Determination of Chlorfenapyr in Surface Water Using Triple Quadrupole GC/MS/MS

1. Scope:

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

2. Principle:

   The chlorfenapyr 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 brought to final volume of 0.5 mL in acetone. The extract is then transfer into an autosampler vial and analyzed by a gas chromatography equipped with triple quadrupole detector.

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 chlorfenapyr 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 Gas Chromatograph equipped with a triple quadrupole

6. Reagents and Supplies:

6.1 Chlorfenapyr CAS#122453-73-0
6.2 Methylene Chloride, nanograde or equivalent pesticide grade
6.3 Acetone, nanograde or equivalent pesticide grade
6.4 Separatory funnel, 1 L
6.5 Boiling flask, 500 mL
6.6 Sodium Sulfate, ACS grade
6.7 Funnels, long stem, 60°, 100 mm I.D.
6.8 Graduated conical tubes with glass stopper, 15 mL
6.9 Glass wool, Pyrex® fiber glass slivers 8 microns
6.10 Disposable Pasteur pipettes, and other laboratory ware as needed
6.11 Recommended analytical columns:
Varian –VF-5ms arylene stabilized phase equivalent to 5% phenyl, 95% dimethylpolysiloxane fused silica column, 30 m x 0.25 mm id x 0.25 um film thickness.

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 acetone.

A working standard of 1 µg/mL was prepared from the 10 µg/mL standard with acetone. The standard was also used to dilute the following concentrations: 0.05, 0.1, 0.25, 0.5, 1.0 and 2.0 µg/mL in acetone. These standards are then added to background matrix.

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 60 ± 5 mL of methylene chloride for 2 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 0.5 mL with acetone, mix well. Transfer the final extract into an autosampler vial. Submit extract for GC-MSMS 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 0.05, 0.1, 0.25, 0 5, 1.0 and 2.0 µg/mL.

10.2 Calibration is obtained using a linear curve 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 GC-MSMS 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 GC-Triple Quadrupole Instrumentation

11.2.1 Gas Chromatograph: Varian CP-3800
Column: Varian Factor Four VF-5ms 30M x 0.25mm x 0.25um.

Temperature Program: initial column temperature 80 °C, hold 1 min., ramp at 50 °C/min. to 210 °C hold for 0 min., ramp at 30 °C/min. to 300 °C hold for 3.0 min..

Injector Temperature: 250°C
Injection Volume: 3 µL
Carrier Gas: Helium 1mL/min.

Triple Quadrupole: Varian Triple Quad 320-MS
Ionization: Positive Electron Impact
Transfer Line: 300°C
Source Temp: 270°C

Collision Gas: Argon @ 1.8 mTorr

Compound	Retention Time (min.)	Precursor Ion	Product Ion	Collision Energy/-ev
Chlorfenapyr	6.4	137	74.6	20
		137	102	20
		247	227	17

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, seven surface water samples are spiked at 0.2ppb 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 chlorfenapyr 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 ± 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 ± 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 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**:**

15.1 A storage stability study was done with this project. The storage stability study consisted of a 2 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, 8, 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 chlorfenapyr within 28 days. Results for the storage study are shown in Appendix 3.

15.2 Solid phase extraction was tried in the early stages of method development. The 500 mL chlorofenapyr fortified surface water samples were filtered, extracted by a HLB cartridge and then eluted with acetonitrile, Since chlorfenapyr has a low solubility in water and it could be bound tightly to particulates, the glass filters used during the filtration were placed in the HLB cartridges for elution with acetonirilte. Recoveries for the trail spikes were in the 50% range. A smaller sample size of 250mL was tried without filtration of the surface water and yielded recoveries in the 70-80%. Further work is needed to determine if loss is occurring only with the capacity of the HLB cartridge or if filtration also contributes.

16. References:

16.1 Hsu, Jean, and White, Jane, “Determination of Ethalfluralin, Trifluralin, Benfluralin, Prodiamine, Pendimethalin, Oxyfluorfen, and Oryzalin in Surface Water”, 2007, Environmental Analysis method, Center for Analytical Chemistry, CDFA

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	Chlorfenapyr ppb
	blank	ND
	0.2ppb spk 1	0.144
	0.2ppb spk 2	0.179
	0.2ppb spk 3	0.194
	0.2ppb spk 4	0.156
	0.2ppb spk 5	0.173
	0.2ppb spk 6	0.194
	0.2ppb spk 7	0.154
	SD	0.0199
Reported	MDL	0.0624
	RL	0.1

Appendix 2

Method Validation Data:

	Spike	Recovery (%)						
Analyte	ppb	Set1	Set2	Set3	Set4	Set5		%
Chlorfenapyr	0.2	88.0	101	102	116	76.5	Mean:	99.0
	0.5	101	99.4	107	137	94.4	SD:	16.65
	1.0	105	105	101	116	138	UCL:	149
	2	97.5	94.0	84.5	92.5	76.5	UWL:	132
	10	87.5	82.5	67.3	109	95.4	LWL:	65.7
							LCL:	49.0

Appendix 3

Storage Study Summary for Chlorfenapyr in Surface Water

Analyte / Recovery %		Day 0	Day 2	Day 4	Day 8	Day 14	Day 21	Day 28
Chlorfenapyr	Blank	ND	ND	ND	ND	ND	ND	ND
	QC spike		94.1	95.5	96.0	107	94.0	101
	Spike 1	84.0	86.5	76.0	89.0	84.5	91.5	94.5
	Spike 2	89.0	88.5	86.0	79.5	84.0	85.5	99.0
	Spike 3	90.0	94.0	94.0	83.0	90.5	89.5	89.0

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