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DETERMINATION OF CARBARYL AND IMIDACLOPRID DESORBED FROM XAD-2 RESIN TUBES

Scope: This method describes the desorption and determination of carbaryl and imidacloprid from XAD-2 resin air sample tubes. The reporting limit of this method is 0.2 µg for carbaryl and 0.5 µg for imidacloprid.

Principle: Carbaryl and imidacloprid in the air that has been absorbed onto XAD-2 resin are desorbed from the resin with methanol. Subsequently, carbaryl and imidacloprid are quantified using a HPLC. Carbaryl is derivatized with OPA in a post colum reaction and detected with a fluorescence detector. Imidacloprid is detected with a UV detector.

Reagents:

1. Carbaryl, CAS# 63-25-2, 1.0 mg/mL in methanol, obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of Food and Agriculture)
2. Imidacloprid, CAS#138261-41-3, 1.0 mg/mL in methanol, obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of Food and Agriculture)
3. Methanol, pesticide residue grade
4. Water, HPLC grade
5. Acetonitrile, HPLC grade
6. Hydrolysis reagent C47™, Pickering Laboratories, part# CB130
7. O-Phthalaldehyde, Pickering Laboratories, part# 0120
8. Thiofluor™, N,N-Dimethyl-2-mercaptoethylamine-Hydrochloride, Pickering Laboratories, part# 3700-2000
9. 2-Mercapto-ethanol, Pickering Laboratories, part# CB910
10. OPA Reagent: Dissolve 100 mg of O-Phthaladehyde in 10 mL 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.

Safety:

Most of the reagents used and analyzed for in this method have not been completely characterized. All general laboratory safety rules must be followed.

Equipment:

1. XAD-2 resin tubes - SKC # 226-30-02 SKC West: phone (714) 992-2780
2. Test tubes, 25 mL, with Teflon-liner caps
3. Assorted pipets and micro-syringes
4. Small triangular file
5. Thermolyne Vortex Maxi Mixer II
6. Forceps
7. Glass syringe, 5 mL
8. Nylon Acrodisc, 0.2 um, Gelman

Instrument:

Carbaryl

1. HPLC: Hewlett-Packard 1090 Liquid Chromatograph with ChemStation and a Hewlett-Packard 1046-A Programmable Fluorescence Detector
   HPLC: Hewlett-Packard 1100 Series with a ChemStation and Fluorescence Detector
2. Post column system: Pickering Laboratories PCX5100 Post-Column Derivatization or Pickering Laboratories PCX5200 Post-Column Derivatization
3. Analytical column: Pickering Laboratories "Carbamates Analysis" C18, 4.6 mm x 25 cm x 5 μm

Imidacloprid

1. HPLC: Hewlett-Packard 1050 Series with ChemStation and UV Detector
2. Analytical column: Beckman Ultrasphere 5μ x 4.6 mm x 25 cm

Interference:

There are no interferences for carbaryl and imidacloprid in XAD-2 resin background or samples at this time.

Standard Preparation:

1. The 1mg/mL standards are diluted to 100ug/mL with methanol for spiking purpose.
2. Dilute the spiking standards into a series of desired standard sets that will be used for spiking, instrument calibration and sample calculation.
3. Keep all prepared standards in the designated refrigerator for storage while not in use.
4. The shelf life of each prepare standard is six months.

Sample Preservation and Storage:

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

PROCEDURE:

1. Remove samples from frozen storage. Allow samples to stand at room temperature for 20-30 minutes before starting extraction of carbaryl and imidacloprid.
2. Fold a sheet of white paper into quarters, reopen and place under the test tube to catch spills.

PROCEDURE: continued

3. Pipette 5 mL of methanol into a labeled test tube.
4. Remove caps from a resin sample tube. Score the tube with a file just above the spring wire and break the glass tube.
5. With a forceps, immediately remove the spring wire and glass wool plug. Place them in the test tube.
6. Placing the large broken end of the resin tube in the mouth of the test tube, insert a a Pasteur pipette from the opposite end and push the cotton plug and resin into the test tube. Immediately, cap the test tube.
7. Extract carbaryl and imidacloprid from resin by mixing for 30 seconds using a vortex mixer.
8. Allow the mixture to stand for 3 minutes. Filter 1.5-2 mL of the mixture through a 0.2 μm Nylon Acrodisc and collect in autosampler vials.
9. Determine carbaryl and imidacloprid using a HPLC.

Preparation of blanks and Spikes

Blank: Score a resin tube with a file just above the spring wire and break glass tube. Next score the tip of the oppsite end of the same tube and break the tube at the end. Follow steps 5-9 above.

Spike: Score a charcoal tube with a file in a similar manner as the blank. Place a micro-syringe needle about 1 cm below the glass wool and slowly add a known amount of carbaryl and imidacloprid onto the resin. After 10-20 seconds, follow steps 5-9 above.

Instrument Conditions:

For Carbaryl,

Instrument: HPLC, Hewlett-Packard Model 1100, controlled by Chemstation or HPLC, Hewlett-Packard Model 1090, controlled by Chemstation

Column: Pickering Laboratories "Carbamates Analysis" C18, 4.6 mm x 25 cm x 5 μm

Mobile Phase:

Time (min.)	Water %	Acetonitrile %
0	100	0
1	100	0
16	30	70
18	30	70
21	100	0
23	100	0

Instrument conditions: continued

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
Fluorescence detector:	Excitation = 340 ηm
	Emission = 450 ηm
Retention Time:	Carbaryl = 15.7 ± 0.2 minutes

For Imidacloprid,

Instrument:	HPLC, Hewlett-Packard Model 1050, controlled by Chemstation
Column:	Beckman Ultrsphere 4.6 mm x 25 cm x 5 µm
Mobile phase:	Isocratic 30% water and 70% acetonitrile
Flow:	1 mL/min.
Injection volume:	20 µL
UV detector:	270 ηm
Retention time:	Imidacloprid = 5.6 ± 0.2 minutes

Both instruments operate in ambient temperate. The retention time of each compound may shift dramatically if temperatures change too much.

Instrument Calibration:

1. Load a method, set the desired condition for analysis on both instruments
2. Run 0.025, 0.05, 0.1, 0.5, and 1 ηg/uL to check the system linearity for carbaryl
3. Run 0.025, 0.05, 0.1, 0.5, and 1 ηg/uL to check the system linearity for imidacloprid

Analysis:

Quality Control:

1. A 5-point calibration curve of 0.025, 0.05, 0.25, 0.5 and 1 ηg/uL for carbaryl and imidacloprid were obtained 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. Results obtained using a calibration curve shall lie within the range of the calibration curve. If results fall outside the calibaration curve, the sample must be concentrated/diluted or the calibration curve extended. A sample set is usually comprised of 10 samples, a blank and a spike.

Method Detection Limit (MDL):

Method Detection Limit (MDL) refers to the lowest concentration of analyte that a method can detect reliably in either a sample or a blank. To determine the MDL, spike 7 samples, with 0.5 µg of carbaryl and imidacloprid and process through the entire method along with a blank. The standard deviation derived from the 7 spike results was used to calculate the MDL using the following equation:

$$MDL = tS$$

Method Detection Limit (MDL):continued

Where: t = the student " t" value for the 99% confidence level with n-1 degrees of freedom (t=3.143 for 6 degrees of freedom). n= the number of replicates.
S = the standard deviation obtained from the 7 replicates analysis

The results for the standard deviations and MDL are in Appendix 1.

Reporting Limit (RL):

RL refers to level above which quantitative results may be obtained. The MDL was used as a guide to determine the RL. The reporting limit for carbaryl is 0.2 μg and 0.5μg for imidacloprid.

Recovery Data:

The analytical method was validated using five sets of spike samples. Each set contained a blank and three levels of spikes. Each set was processed through the entire analytical method. Recoveries of carbaryl and imidacloprid are shown in Appendix 2.

Calculations:

$$\mu g = \frac{(\text{peak ht sample})(\text{response factor, } \eta g)\ (\text{sample final volume, mL})(1000\ \mu L/mL)}{(\text{sample vol. Injected, } \mu L)}$$

$$\text{where: response factor}(\eta g) = \frac{[\ (\text{std. Conc.}_n, \eta g/\mu L\ )(\text{srd. Vol. Injected, } \mu L)/(\text{std. Peak ht..}_n)]}{n}$$

n=number of standards

Acceptance Criteria:

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

   % Change in response = absolute value of [response of (std before - std after)/ std before] x 100

2. The samples were calculated using the response factor average of the curves. 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:

In this project a trapping efficiency study was done for carbaryl. The trapping efficiency study was conducted by the Department of Pesticide Regulation at their warehouse site. Three levels of standard were spiked onto the glass wool contained in XAD-2 resin tubes A, which were connected to tubes B. Tubes A and B were connected to an air sampler, which pumped a flow rate of 3 mL/min for 24 hrs. Three reps were done for each level. Results are shown in Appendix 3.

References:

1. Lew, Robert B., *Determination of Methyl Bromide Desorbed from Charcoal Tubes*,1997, California Department of Food and Agriculture Chemistry Laboratory Services, 3292 Meadowview Road, Sacramento, California 95832.

2. Feng, Hsiao , *HPLC Determination of Carbofuran and Carbaryl in Surface Water*, 1998, California Department of Food and Agriculture, Chemistry Laboratory Services, 3292 Meadowview Road, Sacramento, California 95832.

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Appendix 1:
Carbaryl and Imidacloprid MDL Results (µg) for XAD-2 resin on A tubes

Spike #	Carbaryl	Imidacloprid
1	0.459	0.363
2	0.460	0.380
3	0.453	0.463
4	0.443	0.343
5	0.445	0.420
6	0.455	0.390
7	0.453	0.410
S=	.006	0.0396
MDL = 3.143 x S	.02	0.1243

Carbaryl and Imidacloprid MDL Results (µg) for XAD-2 resin on B tubes

Spike #	Carbaryl	Imidacloprid
1	0.452	0.380
2	0.446	0.429
3	0.423	0.429
4	0.430	0.436
5	0.428	0.373
6	0.441	0.357
7	0.370	0.424
S=	0.027	0.1032
MDL = 3.143 x S	0.085	0.5

Appendix: 2

Carbaryl and Imidacloprid Method Validation Results and Recovery on XAD-2 resin on A tubes

	Carbaryl		Imidacloprid	
Spike Level (µg)	Result (µg)	Recovery (%)	Result (µg)	Recovery (%)
0.5	0.404	80.8	.394	78.8
	0.354	70.7	0.470	94.0
	0.464	92.9	0.419	83.9
	0.382	76.4	0.425	85.0
	0.442	88.5	0.411	82.1
2	1.48	74.0	1.54	77.2
	1.45	72.5	1.60	80.0
	1.68	84.0	1.67	83.3
	1.54	77	1.69	84.5
	1.74	87.1	1.68	83.8
10	7.00	70.0	6.85	68.5
	7.71	77.1	8.26	82.6
	8.30	83.0	8.97	89.7
	7.40	74.0	7.22	72.2
	8.50	85.0	8.03	80.3

Carbaryl and Imidacloprid Method Validation Results and Recovery on XAD-2 resin on B tubes

	Carbaryl		Imidacloprid	
Spike Level (µg)	Result (µg)	Recovery (%)	Result (µg)	Recovery (%)
0.5	0.390	77.9	0.390	78.0
	0.381	76.2	0.428	85.6
	0.425	84.9	0.439	87.8
	0.418	83.6	0.439	87.8
	0.468	93.6	0.395	79.0
2	1.40	70.0	1.50	75.0
	1.50	75.0	1.75	87.6
	1.68	83.8	1.76	87.9
	1.77	88.5	1.86	93.7
	1.80	90.0	1.42	71.0
10	7.90	79.0	8.17	81.7
	7.66	76.6	8.29	82.9
	9.17	83.8	8.76	87.6
	7.75	77.5	7.96	79.6
	8.90	89.0	7.21	72.1

Appendix: 3
Trapping Efficiency results for carbaryl at flow rate 3.0 mL/min and a time of 24 hrs

Sample #	Spike level	µg for A	%	µg for B
197-602	0.5	0.355	71.0	N/D
197-606	0.5	0.345	72.0	N/D
197-607	0.5	0.355	71.0	N/D
197-603	2.0	1.39	69.3	N/D
197-604	2.0	1.44	72.0	N/D
197-630	2.0	1.37	68.5	N/D
197-608	10	8.47	84.7	N/D
197-609	10	7.80	78.0	N/D
197-610	10	8.14	81.4	N/D
197-605	blank	N/D		N/D