California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Analysis Section
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Title: Determination of Bensulide and Imidacloprid in Surface Water

1. Scope:

   This section method (SM) documents Bensulide and Imidacloprid pesticide Residue analysis in surface water. It is to be followed by all authorized section personnel.

2. Principle:

   The surface water sample is extracted with methylene chloride. The extract is passed through sodium sulfate to remove residual water. The anhydrous extract is evaporated to almost dryness on a rotary evaporator and diluted to a final volume of 1.0 mL with methanol. The extract is then analyzed by an Ultra Performance Liquid Chromatography (UPLC) coupled to a triple quadrupole using electrospray ionization in positive ion mode.

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 is no known interference for this analysis.

5. Apparatus and Equipment:

   5.1 Rotary evaporator (Büchi/Brinkman or equivalent)
   5.2 Nitrogen evaporator (Meyer N-EVAP Organomation Model # 112 or equivalent)
   5.3 Vortex-vibrating mixer
   5.4 Balance (Mettler PC 4400) or equivalent
   5.5 Liquid Chromatograph equipped with an ion trap mass spectrometer

6. Reagents and Supplies

6.1 Methylene Chloride, nanograde or equivalent pesticide grade
6.2 Methanol, nanograde or equivalent pesticide grade
6.3 Anhydrous Sodium Sulfate, granular
6.4 Bensulide CAS# 741-58-2
6.5 Imidacloprid CAS# 138261-41-3
6.6 Conical tube with glass stopper, 15-mL graduated, 0.1 mL subdivision
6.7 Separatory funnel, 2 L
6.8 Boiling flask, 500 mL
6.9 Funnel, long stem, 10 mm diameter
6.10 Disposable Pasteur pipettes, and other laboratory ware as needed
6.11 Recommended analytical columns: Waters Symmetry HSS T3 1.8µm 2.1x100 mm column

7. Standards Preparation:

7.1 The individual bensulide and Imidacplorid stock standards of 1.0mg/mL were obtained from the CDFA/CAC Environmental Analysis Standards Repository. The standards were diluted to 10 µg/mL with methanol for identification purposes. A combination standard of 10 µg/mL was prepared from the individual mg/mL standards in methanol. The combination 10 µg/mL standard was used to dilute the following concentrations: 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 µg/mL in methanol.

7.2 Store standards according to manufacturing requirement. Keep all standards in designated refrigerator for storage.

7.3 The expiration date of working standard is six months from the preparation date of the stock standard

8. Sample Preservation and Storage:

All water samples and sample extracts shall be stored in the refrigerator (4 ± 3 °C).

9. Test Sample Preparation:

9.1 Sample Preparation

9.1.1 Remove samples from refrigerator and allow samples to come to room temperature before extraction.

9.1.2 Preparation of matrix blank and matrix spike:

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

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

9.1.2.2 Matrix spike: Weigh out approximate 1000 g of background water. Spike a client requested amount of bensulide/imidacloprid into the background water and let it stand for 1 minute. Follow the test sample extraction procedure.

9.2 Test Sample Extraction

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

9.2.2 Shake with 100 ± 5 mL of methylene chloride for 2 minutes. Vent frequently to relieve pressure.

9.2.3 After phases have separated, drain lower methylene chloride layer through 20 ± 4 g of anhydrous sodium sulfate and glasswool, into a 500 mL boiling flask.

9.2.4 Repeat steps 9.2.2 & 9.2.3 two more times using 80 ± 5 mL of methylene chloride each time. Combine the extracts in the same boiling flask.

9.2.5 After draining the final extraction, rinse the sodium sulfate with 25 ± 5 mL of methylene chloride.

9.2.6 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. Add 2 - 4 mL of methanol and rotoevaporate to 1 - 2 mL. Transfer the extract to a calibrated 15 mL graduated test tube.

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

9.2.8 Evaporate the extract to a volume slightly less than 1 mL in a water bath at 38 ± 2 °C under a gentle stream of nitrogen. Then bring to a final volume of 1.0 mL with methanol, mix well and transfer into two autosampler vials.

9.2.9 Submit extract for LC-MS analysis.

10. Instrument Calibration:

10.1 A calibration standard curve consists of minimum of three levels. Standard concentrations of 0.025, 0.05, 0.1, 0.25, 0.5 or 1.0 ηg/µL are recommended. Calibration is obtained using a linear or quadratic regression with the correlation coefficient (r) equal to or greater than 0.995.

11. Analysis:

11.1 UPLC-MS/MS

11.1.1 UPLC instrument: Waters Acquity Ultra Performance LC
Column: Waters Acquity HSS T3 1.8µm 2.1x100 mm
ColumnTemperature: 50°C
Mobile Phase: Gradient
Solvent 1: Water + 4% acetic acid
Solvent 2: Methanol + 4% acetic acid
Gradient:

Time (min)	Flow rate	Solvent 1	Solvent 2
0	0.50	90.0	10.0
0.5	0.50	90.0	10.0
3.5	0.50	10.0	90.0
4.5	0.50	10.0	90.0
5.0	0.50	90.0	10.0
6.0	0.50	90.0	10.0

Injection Volume: 1.0 µL

11.1.2 Mass Spectrometry and Operating Parameters
Model: Waters Xevo Triple Quadrupole
Ion ProbeType: Electrospray Ionization (ES)
Ion Mode: ESI (+)
Desolvation Temp: 500 °C
Source Temp: 150 °C

Compound	Retention Time (min)	Precursor ion	Product Ion	Dwell (s)	Cone(V)	Collision Energy/-ev
Imidacloprid	2.51	256.08	**175.02**	0.025	24.0	16.0
		256.08	209.1	0.025	24.0	16.0
Bensulide	3.94	398.16	**158.01**	0.061	14.0	34.0
		398.16	314	0.061	46.0	30.0

Quantitation ions are in bold.

12. Quality Control:

12.1 Method Detection Limits (MDL)

The method detection limit refers to the lowest concentration of analyte that a method can detect reliably. To determine the MDL, 7 replicate water samples are spiked at 0.10 ppb. The standard deviation from the spiked sample recoveries are used to calculate the MDL for the analyte using the follow 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 replicate 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):

The reporting limit (RL) refers to the level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. Per client agreement, the RL is chosen in a range 1-5 times the MDL. The reporting limit for Bensulide is 0.04ppb and Imidacloprid is 0.05ppb

12.3 Method Validation

The method validation for bensulide and Imidachloprid consisted of three 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. Spikes levels and recoveries for bensulide and Imidacloprid are shown in Appendix 2.

12.4 Control Charts and Limits

Control charts were generated using the data from the method validation. The upper and lower control limits are set at ± 3 standard deviation of the % recovery, shown in Appendix 2. The control chart range generated from this validation data was narrower than that of the previous method for Bensulide. It was decided that the control charts would be used but the upper and lower control limits would be set with the limits from the previous methods Bensulide 56.7 – 130.6 and Imidacloprid 77.2-121.9. The new data for Bensulide fit within these limits and the data for Imidacloprid was almost the same as the old control limits.

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 per cent 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 external standard (ESTD) calculation using either the peak area or height. The software uses a linear or quadratic curve fit, with all levels weighted equally. Alternatively, at chemist discretion, results may be calculated using the response factor for the standard whose value is closest to the level in the sample.

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

14. Reporting Procedure:

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

15. Discussion:

This SOP combines the analysis of bensulide and Imidacloprid into a single method. In the past both compounds were extracted and analyzed separately.

16. References:

16.1. Lee, Paul; *Determination of Bensulide in Surface Water Using Liquid Chromatography Mass Spectrometry,* 2002, Environmental Monitoring method, Center for Analytical Chemistry, CDFA.

16.2 Hernandez, Jorge; *HPLC Determination of Imidacloprid in Surface and Well Water,* 2001, Environmental Monitoring method, Center for Analytical Chemistry, CDFA.

APPENDIX I

The determination of Method Detection Limit (MDL) data and Reporting Limit (RL) for Bensulide and Imidacloprid in surface water:

Spk\Analyte	Bensulide ppb	Imidacloprid ppb
0.1 ppb spk 1	0.105	0.112
0.1 ppb spk 2	0.107	0.094
0.1 ppb spk 3	0.190	0.100
0.1 ppb spk 4	0.105	0.093
0.1 ppb spk 5	0.106	0.080
0.1 ppb spk 6	0.097	0.095
0.1 ppb spk 7	0.097	0.073
SD	0.00629	0.00125
MDL	0.0198	0.0394
RL	0.04	0.05

APPENDIX II

Method Validation Data and Control Limit

Analyte	Spike ppb	Recovery (%) Set 1	set 2	set 3	%	%
Bensulide	0.1	109	112	92.2	Mean:	102
	0.2	113	104	106	SD:	7.43
	0.5	106	89.2	97.4	UCL:	124.4
	1.0	103	108	102	UWL:	117
	2.0	95.0	103	92.0	LWL:	87.3
					LCL:	79.8
Imidacloprid	0.1	103	97.0	105	Mean:	100
	0.2	108	104	103	SD:	7.78
	0.5	104	87.0	103	UCL:	123.5
	1.0	104	108	108	UWL:	115.7
	2.0	90.0	93.0	84.9	LWL:	84.6
					LCL:	76.8

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