

## **Determination of Azoxystrobin, Kresoxim methyl, Pyraclostrobin and Trifloxystrobin in Surface Water by Ultra Performance Liquid Chromatography Coupled to Tandem Mass Spectrometry**

### 1. Scope:

This section method (SM) provides stepwise procedure for azoxystrobin, kresoxim methyl, pyraclostrobin, and trifloxystrobin analysis in surface water. It is followed by all authorized EA personnel.

### 2. Principle:

The azoxystrobin, kresoxim methyl, pyraclostrobin, and trifloxystrobin are extracted from the surface water sample with methylene chloride. The extract is passed through sodium sulfate to remove residual water. The anhydrous extract is evaporated on a rotary evaporator and then a solvent exchange is performed with methanol. The extract is concentrated to a final volume of 1 mL and then vialled into an autosampler vial for analysis on Ultra Performance Liquid Chromatography (UPLC) coupled to a positive electrospray ionization triple quadrupole mass spectrometry (ES-LC/MS/MS).

### 3. Safety:

- 3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.
- 3.2 Methylene chloride is a regulated and controlled carcinogenic hazardous substance. It must be stored and handled in accordance with California Code of Regulations, Title 8, Subchapter 7, Group 16, Article 110, Section 5202.

### 4. Interferences:

There were no matrix interferences for azoxystrobin, kresoxim methyl, pyraclostrobin, and trifloxystrobin at the time of method development.

### 5. Apparatus and Equipment:

- 5.1 Rotary Evaporator (Buchi/Brinkman or equivalent)
- 5.2 Nitrogen Evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
- 5.3 Balance (Mettler PC 4400 or equivalent)
- 5.4 Vortex-vibrating mixer

5.5 UPLC equipped with a triple quadrupole mass spectrometry and ES ion source.

.6. Reagents and Supplies:

- 6.1 Azoxystrobin CAS#131860-33-8
- 6.2 Kresoxim methyl CAS#143390-89-0
- 6.3 Pyraclostrobin CAS#175013-18-0
- 6.4 Trifloxystrobin CAS#141517-89-0
- 6.5 Methylene Chloride, nanograde or equivalent pesticide grade
- 6.6 Water, MS grade, Burdick & Jackson or equivalent
- 6.7 Methanol, MS grade, Burdick & Jackson or equivalent
- 6.8 Formic Acid, HPLC grade
- 6.9 Ammonium formate, reagent grade or equivalent
- 6.10 Separatory funnel, 2 L
- 6.11 Boiling flask, 500 mL
- 6.12 Sodium Sulfate, ACS grade
- 6.13 Funnels, long stem, 60°, 100 mm I.D.
- 6.14 Volumetric Pipette, 0.5 mL
- 6.15 Graduated conical tubes with glass stopper, 15 mL
- 6.16 Glass wool, Pyrex® fiber glass slivers 8 microns
- 6.17 Disposable Pasteur pipettes, and other laboratory ware as needed
- 6.18 Recommended analytical column:  
Waters Acquity BEH C18 1.7µm, 2.1 x 100 mm
- 6:19 Aqueous Solution: For 500 mL, mix 470 ± 2mL water, 25 ± 0.5 mL methanol, 4.50 ± 0.25 mL 1 M ammonium formate and 0.5 ± 0.05 mL formic acid.
- 6.20 Organic Solution: For 500mL, mix 450 ± 2mL methanol and 45 ± 0.5 mL water with 4.50 ± 0.25 mL 1 M ammonium formate and 0.5 ± 0.05 mL formic acid.

7. Standards Preparation:

7.1 The individual stock standards of 1.0 mg/mL were obtained from the CDFCA/CAC Standards Repository. The standards were diluted to 10 µg/mL with methanol for identification purposes.

A combination standard of 1 µg/mL was prepared from the individual 10 µg/mL standards with methanol. The standard was also used to dilute the following concentrations: 0.025, 0.05, 0.1, 0.25 and 0.5 µg/mL in methanol for instrument calibration.

7.2 Keep all standards in the designated refrigerator for storage.

7.3 The expiration date of each standard is six months from the preparation date.

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a separate refrigerator ( $4 \pm 3$  °C).

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulations (DPR) provides the background water for matrix blank and spikes.

9.2 Preparation of blank and spike

Matrix blank: Weigh out 1000 g of background water and follow the test sample extraction procedure.

Matrix spike: Weigh out 1000 g of background water. Spike a client requested amount of fungicides into the background water, mix well and let it stand for 1 minute. Follow the test sample extraction procedure.

9.3 Test Sample Extraction

9.3.1 Remove samples from the refrigerator and allow them to reach ambient temperature.

9.3.2 Record the weight of water samples to 0.1 g by subtracting the weight of the sample container before and after water has been transferred into a separatory funnel.

9.3.3 Shake with  $100 \pm 5$  mL of methylene chloride for 2 minutes. Vent frequently to relieve pressure.

9.3.4 After phases have separated, drain the lower methylene chloride layer through  $25 \pm 4$  g of anhydrous sodium sulfate and glass wool into a 500 mL boiling flask.

- 9.3.5 Repeat steps 9.3.3 & 9.3.4 two more times using  $80 \pm 5$  mL of methylene chloride and shake for 1 minute each time. Combine the extracts in the same boiling flask.
- 9.3.6 After draining the final extraction, rinse the sodium sulfate with  $25 \pm 5$  mL of methylene chloride.
- 9.3.7 Evaporate the sample extract to 2 - 4 mL on a rotary evaporator using a water bath at  $35 \pm 2$  °C and 15 – 20 inch Hg vacuum. Add 2-4 mL of methanol and rotoevaporate to 1-2 mL. Transfer the extract to a calibrated 15 mL graduated test tube.
- 9.3.8 Rinse flask 3 more times with 2 - 4 mL of methanol and transfer each rinse to the same test tube.
- 9.3.9 Evaporate the sample extract to a volume slightly less than 1 mL in a water bath at  $40 \pm 2$  °C under a gentle stream of nitrogen. Then bring to a final volume of 1.0 mL with methanol, mix well and transfer to an autosampler vial. Submit extract for LC-MS analysis.

## 10. Instrument Calibration:

- 10.1 The calibration standard curve consists of a minimum of three levels. The lowest level must be at or below the corresponding reporting limit. The current working standard levels are 0.025, 0.05, 0.1, 0.25, and 0.5 µg/mL.
- 10.2 Calibration is obtained using a linear or quadratic regression with the correlation coefficient (r) equal to or greater than 0.995, with all levels weighted 1/x.

## 11. Analysis:

### 11.1 Injection Scheme

The LC-MS needs to be conditioned with standard or a sample extract 2 to 5 runs before running the following sequence: A set of calibration standards, a matrix blank, a matrix spike, a set of up to 12 test samples, then a set of standards, etc.

### 11.2 UPLC-MS/MS

11.2.1 UPLC Instrument: Waters Acquity Ultra Performance LC  
Column: Waters Acquity BEH C18 1.7µm, 2.1 x 100 mm  
Column Temperature: 60 °C  
Mobile Phase: Gradient  
Solvent 1: Aqueous Solution  
Solvent 2: Organic Solution  
Gradient:

<u>Time(min)</u>	<u>Flow rate (mL/min)</u>	<u>Solvent 1</u>	<u>Solvent 2</u>
0	0.60	90.0	10.0
0.5	0.60	90.0	10.0
7.0	0.60	10.0	90.0
8.80	0.60	10.0	90.0
9.50	0.60	90.0	10.0
10.5	0.60	90.0	10.0

Injection Volume: 1.0 µL

11.2.2 Mass Spectrometry and Operating Parameters

Model: Waters Xevo Triple Quadrupole  
Ion ProbeType: Electrospray Ionization (ES)  
Ion Mode: ES+  
Source Temp: 150 °C

Compound	Retention Time ( min)	Precursor ion	Product Ion	Dwell (s)	Cone(V)	Collision Energy/-ev
Azoxystrobin	5.53	404.21	<b>344.21</b>	0.028	6.0	24.0
		404.21	372.22	0.028	6.0	14.0
Kresoxim-methyl	6.41	314.16	<b>115.99</b>	0.028	14.0	12.0
		314.23	267.14	0.028	18.0	6.0
Pyraclostrobin	6.68	388.19	<b>163.10</b>	0.028	10.0	26.0
		388.19	194.08	0.028	10.0	12.0
Trifloxystrobin	7.04	409.23	<b>145.00</b>	0.028	10.0	44.0
		409.23	186.06	0.028	10.0	18.0

Quantitation ions are in bold.

## 12. Quality Control:

### 12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 well water samples are spiked at 0.1ppb and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for each analyte using the following equation:

$$\text{MDL} = tS$$

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL,  $t=3.143$ .

The results for the standard deviations and MDL are in Appendix 1.

### 12.2 Reporting Limit (RL)

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The RL is chosen in a range 1-5 times the MDL, as per client agreement. The reporting limit for azoxystrobin, kresoxim-methyl, pyraclostrobin, and trifloxystrobin is 0.05 ppb.

### 12.3 Method Validation

The method validation consisted of five sample sets. Each set included five levels of fortification and a method blank. All spikes and method blanks were processed through the entire analytical method. Spike levels and recoveries for the analytes are shown in Appendix 2.

### 12.4 Control Charts and Limits

Control charts were generated using the data from the method validation for each analyte. The upper and lower warning and control limits are set at  $\pm 2$  and 3 standard deviations of the percent recovery, respectively, shown in Appendix 2.

## 12.5 Acceptance Criteria

12.5.1 Each set of samples will have a matrix blank and a spiked matrix sample.

12.5.2 The retention time should be within  $\pm 2$  percent of that of the standards.

12.5.3 The recoveries of the matrix spikes shall be within the control limits.

12.5.4 The sample shall be diluted if results fall outside of the calibration curve.

## 13. Calculations:

Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. The triple quadrupole LCMS software used a linear curve fit, with all levels weighted  $1/x$ . Alternatively, at the chemist's discretion, sample results may be calculated using the response factor for the standard.

$$\text{ppb} = \frac{(\text{sample peak area or ht}) \times (\text{std conc}) \times (\text{std vol. injected}) \times (\text{final vol of sample})(1000 \mu\text{L/mL})}{(\text{std. peak area or ht}) \times (\text{sample vol injected}) \times (\text{sample wt (g)})}$$

## 14. Reporting Procedure:

Sample results are reported out according to the client's analytical laboratory specification sheets.

## 15. Discussion and References:

A storage stability study was done with this project. The storage stability study consisted of a 1.0 ppb spike level and 3 replicates over a 28 day period. Twenty-one liters of background surface water were spiked and then transferred to twenty-one of the one liter amber bottles. These spiked samples were stored in the refrigerator until analyzed on 0, 2, 4, 7, 14, 21 and 28 days. Along with the storage spikes a blank and method control spike were also extracted. This storage study showed no significant degradation for these compounds within the 28 days. Results for the storage study are shown in Appendix 3.

16. References:

- 16.1 Hsu, J and White, J; *Determination of Azoxystrobin, Azoxystrobin Acid, Azoxystrobin Z-metabolite, Dicloran, Iprodione, Isoiprodione, Vinclozalin and 3,5-Dichloroaniline in Well Water*, 2010 Environmental Analysis Method, Center for Analytical Chemistry, CDFA
- 16.2 *EURL-FV Multiresidue Method using QuEChERS followed by GC-QqQ/MS/MS and LC-QqQ/MS/MS for Fruits and Vegetables*, 2009 European Union Reference Laboratory –Fruit and Vegetables,EURL-FV

## Appendix 1

### The Determination of Method Detection Limit (MDL) and Reporting Limit (RL)

Lab #	Spk\Analyte	Azoxystrobin	Kresoxim methyl	Pyraclostrobin	Trifloxystrobin
2011-0062	blk	nd	nd	nd	nd
2011-0063	0.1ppb spk 1	0.085	0.083	0.082	0.077
2011-0064	0.1ppb spk 2	0.092	0.090	0.091	0.075
2011-0065	0.1ppb spk 3	0.099	0.098	0.097	0.084
2011-0066	0.1ppb spk 4	0.088	0.085	0.084	0.073
2011-0067	0.1ppb spk 5	0.088	0.086	0.085	0.070
2011-0068	0.1ppb spk 6	0.102	0.098	0.099	0.084
2011-0069	0.1ppb spk 7	0.102	0.091	0.092	0.081
	SD	0.00718	0.00604	0.00658	0.00547
<b>Reported</b>	<b>MDL</b>	<b>0.0225</b>	<b>0.0190</b>	<b>0.0207</b>	<b>0.0172</b>
	<b>RL</b>	<b>0.05</b>	<b>0.05</b>	<b>0.05</b>	<b>0.05</b>

All concentrations are expressed in ppb.



### Appendix 3

#### Storage Stability Study

Analyte	Recovery %	Day 0	Day 2	Day 4	Day 7	Day 14	Day 21	Day 28
Azoxystrobin	blank	nd	nd	nd	nd	nd	nd	nd
	QC Spk		83.4%	96.9%	98.8%	91.8%	84.0%	115.0%
	Spike 1	81.6%	87.4%	86.5%	79.9%	88.7%	77.1%	92.8%
	Spike 2	89.1%	79.2%	90.8%	78.3%	95.7%	92.7%	90.6%
	Spike 3	85.4%	83.4%	89.9%	76.6%	97.0%	92.9%	88.4%
Kresoxim-methyl	blank	nd	nd	nd	nd	nd	nd	nd
	QC Spk		81.5%	94.0%	95.3%	82.5%	85.0%	115.0%
	Spike 1	79.8%	83.1%	82.7%	74.2%	86.9%	69.4%	80.9%
	Spike 2	88.6%	76.5%	88.4%	71.8%	86.8%	81.8%	77.8%
	Spike 3	84.8%	77%	87.0%	70.3%	87.8%	82.9%	75.8%
Pyraclostrobin	blank	nd	nd	nd	nd	nd	nd	nd
	QC Spk		80.9%	94.1%	96%	88.4%	82.4%	114%
	Spike 1	78.5%	84.7%	83.2%	75.4%	86.8%	76.3%	89.5%
	Spike 2	86.5%	77.5%	88.1%	73.8%	92.5%	89.8%	88.1%
	Spike 3	83.9%	79.0%	86.4%	72.3%	94.8%	90.6%	86.9%
Trifloxystrobin	blank	nd	nd	nd	nd	nd	nd	nd
	QC Spk		72.3%	86.8%	82.9%	72.1%	75.5%	103.6%
	Spike 1	73.8%	70.0%	72.9%	65%	81.5%	61.1%	69.5%
	Spike 2	77.8%	61.1%	78.4%	62.5%	80.5%	72.8%	71.3%
	Spike 3	74.6%	65.1%	73.4%	62.6%	77.6%	72.1%	72.3%

**Written By:**

Original signed by

3/13/2012

\_\_\_\_\_  
Jean Hsu  
Environmental Scientist

\_\_\_\_\_  
Date

**Written By:**

Original signed by

3/13/2012

\_\_\_\_\_  
Jane White  
Staff Environmental Scientist

\_\_\_\_\_  
Date

**Approved By:**

Original signed by

2/28/2012

\_\_\_\_\_  
Steve Siegel  
Sr. Environmental Scientist

\_\_\_\_\_  
Date

**Approved By:**

Original signed by

2/28/2012

\_\_\_\_\_  
Elaine Wong  
Environmental program Manager I

\_\_\_\_\_  
Date

