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HPLC Determination of Imidacloprid in Surface and Well Water

Scope: This method is for the determination of imidacloprid in surface and well water. The reporting limit for this method is 0.05 ppb for both surface and well water.

Principle: Imidacloprid in water is extracted with methylene chloride. After evaporating the methylene chloride, the extracted residue is redissolved in methanol and separated by HPLC. The analyte is detected with an UV detector.

Safety:

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

Interference:

No background interference for imidacloprid in surface or well water was found in the water used to validate this method.

Reagents, Equipment and Instrument:

Reagents:

1. Imidacloprid Standard, 1.0 mg/mL in methanol, obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of Food and Agriculture)
2. Methylene chloride, pesticide residue grade
3. Methanol, pesticide residue grade
4. Water, HPLC grade
5. Acetonitrile, HPLC grade
6. Sodium sulfate, anhydrous, granular, ACS 10-60 mesh

Equipment:

1. Separatory funnels, 1000 mL
2. Boiling flasks, flat-bottomed, 24/40 joints, 500 mL
3. Rotary evaporator, Büchi-Brinkmann, Model R 110
4. Nitrogen evaporator, Organomation, Model 112
5. Vortex mixer, Thermolyne, Model 37600
6. Acrodisc®, Gelman, 25 mm x 0.2 µm, disposable filter
7. Funnel, long stem, 60°, 100 mm diameter
8. Conical test tubes, graduated, 15 mL

Reagents, Equipment and Instrument: continued

Instrument:

1. HPLC: Hewlett-Packard 1050 Liquid Chromatograph with ChemStation and UV detector
2. Analytical column: Beckman Ultrasphere 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 for storage when not in use.
3. The expiration date of each standard is six months 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 the EMON number from supervisor.
3. Store all samples waiting for extraction in the walk-in refrigerator.
4. Store all samples waiting for analysis in a refrigerator.

Analysis:

Sample Extraction:

1. Remove samples from refrigerated storage and allow them to come to room temperature (± 5 °C).
2. Shake each sample well and weigh out approximately 500 grams by difference. Place this aliquot into a separatory funnel. Record the sample weight.
3. Extract samples by adding 100 mL of methylene chloride and shaking vigorously for one minute. **Vent frequently to relieve pressure.**
4. After phase separation, drain the methylene chloride through ~ 25-30 g of anhydrous sodium sulfate and glass wool in a glass funnel. Collect the extract into a 500 mL boiling flask.
5. Repeat steps 3 and 4 two more times using 80 mL portions of methylene chloride.
6. After draining the final extract, rinse the sodium sulfate with ~ 25 mL of methylene chloride.
7. Concentrate the extract to 2 ~ 3 mL on a rotary evaporator using 30 ~ 35 °C water bath and a vacuum of 15 inches Hg.
8. Filter the extract through a 0.2 μm Acrodisc® unit and collect the filtrate into a calibrated centrifuge tube.
9. Rinse the flask two times with 2 mL aliquots of methylene chloride. Filter through the same Acrodisc® and collect the rinse in the same centrifuge tube.
10. Place extract in a nitrogen evaporator with the water bath set to 35 °C and evaporate just to dryness under a gentle stream of nitrogen.
11. Pipette 1 mL of methanol and mix contents by vortexing for about 15 seconds
12. Transfer the contents to an autosampler vial.
13. Analyze the extract by HPLC.

Preparation of blanks and spikes

Blank: Weigh out 500 ±0.1 g of the homogeneous background sample into a separatory funnel. Follow the sample extraction procedure outlined above.

Spike: Weigh out 500 ±0.1 g of the homogeneous background sample into a separatory funnel. Spike a known amount of imidacloprid into the sample. Mix well and let stand for at least 1 minute, then follow the sample extraction procedure outlined above.

Instrument Conditions

Instrument:	HPLC: Hewlett-Packard 1050, controlled by ChemStation
Column:	Beckman Ultrasphere 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 ~ 5.9 minutes

Instrument calibration:

Load a method and run a set of calibration standards (0.025 ng/μL, 0.05 ng/μL, 0.1 ng/μL, 0.5 ng/μL, and 1.0 ng/μL) to check system linearity.

Method Performance:

Quality Control:

1. A 5-point calibration curve of 0.025, 0.05, 0.1, 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 μg.
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 refrigerated at 5 °C.
4. Sample extracts: All extracts shall be kept refrigerated at 5 °C until analyzed.
5. Refrigerator 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, 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:

$$ppb = \frac{\text{(sample peak ht.)(std. conc., ηg/μL)(std. vol. inj., μL)(sample final vol., mL)(1000μL/mL)}}{\text{(Std. peak ht.)(sample vol. injected, μL)(sample wt., g)}}$$

Method Performance: continued

Recovery Data:

The method was validated by preparing five sets of spiked samples. Each set contained a blank and five levels of spikes. The background waters (American River and well water) were obtained from the Department of Pesticide Regulation. Sets were processed through the entire analytical method on separate days. Calculated recoveries for imidachloprid are tabulated in Appendix I.

Method Detection Limit (MDL):

The MDL refers to the minimun concentration of imidacloprid that can be detected in surface and well waters with 99% confidence. The MDL was computed based on the following procedure:

a) Prepare 7 replicates of imidacloprid at 0.1 ppb 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:

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

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. 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 ppb.

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 correspond 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.

$$\text{ppb} = \frac{(\text{instrument calculated results., } \mu g)\ (1000\ ng/\mu g)}{(\text{sample wt., g})}$$

where instrument calculated results (ICR):

Calculations: continued

$$\text{ICR } (\mu g) = \frac{(\text{sample peak. ht.})\ (\text{RF., } \mu g/mL)\ (\text{std. vol. injected, } \mu L)\ (\text{FV., mL})}{(\text{sample vol. injected, } \mu L)}$$

and

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

n = number of standards

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:

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

2. The samples are calculated based on the calibration curve before the samples using the instrument chemstation software. If the results between the two 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:

The method MDL and validation 1 and 2 were done by Jean Hsu. 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:

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4. Krause, R.T., *Further Characterization and Definment of an HPLC Post Column Fluorometric Labeling Technique for the Determination of Carbamate Insecticide, J. Chromatographic Science*, 1978, 16, 281-288
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Appendix I: Method Validation Results and Recoveries

	Imidachloprid			
	Surface Water		Well Water	
Spike Level (ppb)	Results (ppb)	%	Results (ppb)	%
0.1	0.118	118	0.092	92.0
	0.100	100	0.089	89.0
	0.099	99.0	0.105	105
	0.079	79.0	0.076	76.0
	0.097	97.0	0.081	81.0
0.25	0.259	104	0.232	92.8
	0.254	102	0.240	96.0
	0.240	96.0	0.249	99.6
	0.244	97.6	0.237	94.8
	0.241	96.4	0.214	85.6
0.5	0.510	102	0.485	97.0
	0.500	100	0.308	61.6
	0.486	97.2	0.477	95.4
	0.508	102	0.449	89.8
	0.490	98.0	0.485	97.0
1.0	1.01	101	0.923	92.3
	0.984	98.4	1.06	106
	0.980	98.0	0.914	91.4
	1.12	112	0.974	97.4
	0.854	85.4	0.937	93.7
5.0	5.11	102	5.59	112
	5.04	101	4.10	82.0
	4.54	90.8	5.07	101
	5.29	106	4.99	99.9
	5.14	103	5.00	100

Appendix II: MDL Determination

	Imidachloprid			
	Surface Water		Well Water	
Spike	ppb	%	ppb	%
Blank	ND		ND	
spk 1	0.0986	98.6	0.0978	97.8
spk 2	0.0968	96.8	0.0874	87.4
spk 3	0.0927	92.7	0.0890	89.0
spk 4	0.0898	89.8	0.0971	97.1
spk 5	0.0949	94.9	0.0800	80.0
spk 6	0.0977	97.7	0.0920	92.0
spk 7	0.0970	97.0	0.0912	91.2
STDEV	0.0031		0.0061	
MDL	0.010		0.019	
RL	0.05		0.05	