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DETERMINATION OF CARBARYL AND IMIDACLOPRID ON DISLODGEABLE LEAF PUNCH SAMPLES

Scope: This method is for the determination of carbaryl and imidacloprid on dislodgeable leaf punch samples. The reporting limit of this method is 0.5 µg for carbaryl and 1.0 µg for imidacloprid.

Principle: Carbaryl and imidacloprid are washed from leaf punch samples with water and surfactant. The combined water washings are extracted with methylene chloride. After solvent evaporation, the extract is dissolved in methanol. Subsequently, Carbaryl and Imidaclodrid are quantified using a HPLC. Carbaryl is derivatized with OPA in a post column 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. Methylene chloride, pesticide residue grade
5. Sodium sulfate, anhydrous granular (ACS)
6. 2% Sur-ten solution, (1g/50 mL of Aerosol® OT 75% aqueous) American Cyanamid Co., Wayne, NJ
7. Dislodgeable Washing solution, (0.4 mL of 2% Sur-ten solution in 500 mL distilled water)
8. Water, HPLC grade
9. Acetonitrile, HPLC grade
10. Hydrolysis reagent C47™, Pickering Laboratories, part# CB130
11. O-Phthalaldehyde, Pickering Laboratories, part# 0120
12. Thiofluor™, N,N-Dimethyl-2-mercaptoethylamine-Hydrochloride, Pickering Laboratories, part# 3700-2000
13. 2-Mercapto-ethanol, Pickering Laboratories, part# CB910
14. 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. Rotator, 30 rpm
2. Separatory funnels, 250 mL
3. Boiling flasks, flat bottom, 24/40 joints, 250 mL
4. Funnels, glass short stemmed 100 mm diameter
5. Rotary evaporator, Buchi/Brinkmann, R110
6. Conical test tubes, graduated, calibrated 15 mL
7. Nitrogen evaporator, Organomation, Model 12
8. Syringe, Hypodermic, 5 mL
9. 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 on background leaf punches 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 prepared 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. Samples need to be extracted the same day as received.

Procedure:

1. Add 50 mL of dislodgeable washing solution to the jar containing the leaf punches.
2. Rotate for 30 minutes.
3. Decant the aqueous solution into a 250 mL separatory funnel.
4. Repeat steps 1 through 3 two more times.
5. Extract aqueous solution with 50 mL of methylene chloride and shake for 1 minute.
6. After phase separation, drain the methylene chloride through a glass funnel containing glass wool and ~ 15 grams of sodium sulfate. Collect the extract into a boiling flask.
7. Repeat steps 5 and 6 two more times.
8. Evaporate the methylene chloride to near dryness on a rotary evaporator with the water bath set at 30 °C and 15 inches of vacuum.
9. Add ~ 5 mL of methanol and return the flask to the rotary evaporator for a few minutes. Then quantitatively transfer the extract to a volumetric centrifuge tube and wash the flask with another 5 mL of methanol. Transfer the washes to the corresponding tube.
10. Nitrogen evaporate to a final volume of 10 mL with a gentle stream of nitrogen and a water bath temperature of 35 °C.
11. Filter the extract through a 0.2 μ Acrodisc into 2 autosampler vials.

Preparation of blanks and Spikes

Blank: Background leaves were punched and treated the same as samples.

Spike: Background leaves were punched and spiked after all dislogdeable washes were done, but before methylene chloride was added.

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

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 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 temperature. The retention time of each compound may shift if temperatures change.

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 1 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:

$$MDL = tS$$

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.

Analysis: continued

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 0.5 μg and 1μg for imidacloprid.

Recovery Data:

The analytical method was validated using six 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 method originally bifenthrin was included, but had poor recovery during validaton at the 20 μg and 200 μg spiking level. It was determined that at lower levels bifenthrin could be extracted out of the dislodgeable washing solution, but at the 20μg level it started to give inconsistent results and at the 200 μg level the recovery was low. Further work is needed to solve this problem.

References:

1. Margetich, Sheila, *Carbamates Screen,*,1985, Worker, Health and Safety Methods, 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, Environmental Monitoring Methods, 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 (ug) for dislodgeable leaf punches

Spike #	Carbaryl	Imidacloprid
1	1.08	0.981
2	1.03	1.16
3	1.00	1.23
4	1.04	1.10
5	0.982	1.05
6	1.03	1.01
7	1.00	1.28
S=	0.03	0.113
MDL = 3.143 x S	0.10	0.355

Appendix: 2

Carbaryl and Imidacloprid Method Validation Results and Recovery on dislodgeable leaf punches

	Carbaryl		Imidacloprid	
Spike Level (µg)	Result (µg)	Recovery (%)	Result (µg)	Recovery (%)
2	1.98	99.2	1.99	99.3
	1.99	99.7	2.22	111.2
	1.40	70.1	1.42	71.0
	1.90	95.0	1.81	90.3
	1.86	93.2	2.01	100.5
	1.59	79.6	1.65	82.5
20	17.0	85.1	18.9	94.7
	17.6	88	18.8	93.9
	15.9	79.5	17.7	88.4
	17.9	89.4	18.0	89.9
	17.5	87.5	19.7	98.3
	24.0	119.9	20.6	103.1
200	170	85.1	199	99.6
	185	92.5	195	97.4
	184	92.0	192	95.9
	193	96.3	189	94.4
	178	88.9	186	92.9
	169	84.5	197	98.4