Title: Determination of Chlorantraniliprole in Surface Water by Liquid Chromatography Coupled to Linear Ion Trap Quadrupole

1. Scope:

This section method (SM) provides stepwise procedure for chlorantraniliprole analysis in surface water. It is followed by all authorized EA personnel.

2. Principle:

The chlorantraniliprole is 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 to just dryness on a nitrogen evaporator and diluted to a final volume of 2 mL in methanol/ water (1:1). The extract is then transfer into an autosampler vial and analyzed by Liquid Chromatography coupled to a Linear Ion Trap Quadrupole 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.

3.3 All solvents should be handled with care in a ventilated area.

4. Interferences:

There were no matrix interferences for chlorantraniliprole 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 HPLC coupled to a linear ion trap quadrupole mass spectrometry.

6. Reagents and Supplies:

6.1 Chlorantraniliprole CAS#500008-45-7
6.2 Methylene Chloride, nanograde or equivalent pesticide grade
6.3 Water, MS grade, Burdick & Jackson or equivalent
6.4 Methanol, MS grade, Burdick & Jackson or equivalent
6.5 Formic Acid, HPLC grade
6.6 Ammonium formate, reagent grade or equivalent
6.7 Separatory funnel, 1 L
6.8 Boiling flask, 500 mL
6.9 Sodium Sulfate, ACS grade
6.10 Funnels, long stem, 60°, 100 mm I.D.
6.11 Graduated conical tubes with glass stopper, 15 mL
6.12 Glass wool, Pyrex® fiber glass slivers 8 microns
6.13 Disposable Pasteur pipettes, and other laboratory ware as needed
6.14 Recommended analytical column:
Waters SymmetryShieldRP_{18} 5 µm, 3.9 x 150 mm column or equivalent
6:15 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.
6.16 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 An individual stock standard of 1.0 mg/mL was obtained from the CDFA/CAC Standards Repository. The standard was diluted to10 µg/mL with methanol.

A working standard of 1 µg/mL was prepared from the 10 µg/mL standard with methanol. The standard was also used to dilute the following concentrations: 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5 µg/mL in methanol. These standards were then diluted in half with water to make the following concentrations: 0.005, 0.0125, 0.025, 0.05, 0.125, 0.25 ug/mL 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 ± 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 500 g of background water and follow the test sample extraction procedure.

Matrix spike: Weigh out 500 g of background water. Spike a client requested amount of insecticide into the background water, mix well and let it stand for one 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 Mix sample well before weighing aliquot. Weight 500 ± 0.1 g of water samples by subtracting the weight of the sample container before and after water has been transferred into a separatory funnel.

9.3.3 Shake with 80 ± 5 mL of methylene chloride for 1 minute. Vent frequently to relieve pressure.

9.3.4 After phases have separated, drain the lower methylene chloride layer through 25 ± 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 60 ± 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 ± 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 ± 2 °C and 15 – 20 inch Hg vacuum. Transfer the extract to a calibrated 15 mL graduated test tube.

9.3.8 Rinse flask 3 more times with 2 - 4 mL of methylene chloride and transfer each rinse to the same test tube.

9.3.9 Evaporate the sample extract to just dryness in a water bath at 40 ± 2 °C under a gentle stream of nitrogen. Then bring to a volume of 1.0 mL with methanol, mix well and add 1.0 mL of water, mix well. Transfer the final extract into 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 0.005, 0.0125, 0.025, 0.05, 0.125 and 0.25 µg/mL.

10.2 Calibration is obtained using a quadratic regression with the correlation coefficient (r) equal to or greater than 0.995, with all levels weighted none.

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 Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer

11.2.1 LC Instrument: Shimadzu LC30
Column: Waters SymmetryShieldRP_{18} 5 µm, 3.9 x 150 mm column
Column Temperature: 40 °C
Mobile Phase: Gradient
Solvent 1: Aqueous Solution

Solvent 2: Organic Solution
Gradient:

Time(min)	Flow rate (mL/min)	Solvent 1	Solvent 2
0.1	0.8	90.0	10.0
5.00	0.8	10.0	90.0
10.0	0.8	10.0	90.0
10.1	0.8	90.0	10.0
13.0	0.8	90.0	10.0

Injection Volume: 4.0 µL

11.2.2 Mass Spectrometry and Operating Parameters

Model:	ABSciex QTRAP 5500
Ion ProbeType:	Electrospray Ionization (ESI)
Ion Mode:	Positive
Curtain Gas:	40.00
Ion Spray Voltage:	5500.0
Temp:	500.0
Ion Source Gas 1	40.0
Ion Source Gas 2	40.0
Collision:	Medium
Declustering Potential:	46.0
Entrance Potential:	10.0
Electron Multipler:	2400.0

Compound	Retention Time (min)	Precursor ion	Product Ion	Dwell (msec)	Collision Energy	Exit Potential
Chlorantraniliprole	6.84	484.000 484.000 484.000	**453.000** 286.000 112.000	150.00 150.00 150.00	21.00 19.00 81.00	36.00 20.00 8.00

Quantitation ion is 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 surface 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 using the following equation:

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 chlorantraniliprole is 0.1 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 analyte are shown in Appendix 2.

12.4 Control Charts and Limits

A control chart was generated using the data from the method validation. 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 Linear Ion Trap Quadruple LCMS software used a quadratric curve fit, with all levels weighted none. Alternatively, at the chemist's discretion, sample results may be calculated using the response factor for the standard.

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

14. Reporting Procedure**:**

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

15. Discussion and References**:**

15.1 A storage stability study was done with this project. The storage stability study consisted of a 10 ppb spike level and 3 replicates over a 28 day period. Twelve liters of background surface water were spiked and then transferred to twelve one liter amber bottles. These spiked samples were stored in the refrigerator until analyzed on 0, 2, 4, 7, 15, 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 chlorantraniliprole within 28 days. Results for the storage study are shown in Appendix 3.

15.2 In the final step of the extracting procedure we found that it was critical to add the methanol first to the test tube to reconstitute the analyte before adding DI water to make the final volume 1:1 with methanol/water. If we only added 1:1 methanol/water solution to the test tube, our recoveries were in the 50% -60% ranges. The water was added to help with response reproducibility of samples and standard on the instrument.

16. References:

16.1 Schwarz, Timo; Snow, Timothy A.; Santee, Christopher J.; Mulligan, Christopher C.; Class, Thomas; Wadsley, Michael P.; and Nanita, Sergio C., "QuEChERS Multiresidue Method Validation and Mass Spectrometric Assessment for the Novel Anthranilic Diamide Insecticides Chlorantraniliprole and Cyantraniliprole", J. Agric. Food Chem. 2011, 59, 814-821

16.2 "Crop Protection Handbook, 2010", MeisterPro Executive Office 27722 Euclid Ave., Willoughby, OH

Appendix 1

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

	Spk\Analyte	Chlorantraniliprole ppb
	blk	nd
	0.1ppb spk 1	0.102
	0.1ppb spk 2	0.098
	0.1ppb spk 3	0.100
	0.1ppb spk 4	0.098
	0.1ppb spk 5	0.080
	0.1ppb spk 6	0.084
	0.1ppb spk 7	0.072
	SD	0.0118
Reported	MDL	0.0370
	RL	0.1

Appendix 2

Method Validation Data:

Analyte	Spike ppb	Recovery (%) Set1	Set2	Set3	Set4	Set5		%
Chlorantraniliprole	0.1	94.5	93.3	97.2	96.3	95.2	Mean:	97.2
	0.25	100	102	100	102	107	SD:	4.26
	0.5	92.4	89.6	95.0	104	90.8	UCL:	110
	1	99.6	95.0	101	99.6	99.6	UWL:	106
	5	97.4	91.6	93.8	95.0	97.8	LWL:	88.7
							LCL:	84.4

Appendix 3

Storage Study Summary for Chlorantraniliprole in Surface Water

Analyte / Recovery %		Day 0	Day 2	Day 4	Day 7	Day 15	Day 21	Day 28
Chlorantraniliprole	blank	ND	ND	ND	ND	ND	ND	ND
	QC spike		91.2%	85.0%	90.9%	92.7%	87.4%	91.5%
	Spike 1	96.0%	90.1%	90.8%	87.3%	91.5%	89.5%	99.6%
	Spike 2	88.3%	89.8%	84.6%	95.9%	90.7%	95.0%	82.7%
	Spike 3	84.8%	94.0%	93.7%	91.9%	93.4%	90.0%	93.7%

Written By:

Original Signed by — 2/20/2013

Jean Hsu — Date
Staff Environmental Scientist

Written By:

Original Signed by — 2/20/2013

Jane White — Date
Staff Environmental Scientist

Approved By:

Original Signed by — 2/25/2013

Steve Siegel — Date
Sr. Environmental Scientist

Approved By:

Original Signed by — 2/25/2013

Elaine Wong — Date
Environmental Program Manager I

Revision Log:

Date	What was revised? Why?