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Method #: EM 12.5
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Revised:

Determination of Carbaryl in Selected Fruits and Vegetables using Liquid Chromatography

Scope: This method is for the determination of the pesticide carbaryl for specific classes of fruits and vegetables. The reporting limit (RL) for carbaryl on tomatoes is 0.08 ppm and on stone fruits, grapes, citrus and zucchini is 0.05 ppm.

Principle: The sample materials are cut into small pieces and homogenized with dry ice using a cusinart . A portion of the homogeneous sample is extracted with acetonitrile. An aliquot of the acetonitrile extract is evaporated to near dryness on a nitrogen steambath. If needed, the extract is cleaned up. The final extract is analyzed by reverse-phase liquid chromatography using a post-column derivatization system and a fluorescence detector.

Safety:

All general laboratory safety rules for sample preparation and analysis shall be followed.

Interference:

There are no background interference for carbaryl on the stone fruits, grapes, citrus, and zucchini on the samples analyzed. Clean up is necessary for tomato samples due to their background interference with carbaryl.

Equipment, Reagents, and Instruments:

Equipment:

1. Cuisinart™ food processor, model DLC 7
2. Mason jar: One pint, narrow mouth with cap
3. Blender: Omni-Mixer
4. Vacuum manifold: Vac Elut SPS24. Varian
5. Vortex-vibrating mixer
6. Filter paper, Sharkskin, 18.5 cm
7. Bond Elut®: Aminopropyl Bond Elut 10 cc/500 mg. Varian®
8. Acrodisc®, 0.2 µm filter. Gelman Sciences
9. Conical test tube with glass stopper, 15 mL, graduated
10. Funnel, short stem, 60°, 100 mm
11. Disposable Pasteur pipettes, 5.75 inches
12. Balance, Mettler PC 4400
13. Nitrogen evaporator, Organomation Model # 112
14. Brown bottle: 8 oz with cap

Equipment, Reagents, and Instruments: continued

Reagents:

1. Acetonitrile, HPLC - Residue and Pesticide Analysis
2. Methylene Chloride, HPLC - Residue and Pesticide Analysis
3. Methanol, HPLC - Residue and Pesticide Analysis
4. Sodium Chloride, granular, anhydrous
5. Dry ice
6. Carbaryl CAS# 63-25-2, 1 mg/mL in methanol, stock standard solutions. Obtain standards from Standard Repository, Center for Analytical Chemistry.
7. Hydrolysis reagent C47™, Pickering Laboratories, part# CB130.
8. O-Phthalaldehyde, Pickering Laboratories, part# CB 0120.
9. Thiofluor™, N,N-Dimethyl-2-mercaptoethylamine-Hydrocloride, Pickering Laboratories, part# 3700-2000.
10. 2-Mercapto-ethanol, Pickering Laboratories, part# CB910.
11. OPA Reagent: Dissolve 100 mg of O-Phthalaldehyde in 10 mL of methanol. Add this mixture to 950 mL O-Phthalaldehyde diluent and mix well. Pour the solution into the reagent reservoir and add 2 g of thiofluor or 1 mL of 2-Mercapto-ethanol directly into it.

Instrument:

Hewlett Packard 1090 or 1050 HPLC with Pickering post-column derivatization system and a Carbonate Analysis C_{18} column (5 µm x 0.4.6 mm x 250 mm).
Fluorescence detector

Standard Preparation:

1. Dilute the 1 mg/mL carbaryl standard with methanol into a series standard solutions that will be used for spiking, instrument calibration and sample calculation.
2. Keep all prepared standards in the designated refrigerator for storage while not in use.
3. The shelf life of each prepared standard is six months.

Sample Preservation and Storage:

1. Check the temperature of ten percent of the samples upon arrival and record it.
2. Sign the sample chain of custody and obtain the EMON number from supervisor.
3. Store all samples waiting for extraction in the walk-in freezer.
4. Store all samples waiting for analysis in a refrigerator.

Analysis:

Sample Extraction:

1. Cut the entire sample into small pieces. Homogenize the sample with dry ice using a cuisinart until obtaining a finely chopped sample. Store the sample in a freezer overnight with a loose cap to allow the CO_2 to dissipate.
2. Weigh out 20.0 g of the frozen homogeneous sample into a narrow mouth, one pint mason jar. Add 100 mL of acetonitrile into the jar and blend for 2 minutes using an Omni-Mixer set at medium speed (@ 3.5).

Sample Extraction: continued

3. Decant the liquid layer through a funnel with a sharkskin filter paper into a brown bottle containing 15 g of Sodium Chloride. Cap bottle and shake it vigorously for 2 minutes. Let bottle set at room temperature for 30 minutes to allow the phases to separate.
4. Pipette 10 mL of the acetonitrile extract (upper layer) into a 15 mL graduated test tube calibrated to 1.0 mL. Evaporate to less than 0.1 mL in a nitrogen evaporator. *If needed proceed with clean up procedure.* Clean up is needed for all tomatoes samples.
5. Bring sample to a final volume of 1.0 mL with methanol and vortex for 20 seconds. Filter through a 0.2 μm acrodisc filter into an autosampler vial.
6. Analyze by HPLC with a post-column derivatization system.

Clean up Procedure

1. From step 4 of sample extraction above, resuspend the residue with 2 mL of 1% methanol in methylene chloride. Vortex for 15 sec.
2. Condition an Aminopropyl Bond Elut (NH_2) cartridge with 5 mL of 1% methanol in methylene chloride using a vacuum manifold with no vacuum applied. *Do not allow cartridge to dry.*
3. Load the sample in the preconditioned NH_2 cartridge and collect the eluent in a 15 mL graduated conical test tube calibrated to 1.0 mL.
4. Rinse the test tube well with 4 mL of 1% methanol in methylene chloride and vortex for 15 sec. Load the rinsing on the same cartridge and combine the eluent with the previous one.
5. Repeat step four one more time.
6. Evaporate sample to less than 0.1 mL in a nitrogen evaporator but do not go to dryness.
7. Proceed with step 5 of above sample extraction.

Preparation of blanks and spikes

Blank: Weigh out 20.0 ±0.01 g of the homogeneous background sample into a narrow-mouth, one pint mason jar. Follow the sample extraction procedure outline above.

Spike: Weigh out 20.0 ±0.01 g of the homogeneous background sample into a narrow-mouth, one pint mason jar. Spike a known amount of carbaryl over the sample. Let stand for at less 1 minute, then follow the sample extraction procedure outlined above.

Instrument Conditions

Instrument: Hewlett Packard 1090 or 1050, controlled by Chemstation
Column: Pickering Laboratories, "Carbamate Analysis", C18, 4.6 mm x 25 cm x 5 μm
Mobile phase: Isocratic 40 % acetonitrile 60% water
Flow: 1 mL/min
Injection volume: 25 μL
Post column system: Pickering Laboratories PCX5100 Post-Column Derivatization
Column Temperature = 42 °C
Reagent 1 = Hydrolysis Reagent C47™, Reactor Temperature = 100 °C
Reagent 2 = OPA Reagent

Instrument Conditions: continued

Fluorescence Detector:	Excitation = 340 nm
	Emission = 450 nm
	Time constant = 0.3 sec
Carbaryl retention time:	10.725 min

Instrument calibration:

Load a method and run a set of calibration standards (0.05 ng/µL, 0.1 ng/µL, 0.5 ng/µL, 1.0 ng/µL, and 2.0 ng/µL) to check system linearity.

Method Performance:

Quality Control:

1. A five-point calibration curve (0.05 ng/µL, 0.1 ng/µL, 0.5 ng/µL, 1.0 ng/µL, and 2.0 ng/µL) is run at the beginning and the end of each set of samples..
2. Each sample shall be injected two times to insure reliability of the analysis. If a sample signal is greater than the highest standard, dilute the sample. Reinject the diluted sample with standards as directed above.
3. Sample storage: All field samples shall be keep frozen at -10 °C. Thaw the samples in a refrigerator overnight before grinding.
4. Sample extracts: All extract shall be kept in a refrigerator at 5 °C until analyzed.
5. Freezer and refrigerators temperature shall be monitored and recorded daily.
6. For each set of samples, one matrix blank and one matrix spike shall be included. Each set of samples shall not contain more than twelve samples.
7. To avoid-cross contamination, all grinding equipment shall be rinsed with water several times followed by an acetone or methanol rinse and dried before grinding the next sample. Glassware shall be washed following Environmental Monitoring standard operation procedure (SOP 502.6)

Recovery Data:

The analytical method was validated using tomatoes, grapes, zucchini (for zucchini and squash), oranges (for citrus) and apricots (for stone fruits). Three sets of fortified samples were prepared for each matrix. Each set contained three levels of fortification and a matrix blank. All fortified and matrix blank samples were processed through the entire analytical method. The results are tabulated on appendix I.

Method Detection Limit:

Data used to calculate the method detection limits (MDL) are in appendix II. The MDLs are as follows:

Compound	STD DEV (ppm)	MDL (ppm)
Tomatoes	0.005	0.016
Zucchini	0.004	0.013
Grapes	0.011	0.035
Oranges	0.006	0.019
Apricots	0.011	0.035

Method Performance: continued

The MDL is the minimum concentrations of carbaryl in the above matrices that can be reported with 99% confidence. The method detection limit (MDL) was computed based on the following procedure:

a) Prepare 7 replicates of carbaryl at 0.1 ppm for each matrix.
b) Calculate the standard deviation (S) for the percent recovery for each matrix.
c) Compute the MDL as follows:

$$\mathbf{MDL} = t \times S$$

where;
t is the Student 't' value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - $\alpha = 0.99$) where n represents the number of replicates.
S denotes the standard deviation obtained from replicate analyses.

Reporting Limit:

The carbaryl reporting limit (RL) for tomatoes is 0.08 ppm and for zucchini, grapes, citrus and stone fruits is 0.05 ppm. The MDL is used as a guide to determine the RL for this method. The RL is one to five times the MDL.

Calculations:

$$\textbf{ppm } (\mu g/g) = \frac{\mu g/mL \text{ (from the standard curve) x aliquot final volume (mL)}}{\text{Aliquot sample weight (g)}}$$

For this method, the aliquot final volume is 1 mL and aliquot sample weight is 2.0 g.

Acceptance Criteria:

1. The standard curves at the beginning and the end of each sample set should not have a percent change greater than 15%. The % change in response was calculated as follows:

$$\textbf{\% change in response} = \frac{\text{Absolute value of response (std before - std after)}}{\text{Std before}} \times 100$$

2. The samples were calculated based on the calibration curve before the samples using the instrument chem-station software. If the results between the two injections differ less than 10 % either result can be reported. A change greater than 10 % with no known reason requires a third injection.

Discussion:

The MDL and validation samples for stone fruits, citrus and grapes were prepared by Tina Monk. The clean-up procedure can be used with any of the samples but produce slightly lower recoveries results. Stone fruits, grapes, citrus, and zucchini in most cases do not need clean up and the method was validated without it. Clean-up is needed for tomatoes because of baseline raise due to background interference. The reporting level for tomatoes was set higher than the other fruits and vegetables due to interference.

Reference:

1. Paul Lee and Silvia Richman. *Method EM 11.0, Determination of N-Methyl Carbamates in River Water by HPLC.* April 1990.
2. Duc Tran. *Method EM 34.3, Determination of Hexazinone in Plant Material by HPLC.* December 1995.
3. Jane White. *Method EM 12.0, HPLC Determination of Carbaryl in Well Water.* October 1990.
4. *SOP QAQC001.0,* California Department of Pesticide Regulation, Environmental Hazards Assessment Program, 1995.

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APPENDIX I

Recovery data of method validation of carbaryl in tomatoes

Fortified Levels (ppm)	Results (ppm)	Recovery (ppm)
1.0	0.824	82.4
1.0	0.872	87.2
1.0	0.820	82.0
10.0	8.63	86.3
10.0	8.21	82.1
10.0	8.38	83.8
100	82.9	82.9
100	83.3	83.3
100	81.7	81.7

Recovery data of method validation of carbaryl in zucchini

Fortified Levels (ppm)	Results (ppm)	Recovery (%)
1.0	0.931	93.1
1.0	0.760	76.0
1.0	0.903	90.3
10.0	9.53	95.3
10.0	8.98	89.8
10.0	9.39	93.9
100	87.0	87.0
100	86.0	86.0
100	84.1	84.1

Recovery data of method validation of carbaryl in grapes

Fortified Levels (ppm)	Results (ppm)	Recovery (%)
1.0	0.824	82.4
1.0	0.911	91.1
1.0	0.907	90.7
10.0	9.10	91.0
10.0	9.92	99.2
10.0	9.34	93.4
100	87.5	87.5
100	93.4	93.4
100	93.6	93.6

APPENDIX I: continue

Recovery data of method validation of carbaryl in oranges

Fortified Levels (ppm)	Results (ppm)	Recovery (%)
1.0	0.859	85.9
1.0	0.893	89.3
1.0	0.858	85.8
10.0	9.33	93.3
10.0	10.0	100
10.0	8.92	89.2
100	83.0	83.0
100	87.2	87.2
100	85.1	85.1

Recovery data of method validation of carbaryl in apricots

Fortified Levels (ppm)	Results (ppm)	Recovery (%)
1.0	0.966	96.6
1.0	0.914	91.4
1.0	0.842	84.2
10.0	9.40	94.0
10.0	8.14	81.4
10.0	8.27	82.7
100	88.6	88.6
100	86.5	86.5
100	86.9	86.9

APPENDIX II

Carbaryl recovery data for the determination of method detection limits

	Fortified Level	Tomatoes	Zucchini	Grapes	Oranges	Apricots
	ppm	ppm	ppm	ppm	ppm	ppm
MDL 1	0.10	0.090	0.083	0.089	0.078	0.089
MDL 2	0.10	0.085	0.085	0.114	0.072	0.114
MDL 3	0.10	0.085	0.089	0.106	0.077	0.106
MDL 4	0.10	0.075	0.090	0.090	0.067	0.090
MDL 5	0.10	0.080	0.083	0.091	0.068	0.091
MDL 6	0.10	0.083	0.086	0.088	0.071	0.088
MDL 7	0.10	0.081	0.092	0.086	0.085	0.086
STDEV		0.005	0.004	0.011	0.006	0.011
MDL		0.016	0.013	0.035	0.019	0.035
RL		0.08	0.05	0.05	0.05	0.05