California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Analysis Section
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Title: Determination of Acephate and Methamidophos in Surface Water by LC-MS

1. Scope:

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

2. Principle:

Water samples are passed through large Extrelut solid phase extraction columns (a pair of columns is used for each sample to provide more capacity). The analytes are eluted with methylene chloride. The eluant is collected and evaporated to just dryness. Residues are dissolved in 0.5 mL of solvent and are analyzed by APCI/LCMS.

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 that caused quantitative problems during method development and validation.

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 Liquid Chromatograph equipped with an ion trap (LCMS)

6. Reagents and Supplies:

6.1	Acephate	CAS#30560-19-1
6.2	Methamidophos	CAS#10265-92-6

6.3 Methylene Chloride, nanograde or equivalent pesticide grade
6.4 Water, MS grade, Burdick & Jackson or equivalent
6.5 Methanol, MS grade, Burdick & Jackson or equivalent
6.6 Acetic Acid, Glacial, ACS Grade
6.7 Boiling flask, 500 mL
6.8 Ammonium Sulfate, ACS grade
6.9 Volumetric Pipette, 0.5 mL
6.10 Graduated conical tubes with glass stopper, 15 mL
6.11 Disposable Pasteur pipettes, and other laboratory ware as needed
6.12 Extrelut QE, EMD Chemical Inc. Catalog number: 902050-1
6.13 Recommended analytical column:
For HPLC/MS – Waters SymmetryShieldRP_{18} 5 µm, 3.9 x 150 mm cartridge
Guard column: Waters SymmetryShieldRP_{18} 5 µm, 3.9 x 20 mm cartridge
Guard column holder: Waters Sentry guard holder universal.

7. Standards Preparation:

7.1 The Acephate and Methamidophos stock standards of 1.0 mg/mL were obtained from the CDFA/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 10ug/mL standards with solvent (Methanol / Water = 1 / 3). The standard was also used to dilute the following concentrations: 0.025, 0.05, 0.1, 0.2, and 0.5µg/mL.

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 or the expiration date of stock standards whichever comes first.

8. **Sample Preservation and Storage**:

Store all samples waiting for extraction in a separate refrigerator (0 - 5 °C).

9. **Test Sample Preparation**:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the background surface water for method determination, validation and QC.

9.2 Preparation of blank and spike

Matrix blank: Weigh out 100 g of background water and follow the test sample extraction procedure.

Matrix spike: Weigh out 100 g of background water. Spike a client requested amount of insecticides into the background water and let it stand for 1 minute. Divide this spike into two 50 mL aliquots since the Extrelut can hold 50 mL each. The two Extrelut eluates comprising a single sample are recombined during the rotary evaporation step. Follow the test sample extraction procedure.

9.3 Test Sample Extraction

To obtain the sensitivity needed for this project 100 mL water sample was used. Since the capacity of Extrelut QE column is 50 mL, two 50 mL aliquots of each water sample were loaded onto two different Extrelut QE columns. The eluents were combined together during the rotary evaporation step.

9.3.1 Weigh out two 50 g aliquots of water sample into 125 mL beakers and add 12.5 g ammonium sulfate to each aliquot. Mix well.

9.3.2 Load the sample aliquots onto 2 Extrelut QE columns. Allow the sample to be absorbed by the columns by waiting 15 min.

9.3.3 Elute the column with 250 mL of methylene chloride and collect the eluents in 500 mL flat bottom flasks.

9.3.4 After the columns have finished draining, apply air pressure to remove any remaining solvent.

9.3.5 Evaporate the methylene chloride in one flask to ~ 2-3 mL under vacuum at approximately 17-20 inch Hg in a water bath at 35° C and then add the remaining 250 mL from the other flask to it. Rinse the flask 2 times with methanol to transfer the sample. Continue to rotary evaporate the sample to ~ 2-3 mL

9.3.5 Transfer the extract to a 15 mL graduated test tube. Rinse flask 3 times with approximately 2 mL of methanol and transfer each rinsate to the same test tube.

9.3.6 Place the test tube on nitrogen evaporator under a gentle stream of nitrogen with water bath set at 40°C and evaporate the extract to just dryness.

9.3.8 Pipette 0.5 mL of solvent (Methanol / Water = 1 / 3) into the test tube and vortex well. Transfer extract to an autosampler vial with insert to analyze on LCMS.

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 limits.

10.2 The LCMS calibration curves were obtained using linear regression.

11. **Analysis:**

11.1 HPLC-MS

11.1.1 HPLC Instrument: Waters model 2695 HPLC and auto-sampler with column heater and remote control through Thermo Finnigan Xcalibur system.

Column: Waters SymmetrySheid RP_{18} 5 µm, 3.9 x 150 mm column
Column Temperature: 40 °C
Mobile Phase: 425 mL water, 75 mL methanol, 0.5 mL Acetic acid

Time(min)	Flow rate	Mobile Phase
0	0.75	100
7.0	0.75	100

Injection Volume:40 µL

11.1.2 Liquid Chromatograph Mass spectrometer (LC-MS) and Operating Parameters

Model:	Finnigan Model DECA ion trap MS
Ion Source Type:	Atmospheric pressure Ionization (APCI)
Source Polarity:	Positive
APCI Vaporizer Temp:	450 °C

Capillary Temperature: 220 °C
Sheath Gas Flow Rate: 23
Auxiliary Gas Flow Rate: 10
Mode of operation: MS/MS

Compound Name	Retention Time (min.)	Molecular Weight	Mass Range	Product Ions
Methamidophos	2.83	141.13	100-200	112
Acephate	3.42	183.16	100-200	143

Note: The column conditions, temperature, mobile phase, etc. may slightly shift retention time.

11.1.3 Operating parameter

Compound Name	Parent Mass (m/z)	Isolation Width (m/z)	Normalized Collision Energy (%)	Activation Q (msec.)	Activation Time (msec.)
Methamidophos	142	2.0	28.0	0.250	30.0
Acephate	184	3.0	24.0	0.250	30.0

12. **Quality Control**:

12.1 Method Detection Limit (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 0.25ppb 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:

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 acephate and Methamidophos is 0.25 ppb.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included four levels of fortification and a method blank. All spikes and method blanks were processed through the entire analytical method. Spike levels and recoveries for acephate and Methamidophos 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 average % recovery, respectively.
The control limits are tabulated 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 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 an external standard (ESTD) calculation using either the peak area or height. The LCMS software used a linear curve fit, with all levels weighted equally. 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 Acephate and its metabolite, Methamidophos, are both highly polar organophosphorus pesticides and are therefore highly soluble in water. This leads to difficulties when using traditional methods of extraction, such as liquid/liquid extraction. To improve the partition of acephate and Methamidophos into the organic phase, 20% of sodium chloride was added to the aqueous phase. Even with this enhancement the recoveries for both analytes were less than 15%.

15.2 Solid phase extraction was attempted using Oasis HLB cartridge. The recovery from the first experiment was ~100%, however we were unable to get consistently good results because of large losses in instrument sensitivity after the sample injections. We noticed that significant amounts of water were present in the final eluates from HLB cartridges. The eluate likely contains ammonium sulfate, which cause the observed suppression of LCMS sensitivity. We tried using different sizes of HLB extraction cartridges and a longer dry process to eliminate water in sample extract, but this was only partly successful.

15.3 Finally the Extrulet QE column was tried and gave acceptable results, which are presented in Appendix 1 and 2. Sensitivity needed to be increased, but was limited by the 50 mL capacity of Extrelut QE column. To obtain the sensitivity needed for this project 100 mL water samples were used but they were divided into two 50 mL aliquots, each of which was passed through a separate Extrelut QE column. The pair of eluates obtained for same sample were combined together during the rotary evaporation step.

15.4 A storage stability study was done with this project. The storage stability study consisted of a 2.5ppb spike concentrate and 3 replicates over 26 day period at pH3 and pH 8 for acephate. Twelve liters of background American River water pH adjusted to 3 was spiked and then transferred to twelve 1 liter amber bottles. Then twelve liters of background American River water pH adjusted to 8 was spiked and then transferred to twelve 1 liter amber bottles .The spikes storage samples were refrigerated until analyzed on 0, 5, 14, and 26 days. Along with

the storage spikes a blank and method control spike were extracted. The storage spikes were analyzed for both acephate and Methamidophos. This same procedure was repeated again, but this time Methamidophos was spiked. The results for this study are presented in Appendix 3.

15.5 References:

15.61 Waters Oasis, Acephate in River Water, Application Notes, 2003

15.62 Residue Lab.; Extraction Procedure for Milk, Application notes, Center for Analytical Chemistry, CDFA

15.63 Lee P., *HPLC determination of Aldicarb, Aldicarb Sulfoxide and Aldicarb* , *Sulfone in groundwater*, 1989, Environmental Monitoring Method, Center for Analytical Chemistry, CDFA.

Appendix 1

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

Results:	Finnigan LCMS	
Spk\Analyte	Acephate ppb	Methamidophos ppb
0.25ppb spk 1	0.178	0.082
0.25ppb spk 2	0.204	0.100
0.25ppb spk 3	0.190	0.156
0.25ppb spk 4	0.214	0.166
0.25ppb spk 5	0.197	0.181
0.25ppb spk 6	0.188	0.185
0.25ppb spk 7	0.190	0.163
SD	0.0118	0.0403
MDL	0.0370	0.126
RL	0.25	0.25

Appendix 2

Method Validation Data

Results: **Finnigan LCMS**

Analyte	Spike ppb	Recovery Set 1	(%) set 2	set 3	set 4	set 5	%	%
Acephate	0.5	79.6	60.4	77.8	64.6	93.0	Mean:	82.9
	1.0	89.8	74.9	85.5	67.8	81.5	SD:	10.8
	2.0	83.0	71.0	80.5	96.6	88.2	UCL:	115.3
	5.0	97.0	93.4	94.0	90.0	89.6	UWL:	104.5
							LWL:	61.3
							LCL:	50.5
Methamidophos	0.5	61.6	57.6	61.6	59.6	57.0	Mean:	58.7
	1.0	54.1	52.6	57.6	65.7	54.2	SD:	6.50
	2.0	60.0	71.5	51.0	64.5	49.1	UCL:	78.2
	5.0	71.4	64.0	55.8	48.6	57.0	UWL:	71.7
							LWL:	45.7
							LCL:	39.2

Appendix 3

pH 3

Sample	Acephate	
day 0	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.448	89.6%
Acephate-1 pH3 Day0	2.45	98.0%
Acephate-2 pH3 Day0	2.44	97.6%
Acephate-3 pH3 Day0	2.43	97.2%
day 5	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.464	92.8%
Acephate-1 pH3 Day5	2.07	82.6%
Acephate-2 pH3 Day5	2.18	87.3%
Acephate-3 pH3 Day5	2.25	90.1%
day 14	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.362	72.4%
Acephate-1 pH3 Day14	2.13	85.2%
Acephate-1 pH3 Day14	2.06	82.4%
Acephate-1 pH3 Day14	2.05	82.0%
day 26	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.412	82.4%
Acephate-1 pH3 Day26	1.96	78.4%
Acephate-1 pH3 Day26	1.74	69.6%
Acephate-1 pH3 Day26	1.96	78.4%

Sample	**Methamidophos**	
day 0	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.364	72.8%
Methamidophos-1 pH3 Day0	1.51	60.3%
Methamidophos-2 pH3 Day0	1.65	65.9%
Methamidophos-3 pH3 Day0	1.40	56.0%
day 5	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.210	42.0%
Methamidophos-1 pH3 Day5	1.13	45.2%
Methamidophos-2 pH3 Day5	1.54	61.7%
Methamidophos-3 pH3 Day5	1.45	58.2%
day 14	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.285	57.0%
Methamidophos-1 pH3 Day14	1.42	56.8%
Methamidophos-2 pH3 Day14	1.58	63.2%
Methamidophos-3 pH3 Day14	1.35	54.0%
day 26	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.360	72.0%
Methamidophos-1 pH3 Day26	1.42	56.8%
Methamidophos-2 pH3 Day26	1.26	50.4%
Methamidophos-3 pH3 Day26	0.992	39.7%

pH 8

Sample	Acephate	
day 0	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.468	93.6%
Acephate-1 pH8 Day0	2.48	99.0%
Acephate-2 pH8 Day0	2.39	95.6%
Acephate-3 pH8 Day0	2.75	110.0%
day 5	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.527	105%
Acephate-1 pH8 Day5	2.19	87.6%
Acephate-2 pH8 Day5	2.58	103.2%
Acephate-3 pH8 Day5	2.49	99.6%
day14	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.457	91.4%
Acephate-1 pH8 Day14	1.97	78.8%
Acephate-2 pH8 Day14	2.04	81.6%
Acephate-3 pH8 Day14	2.05	82.0%
day26	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.387	77.4%
Acephate-1 pH8 Day26	1.87	74.8%
Acephate-2 pH8 Day26	1.62	64.8%
Acephate-3 pH8 Day26	2.12	84.8%

Sample	Methamidophos	
day 0	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.339	67.8%
Methamidophos-1 pH8 Day0	1.69	67.6%
Methamidophos-2 pH8 Day0	1.55	62.2%
Methamidophos-3 pH8 Day0	1.51	60.2%
day 5	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.362	72.4%
Methamidophos-1 pH8 Day5	1.41	56.4%
Methamidophos-2 pH8 Day5	1.68	67.2%
Methamidophos-3 pH8 Day5	1.56	62.4%
day 14	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.271	54.2%
Methamidophos-1 pH8 Day14	1.65	66.0%
Methamidophos-2 pH8 Day14	1.39	55.6%
Methamidophos-3 pH8 Day14	1.05	42.0%
day 26	Result ppb	% Recovery
Blank	nd	
QC Spike, 0.5ppb	0.281	56.2%
Methamidophos-1 pH8 Day26	1.29	51.6%
Methamidophos-2 pH8 Day26	1.26	50.4%
Methamidophos-3 pH8 Day26	1.53	61.2%

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Revision Log:

Date	What was revised? Why?
5/1/09	**Changed pesticide name from monitor to methamidophos**