

1. INTRODUCTION

1.1 Scope of the Method

Smithers Viscient analytical methods 14118.6100 and 14118.6101 were developed to determine the residues of pyrethrins I and Pyrethrins II, in water, soil and sediment matrices using LC-MS/MS validated at Smithers Viscient, Wareham, Massachusetts (Reference 1). This report represents the validation by an independent laboratory, Concord Biosciences, Concord, OH.

The LOQ was defined as the lowest fortification level tested. For surface water and ground water the LOQ for PYR I/PYR II of the method 14118.6100 was 0.1/0.0818 µg/L. For soil and sediment the LOQ for PYR I/PYR II of the method 14118.6101 was 8.00/6.54 µg/kg. This independent lab validation was conducted using two fortification levels enforcement limit of quantitation and one ten times of limit of quantitation for all matrices. For each fortification level and matrix, five replicates were analyzed. Additionally, one reagent blank and two replicates of unfortified samples of each matrix were analyzed.

1.2 Principle of the Method

The water samples (40 mL) were extracted twice with 5 mL of dichloromethane. After extraction, combined extract was evaporated and reconstituted with acetonitrile and then diluting with water to bring final composition in 50:50 acetonitrile:water. An aliquot of resulting solution was analyzed to determine the residues of PYR I and PYR II using LC-MS/MS. For soil and sediment, 5 g of soil and sediment (dry weight) were extracted twice with 20 mL of acetonitrile and centrifuged. The combined extracts were transferred to 50 mL flask and bring to final volume of 50 mL. The recovery sample extracts were further diluted into the calibration standard range with 50:50 acetonitrile:water. The transitions for PYR I and PYR II at m/z 329 → 161 and at m/z 373 → 161 were monitored in positive mode, respectively.

2. REFERENCE SUBSTANCE AND SAMPLING HISTORY

2.1 Test Systems and Sample Handelling

The test systems considered in this study were ground water, surface water, soil and sediment.

The control samples soil and sediment were received Agvise laboratories. The sediment and soil was collected from Golden lake, ND and Grand Forks, ND, respectively. Ground water (Well water) was collected locally from 3254, Callender Road, Rock Creek, OH. River water was collected from Grand River, Hidden Valley Lake Metro Park, Madison, OH. Characterization report for water, soil and sediment are presented in Appendix A.

2.2 Test Substances

The test substance, pyrethrum Concentrate (Stewardship Blend), consists of six different esters that are referred to, collectively, as pyrethrins I (PYR I) and pyrethrins II (PYR II). Test substance was received from MGK, Minneapolis, MN and stored at ambient temperature.

Compound	Pyrethrum Concentrate (Stewardship Blend)
Synonym Name:	BAS 383 HB I
Lot No:	230-089
Purity:	Pyrethrin I (29.46 %) Pyrethrin II (24.02 %)
Expiration Date:	09/27/2017

Certificate of analysis is presented in [Appendix A](#).

3. ANALYTICAL METHOD

Smithers Viscient analytical methods 14118.6100 and 14118.6101, "Validation of an Environmental Chemistry Method for the Determination of Pyrethrins in Groundwater and Surface Water" and "Validation of an Environmental Chemistry Method for the Determination of Pyrethrins in Soil and Sediment" was used for the analysis of the samples (Reference 1 and 2). Instrument parameters are described in [Table A - 9](#).

3.1 Method (Ground Water and Surface Water)

The residues of PYR I and PYR II were extracted from 40mL of surface water and ground water as per the procedure given below:

Fortification (Section 2.9 of Reference Method)

Approximately 40 mL (surface water and ground water). Two Controls, 5 control samples spiked at 0.100/0.0818 µg/L (LOQ) and 5 control samples spiked at 1.00/0.818 µg/L (10X LOQ) of PYR I/PYR II. LOQ and 10X LOQ samples fortified with appropriate amount of fortification solution as per short below and cap, vortex and proceed immediately to extraction.

Sample ID	Stock Fortification Conc. PYR I/PYR II (µg/mL)	Fortification Amount (mL)	Fortification Volume (mL)	Fortified Concentration PYR I/PYR II (µg/L)
Reagent Blank	--	--	--	--
Control 1 and 2	--	--	40.0	--
LOQ-1,2,3,4 and 5	0.100/0.0818	0.04	40.0	0.100/0.0818
10X LOQ-1,2,3,4 and 5	0.100/0.0818	0.4	40.0	1.00/ 0.818

Extraction of Sample Material (Section 2.10 of Reference Method)

Add 5 mL DCM to each vial. Cap and shake for ~1 min.
Transfer bottom layer of DCM to 15 mL glass vial. Take care to avoid transfer of water.
Add another 5 mL DCM to each vial. Cap and shake for ~ 1 min.
Carefully transfer bottom layer of DCM to same 15 mL glass vial to combine extracts.
Evaporate to ~100 μ L under gentle stream of nitrogen in turbovap set at 40 °C. Do not allow to go to dryness.
Add 7.5 mL ACN. Cap and vortex ~30 sec. Sonicate 5 min. Pour into 20 mL amber glass vial.
Add 7.5 mL H ₂ O. Cap and vortex ~30 sec. Sonicate 5 min. Pour into same 20 mL amber glass vial. Vortex well.
Dilute 10X LOQ samples 1:10 with 50:50 ACN:H ₂ O. (0.5 mL sample + 4.5 mL 50:50 ACN:H ₂ O)
Aliquot ~1 mL to glass LC vial, cap and submit for LC-MS/MS analysis or store refrigerated.

The following table summarizes the extraction procedure for each sample.

Sample ID	Fortified Concentration PYR I/PYR II (μ g/mL)	Sample Volume (mL)	Reconstituted Volume (mL)	Fortified Concentration (μ g/L) as (PYR I/PYR II)	Dilution Factor
Reagent Blank	0.00	NA	15.0	NA	0.375
Control 1 and 2	0.00	40.0	15.0	NA	0.375
LOQ-1,2,3,4 and 5	0.100/0.0818	40.0	15.0	0.100/0.0818	0.375
10X LOQ-1,2,3,4 and 5	0.100/0.0818	40.0	15.0	1.00/ 0.818	3.75

NA = Not Applicable

3.2 Method (Soil and Sediment)

The residues of PYR I and PYR II were extracted from soil and sediment (5.00 g dry weight) as per the procedure given below:

Fortification (Section 2.9 of Reference Method)

Approximately 5 g of soil and sediment (5.00 g dry weight). Two Controls, 5 control samples spiked at 8.00/6.54 µg/kg (LOQ) and 5 control samples spiked at 80.0/65.4 µg/kg (10X LOQ) of PYR I/PYR II. LOQ and 10X LOQ samples fortified with appropriate amount of fortification solution as per short below and cap, vortex and proceed immediately to extraction.

Sample ID	Stock Fortification Conc. PYR I/PYR II (µg/mL)	Fortification Amount (mL)	Dry Weight (g)	Fortified Concentration PYR I/PYR II (µg/L)
Reagent Blank	NA	NA	0.00	0.00
Control 1 and 2	NA	NA	5.00	0.00
LOQ-1,2,3,4 and 5	0.100/0.0818	0.4	5.00	8.00/6.54
10X LOQ-1,2,3,4 and 5	10.0/8.18	0.04	5.00	80.0/65.4

NA = Not Applicable

Extraction of Sample Material (Section 2.10 of Reference Method)

Add 20 mL ACN to each sample. Cap and shake for 20 min.
Centrifuge @ 3000 rpm for 10 minutes.
Transfer extract to a 50 mL volumetric flask.
Repeat steps 1-3.
Combine extracts, bring to final volume (50mL), cap and mix well.
Dilute RB, Controls and LOQ samples: 1.25 mL sample + 8.75 mL 50:50 ACN:H ₂ O
Dilute 10X LOQ samples: 0.375 mL sample + 9.625 mL 50:50 ACN:H ₂ O
Aliquot ~1 mL to glass LC vial, cap and submit for LC-MS/MS analysis or store refrigerated.

The following table summarizes the extraction procedure for each sample.

Sample ID	Fortified Concentration PYR I/PYR II (µg/L)	Dry Weight (g)	Final Volume (mL)	Sample Volume for Dilution (mL)	Diluted Final Volume (mL)	Dilution Factor
Reagent Blank	0.00	NA	50.0	1.25	10.0	80.0
Control 1 and 2	0.00	5.00	50.0	1.25	10.0	80.0
LOQ-1,2,3,4 and 5	8.00/6.54	5.00	50.0	1.25	10.0	80.0
10X LOQ-1,2,3,4 and 5	80.0/65.4	5.00	50.0	0.375	10.0	267

NA = Not Applicable

STATISTICS AND DATA INTEGRITY

Statistical treatment of the data included simple descriptive statistics, such as determinations of averages, standard deviation and/or relative standard deviation (RSD) for the procedural recoveries and area counts and calculation of the calibration curve and correlation coefficient (r) by linear regression of the instrument responses for the reference standards. The statistical calculations throughout this report were performed using spreadsheet (Microsoft Excel®) and were peer-reviewed.

Several measures were taken to ensure the quality of the study results. The quality assurance unit at Concord Biosciences, inspected the analytical procedures for compliance with Good Laboratory Practices that included adherence to the protocol. The dates inspected are detailed in the quality assurance unit statement. Study samples and test and reference items were maintained in a secured laboratory with limited access.

5. SUMMARY OF METHOD

Summaries of the method parameters and characteristics are provided below.

Summary of Method

Type of Method	LC-MS/MS
Test Systems	Surface water, ground water, soil and sediment
Selected mass transitions (m/z)	

Instrument Parameters	Pyrethrin I	Jasmolin I	Cinerin I	Pyrethrin II	Jasmolin II	Cinerin II
Q1/Q3 Masses (amu)	329.0/161.0	331.0/163.0	317.0/149.0	373.0/161.0	375.0/163.0	361.0/149.0

Analytical Procedure

Smithers Viscient analytical methods 14118.6100 and 14118.6101, "Validation of an Environmental Chemistry Method for the Determination of Pyrethrins in Groundwater and Surface Water" and "Validation of an Environmental Chemistry Method for the Determination of Pyrethrins in Soil and Sediment"

Method of Quantitation

The quantitation is based on the monitoring of transitions for Pyrethrins I (pyrethrin I, jasmolin I and cinerin I) and Pyrethrins II (pyrethrin II, jasmolin II and cinerin II). Recovery data was reported for sum of mass transitions of corresponding pyrethrins I and II.

LOD	0.0132 and 0.0181 µg/L for PYR I and PYR II (Surface water and Ground water) 1.03 and 1.21 µg/kg for PYR I and PYR II (Soil and sediment)
LOQ	0.100 and 0.0818 µg/L for PYR I and PYR II (Surface water and Ground water) 8.00 and 6.54 µg/kg for PYR I and PYR II (Soil and sediment)
Levels of Fortification	0.100 and 1.00 µg/L for PYR I and 0.0818 and 0.818 µg/L for PYR II (Surface water and Ground water) 8.00 and 80.0 µg/kg for PYR I and 6.54 and 65.4 µg/kg for PYR II (Soil and sediment)
Time Required	A set of 13 samples requires approximately 8 hours of work (calculation of the results included).

Table A - 9. Instrument Conditions and Parameters

UPLC Conditions			
Chromatographic System:	Shimadzu Nexera UHPLC		
Column:	Agilent Poroshell 120 EC-C8; 2.7 μ m, 3.0 x 50 mm		
Temperature:	25 °C		
Flow rate (μ L/min)	600		
Gradient:	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
	0.01	98.0	2.0
	0.50	98.0	2.0
	2.00	30.0	70.0
	5.00	2.0	98.0
	6.00	2.0	98.0
	6.10	98.0	2.0
	7.00	98.0	2.0
Mobile Phase A:	0.1% formic acid in water		
Mobile Phase B:	0.1% formic acid in acetonitrile		
Injection Volume:	100 μ L		

MS/MS Conditions						
Detection System:	AB SCIEX 4000					
Ionization:	Turbo Spray					
Polarity:	Positive					
Curtain gas (CUR):	25					
Temperature (TEM):	500 °C					
Collision gas setting (CAD):	Medium					
GS1:	50.0					
GS2:	60.0					
Entrance potential (EP):	10.0					
Scan type:	MRM					
MRM Conditions	Transition (m/z)	Dwell (msec)	DP	CE	CXP	Retention Time (min)
Pyrethrin I	329.0/161.0	50	70	14.0	15	~4.07
Jasmolin I	331.0/163.0		70	14.0	15	~4.31
Cincerin I	317.0/149.0		70	15.0	15	~4.04
Pyrethrin II	373.0/161.0	50	70	15.0	15	~3.49
Jasmolin II	375.0/163.0		70	16.0	15	~3.65
Cincerin II	361.0/149.0		70	14.0	15	~3.45

Figure 49: Residue Calculations for Water, Soil and Sediment

Peak integration and quantitation were performed within Analyst® 1.6.2 software; using the calibration curve equation to determine the amount of analyte found (ng) during sample analysis and analyst raw data table is presented in Appendix D. Recovery results and additional sample concentrations were calculated using Microsoft® Excel spreadsheet, which are presented in Table A - 1 through Table A - 9 and Table C - 1 through Table C - 12.

The following equations are used for residue and recovery calculations for PYR I and PYR II in water, sediment and soil.

A calibration curve was generated by plotting the analyte concentration ($\mu\text{g/L}$) of the calibration standards against sum of peak area of Pyrethrin I, Jasmolin I and Cinerin I for Pyrethrins I (PYR I) and Pyrethrin II, Jasmolin II and Cinerin II for Pyrethrins II (PYR II) The concentration of test substance in each recovery sample was calculated using the slope and intercept from linear regression analysis, the detector response and the dilution factor as shown in equation a and b:

a) Calibration curve: $y = mx + b$

Solving for x:

$$\text{Detected Concentration (DC}_x\text{)}(\mu\text{g/L}) = \frac{y-b}{m}$$

Where, m = slope
 b = y-intercept
 DC_x = Amount found ($\mu\text{g/L}$)
 y = Peak area

b) Analyte concentration $\mu\text{g/L} = \text{DC} \times \text{DF}$

Where, DF = Dilution factor (Final volume of the sample divided by the original sample mass)

For water recovery sample,

Dilution factor (LOQ sample) = $15 \text{ mL}/40 \text{ mL} = 0.375$
 Dilution factor (10X LOQ sample) = $150 \text{ mL}/40 \text{ mL} = 3.75$

Final Volume for LOQ Sample = 15 mL
 Final Volume for 10XLOQ Sample = 150 mL
 Original Sample Volume = 40 mL

For soil and sediment recovery sample,

Dilution factor = Final volume of the sample*(Extraction Volume/Aliquot Volume) divided by the original mass

Final Volume of Sample = 10 mL
 Extraction Volume = 50 mL
 Aliquot Volume (LOQ Sample) = 1.25 mL
 Aliquot Volume (LOQ Sample) = 0.375 mL
 Original Sample mass = 5 g

The recoveries of spiked compounds are calculated according to equation C.

$$\text{c) Recovery (\% for Water)} = \frac{\text{Residue in sample } (\mu\text{g/L})}{\text{Amount fortified } (\mu\text{g/L})} \times 100$$

$$\text{Recovery (\% for Soil/Sediment)} = \frac{\text{Residue in sample } (\mu\text{g/kg})}{\text{Amount fortified } (\mu\text{g/kg})} \times 100$$

As an example, calculations to obtain PYR I recovery result in ground water at LOQ level is shown below:

Calibration curve: $y = 139175x + 223.73$

a)

Solving for x (Concentration of analyte $\mu\text{g/L}$): **Error! Bookmark not**

$$\text{defined. } x = \frac{29280 - 223.73}{139175} = 0.20878$$

$$\text{b) Residue } (\mu\text{g/L}) = \frac{15 \text{ mL} \times 0.20878 \mu\text{g/L}}{40 \text{ mL}} = 0.0783 \mu\text{g/L}$$

$$\text{c) Recovery (\%)} = \frac{0.0783 \mu\text{g/L}}{0.10 \mu\text{g/L}} \times 100 = 78.3\%$$

Statistical treatment of the data included calculation of means, standard deviations (SD), and percent relative standard deviations (%RSD). These calculations were performed using Microsoft® Excel. Results were rounded only for reporting purposes. No calculations were made with rounded numbers.