ABSTRACT

The purpose of this study is to conduct an independent laboratory validation (ILV) of environmental chemistry methods for determination of pyrimisulfan and metabolites M1, M15, and Imino M18 in soil, sediment, and natural and surface water according to validated methods described in Ricerca Study 032005 [1]. The independent laboratory validation was conducted on the same test systems (soil, sediment, natural water, and surface water) used in Ricerca Study 032005 to meet criteria for acceptance as defined by U.S. EPA and/or the European Commission for General Health and Consumer Protection.

The independent laboratory validation (ILV) followed the U.S. EPA guidelines found in OCSPP 850.6100 [2] and was conducted separately from the validation laboratory. Study personnel, equipment, instruments, standard aliquots and subsequent solutions and supplies (i.e., glassware, solvents, reagents, etc.) for this ILV were separate from the ECM validation laboratory. The ILV also followed the guidelines found in EU Council Directive 91/414/EEC with particular regard to the European Commission Guidance Document SANCO/825/00 rev. 8.1, Directorate General Health and Consumer Protection dated November 16, 2010 [3].

The Ricerca Study 032005 validated methods were independently validated. Soil, sediment, natural water, and surface water samples were fortified with a mixture of pyrimisulfan M1, M15, and Imino M18 at two recovery levels: the Limit of Quantification (LOQ), 2 ppb, and 10x the LOQ, 20 ppb. In addition, two control samples were analyzed for presence of background. Tables 1-10 summarize the recoveries:

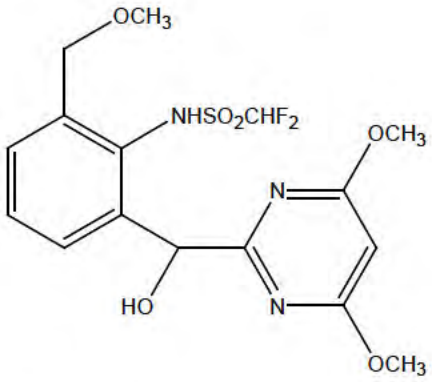
OBJECTIVE/PURPOSE

The purpose of this study is to conduct an Independent Laboratory Validation (ILV) of environmental chemistry methods for the determination of pyrimisulfan and metabolites M1, M15, and Imino M18 in soil, sediment, natural water, and surface water according to validated methods described in Ricerca Study 032005.

TEST SUBSTANCES

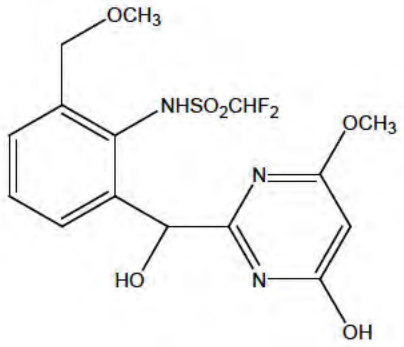
Pyrimisulfan was supplied by Sponsor. M1, M15, and Imino M18 were synthesized by Ricerca Synthetic Chemistry group. Information concerning the test substances including purity is provided as follows. The certificates of analysis (CoA) of the test substances are included in Appendix A.

- **Pyrimisulfan**



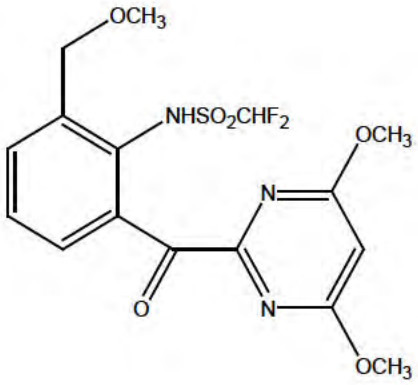
Common Name:	Pyrimisulfan
Chemical Name:	(RS)-2'-(4,6-dimethoxypyrimidin-2-yl)hydroxymethyl-6'-methoxymethyl-1,1-difluoromethanesulfonamide
Molecular Formula:	$C_{16}H_{19}F_2N_3O_6S$
Ricerca Code:	CS_20003 and CS_20017
Lot Number:	13J016
Molecular Weight:	419.4 g/mole
Purity:	100%
Storage:	Ambient
Expiration Date:	April 24, 2017

- **M1**



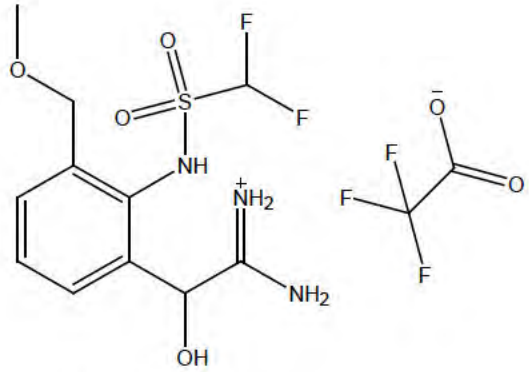
Common Name:	M1
Chemical Name:	(*RS*)-2'-(4-hydroxy-6-methoxypyrimidin-2-yl)hydroxymethyl-6'-methoxymethyl-1,1-dilfuoromethanesulfonanilide
Molecular Mass:	405.37
Molecular Formula:	$C_{15}H_{17}F_2N_3O_6S$
Lot Number:	55269-7-36
Stated Chemical Purity:	100.0%
Storage:	Refrigerated

- **M15**



Common Name:	M15
Chemical Name:	2'-(4,6-Dimethoxypyrimidin-2-yl)carbonyl-6'-methoxymethyl-1,1-difluoromethanesulfonanilide
Molecular Mass:	417.38
Molecular Formula:	$C_{16}H_{17}F_2N_3O_6S$
Lot Number:	55231-12-16
Stated Chemical Purity:	96.6%
Storage:	Refrigerated

- **Imino M18 TFA salt**



Common Name:	Imino M18 TFA salt
Chemical Name:	2-[2-(difluoromethylsulfonamido)-3-(methoxymethyl)phenyl]-2-hydroxyacetamide
Molecular Mass:	324.3
Molecular Formula:	$C_{13}H_{16}F_5N_3O_6S$
Lot Number:	55357-16-35
Stated Chemical Purity:	93.6%
Storage:	Refrigerated

TEST SYSTEMS

The Sponsor provided the same test systems (soil, sediment, and natural and surface water, described in the table below) used in the method validation (Ricerca Study 032005) for this ILV. The characterization reports of the test systems are included in Appendix B.

Table 11: Test Systems

Test System	Source	Characteristics	pH
Soil	Fresno, CA, 0-6" Soil From Ricerca Study 031852	Loamy sand (79% sand, 14% silt, 7% clay)	7.8
Sediment	Golden Lake Sediment (Golden Lake, ND) N47.33744, W97.38352	Sand (88% sand, 10% silt, 2% clay)	8.1
Natural Water	Golden Lake Water (Golden Lake, ND) N47.33744, W97.38352	-	-
Surface Water	Smokey Oaks Pond Water (North Bloomfield, Trumbull County, OH 44450) N41.495446, W80.747337	-	8.0

SOLVENTS AND REAGENTS

Formic acid, Fisher Optima LC/MS Grade
Acetonitrile (ACN), Fisher Optima Grade or Sigma HPLC Grade
Water, Fisher HPLC Grade or Sigma HPLC Grade
0.1% Formic acid in water: formic acid (1 mL) and HPLC water (1 L) were mixed.
0.1% Formic acid in acetonitrile: formic acid (1 mL) and acetonitrile (1 L) were mixed.
Acetonitrile-water (80:20, v:v): acetonitrile (800 mL) and HPLC water (200 mL) were mixed.
Acetonitrile-water (20:80, v:v): acetonitrile (200 mL) and HPLC water (800 mL) were mixed.

STOCK, FORTIFICATION, AND CALIBRATION SOLUTIONS

All standard solutions (stock, fortification, and calibration solutions) were freshly prepared for use in this study only.

STOCK SOLUTIONS

Two pyrimisulfan stock solutions were prepared by weighing 10.0 mg (**R1**) and 10.0 mg (**R2**) of pyrimisulfan in two separate 10-mL volumetric flasks and acetonitrile was added to volume. The stated chemical purity of pyrimisulfan is 100% (CoA in Appendix A). The concentrations of the pyrimisulfan stock solutions were 1000 µg/mL (**R1**) and 1000 µg/mL (**R2**) after corrected for purity. The stock solutions were stored in a freezer (< -15 °C) when not in use.

Two **M1** stock solutions were prepared by weighing 10.0 mg (**R1**) and 10.0 mg (**R2**) of **M1** in two separate 10-mL volumetric flasks and acetonitrile was added to volume. The stated chemical purity of **M1** is 100% (CoA in Appendix A). The concentrations of the **M1** stock solutions were 1000 µg/mL (**R1**) and 1000 µg/mL (**R2**) after corrected for purity. The stock solutions were stored in a freezer (< -15 °C) when not in use.

Two **M15** stock solutions were prepared by weighing 10.0 mg (**R1**) and 10.0 mg (**R2**) of **M15** in two separate 10-mL volumetric flasks and acetonitrile was added to volume. The stated chemical purity of **M15** is 96.6% (CoA in Appendix A). The concentrations of the **M15** stock solutions were 966 µg/mL (**R1**) and 966 µg/mL (**R2**) after corrected for purity. The stock solutions were stored in a freezer (< -15 °C) when not in use.

Two **Imino M18** stock solutions were prepared by weighing 10.0 mg (**R1**) and 10.1 mg (**R2**) of **Imino M18** in two separate 10-mL volumetric flasks and acetonitrile was added to volume. The stated chemical purity of **Imino M18** is 93.6% (CoA in Appendix A). The concentrations of the **Imino M18** stock solutions were 936 µg/mL (**R1**) and 945 µg/mL (**R2**) after corrected for purity. The stock solutions were stored in a freezer (< -15 °C) when not in use.

FORTIFICATION SOLUTIONS

The fortification solutions were prepared by mixing and serial dilution of the **R1** stock solutions with acetonitrile as detailed below:

R1 10 µg/mL Mixed Standard Solution:
Aliquots of pyrimisulfan, M1, M15, and Imino M18 stock solutions (**R1**) were transferred to a 10-mL Class A volumetric flask and brought to volume with acetonitrile, as described in the table below:

Source Solution ID	Source Solution Conc. (µg/mL)	Aliquot Volume (µL)	Final Volume (mL)	Standard Solution Conc. (µg/mL)
Pyrimisulfan Stock Solution R1 (5/6/16 RLS)	1000	100	10	10
M1 Stock Solution R1 (5/6/16 RLS)	1000	100		10
M15 Stock Solution R1 (5/6/16 RLS)	966	104		10
Imino M18 Stock Solution R1 (5/6/16 RLS)	936	107		10

The standard solution was stored in a freezer (< -15 °C) when not in use.

R1 2,000 ng/mL and 200 ng/mL Fortification Solutions:
Aliquots of the mixed standard solutions were transferred to 10-mL Class A volumetric flasks and brought to volume with acetonitrile, as described in the table below:

Source Solution ID	Source Solution Conc. (µg/mL)	Aliquot Volume (mL)	Final volume (mL)	Standard Solution Conc. (ng/mL)
R1 10 µg/mL Mixed Solution (5/6/16 RLS)	10	2.0	10	2,000
R1 2,000 ng/mL Mixed Solution (5/6/16 RLS)	2,000	1.0	10	200

The fortification solutions were stored in a freezer (< -15 °C) when not in use.

CALIBRATION SOLUTIONS
The calibration solutions were prepared by mixing and serial dilution of the **R2** stock solutions as detailed below:

R2 10 µg/mL Mixed Standard Solution:
Aliquots of pyrimisulfan, **M1**, **M15**, and **Imino M18** stock solutions (**R2**) were transferred to a 10-mL Class A volumetric flask and brought to volume with acetonitrile, as described in the table below:

Source Solution ID	Source Solution Conc. (µg/mL)	Aliquot Volume (µL)	Final Volume (mL)	Standard Solution Conc. (µg/mL)
Pyrimisulfan Stock Solution R2 (5/6/16 RLS)	1000	100	10	10
M1 Stock Solution R2 (5/6/16 RLS)	1000	100		10
M15 Stock Solution R2 (5/6/16 RLS)	966	104		10
Imino M18 Stock Solution R2 (5/6/16 RLS)	945	106		10

The standard solution was stored in a freezer (< -15 °C) when not in use.

R2 100 ng/mL and 10 ng/mL Intermediate Solutions:
Aliquots of the mixed standard solutions were transferred to 10-mL Class A volumetric flasks and brought to volume with acetonitrile, as described in the table below:

Source Solution ID	Source Solution Conc.	Aliquot Volume (mL)	Final volume (mL)	Standard Solution Conc. (ng/mL)
R2 10 µg/mL Mixed Solution (5/6/16 RLS)	10 µg/mL	0.1	10	100
R2 100 ng/mL Mixed Solution (5/6/16 RLS)	1,000 ng/mL	1.0	10	10

The fortification solutions were stored in a freezer (< -15 °C) when not in use.

R2 Calibration Solutions:
Aliquots of the mixed standard solutions were transferred to 10-mL Class A volumetric flasks and brought to volume with acetonitrile-water (20:80, v:v), as described in the table below:

Source Solution ID	Source Solution Conc.	Aliquot Volume (mL)	Final volume (mL)	Standard Solution Conc. (ng/mL)
R2 100 ng/mL Mixed Solution (5/6/16 RLS)	100 ng/mL	0.50	10	5.0
R2 100 ng/mL Mixed Solution (5/6/16 RLS)	100 ng/mL	0.30	10	3.0
R2 100 ng/mL Mixed Solution (5/6/16 RLS)	100 ng/mL	0.20	10	2.0
R2 100 ng/mL Mixed Solution (5/6/16 RLS)	100 ng/mL	0.10	10	1.0
R2 10 ng/mL Mixed Solution (5/6/16 RLS)	10 ng/mL	0.50	10	0.50
R2 10 ng/mL Mixed Solution (5/6/16 RLS)	10 ng/mL	0.20	10	0.20
R2 10 ng/mL Mixed Solution (5/6/16 RLS)	10 ng/mL	0.15	10	0.15
R2 10 ng/mL Mixed Solution (5/6/16 RLS)	10 ng/mL	0.10	10	0.10

The calibration solutions were stored a freezer (< -15 °C) when not in use.

STANDARD CONCENTRATION VERIFICATION

The procedure for standard concentration verification follows the same procedure listed in Appendix C of Ricerca Study 032005 report. The R1 10 µg/mL mixed standard solution was diluted 10,000x to 1 ng/mL nominal concentration and R2 10 µg/mL mixed standard solution diluted 8,000x to 1.25 ng/mL nominal concentration with acetonitrile-water (20:80, v:v) for concentration verification. The diluted solutions were analyzed against R2 calibration standards at 0.1 - 5 ng/mL. The Analyst® result tables are copied in Appendix C of this report.

ANALYTICAL METHODOLOGY

FORTIFICATION OF SOIL

Twelve Fresno, CA soil samples (10.0 g each in 50-mL polypropylene centrifuge tubes) were fortified with pyrimisulfan, **M1**, **M15**, and **Imino M18** mixed standard solutions in acetonitrile as described in Table 13. Two Fresno, CA soil samples (10.0 g each in 50-mL polypropylene centrifuge tubes) serving as controls were not fortified.

Table 13: Preparation Scheme for Soil Fortifications (2 ppb and 20 ppb)

Sample ID	Weight (g)	Fortification Solutions and Volume	Fort. Level (ppb)
Soil Control-1	10.0	None	None
Soil Control-2	10.0	None	None
Soil LOQ-1	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Soil LOQ-2	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Soil LOQ-3	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Soil LOQ-4	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Soil LOQ-5	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Soil LOQ-6	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Soil LOQ-7	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Soil 10XLOQ-1	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Soil 10XLOQ-2	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Soil 10XLOQ-3	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Soil 10XLOQ-4	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Soil 10XLOQ-5	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20

FORTIFICATION OF SEDIMENT

Twelve Golden Lake sediment samples (10.0 g each in 50-mL polypropylene centrifuge tubes) were fortified with pyrimisulfan, **M1**, **M15**, and **Imino M18** mixed standard solutions

in acetonitrile as described in Table 14. Two Golden Lake sediment samples (10.0 g each in 50-mL polypropylene centrifuge tubes) serving as controls were not fortified.

Table 14: Preparation Scheme for Sediment Fortifications (2 ppb and 20 ppb)

Sample ID	Weight (g)	Fortification Solutions and Volume	Fort. level (ppb)
Sediment Control-1	10.0	None	None
Sediment Control-2	10.0	None	None
Sediment LOQ-1	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Sediment LOQ-2	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Sediment LOQ-3	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Sediment LOQ-4	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Sediment LOQ-5	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Sediment LOQ-6	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Sediment LOQ-7	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Sediment 10XLOQ-1	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Sediment 10XLOQ-2	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Sediment 10XLOQ-3	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Sediment 10XLOQ-4	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Sediment 10XLOQ-5	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20

FORTIFICATION OF NATURAL WATER

Twelve Golden Lake water samples (10.0 g each in 50-mL polypropylene centrifuge tubes) were fortified with pyrimisulfan, **M1**, **M15**, and **Imino M18** mixed standard solutions in acetonitrile as described in Table 15. Two Golden Lake water samples (10.0 g each in 50-mL polypropylene centrifuge tubes) serving as controls were not fortified.

Table 15: Preparation Scheme for Natural Water Fortifications (2 ppb and 20 ppb)

Sample ID	Weight (g)	Fortification Solutions and Volume	Fort. Level (ppb)
Natural Water Control-1	10.0	None	None
Natural Water Control-2	10.0	None	None
Natural Water LOQ-1	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Natural Water LOQ-2	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Natural Water LOQ-3	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Natural Water LOQ-4	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Natural Water LOQ-5	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Natural Water LOQ-6	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Natural Water LOQ-7	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Natural Water 10XLOQ-1	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Natural Water 10XLOQ-2	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Natural Water 10XLOQ-3	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Natural Water 10XLOQ-4	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Natural Water 10XLOQ-5	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20

FORTIFICATION OF SURFACE WATER

Twelve Smokey Oaks Pond water samples (10.0 g each in 50-mL polypropylene centrifuge tubes) were fortified with pyrimisulfan, **M1**, **M15**, and **Imino M18** mixed standard solutions in acetonitrile as described in Table 16. Two Smokey Oaks Pond water samples (10.0 g each in 50-mL polypropylene centrifuge tubes) serving as controls were not fortified.

Table 16: Preparation Scheme for Surface Water Fortifications (2 ppb and 20 ppb)

Sample ID	Weight (g)	Fortification Solutions and Volume	Fort. Level (ppb)
Surface WaterControl-1	10.0	None	None
Surface Water Control-2	10.0	None	None
Surface Water LOQ-1	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Surface Water LOQ-2	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Surface Water LOQ-3	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Surface Water LOQ-4	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Surface Water LOQ-5	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Surface Water LOQ-6	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Surface Water LOQ-7	10.0	200 ng/mL mixed standard solution (R1), 100 µL	2
Surface Water 10XLOQ-1	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Surface Water 10XLOQ-2	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Surface Water 10XLOQ-3	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Surface Water 10XLOQ-4	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20
Surface Water 10XLOQ-5	10.0	2,000 ng/mL mixed standard solution (R1), 100 µL	20

EXTRACTION (SOIL AND SEDIMENT)

1. Fifteen (15) mL of the extraction solvent (80:20 (v:v) acetonitrile:water) was added to the samples using a 15 mL Class A volumetric pipet.
2. The samples were shaken for approximately one hour on a Burrell Wrist-Action Shaker model 75.
3. The samples were centrifuged for 10 minutes at ~ 4,000 rpm using a Sorvall T6000B Centrifuge.
4. The supernatant was transferred into a 50 mL polypropylene centrifuge tube.
5. The samples were re-extracted with additional 15 mL of the extraction solvent and the extraction procedure was repeated by shaking and centrifugation.
6. The supernatants were transferred into the respective 50 mL polypropylene centrifuge tubes.
7. The volume of the final extract was adjusted to 30 mL with the extraction solvent

PREPARATION OF SOIL AND SEDIMENT EXTRACTS FOR LC-MS/MS ANALYSIS

The LOQ extracts were diluted four times with water (0.25 mL of the extract and 0.75 mL of water) and the 10XLOQ extracts were diluted five times with water (0.20 mL of the extract and 0.80 mL of water) in a glass autosampler vial and analyzed by LC-MS/MS.

For quantification of pyrimisulfan in the LOQ samples, the soil and sediment extracts were analyzed by LC-MS/MS without dilution.

PREPARATION OF WATER SAMPLES FOR LC-MS/MS ANALYSIS

The LOQ samples were diluted 10 times (0.1 mL of LOQ sample and 0. 9 mL of acetonitrile-water (20:80, v:v)) and the 10XLOQ samples were diluted 100 times (0.1 mL of 10XLOQ sample and 9.9 mL of acetonitrile-water (20:80, v:v)) with acetonitrile-water (20:80, v:v) and analyzed by LC-MS/MS.

For quantification of pyrimisulfan in the water samples, the LOQ and 10XLOQ samples were diluted 2 times and 20 times, respectively, with acetonitrile-water (20:80, v:v) and analyzed by LC-MS/MS.

LC-MS/MS ANALYSIS

Separation of the analytes from the matrix was achieved by high performance liquid chromatography (HPLC). Quantitative LC-MS/MS analysis of pyrimisulfan, **M1**, **M15**, and **Imino M18** in the samples utilized a highly specific and sensitive MRM (Multiple Reaction Monitoring) method. Pyrimisulfan precursor ion (m/z 420) was monitored in Q1 and two fragment ions were monitored in Q3 (m/z 370 and 255). **M1** precursor ion (m/z 406) was monitored in Q1 and two fragment ions were monitored in Q3 (m/z 356 and 241). **M15** precursor ion (m/z 418) was monitored in Q1 and three fragment ions were monitored in Q3 (m/z 386, 272, and 243). **Imino M18** precursor ion (m/z 324) was monitored in Q1 and three fragment ions were monitored in Q3 (m/z 292, 161, and 160). The analytes were identified by the coincidence of the retention times with those of the calibration standards, and quantified by integration of the peak area relative to the calibration curves.

The following are the LC-MS/MS systems and the parameters used.

LC-MS/MS systems: LC-MS “J”, LC-MS “G”, and LC-MS “R” with identical components.

(LC-MS systems “J” and “G” were used for ILV and LC-MS system “R” was used for Standard Concentration Verification.)

- UHPLC: Two Shimadzu LC-30AD pumps and a Shimadzu SIL 30 ACMP Autosampler
- MS: SCIEX API4000
- Computer software: Analyst™ version 1.6.2

Column: Phenomenex Synergi Hydro RP 2.5 µm 50 mm x 2.0 mm
Column Temperature: 30 °C
Injection Volume: 10 µL (LC-MS “J” nd “G”), 20 or 25 µL (LC-MS “R”)
Autosampler Sample Tray Temperature: 5 °C

Solvent System:
Solvent A = 0.1% Formic acid in water
Solvent B = 0.1% Formic acid in Acetonitrile

Solvent Program:

Time (minutes)	Flow Rate (mL/min)	%A	%B
0.0	0.65	98	2
1.0	0.65	98	2
4.5	0.65	20	80
4.6	0.65	5	95
5.6	0.65	5	95
5.7	0.65	98	2
7.0	0.65	98	2

The LC flow was diverted to the MS between 0.9 and 4.7 min and to waste between 0.0 and 0.9 min and between 4.7 and 7.0 min.

Retention times: **Pyrimisulfan**: 3.85 min ("J"), 4.08 min ("G"), 4.20 min ("R")
M1: 3.06 min ("J"), 3.36 min ("G"), 3.38 min ("R")
M15: 3.71 min ("J"), 3.94 min ("G"), 4.04 min ("R")
Imino M18: 2.29 min ("J"). 2.48 min ("G"), 2.54 min ("R"),

Mass Spectrometer settings:

Scan Type:	MRM
Polarity:	Positive
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	Unit
Ion Source Gas 1 (GS1):	50 psi
Ion Source Gas 2 (GS2):	60 psi
Curtain Gas (CUR):	30 psi
Collision Gas (CAD):	10 psi
IonSpray Voltage (IS):	5000 V
Temperature (TEM):	500 ºC
Declustering Potential (DP):	45 V
Entrance Potential (EP):	10 V

MRM Transitions:

Analyte ID	Q1 Mass (amu)	Q3 Mass (amu)	Collision Energy (CE)	Collision Gas Exit Potential (CXP)	Dwell Time (msec)
Pyrimisulfan-370	420	370	29	15	100
Pyrimisulfan-255	420	255	39	15	100
M1-356	406	356	26	15	100
M1-241	406	241	38	15	100
M15-386	418	386	25	13	100
M15-272	418	272	34	8	100
M15-243	418	243	47	7	100
Imino-M18-292	324	292	21	15	100
Imino-M18-161	324	161	31	15	100
Imino-M18-160	324	160	38	15	100

LIMIT OF QUANTIFICATION AND DETECTION (LOQ AND LOD)

The Limit of Quantification, LOQ, is 2 ppb for all four analytes (pyrimisulfan, M1, M15, and imino M18) in all four matrices (soil, sediment, natural water, and surface water).

The Limit of Detection (LOD) for pyrimisulfan, **M1**, **M15**, and **Imino M18** in soil, sediment, natural water, and surface water was calculated in Ricerca Study 032005 in the table below.

Table 17: Limit of Detection (LOD) for pyrimisulfan, M1, M15, and imino M18 in soil, sediment, natural water, and surface water

Analyte	Q1 m/z	Q3 m/z	Method Detection Limit (ppb)			
			Soil	Sediment	Natural Water	Surface Water
Pyrimisulfan	420	370	1.22	0.23	0.19	0.26
Pyrimisulfan	420	255	1.36	0.54	0.60	0.40
M1	406	356	0.34	0.28	0.26	0.29
M1	406	241	0.41	0.62	0.55	0.45
M15	418	386	1.37	0.41	0.16	0.21
M15	418	272	1.34	0.38	0.29	0.48
M15	418	243	1.22	0.73	0.53	0.73
Imino M18	324	292	0.21	0.23	0.24	0.15
Imino M18	324	161	0.31	0.94	0.55	0.40
Imino M18	324	160	0.41	0.48	0.42	0.26

METHODS OF CALCULATION

The recoveries of analytes (pyrimisulfan, **M1**, **M15**, and **Imino M18**) from fortified samples were calculated relative to the calibration curve generated with each set:

Linear regression formula from calibration curve y = mx + b

$$\text{ng/mL Analyte} = \frac{y - b}{m}$$

Where y = Sample peak area

b = Calibration intercept

m = Calibration slope

$$\text{Sample Concentration (ng/mL)} = \frac{\text{Sample peak area - intercept}}{\text{Slope}}$$

$$\text{ppb Analyte} = \frac{\text{Sample Conc. (ng/mL) x Final Sample Vol. (mL) x Dilution Factor}}{\text{Sample weight (g)}}$$

$$\text{Percent Recovery} = \frac{\text{Conc. of Fortified Sample (ppb) - Conc. of Control (ppb)}}{\text{Fortification Level (ppb)}} \text{ x } 100$$

An example calculation for the recovery of **Imino-M18-292** (20 ppb fortification) from sediment (sample **Sediment 10XLOQ-1**, in Figure 186) is shown below:

The **Imino-M18** calibration curve equation was y = 55800x + 274 (r = 0.9998)

$$\text{ng/mL } \textbf{Imino-M18} = \frac{72540 - 274}{55800} = 1.295 \text{ ng/mL}$$

The ppb **Imino-M18** in sediment was calculated as follows:

$$\text{ppb } \textbf{Imino-M18} = \frac{1.295 \text{ ng/mL x } 30 \text{ mL x } 5}{10 \text{ g}} = 19.43 \text{ ppb}$$

The percent recovery of **Imino-M18** was calculated as follows:

$$\text{Percent recovery} = \frac{19.43 \text{ ppb} - 0 \text{ ppb}}{20 \text{ ppb}} \text{ x } 100 = 97.2\%*$$

*Slight difference from reported value (97.4%)

An example calculation for the recovery of **M1-356** (20 ppb fortification) from natural water (sample **Natural Water 10XLOQ-5**, in Figure 69) is shown below:

The **M1** calibration curve equation was y = 45800x + 371 (r = 0.9997)

$$\text{ng/mL } \mathbf{M1} = \frac{9588 - 371}{45800} = 0.2012 \text{ ng/mL}$$

The ppb **M1** in natural water was calculated as follows:

$$\text{ppb } \mathbf{M1} = \frac{0.2012 \text{ ng/mL} \times 10 \text{ mL} \times 100}{10 \text{ g}} = 20.12 \text{ ppb}$$

The percent recovery of **M1** was calculated as follows:

$$\text{Percent recovery} = \frac{20.12 \text{ ppb} - 0 \text{ ppb}}{20 \text{ ppb}} \times 100 = 101\%$$

The ILV in soil, sediment, natural water, and surface water passed in the first trial. A total of 24 hours were needed to prepare and analyze all four sets of samples by LC-MS/MS (including standard preparation and analysis).

SIGNIFICANT OBSERVATION

When analyzed pyrimisulfan in soil, sediment, natural water, and surface water in this ILV, the soil extracts (LOQ only), sediment extracts (LOQ only), natural water, and surface water were diluted differently from Ricerca Proj032005 method, as shown in the table below. The dilution factors for analysis of M1, M15, and Imino M18 in soil extracts, sediment extracts, natural water, and surface water remained the same as those in Ricerca Proj032005 methods.

Table 18: Dilution Factors for Analysis of Pyrimisulfan, M1, M15, and Imino M18 in Soil Extracts, Sediment Extracts, Natural Water, and Surface Water

Matrix	Fort. Level	Pyrimisulfan		M1, M15, Imino M18	
		Original Method	ILV	Original Method	ILV
Soil	2 ppb	4x	1x	4x	4x
	20 ppb	5x	5x	5x	5x
Sediment	2 ppb	4x	1x	4x	4x
	20 ppb	5x	5x	5x	5x
Natural Water	2 ppb	10x	2x	10x	10x
	20 ppb	100x	20x	100x	100x
Surface Water	2 ppb	10x	2x	10x	10x
	20 ppb	100x	20x	100x	100x

The reason for this modification is due to poor recoveries of pyrimisulfan in soil, sediment, natural water, and surface water following the dilution factors in the original methods. The linearity of the pyrimisulfan calibration standards that were prepared by sequential dilution following Ricerca Study 032005 methods to 0.1 ng/mL is demonstrated. However, the MS response is not linear at lower pyrimisulfan concentrations in samples (for water ILV, 1 ng/mL is still in the linear range but not 0.2 ng/mL; for soil and sediment ILV, 0.667 ng/mL is still in the linear range but not 0.167 ng/mL). Therefore, to analyze pyrimisulfan in soil, sediment, natural water, and surface water following Ricerca Proj032005 method, it may be necessary to modify the dilution factors in the methods unless quantitative recovery of pyrimisulfan can be demonstrated.

REFERENCES

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