California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Monitoring Section
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Title: Determination of Molinate, EPTC, Vernolate, Pebulate, Cycloate, Thiobencarb, Butylate and TriallateThiocarbamates in Ground Water by High Performance Liquid Chromatography with Tandem Mass Spectrometry

1. Scope

This section method (SM) is for the analysis of thiocarbamates in ground water. These pesticides are molinate, EPTC, vernolate, pebulate, cycloate, thiobencarb, butylate and triallate. It is to be followed by all authorized section personnel. The reporting limits are 0.05 ppb for all compounds.

2. Principle:

Residues of thiocarbamates are extracted from sample using petroleum ether. Volatile solvent as petroleum ether provides advantage of easy evaporation resulting in high recoveries for all analytes. All eight compounds are determined by the injection of sample extract into an HPLC equipped with a C-18 column and a mass spectrometer (LC-MS). The confirmation of compound identity on LC-MS is achieved simultaneously with collision-induced dissociation to produce a product ion for each analyte.

3. Safety:

3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.
3.2 All solvents should be handled with care in a ventilated area.

4. Interferences:

Pebulate and vernolate are isomers. They have same molecular ion (ion 204), same major product ion (ion 128) and almost same retention time on C-18 column. However, the minor product ions are different: pebulate (ion 57) and vernolate (ion 86).

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 Separatory funnel, 2000 mL
5.5 Conical tube with glass stopper, 15-mL graduated
5.6 Boiling flask, 500-mL
5.7 Funnel, 15 cm diameter
5.8 Disposable Pasteur pipettes, and other laboratory ware as needed
5.9 Liquid chromatograph (Thermo Finnigan Surveyor HPLC) equipped with a Thermo Finnigan TSQ Quantum Mass detector.

6. Reagents and Supplies: (All reagents shall meet the minimum requirement in HPLC, residue and pesticide analysis)

6.1 Formic acid, HPLC grade (Fisher #A35-500 or equivalent)
6.3 Methanol, (Burdick &Jackson, MS grade, or equivalent)
6.4 Nitrogen, refrigerated liquid or nitrogen generator with capacity of delivering 20 liters per minute.
6.5 Petroleum ether
6.6 Standards: The individual 1.0 mg/mL stock standards of each compound were obtained from the CDFA/CAC Standard Repository.

Molinate	CAS Number 2212-67-1
EPTC	CAS Number 759-94-4
Vernolate	CAS Number 1929-77-7
Pebulate	CAS Number not available
Cycloate	CAS Number 1134-23-2
Thiobencarb	CAS Number 28249-77-6
Butylate	CAS Number 2008-41-5
Triallate	CAS Number 2303-17-5

6.7 Water, (HPLC grade, Burdick & Jackson Cat #AH365-4 or equivalent)
6.8 Analytical column: Waters Xterra® MS C18, 3.5 μm, 2.1x50mm column (part no.188000400)
6.9 Guard column: Waters Symmetryshield RP 18 5 μm, 3.9 x 20 mm cartridge (part number, 186000107).
6.10 Guard column cartridge holder: Waters Sentry guard holder universal (P/N wat064610)

7. Standards Preparation:

7.1 Dilute the 1.0 mg/mL standards, obtained from the CDFA/CAC Standards Repository, with methanol. The concentration of each diluted individual standard is 10 ng/μL.

7.2 Prepare the combination standards of the 8 compounds by mixing equal volume of each individual 10 μg/mL and two volumes of methanol. This combination standard is 1.0 μg/mL of each compound.

7.3 Working standards are diluted from the 1.0 μg/mL combination standards. The working standards are 0.05, 0.1, 0.25, 0.5 and 1.0ng/uL.

8. Sample Preservation and Storage:

All samples and sample extracts shall be stored in the refrigerator (0-5 ^{o}C).

9. Test Sample Preparation:

9.1 Sample Preparation

9.1.1 Remove samples from refrigerator and allow them to reach ambient temperature.

9.1.2 Measure 1000 mL of water sample into a 2 liter separatory funnel. Add 100 mL of petroleum ether.

9.1.3 Put the stopper on the flask and shake for 1 minute. Let the two layers separate clearly.

9.1.4 Drain the lower layer to a 1liter beaker. Pour the petroleum ether layer to a 500 mL flask thru a funnel containing a layer of glass wool and 20 grams of anhydrous sodium sulfate.

9.1.5 Using 80 mL of petroleum ether repeat 9.1.3 and 9.1.4 two times

9.1.6 Rinse the sodium sulfate with 50 mL petroleum ether.

9.1.7 Evaporate the extract to approximately 10 mL on a rotary evaporator at 35 °C water bath and 15 “ vacuum

9.1.8 Quantitatively transfer the solution with petroleum ether to a 15 mL conical tube.

9.1.9 Evaporate with a gentle stream of nitrogen on a 35 °C water bath to about 1 mL

9.1.10 Add 4 mL methanol, mix and evaporate to about 1 mL. Repeat one more time.

9.1.11 Adjust final volume to 1.0 mL with methanol. Vortex for 20 seconds and transfer to two auto-sampler vials, one with insert for analysis and one without insert for storage for in case of re-analysis.

10 Instrument Calibration:

10.1 The calibration standard curves consist of a minimum of three levels. The lowest level must be at or below the corresponding reporting limits. (The current working standard levels are 0.05, 0.1, 0.25, 0.5, and 1.0 ng/μL as prepared in 7.3)

10.2 The calibration curves for the LC-MS are generally obtained using linear regression. Quadratic fit may be used if the response of certain compounds exhibited quadratic behavior. A minimum of 5 levels of standards are needed When a quadratic curve is used

11 Analysis:

11.1 Injection Scheme

A set of calibration standards, a matrix blank, a matrix spike, a set of up to 10 test samples, then a set of standards in matrix, etc.

11.2 HPLC-MS Instrumentation

11.2.1 Thermo Finnigan model Surveyor HPLC and auto-sampler with column heater and remote control through Thermo Finnigan Xcalibur system.

11.2.2 Column: Waters Xterra® MS C18, 3.5 μm, 2.1 x 50 mm column (part no. 188000400)

11.2.3 Guard column cartridge Holder and cartridge: Waters Sentry guard holder universal (P/N wat064610); Waters SymmetryShield RP 18 5μm, 3.9 x 20mm.

11.2.4 Column Temperature: 40 °C

11.2.5 Mobile Phase: Gradient
Solvent B: 0.1% formic acid in water
Solvent C: 0.1% formic acid in methanol
Flow rate: 300 μL/min
Gradient:

Time(min)	Flow rate (μL/min)	A	B	C	D
0	300	0	90	10	0
3	300	0	90	10	0
20	300	0	10	90	0
22	300	0	10	90	0
25	300	0	90	10	0
30	300	0	90	10	0

11.2.6 Injection Volume: 10 μL

11.2.8 Mass spectrometer (LC-MS) and Operating Parameters

Model:	Finnigan Model TSQ Quantum MS
Ion Source Type:	Electron spray Ionization (ESI)
Source Polarity:	Positive
Capillary Temperature:	200°C
Sheath Gas	44
Auxiliary Gas:	3
Mode of Operation:	MS/MS

Retention time, molecular mass, ion filter range and product ions are listed below:

Compound Name	Retention Time (min)	Molecular Masses	Ion Filter Range	Product Ions
Molinate	17.97	187	187.32±2	188,126
EPTC	19.11	189	190±1	128,86,43
Vernolate	20.24	203	203±1	128,57,72
Pebulate	20.23	203	203±1	128,57,72
Cycloate	20.33	215	215.86±1	153.84,82.88
Thiobencarb	20.39	257,259	257±1	258,126,128
Butylate	20.82	217	217±1	156,218
Triallate	21.90	303,305,307	M±1	M+1, 128, 86

Note: The real retention times are expected within 30 seconds of that stated above when the column is new. The column conditions, temperature, mobile phase, etc. may slightly shift retention time.

11.2.9 Operating parameter details are listed in Appendix 1

12. Quality Control:

12.1 Each set of samples shall have a matrix blank and minimum of one matrix spike sample. Each set contains up to 12 samples.

12.2 The matrix blank shall be free of target compounds.

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

12.4 The retention time shall be within ± 20 seconds of that of the standard.

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

12.6 Add additional levels when there is a need to extend the standard curve range.

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 samples are spiked at 0.1ppb. The standard deviations from the spiked samples are used to calculate the MDL for each 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. In general, the RL is chosen in a range 1-5 times the MDL. The response reproducibility of each compound is also considered to determine the RL

MDL data and the RL are tabulated in Appendix 2 and 3.

12.9 Method Validation Recovery Data and Control Limits:

12.9.1 The method validation consisted of five sample sets. Each set included 4 levels of fortification (0.1, 0.5, 1, 2 ppb) and a method blank. All spikes, method blank and 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.

12.9.3 Method validation results and control limits are tabulated in appendix 4.

13. Calculations:

13.1 The quantification is based on the sum of area counts of the product ion and the precursor of the compound analyzed. The calculation is based on external standard (ESTD).

13.2 The software LCQuan in the Xcalibur system is used to establish the standard curve and to calculate the analytes in the samples. The correlation coefficient, slope, intercept of the linear regression line are calculated once the calibration standards are defined. The equation for calculating analytes is as follows:

$$y = mx+b$$

Where: y = peak response
m = slope
b = intercept
x = concentration of compound

When the unit and the dilution factor are entered correctly in the analysis sequence, the software will then correctly generate the results.

13.3 Results can be manually calculated by a single point standard. The unit is μg/kg (or ppb). This calculation is to verify the results derived from the software

The general equation is as follows:

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

14. Reporting Procedure:

14.1 Perform Quantification with LCQuan:

14.1.1 Create a new Processing method
Open a raw file
Select calibration options
Identify components
Define calibration settings. Or

14.1.2 Open an existing quantification method and save to an appropriate sub-directory with a new name

14.1.3 Open the sequence and review the sequence. Or

14.1.4 Go to the appropriate sub-directory and select the raw files to be used as standards. Place the standard raw files to the appropriate calibration levels. Select the unknown raw files to be calculated.

14.1.5 Review the calibration

14.1.6 Review all calculated results

14.1.7 Create, review, and print peak integration report, calibration report, and summary report

14.2 Acceptance Criteria:

14.2.1 Peak retention time between standards, QC spikes and unknowns shall be within 20 seconds. If there is a known reason for retention time shifting, an explanation memo shall be included.

14.2.2 Peak response shall be within the calibration range

14.2.3 The R^2 of calibration curve or overlay calibration curves shall be 0.990 or better.

14.2.4 Recoveries of spike QC shall be within the established control range, otherwise a rerun of the entire set shall be performed. If problems remain, an explanation memo shall be included.

14.2.5 The ratio of product ion and precursor ion between standard and unknown shall be consistent and the variation of the ratio between standard and unknown shall be within ±20 %.

14.2.6 Manual single point calculation result is acceptable with explanation

14.3 Reporting:

14.3.1 Sample results are reported out according to the client's analytical laboratory specification sheet.
14.3.2 Fill out COC, QC sheet, and control chart.
14.3.3 Prepare data package. Peer review. Report.

APPENDIX I Operating parameters

Creator: User Last modified: 8/5/2010 by User

MS Run Time (min): 30.00

TSQ MS Method Settings:

Segment	1	2	3	4
Duration (min)	15.90	3.94	1.43	8.73
Scan Events	2	2	5	1

Segment 1:
Tune Method C:\Xcalibur\methods\ESI Calib 12-1-04 triallate.TSQTune
Chrom filter: Not used
Q2 Gas Pressure: 1.5
Syringe Pump: Off
Scan Events:
1: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
303.700	142.600	2.000	0.50	36	0.70	0.70	Tuned Value
303.700	85.900	2.000	0.50	24	0.70	0.70	Tuned Value
303.700	82.800	2.000	0.50	58	0.70	0.70	Tuned Value

2: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
215.860	153.840	2.000	0.50	18	0.70	0.70	Tuned Value
215.860	82.880	2.000	0.50	24	0.70	0.70	Tuned Value
215.860	54.930	2.000	0.50	38	0.70	0.70	Tuned Value

Segment 2:
Tune Method C:\Xcalibur\methods\ESI Calib 12-1-04 triallate.TSQTune
Chrom filter: Not used
Q2 Gas Pressure: 1.5
Syringe Pump: Off
Scan Events:
1: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
187.320	188.000	2.000	0.10	15	0.70	0.70	Tuned Value
187.320	126.000	2.000	0.10	15	0.70	0.70	Tuned Value

2: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
189.000	190.000	2.000	0.10	18	0.70	0.70	Tuned Value

189.000	128.000	2.000	0.10	18	0.70	0.70	Tuned Value
189.000	86.000	2.000	0.10	18	0.70	0.70	Tuned Value
189.000	43.000	2.000	0.10	18	0.70	0.70	Tuned Value

Segment 3:
Tune Method C:\Xcalibur\methods\ESI Calib 12-1-04 triallate.TSQTune
Chrom filter: Not used
Q2 Gas Pressure: 1.5
Syringe Pump: Off
Scan Events:
1: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
203.000	204.000	2.000	0.10	16	0.70	0.70	Tuned Value
203.000	128.000	2.000	0.10	16	0.70	0.70	Tuned Value
203.000	57.000	2.000	0.10	16	0.70	0.70	Tuned Value

2: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
215.860	153.840	2.000	0.10	16	0.70	0.70	Tuned Value
215.860	82.880	2.000	0.10	16	0.70	0.70	Tuned Value
215.860	216.000	2.000	0.10	16	0.70	0.70	Tuned Value

3: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
257.000	258.000	2.000	0.10	16	0.70	0.70	Tuned Value
257.000	126.000	2.000	0.10	16	0.70	0.70	Tuned Value
259.000	260.000	2.000	0.10	16	0.70	0.70	Tuned Value
258.000	128.000	2.000	0.10	16	0.70	0.70	Tuned Value

4: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
218.000	57.000	2.000	0.10	16	0.70	0.70	Tuned Value
218.000	156.000	2.000	0.10	16	0.70	0.70	Tuned Value
218.000	218.000	2.000	0.10	16	0.70	0.70	Tuned Value

5: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
203.000	127.900	2.000	0.10	16	0.70	0.70	Tuned Value
203.000	86.000	2.000	0.10	16	0.70	0.70	Tuned Value
203.000	43.000	2.000	0.10	16	0.70	0.70	Tuned Value
203.000	204.000	2.000	0.10	16	0.70	0.70	Tuned Value

Segment 4:
Tune Method C:\Xcalibur\methods\ESI Calib 12-1-04 triallate.TSQTune
Chrom filter: Not used
Q2 Gas Pressure: 1.5
Syringe Pump: Off
Scan Events:
1: + c SRM

Parent	Center	Width	Time	CE	Q1 PW	Q3 PW	Tube Lens
303.840	303.840	2.000	0.10	18	0.70	0.70	Tuned Value
303.840	128.000	2.000	0.10	18	0.70	0.70	Tuned Value
303.840	86.000	2.000	0.10	18	0.70	0.70	Tuned Value
305.820	305.820	2.000	0.10	18	0.70	0.70	Tuned Value
305.820	128.000	2.000	0.10	18	0.70	0.70	Tuned Value
305.820	86.000	2.000	0.10	18	0.70	0.70	Tuned Value

Syringe pump not in use

Divert Valve: in use during run

Divert Time (min)	Valve State
0.00	Inject \ Waste
14.30	Load \ Detector
28.09	Inject \ Waste

Appendix 2

Method Detection Limit (MDL) data

	Molinate	EPTC	Vernolate /Pebulate	Cycloate	Thiobencarb	Butylate	Triallate
	Spiked(ppb)						
	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Found (ppb)						
Spike1	0.095	0.091	0.091	0.105	0.107	0.075	0.09
Spike2	0.103	0.096	0.090	0.114	0.109	0.081	0.108
Spike3	0.102	0.1	0.092	0.108	0.108	0.084	0.097
Spike4	0.104	0.095	0.097	0.109	0.121	0.081	0.097
Spike5	0.103	0.102	0.092	0.113	0.126	0.081	0.114
Spike6	0.097	0.092	0.094	0.108	0.108	0.082	0.096
Spike7	0.097	0.097	0.087	0.104	0.093	0.08	0.088
Average	0.100	0.096	0.092	0.109	0.110	0.081	0.099
Stdev	0.0037	0.0040	0.0031	0.0037	0.0107	0.0028	0.0093
MDL=3.143*Stdev	0.012	0.012	0.010	0.012	0.034	0.009	0.029
RL	0.05ppb	0.05ppb	0.05ppb	0.05ppb	0.05ppb	0.05ppb	0.05ppb

Appendix 3

Method Validation Data

	Spike level (ppb)	Molinate (%recovery)	EPTC (%recovery)	Vernolate /Pebulate (recovery)	Cycloate (%recovery)	Thiobencarb (%recovery)	Butylate (%recovery)	Triallate (%recovery)
Set 1	0.1	93.0	84.0	76.0	89.0	102	70.0	106
	0.5	93.6	92.4	96.4	98.8	103	89.4	105
	1.0	89.2	87.3	92.6	94.9	98.4	87.9	102.9
	2	82.3	78.5	85.8	87.9	89.8	81.1	95.8
Set 2	0.1	101	95	81	93	105	79	102
	0.5	91.4	89.8	89.6	94.8	101	86.2	106.6
	1.0	93.2	91.1	95.7	100	106.1	91.6	110.6
	2	81.7	80.65	83.25	88.5	89.15	83.05	95.05
Set 3	0.1	95.0	93.0	90.0	96.0	86.0	67.0	91.0
	0.5	88.8	89.2	85.8	90	87	68.6	81.6
	1.0	92.8	97.4	99.8	87.4	79.1	82.9	93.4
	2	87.9	91	91.5	80.5	81	79.5	84.5
Set4	0.1	97	103	99	96	91	90	108
	0.5	97.2	98.8	101.8	92	94.6	84.4	100.8
	1.0	89.4	95.1	96.5	87	91	82.2	93.7
	2	91.5	97.5	97.5	86	89.5	85	96
Set 5	0.1	100	97	94	103	93	86	99
	0.5	91.2	92.6	93.0	95	102.4	80.6	92.2
	1.0	90.2	90.4	94.3	89.5	101.6	81.2	90.1
	2	84	85.3	87	82.65	88.9	78.7	83.3
Set 6	0.1	75	75	91	87	92	62	89
	0.5	80	79.8	93.2	90	98	60.8	86.6
	1.0	78.4	76.4	88.9	86.7	98.9	63.3	83
	2	79.85	80.35	92.45	86.15	97.25	62.3	87.5
	average	89.3	89.2	91.5	90.9	94.4	78.4	95.2
	stdev	7.0	7.7	6.2	5.5	7.4	9.7	8.6
	UCL	110.2	112.2	110.0	107.5	116.6	107.5	121.1
	UWL	103.2	104.6	103.8	102.0	109.2	97.8	112.4
	LWL	84.4	83.5	87.3	87.4	89.0	70.8	88.5
	LCL	68.4	66.1	73.0	74.3	72.1	49.4	69.2

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