California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Analysis Section
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Title: Determination of Bensulide in Surface Water Using Liquid Chromatography-Mass Spectrometry

1. Scope:

   This section method (SM) documents Bensulide 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 a liquid chromatograph equipped with a mass spectrometer.

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 Conical tube with glass stopper, 15-mL graduated, 0.1 mL subdivision
6.6 Separatory funnel, 2 L
6.7 Boiling flask, 500 mL
6.8 Funnel, long stem, 10 mm diameter
6.9 Disposable Pasteur pipettes, and other laboratory ware as needed
6.10 Recommended analytical columns: Any common analytical C-18 column shall do the job. We use the Waters Symmetry C-18 5µm 4.6x250 mm column

7. Standards Preparation:

7.1 Dilute the 1 mg Bensulide standards obtained from the CDFA/CAC Environmental Analysis Standards Repository with methanol to make up a series of working standards (see 10.2). These standards shall be prepared to cover the linear range from 0.05ηg/µL to 1.0 ηg/µL.

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) provides 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 organophosphate pesticides 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 0.05, 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 Injection Scheme

Follow the sequence of Solvent, Calibration standards, Solvent, Matrix Bank, Matrix Spike, Test Samples (maximum of 10-12 samples) and Calibration standards. Injection of an old sample or matrix blank before the sequence analysis to condition the instrument is recommended.

11.2 LC-MS Instrumentation

11.2.1 Analyze bensulide pesticides by a liquid chromatograph equipped with a mass spectrometer.

11.2.2 Recommended column: The column we used was a Waters symmetry C-18. Other reverse phase analytical column can be used as long as it produces equivalent result.

11.2.3 Mobile phase: We used 0.1% acetic acid in water and 0.1% acetic acid in methanol gradient as listed in the following'

11.2.4 Injection volume 10 or 20 µL.

11.2.5 MS detector setting:

11.2.5.1 Duration (min): 15.00
11.2.5.2 Number of Scan Events: 2

11.2.5.3 Tune Method: APCI Bensulide2-24-09

11.2.5.4 Scan Event Details:
11.2.5.4.1.1 + c norm ·(398.0)->o(105.0-420.0)
11.2.5.4.1.2 MS/MS: Amp. 18.0% Q 0.250 Time 30.000 IsoW 3.0 + c norm o(200.0-420.0)

12. Quality Control:

12.1 Each set of samples shall have a matrix blank and minimum of one matrix spike sample.

12.2 The matrix blank should be free of target compounds.

12.3 The recoveries of the matrix spike shall be within the control limits.

12.3.1 When spike recoveries fall outside the control limits, the chemist must investigate the cause. The entire extraction set of samples is re-analyzed. If the spike recoveries fall within the limit, then the results from the re-analyzed samples shall be reported.

12.3.2 If the spike recoveries still fall outside the control limits, the client will be notified. The backup samples will be re-extracted for analysis.

12.4 The retention time should be within ±0.1 minute of that of the standard.

12.5 The sample must be diluted if results fall outside the linear range of the standard curve.

12.6 Bracketing standard curves should have a percent change less than 20 %.

12.7 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.

12.8 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 except in special cases. (See 15.5)

MDL data and the RL are tabulated in Appendix I

12.9 Method Validation Recovery Data and Control Limits:

12.9.1 The method validation consisted of five sample sets. Each set included seven levels of fortification (0.05, 0.1, 0.25, 0.5, 1.0, 2.0, and 5.0 ppb) and a method blank. All spikes and method blank samples were processed through the entire analytical method.

12.9.2 Upper and lower warning and control limits are set at $\pm$ 2 and $\pm$ 3 standard deviations of the average % recovery, respectively.

Method validation results and control limits are tabulated in Appendix II

12.10 Estimated Measurement Uncertainty:

Total uncertainty for this method is not done.

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, concentrations 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:

14.1 Identification of Analyte

The specific ion 356^{+} is an identification of analyte. For responses within calibration range, compare the retention time of the peaks with the retention time of standards. For positive results retention times shall not vary from the standards more than 0.1 minute.

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

15. Discussion and References:

15.1 Bensulide has molecular ion 398^{+}. About a little more than 50% of them were broken down to 356^{+} in the ion source. We collected two sets of information. In the ms event, the ions 398^{+}, 356^{+} and background ions were collected. In the msms event, the remaining 398^{+} were chosen and fragmented in the ion trap to 356^{+} under the controlled conditions. Based on the validation data, the ms result appeared better than the result of msms in the terms of recovery, accuracy and precision. But in the terms of the signal/noise ratio and specificity, the result of msms is better than that of ms. After running a set of real samples, we observed the signal noise ratio of the ms became so poor that the result of low level is no longer accurate. For this reason we concluded that the validation data from msms event should be used.

16. References:

APPENDIX I

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

Spk \ Analyte	**Bensulide By MS data**	**Bensulide By MSMS data**
0.1 ppb spk1	0.101	0.066
0.1 ppb spk2	0.101	0.078
0.1 ppb spk3	0.094	0.067
0.1 ppb spk4	0.102	0.075
0.1 ppb spk5	0.109	0.073
0.1 ppb spk6	0.101	0.073
0.1 ppb spk7	0.113	0.073
SD	0.006191	0.004259
MDL	0.01946	0.013387
RL	0.05	0.05

All concentrations are expressed in ppb

APPENDIX II

Method Validation Data and Control Limit

By MS Data										
	Set 1		Set 2		Set 3		Set 4		Set 5	
Spike level	Found	%	Found	%	Found	%	Found	%	Found	%
0.10ppb	0.097	97%	0.098	98%	0.100	100%	0.093	93%	0.107	107%
0.20ppb	0.204	102%	0.219	110%	0.212	106%	0.207	104%	0.208	104%
0.50ppb	0.459	92%	0.516	103%	0.521	104%	0.476	95%	0.565	113%
1.00ppb	0.977	98%	0.95	95%	0.971	97%	0.964	96%	1.07	107%
2.00ppb	2.16	108%	2.03	102%	2.04	102%	2.06	103%	2.09	105%
Average		101.6%								
Stdev		5.39%								
UCL		117.7%								
LCL		85.4%								
By MS-MS Data										
	Set 1		Set 2		Set 3		Set 4		Set 5	
Spike level	Found	%	Found	%	Found	%	Found	%	Found	%
0.10ppb	0.07	70%	0.076	76%	0.073	73%	0.068	68%	0.080	80%
0.20ppb	0.204	102%	0.183	92%	0.186	93%	0.187	93.6%	0.170	85%
0.50ppb	0.442	88%	0.576	115%	0.517	103%	0.464	92.8%	0.521	104%
1.00ppb	1.037	104%	0.94	94%	0.996	100%	1.020	102%	1.02	102%
2.00ppb	2.21	111%	1.99	99%	1.93	96%	1.83	91.5%	2.07	103%
Average		93.5%								
Stdev		12.38%								
UCL		130.6%								
LCL		56.4%								

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Revision Log:

Date	What was revised? Why?
6/15/09	Add the section 15.1, MS validation data do not meet our need for dirty samples. Data from MSMS method is a better choice for this problem.
6/12/09	Appendix II. Spike levels were typed wrong. They are correct now.
6/15/09	Re-word a sentence in the section 15.1
6/15/09	Update signatories