93.2	0.763	0.819
Pyrethrin II	0.0818 (LOQ)	7	81.9-99.6	92.3	7.03	7.61
	0.818	5	93.1-102	95.9	3.76	3.92
Surface (River) Water						
Pyrethrin I	0.10 (LOQ)	7	80.6-94.4	87.3	4.39	5.03
	1.0	5	65.3-92.8	80.8	10.9	13.5
Pyrethrin II	0.0818 (LOQ)	7	88.7-99.5	92.8	3.84	4.13
	0.818	5	78.7-94.9	86.2	7.46	8.66

Data (uncorrected recovery results, pp. 20-21) were obtained from Tables 1-4, pp. 29-32 of MRID 50475402.

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 Surface (river) water (pH 7.6, 85 mg equiv. CaCO_3/L , 0.53 mmhos/cm conductivity) obtained from Taunton River, Taunton, Massachusetts, and ground (well) water (pH 6.24, 84 mg equiv. CaCO_3/L , 20 mg/L alkalinity, 642 $\mu\text{S}/\text{cm}$ conductivity) obtained from Rock Creek, Ohio, were used (p. 12). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

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

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground (Well) Water						
Pyrethrin I	0.10 (LOQ) ⁴	6	20.2-81.9	67.8	23.5	34.6
	1.0	5	94.6-100	97.7	2.4	2.5
Pyrethrin II	0.0818 (LOQ) ⁴	6	30.6-80.3	70.7	19.9	28.1
	0.818	5	87.9-107	95.3	8.1	8.5
Surface (River) Water						
Pyrethrin I	0.10 (LOQ) ⁴	7	67.0-213	112	49	44
	1.0	5	87.1-104	93.6	7.8	8.3
Pyrethrin II	0.0818 (LOQ) ⁴	7	76.2-202	117	41	35
	0.818	5	91.5-105	98.9	5.8	5.9

Data (uncorrected recovery results, Tables A-1 to A-4, pp. 29-31) were obtained from Tables 1-2, pp. 20-21 and Tables A-1 to A-4, pp. 29-31 of MRID 50475401 and DER Attachment 2.

Red values indicate discrepancies with meeting guideline requirements.

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 Surface (river) water (EFS-629; pH 8.3, 104 mg equiv. CaCO₃/L, 24 ppm total dissolved solids) obtained from Grand River, Madison, Ohio, and ground (well) water (EFS-625; pH 8.0, 242 mg equiv. CaCO₃/L, 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 14; Appendix A, pp. 91-92). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

4 Means, standard deviations and RSDs were reviewer-calculated based on all reported recovery values. Rules of significant figures were followed when reporting results. The study author only used five values for statistics.

Recovery values which were deemed outliers were omitted, and the samples were replaced with additional samples.

III. Method Characteristics

In the ECM and ILV, the method LOQs in water were 0.10 µg/L for Pyrethrin I (pyrethrin I, jasmolin I, and cinerin I) and 0.0818 µg/L for Pyrethrin II (pyrethrin II, jasmolin II, and cinerin II; pp. 19-22, 25-26 of MRID 50475402; 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 0.0132 µg/L in ground water and 0.0138 µg/L in surface water for Pyrethrin I and 0.0181 µg/L in ground water and 0.00986 µg/L in surface water 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 Water.

Analyte ¹		Pyrethrin I		Pyrethrin II	
Limit of Quantitation (LOQ)	ECM	0.10 µg/L		0.0818 µg/L	
	ILV				
Limit of Detection (LOD)	ECM	Nominal	Not reported		
		Calculated	0.0132 µg/L (ground) 0.0138 µg/L (surface)	0.0181 µg/L (ground) 0.00986 µg/L (surface)	
	ILV ²	0.0132 µg/L (ground) 0.0138 µg/L (surface)	0.0181 µg/L (ground) 0.00986 µg/L (surface)		
Linearity (calibration curve r ² and concentration range)	ECM	Ground	r ² = 0.99625	r ² = 0.99435	
		Surface	r ² = 0.99290	r ² = 0.99768	
	ILV	Ground	r ² = 0.9939³	r ² = 0.9983	
		Surface	r ² = 0.9964 ³	r ² = 0.9985 ³	
	Range	0.05-0.50 µg/L		0.0409-0.409 µg/L 0.0409-0.327 µg/L (ILV/surface)	
Repeatable	ECM ⁴		Yes at LOQ and 10×LOQ in one surface water and one ground water matrices (characterized).		
	ILV ^{5,6}		Yes at 10×LOQ in one surface water and one ground water matrices (characterized).		
			No at LOQ [mean 67.8% and RSD 34.6% (ground); surface water, RSD 44% (surface)].	No at LOQ [RSD 28.1% (ground); RSD 35% (surface)].	
Reproducible		No at LOQ Yes at 10×LOQ			
Specific	ECM		Yes, matrix interferences were < 7% of the LOQ (based on peak area) for each of the three components. Baseline noise interference was more significant in chromatograms of jasmolin I/II.		
	ILV		Yes, matrix interferences were < 6% of the LOQ (based on peak area) for each of the three components. Baseline noise interference was more significant in chromatograms of jasmolin I/II and cinerin I/II.		

Data were obtained from pp. 19-22, 25-26 (LOQ/LOD); Tables 1-4, pp. 29-32 (recovery results); Figures 1-20, pp. 33-52 (chromatograms); Figures 21-24, pp. 53-56 (calibration curves) of MRID 50475402; pp. 6, 25 (LOQ/LOD); Tables 1-2, pp. 20-21 and Tables A-1 to A-4, pp. 29-31 (recovery results); Figures 1-4, pp. 38-41 (calibration curves); Figures 9-28, pp. 46-65 (chromatograms) of MRID 50475401; DER Attachment 2. 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 ILV coefficient of determination (r²) values were reviewer-generated from reported regression data from Figures 1-4, pp. 38-41 of MRID 50475402 since the study author did not report these values (DER Attachment 2). Linear regression equations were used.

4 In the ECM, surface (river) water (pH 7.6, 85 mg equiv. CaCO₃/L, 0.53 mmhos/cm conductivity) obtained from Taunton River, Taunton, Massachusetts, and ground (well) water (pH 6.24, 84 mg equiv. CaCO₃/L, 20 mg/L alkalinity, 642 µS/cm conductivity) obtained from Rock Creek, Ohio, were used (p. 12 of MRID 50475402). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

5 In the ILV, surface (river) water (EFS-629; pH 8.3, 104 mg equiv. CaCO₃/L, 24 ppm total dissolved solids) obtained from Grand River, Madison, Ohio, and ground (well) water (EFS-625; pH 8.0, 242 mg equiv. CaCO₃/L, 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 14; Appendix A, pp. 91-92 of MRID 50475401). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

6 The ILV validated the ECM in the second trial for pyrethrins in water with insignificant modifications of the analytical instrumentation and parameters; however, through the failure of the LOQ fortification in the first trial, the ILV learned that Fisher acetonitrile must be used for the sample processing since problems have occurred when other sources of acetonitrile have been used for pyrethrins (pp. 15-16, 18, 24-25; Appendix E, pp. 127-130 of MRID 50475401). After switching from Sigma-Aldrich to Fisher brand acetonitrile, the ILV successfully validated the ECM. 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.

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. After the failure of the LOQ fortification in the first trial, 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. 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. ILV performance data at the LOQs was unacceptable in both water matrices: Pyrethrin I (ground water, mean 67.8% and RSD 34.6%; surface water, RSD 44%) and Pyrethrin II (ground water, RSD 28.1%; surface water RSD 35%; Tables 1-2, pp. 20-21; Tables A-1 to A-4, pp. 29-31; DER Attachment 2). LOQ recovery statistics were reviewer-calculated based on all reported recovery values ($n = 6$ or 7). The study author only used five values for statistics. Recovery values which were deemed outliers were omitted, and the samples were replaced with additional samples; subsequent statistics calculated by the study author were acceptable. OCSPP guidelines state that acceptable mean recoveries and relative standard deviations (RSD) are 70-120% and $\leq 20\%$, respectively, at the LOQ and higher fortifications.

Since the ILV did not provide acceptable data at the LOQ for the analytes in either water matrix, the reproducibility of the method was not demonstrated in the submitted method validation set.

3. ILV linearity was not satisfactory for Pyrethrin I in ground water, $r^2 = 0.9939$ (Figures 1-4, pp. 38-41 of MRID 50475401; DER Attachment 2). Coefficient of determination (r^2) values were reviewer-generated for three of the four calibration curves since the study author did not report these values. Linear regression equations from reported regression data were used.

ECM linearity was not satisfactory for Pyrethrin I in surface water, $r^2 = 0.9929$, and was not satisfactory for Pyrethrin II in ground water, $r^2 = 0.99435$ (Figures 21-24, pp. 53-56 of MRID 50475402).

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. 19-22, 25-26 of MRID 50475402; 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 typographical error: PYR I in the Figure Title should have been PYR II (Figure 20, p. 52 of MRID 50475402).
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.

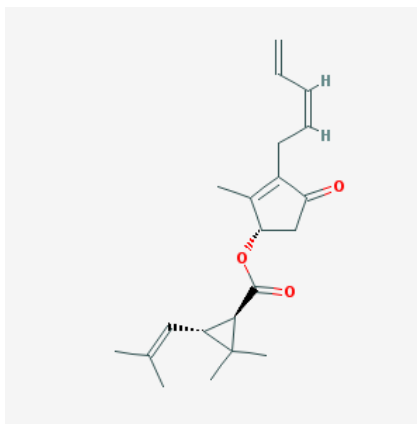
DER Attachment 1: Chemical Names and Structures.**Pyrethrin I**

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

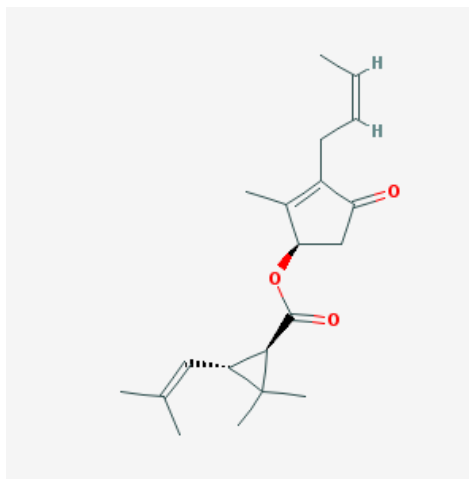
**Cinerin I**

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



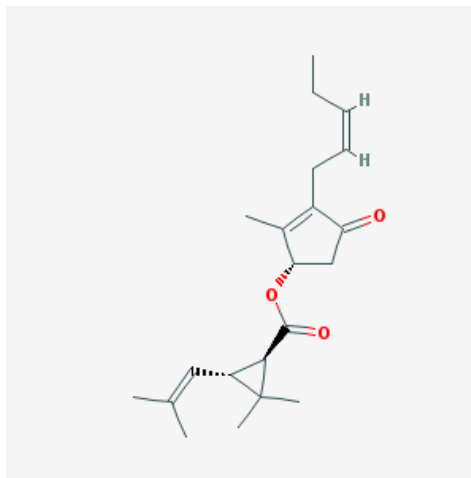
Jasmolin I

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

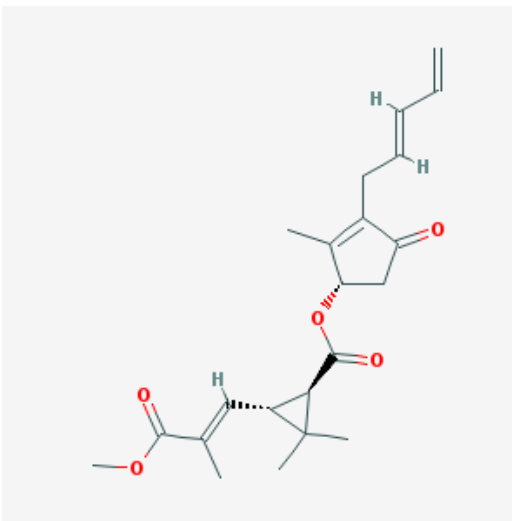
**Pyrethin II**

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



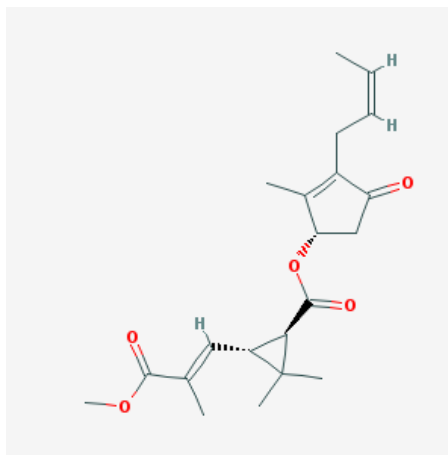
Cinerin II

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

