CALIFORNIA DEPT. OF FOOD & AGRICULTURE
Center for Analytical Chemistry
Environmental Monitoring Section
3292 Meadowview Road
Sacramento, CA 95832
(916) 262-2080 FAX (916) 262-1572

Method #: EM 12.7
Original Date: October 1, 2002
Revised Date:

HPLC Determination of Total Imidacloprid in Vegetation

Scope:

This method is for the determination of total imidacloprid in vegetation. The reporting limit for this method is 0.05 ppm for oleander sprig and 1.0 ppm for liquid amber sprig.

Principle:

Sample is ground with acetonitrile. The extract is then cleaned up with a series of solid phase cartridges. The final extract is analyzed by reverse-phase using HPLC with a UV detector. Imidacloprid at these levels can be confirmed by LC/MS/MS using triple quadropole or ion trap mass spectrometer.

Safety:

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

Interference:

A clean-up procedure is necessary to eliminate interferences found in the material used to validate this method.

Reagents, Equipment and Instrument:

Reagents:

1. Imidacloprid Standard; 1.0 mg/mL in methanol, obtained from CDFA Standard Repository section; CAS registry number: 105827-78-9
2. Acetonitrile, HPLC grade
3. Methanol, HPLC grade
4. Methylene Chloride, HPLC
5. Water, HPLC grade
6. Sodium Chloride, granular, anhydrous

Equipment:

1. Blender with stainless steel cup
2. Amber bottles with cap, 250 mL
3. Nitrogen evaporator, N-EVAP™, Organomation Associates, Inc; Model 112
4. Vortex mixer, Thermolyne, Model 37600
5. Disposable filter, Acrodisc®, Gelman, 25 mm x 0.2 μm
7. Funnel, long stem, 60°, 100 mm diameter

Reagents, Equipment and Instrument: continued

8. Conical test tubes, graduated, 15 mL
9. C_{18} Bond-Elute cartridge
10. NH_2 Bond-Elute cartridge
11. Filter paper, E&K Scientific, sharkskin, 18.5 cm
12. Variable transformer, Superior, Powerstat

Instrument:

1. HPLC: Hewlett-Packard 1050 Liquid Chromatograph with ChemStation and UV detector
2. Analytical column: Beckman Ultrasphere, ODS, 5μ x 4.6 mm x 25 cm

Standard Preparation:

1. Dilute the 1 mg/mL imidacloprid standard with methanol to make up a series of standard solutions. These standard solutions will be used for spiking, instrument calibration and sample calculation.
2. Keep all prepared standards in the designated refrigerator or freezer for storage when not in use.
3. The expiration date of each standard is six months from the preparation date.

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 an EMON number.
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. Bring sample to room temperature if frozen.
2. Cut sample into small pieces (including stem and twigs as applicable).
3. Weigh-out 50.0 grams of sample and put in stainless steel blender cup.
4. Add 200 mL acetonitrile.
5. Blend at eighty setting on variable transformer dial for two minutes.
6. Filter the extract through sharkskin filter paper into 250 mL bottles with 10 grams of sodium chloride.
7. Shake vigorously for 2 minutes and let stand 30 to 40 minutes.

Sample clean up for Oleander Sprig

a) Take 10 mL aliquot of extract and concentrate to less than 0.1 mL on the N-EVAP™.
b) Suspend the extract in 2 to 3 mL of methanol.
c) Condition a C_{18} Bond-Elute cartridge by rinsing with 5.0 mL of methanol on a vacuum manifold.
d) Load the extract suspension on the cartridge and collect the eluant in a test tube.
e) Rinse the test tubes 2 times with 3 mL methanol. Collect each of the cartridge rinses in the same test tube.

Analysis: continued

f) Concentrate the eluant to less than 0.1 mL on the N-EVAP™.
g) Suspend the extract in 2 mL of 1% methanol in methylene chloride.
h) Condition a NH_2 Bond-Elute cartridge by rinsing with 5 mL of 1% methanol in methylene chloride on a vacuum manifold.
i) Load the extract suspension on the cartridge and collect the eluant in a test tube.
j) Rinse the test tubes 2 times with 3 mL 1% methanol in methylene chloride. Collect each of the cartridge rinses in the same test tube.

Sample clean up for Liquid Amber Sprig

a) Take 1 mL aliquot of extract and concentrate to less than 0.1 mL on the N-EVAP™.
b) Suspend the extract in 2 to 3 mL of methanol.
c) Condition a C_{18} Bond-Elute cartridge with 5.0 mL of methanol on a vacuum manifold.
d) Load the extract suspension on the cartridge and collect the eluant in a test tube.
e) Rinse the test tubes 2 times with 3 mL methanol. Collect each of the cartridge rinses in the same test tube.
f) Concentrate the eluant to less than 0.1 mL on the N-EVAP™.
g) Suspend the extract in 2 mL of 10% methanol in methylene chloride.
h) Condition a NH_2 Bond-Elute cartridge by rinsing with 5 mL of 10% methanol in methylene chloride on a vacuum manifold.
i) Load the extract suspension on the cartridge and collect the eluant in a test tube.
j) Rinse the test tubes 2 times with 3 mL 1% methanol in methylene chloride. Collect each of the cartridge rinses in the same test tube.

8) Concentrate the extract to less than 0.1 mL and bring to 1.0 mL of methanol.
9) Filter the extract through 0.2 µm Acrodisc® into an auto sampler vial.

Preparation of blanks and spikes

Blank: Weigh out 50 g of the homogeneous background sample into a blender. Follow the sample extraction procedure outlined above.

Spike: Weigh out 50 g of the homogeneous background sample into a blender. Spike a known amount of imidacloprid into the sample. Mix well and let stand for at least one minute, them follow the sample extraction procedure outlined above.

Instrument Conditions

Instrument:	HPLC: Hewlett-Packard 1050, controlled by ChemStation
Column:	ODS 5µ x 4.6 mm x 25 cm
UV Detector:	270 ηm
Mobile phase:	Isocratic (70 % Water - 30 % Acetonitrile)
Flow:	1.0 mL/min.
Injection volume:	20 µL
Retention Time:	Imidacloprid ~ 5.5 minutes

Instrument calibration:

Load the method and run a set of calibration standards (0.1 ng/μL, 0.25 ng/μL, 0.5 ng/μL, and 1.0 ng/μL) to check system linearity.

Method Performance:

Quality Control:

1. A 4-point calibration curve of 0.1, 0.25, 0.5 and 1.0 ηg/μL is obtained at the beginning and the end of each sample set. The chemstation software is used to calculate sample result in ppm.
2. Each sample shall be injected two times to insure reliability of the analysis. If the signal of a sample is greater than that of the highest standard in the calibration curve, dilute the sample. Re-inject the diluted sample two more times together with standards.
3. Sample storage: All field samples shall be kept frozen at -10 °C.
4. Sample extracts: All extracts shall be kept refrigerated at 5 °C until analyzed.
5. Refrigerator and freezer temperature shall be monitored and recorded daily.
6. For each set of samples, a minimum of 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, glassware shall be washed following Environmental Monitoring standard operation procedure (SOP 502.6).
8. At least ten percent of the sample results shall be manually calculated to check instrument results. Hand calculated results are based on a single calibration point using the following equation:

$$\text{ppm} = \frac{\text{(sample peak ht.)(std. conc., μg/mL)(std. vol. inj., μL)(sample final vol., mL)}}{\text{(Std. peak ht.)(sample vol. injected, μL)(sample wt., g)}}$$

Recovery Data:

The method was validated by preparing three sets of spiked samples. Originally, each set contained a blank and three levels of spikes at 1.0 ppm, 10 ppm and 100 ppm. Later, two more levels of spike were added for oleander sprig at 0.1 ppm and 0.5 ppm. The background plant materials were specified by the Department of Pesticide Regulation and samples collected from the Meadowview complex. Sets were processed through the entire analytical method on separate days. Calculated recoveries for imidacloprid are tabulated in Appendix I.

Method Detection Limit (MDL):

The MDL refers to the minimum concentration of imidacloprid that can be detected in the material with 99% confidence. The MDL was computed based on the following procedure:

a) Prepare 7 replicates of imidacloprid at 0.1 ppm for each matrix.
b) Process each sample through the entire method along with a blank.
c) Calculate the percent recovery for each sample.
d) Calculate the standard deviation (S) for the percent recovery.
c) Compute the MDL as follows:

Method Performance: continued

MDL = t x S

where;

t is the Student 't' value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - α = 0.99) where n represents the number of replicates. For n=7, t=3.143.

S denotes the standard deviation obtained from replicate analyses.

The results for the standard deviations and MDL calculations are tabulated in Appendix II.

Reporting Limit (RL):

The RL refers to level above which quantitative results are reported. The MDL is used as a guide to determine the RL. In this method, the RL is set at 5 times or less the MDL. The imidacloprid RL is 0.05 ppm in oleander spring and 1.0 ppm in liquid amber.

Calculations:

Samples are calculated using a multilevel calibration curve of response versus concentration. The multilevel calibration curve is used to confirm linearity over the calibration range. The method of linear fit ignoring the origin is selected to create the curve. Each calibration level corresponds to a calibration standard of known concentration. Calibration standards should be prepared so that the imidacloprid concentration varies across the range of concentrations expected in the samples. The instrument calculate results (ICR) are obtained using the following equation:

$$\text{ICR (ppm)} = \frac{\text{(sample peak. ht.) (RF., µg/mL) (std. vol. injected, µL) (FV., mL)}}{\text{(sample vol. injected, µL) (sample wt., g)}}$$

and

$$\text{RF (µg/mL)} = \frac{\Sigma[\text{ (std. conc.}_n\text{, µg/mL) / (std. peak ht.}_n\text{)}]}{n}$$

n = number of standards

For oleander, the sample weight is 2.5 grams and the final volume is 1 mL (if no dilution is made). Therefore, a multiplier factor of 0.4 mL/g is put on the sequence table to account for final volume and sample weight on the ICR equation. For liquid amber, the sample weight is 0.25 grams and the final volume is 1 mL (if no dilution is made). Therefore, a multiplier factor of 4.0 mL/g is put on the sequence table. If sample has to be diluted, the sequence table dilution column needs to be entered with the proper dilution factor.

Acceptance Criteria:

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

Acceptance Criteria: continued

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

2. The samples are calculated based on the calibration curve before the samples are analyzed using the instrument chemstation software. If the results between the two duplicate injections differ by less than 10% either result can be reported. A change greater than 10 % with no known reason requires a third injection to check results agreement. More work shall be done if needed to obtain reproducible results.

Discussion:

David Hirano developed this method. David did the method MDL and validation for the 1 ppm, 10 ppm, 100 ppm spike levels. Jorge L Hernandez did the validation 0.1 ppm and 0.5 ppm spikes levels and wrote the method. It is our experience that imidacloprid is heat sensitive. To achieve acceptable recoveries, prolonged heating must be avoided and the recommended temperature must be followed during evaporation to prevent low recoveries. The imidacloprid calibration curve is linear in the selected range and any of the calibration points may be used for manual calculation. However, it is recommended for this calculation to use a standard with a peak height close to the peak height of the calculated sample.

References:

1. Duc Tran., *Determination of of Hexazinone in Plant Material by HPLC. Center for Analytival Chemist, Enviromental Monitoring Method# 11.3*
2. Ting, K.C., *High Performance Liquid Chromatographic Method for Determination of Six N-Methylcarbamates in Vegetables and Fruits, Bull. Environmental Contamination & Toxicology*, 1984, 33, 538-547
3. Keith, Lawrence H., etc., *Principals of Environmental Analysis, Anal. Chem.*, 1983, 55, 2210-2218

WRITTEN BY:

David Hirano and Jorge L. Hernandez

David Hirano

TITLE: Agricultural Chemist II

Jorge L Hernandez

TITLE: Agricultural Chemist II

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE: Laboratory Supervisor
Environmental Monitoring

Appendix I: Method Validation Results and Recoveries

	Imidacloprid Total			
	Oleander Sprig		Liquid Amber Sprig	
Spike Level (ppm)	Results (ppm)	%	Results (ppm)	%
0.1	0.099	99.0		
	0.092	92.0		
	0.088	88.0		
0.5	0.471	94.1		
	0.431	86.2		
	0.441	88.1		
1	0.756	75.6	0.901	90.1
	0.708	70.8	1.050	105.0
	0.803	80.3	1.015	101.5
10	7.207	72.1	9.401	94.0
	7.262	72.6	7.894	78.9
	9.212	92.1	7.752	77.5
100	82.831	82.8	86.795	86.8
	78.179	78.2	82.613	82.6
	88.651	88.7	70.398	70.4

Appendix II: MDL Determination

	Imidacloprid			
	Oleander Sprig		Liquid Amber Sprig	
Spike	ppm	%	ppm	%
Blank	ND		ND	
Spike 1	0.0808	80.8	0.6120	61.2
Spike 2	0.0795	79.5	0.6400	64.0
Spike 3	0.0896	89.6	0.4680	46.8
Spike 4	0.0763	76.3	0.5700	57.0
Spike 5	0.0798	79.8	0.5980	59.8
Spike 6	0.0743	74.3	0.6680	66.8
Spike 7	0.0699	69.9	0.6930	69.3
STDEV	0.0062	6.16	0.0742	7.42
MDL	0.0193		0.233	
RL	0.05		1.0	