California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Analysis Section
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Title: Determination of N-methylcarbamate Pesticides in Surface Water using High Performance Liquid Chromatography and Post-column derivatization

1. Scope:

   This section method (SM) documents the selected N-methylcarbamate pesticides analysis in surface water 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 then diluted to a final volume of 0.40 mL with methanol. The extract is then analyzed by HPLC. The analytes are derivatized with OPA (ortho-phthaladehyde) in a post column reaction and detected with a fluorescence detector. The reporting limit for this method is 0.05 ppb for all compounds.

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 are matrix interferences that cause quantitative problems. Therefore the calibration standards will be made up in appropriate matrix.

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 HPLC with post column derivatization system and fluorescence detector.

6. Reagents and Supplies

6.3 Methylene Chloride, nanograde or equivalent pesticide grade
6.4 Methanol, nanograde or equivalent pesticide grade
6.5 Anhydrous Sodium Sulfate, granular
6.6 Aldicarb Sulfoxide CAS# 1646-87-3
6.7 Aldicarb Sulfone CAS# 1646-88-4
6.8 Oxamyl CAS# 23135-22-0
6.9 Methomyl CAS# 16752-775
6.10 3-OH-Carbofuran CAS# 16655-82-6
6.11 Aldicarb CAS# 116-06-3
6.12 Carbofuran CAS# 1563-66-2
6.13 Carbaryl CAS# 63-25-2
6.14 Methiocarb CAS# 2032-65-7
6.15 Hydrolysis reagent (Pickering Laboratories CB130 or equivalent)
6.16 O-phthaladehyde (Pickering Laboratories 012 or equivalent)
6.17 O-phthaladehyde diluent (Pickering Laboratories CB910 or equivalent)
6.18 2-mercaptoethanol
6.19 OPA Reagent- Dissolve 100mg O-Phthaladehyde in 10mL methanol. Add this mixture to 950 mL O-Phthaladehyde diluent and mix well. Add 1 mL 2-mercaptoethanol and pour solution into reagent reservoir.
6.20 Conical tube with glass stopper, 15-mL graduated, 0.1 mL subdivision
6.21 Separatory funnel, 250 mL
6.22 Boiling flask, 500 mL
6.23 Funnel, long stem, 10 mm diameter
6.24 Nitrogen Evaporator, Organomation
6.25 Disposable Pasteur pipettes, and other laboratory ware as needed
6.26 0.2µ nylon filters (Acrodisc 28143-274 or equivalent)
6.27 Recommended analytical columns:
Carbamate analysis C18 4.6mm ID X 250 mm. (Pickering Laboratories 1846250 or equivalent)

7. Standards Preparation:

7.1 Dilute the 1 mg/mL Carbamate standards obtained from the CDFA/CAC Environmental Analysis Standards Repository with methanol to make up a series of

mixed working standards (see 10.2). These standards shall be prepared to cover the linear range from 0.0125 ηg/μL to 0.5 ηg/μL for the carbamate screen.

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

7.3 The expiration date of each mixed working standard is six months from the preparation date or same as stock standards, if sooner.

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 100 grams of background water and follow the test sample extraction procedure.

9.1.2.2 Matrix spike: Weigh out 100 grams of background water. Spike a client requested amount of carbamate 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 Shake each sample then weigh out 100 grams of sample and transfer to 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 100 ± 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. Pass sample through 0.2µ filter into a calibrated 15 mL graduated test tube.

9.2.7 Rinse flask 2-3 more times with 2 - 4 mL of methylene chloride and filter the rinse into the same test tube.

9.2.8 Evaporate the extract to a volume slightly less than 0.5 mL in a water bath at 38 ± 2 °C under a gentle stream of nitrogen. Add in approx. 1 mL methanol. Evaporate the extract to less than 300 µL. Transfer extract to a calibrated vial insert. Wash the tube with a few drops of Methanol and add to insert. Adjust the final volume of 0.4 mL with methanol.

9.2.9 Submit extract for HPLC analysis.

10. Instrument Calibration:

10.1 A calibration standard curve consists of minimum of three levels. Standard concentrations of 0.0125, 0.025, 0.05, 0.1, and 0.5 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.

10.2 Compositions of calibration mixed standards are as follows:

CB-A Mixed Standard
Aldicarb Sulfoxide
Aldicarb Sulfone
Methomyl
3-Hydroxycarbofuran
Aldicarb
Carbofuran
Carbaryl

CB-B Mixed Standard
Oxamyl
Methiocarb

11. Analysis:

11.1 Injection Scheme

Follow the sequence of calibration standards, QC samples, test samples (maximum of 10-12 samples) and final calibration standards.

11.2 HPLC Instrumentation

11.2.1 Analyze carbamate pesticides by HPLC equipped with post column reaction module and a fluorescence detector.

11.2.2 Recommended instrument HPLC gradient::

A= 1% methanol in water B= acetonitrile

Time (min)	% A	%B
0.00	98.0	2.0
1.00	98.0	2.0
16.00	30.0	70.0
18.00	30.0	70.0
22.00	100.0	0.0
25.00	100.0	0.0
25.10	98.0	2.0
30.00	98.0	2.0

11.2.3 Injection volume 25 µL.

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 $\pm$ 2 percent 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 % for all compounds.

12.7 Method Detection Limits (MDL)

The method detection limit refers to the lowest concentration of analytel that a method can detect reliably. To determine the MDL, 7 replicate water samples are spiked at 0.05 ppb for OP screen and 7 replicate water samples are spiked at 10 ppt for low level diazinon and chloripyrifos. The standard deviation from the spiked sample recoveries 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. 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 IA and IB.

12.9 Method Validation Recovery Data and Control Limits:

12.9.1 The method validation consisted of three sample sets. Each set included five levels of fortification (0.0125, 0.025, 0.05, 0.1, 1.0 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.

12.10 Estimated Measurement Uncertainty:

12.11 Trend Identification

12.11.1 All matrix spike recoveries for carbamate analysis will be put into control

Charts and monitored for trends. Three trend characteristics will be evaluated at least bi-yearly by the supervisor or designee.
2 of 3 points above or below 2/3 of the UCL or LCL.
7 continuous points above or below the center line (CL)
14 points alternating above and below the CL.

12.11.2 When results indicate an out of control situation the supervisor or designee will indicate this on the control chart and take appropriate corrective action, which may include monitoring the results more closely to initiating a formal corrective action with root cause investigation.

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.

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

14. Reporting Procedure:

14.1 Identification of Analyte

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 2 percent.

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

15. References:

Muth, G.L., Erro, F. A Rapid Carbamate Multiresidue Procedure of Vegetable Crops Environmental Contamination & Toxicology, 1980, 24, 759-765

Keith, Lawrence H., Principles of Environmental Analysis, Anal Chem, 1983, 55, 2210-2218

APPENDIX IA

The determination of Method Detection Limit (MDL) data and Reporting Limit (RL)

	Aldicarb sulfoxide	Aldicarb sulfone/Oxamyl	Methomyl	3-OH Carbofuran	Aldicarb	Carbofuran	Carbaryl	Methiocarb
MDL#1	0.02433	0.06784	0.02939	0.0322165	0.024847	0.02892	0.03208	0.03113
MDL#2	0.02126	0.05778	0.02545	0.02704	0.02244	0.0267	0.02718	0.02444
MDL#3	0.0248	0.06608	0.02709	0.03169	0.02316	0.02691	0.02924	0.02875
MDL#4	0.02172	0.05155	0.02367	0.02685	0.02164	0.02417	0.0245	0.03718
MDL#5	0.01686	0.05218	0.02204	0.02594	0.01776	0.02235	0.02388	0.02301
MDL#6	0.02388	0.05906	0.029	0.03161	0.02579	0.02815	0.02841	0.02617
MDL#7	0.026	0.06423	0.03	0.035	0.0245	0.03114	0.03286	0.02866
SD	0.00307	0.00651	0.00306	0.00343	0.00268	0.00294	0.00344	0.00473
3.1416 xSD	0.01026	0.01882	0.00967	0.01133	0.00871	0.00964	0.01038	0.01578
MDL	0.011	0.020	0.010	0.011	0.010	0.010	0.011	0.016

All concentrations are expressed in ppb.

APPENDIX IIA

Method Validation Data and Control Limit for Carbamates Table 1

Level **µg/L** (ppb)	Aldicarb Sulfoxide	Percent recovery	Aldicarb Sulfone	Percent recovery	Methomyl	Percent recovery	3-OH-Carbofuran	Percent recovery
0.0125	0.0089	71.2	0.0114	91.2	0.0070	88.8	0.0144	115
	0.0093	74.4	0.0109	87.6	0.0140	84.4	0.0123	98.4
	0.0108	86.2	0.0115	91.8	0.0114	86.4	0.0124	99.2
0.025	0.0196	78.5	0.0211	84.2	0.0201	78.1	0.0214	85.7
	0.0216	86.6	0.0232	92.8	0.0268	90.4	0.0231	92.5
	0.0238	95.2	0.0274	110	0.0213	97.6	0.0303	121
0.05	0.0495	99.2	0.0470	94.0	0.0438	87.2	0.0541	108
	0.0467	93.6	0.0459	91.8	0.0404	87.4	0.0481	96.2
	0.0248	85.7	0.0439	87.7	0.0440	85.0	0.0440	87.9
0.10	0.0944	94.4	0.0978	97.8	0.0948	94.8	0.0954	95.4
	0.0904	90.4	0.1031	103	0.0928	92.8	0.1097	110
	0.0896	89.6	0.1033	103	0.0996	99.6	0.1102	110
1.00	0.8064	80.6	0.9223	92.2	0.8858	88.6	0.9238	92.4
	0.8259	82.6	0.9318	93.2	0.8752	87.5	0.9300	93.0
	0.8578	85.8	0.9842	98.4	0.9673	96.7	0.9859	98.6
SD		7.88		6.79		5.73		10.30
SD X 3		23.64		20.38		17.19		30.89

Table 2

Level **µg/L** (ppb)	Aldicarb	Percent recovery	Carbofuran	Percent recovery	Carbaryl	Percent recovery
0.0125	0.0119	95.2	0.0138	110	0.0132	106
	0.0108	86.4	0.0119	95.2	0.0119	95.6
	0.0107	85.8	0.0118	94.4	0.0119	95.2
0.025	0.0188	75.3	0.0208	83.0	0.0213	85.1
	0.0222	88.9	0.0234	93.5	0.0234	93.6
	0.0252	101	0.0284	114	0.0276	110
0.05	0.0416	83.2	0.0488	97.6	0.0480	96.0
	0.0418	83.6	0.0462	92.4	0.0454	90.8
	0.0397	79.4	0.0436	87.2	0.0435	86.9
0.10	0.0886	88.6	0.0956	95.6	0.0946	94.6
	0.0984	98.4	0.1018	102	0.1023	102
	0.1038	102	0.1063	106	0.1049	105
1.00	0.8776	87.8	0.9238	92.4	0.9178	91.8
	0.8309	83.1	0.9122	91.2	0.9267	92.7
	0.9291	92.9	0.9743	97.4	0.9711	97.1
SD		7.79		8.26		6.97
3 X SD		23.38		24.79		20.90

Table 3

Level **µg/L** (ppb)	Oxamyl	Percent recovery	Methiocarb	Percent recovery
0.0125	0.0116	92.8	0.0124	99.2
	0.0118	94.8	0.0124	99.2
	0.0103	82.4	0.0105	83.6
0.025	0.0242	96.9	0.0247	98.9
	0.0233	93.2	0.0237	94.7
	0.0248	99.2	0.0232	92.8
0.05	0.0462	92.4	0.0424	84.8
	0.0449	89.8	0.0412	82.4
	0.0458	91.5	0.0442	88.3
0.10	0.0940	94.0	0.0949	94.9
	0.0848	84.8	0.0992	99.2
	0.0964	96.4	0.1042	104
1.00	0.9099	91.0	0.9043	90.4
	0.8909	89.1	0.8887	88.9
	0.9159	91.6	0.9263	92.6
SD		4.37		6.50
3 X SD		13.12		19.50

Written BY:

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Revision Log:

Date	What was Revised? Why?
3/11/09	Changed method validation results to the validation results done in June 2007.
3/3/2011	Change validation results to the validation done in November 2007.