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Method #: 50.6
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Revised:

Oryzalin in Surface Water

Scope: This method is for the determination of oryzalin in surface water. The reporting limit for oryzalin is 0.08 µg/L.

Principle: Residue of oryzalin is extracted from surface water with dichloromethane. The extract is evaporated to dryness on a rotary evaporator and brought to 1 mL final volume with methanol. The oryzalin residue is analyzed by HPLC with a UV detector at 280 ηm.

Reagents:

1. Dichloromethane (pesticide residue grade)
2. Methanol (pesticide residue grade)
3. Oryzalin, CAS registry number 333-41-5, 1 mg/mL in acetone obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of food and Agriculture).
4. Sodium sulfate, anhydrous, granular (ACS)

Safety:

All general laboratory safety rules must be followed.

Equipment:

1. Rotary evaporator (Büchi/Brinkman, R110)
2. Nitrogen evaporator (Organomation Model #112)
3. Vortex vibrating mixer for test tubes
4. Balance (Mettler AC100)
5. Separatory funnels, 2000 mL
6. Flasks, flat-bottomed, evaporating, 500 mL
7. Funnels, 60°, 100 mm, short-stem
8. Test tubes, 15 mL, glass-stoppered, graduated, calibrated
9. Pipettes, volumetric, assorted sizes
10. Beakers, assorted sizes
1. Nylon Acrodisc®, 0.2 micron, Gelman Sciences

Instrument:

Hewlett Packard HPLC Model 1050 with autosampler and variable wave length UV-Visible detector.

Interference:

There are no interferences for oryzalin in the background at this time.

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 refrigerator.

Procedure:

1. Remove sample from refrigerated storage and allow coming to room temperature.
2. Mix sample by inverting several times.
3. Weigh approximately 800 g sample into a 2000 mL separatory funnel.
4. Add 100 mL dichloromethane to the separatory funnel. Stopper the funnel and shake funnel for 1.5 minutes, venting often to relieve pressure.
6. Allow phases to separate completely. Drain the dichloromethane layer into a 500 mL boiling flask through a glass wool-plugged funnel containing 20 g sodium sulfate.
7. Repeat the extraction two more times, using 80 mL dichloromethane each time. Combine the extracts into the 500 mL boiling flask.
8. Rinse the sodium sulfate with ~ 10 mL dichloromethane after the last extraction.
9. Evaporate the extract on a rotary evaporator in a 35°C - 40°C water bath with a vacuum of 15 - 20 inches Hg and evaporate just to dryness. Immediately after evaporation, add 4 mL methanol to the flask and swirl the solvent to rinse the sides of the flask.
10. Filter the extract through a 0.2 micron acrodisc and transfer the extract to a 15 mL graduated test tube.
11. Rinse the flask two times with ~ 3 mL methanol each rinse. Filter through the same acrodisc and collect in the same tube.
12. Place sample extract on a nitrogen evaporator with water bath set at 35°C - 40°C and evaporate to less than 1 mL under a gentle stream of nitrogen. Adjust to a final volume of 1.0 mL with methanol.
13. Stopper the test tube and mix sample with a vortex mixer for approximately 10 seconds.
14. Transfer the sample extract into two autosampler vials with insert for HPLC analyses.

Instrument conditions:

Hewlett Packard HPLC model 1050 with UV-Visible detector
Column: Beckman ODS 5 μ, 4.6 mm x 25 cm
Mobile phase: 43% water and 57% Acetonitrile.
Flow rate: 1 mL/min.
Injection volume: 20 μL
Detector wave length: 280 ηm
Retention time: Oryzalin, 10.4 ± 0.2 min.

Calculations:

$$\textbf{ppb} = \frac{(\text{Area Sample Peak})(\text{Final Volume, mL})(1000\ \text{g/Kg})}{(\text{Response Factor})(\text{Sample weight, g})}$$

Where Response Factor = $[\sum(\text{Area of Standard Peak/Conc. of Standard, } \mu g/mL)]/ n$
Where: n = number of standards

Analysis:

Quality Control:

1. A five-point calibration curve of 0.04, 0.08, 0.16, 0.25,.and 0.5 ηg/μL oryzalin was obtained at the beginning and the end of each set of samples.
2. Each sample was analyzed two times to insure reliability of the chromatography. If the signal of the sample was greater than that of the highest concentration of the calibration curve, the sample was diluted within the calibration range and reanalyzed.
3. For each set of samples, one matrix blank and one matrix spike were included, and each set of samples did not contain more than twelve samples.

Method Detection Limit (MDL):

Method Detection Limit refers to the lowest concentration of analyte that a method can detect reliably in either a sample or blank. To determine the MDL, seven-800 g blank background river water samples were fortified with 0.08 μg of oryzalin. The background river water was provided by Department of Pesticide Regulation. These spiked samples along with a blank were analyzed using the described method. The standard deviation derived from the seven spike samples was used to calculate the MDL using the following equation:

$$\textbf{MDL} = t\,S$$

where:

t is the Student 't' value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - $\alpha = 0.99$), which is 3.143. n represents the number of replicates.

S denotes the standard deviation obtained from replicate analyses.

MDL Recoveries for Oryzalin:

Spike	Oryzalin Result (μg/L)
1	0.092
2	0.099
3	0.088
4	0.092
5	0.095
6	0.095
7	0.083

The standard deviation for oryzalin is 0.0050 μg/L. The MDL is 0.0157 μg/L

Reporting Limit (RL):

It refers to the level above which quantitative results may be obtained. The MDL was used as a guide for determining the RL. The reporting limit for oryzalin is 0.08 µg/L.

Recovery Data:

Method validation was performed by spiking 800 ± 1 g of background surface water with four different levels (0.1, 0.2, 0.5 and 1.0 µg/L) of oryzalin for five replicates. Spike samples at two levels, 0.1 and 1.0 µg/L, were validated by P. Lee in September 1992. Spike samples at the other levels, 0.2 and 0.5 µg/L, were validated by J. Hernandez in December 2000. Recovery results are summarized in the table below.

Method Validation Recoveries of Oryzalin:

Analyte	**Spike Level** (µg/L)	**Result** (µg/L)	**Recovery** (%)
Oryzalin	0.10	0.092	92.0
		0.096	96.0
		0.10	100
		0.10	100
		0.089	89.0
	0.20	0.190	95.0
		0.192	96.0
		0.188	94.0
		0.187	93.5
		0.185	92.5
	0.50	0.452	90.4
		0.447	89.4
		0.503	100.6
		0.483	96.6
		0.463	92.6
	1.00	0.91	91.0
		0.80	80.0
		0.94	94.0
		0.96	96.0
		0.95	95.0

Discussion:

The background American river water used for the determination of Method Detection Limit and Method Validation was very clean at this time. Therefore, the MDL is low and the quality control chart is tight. When the water samples have significant interference, the Reporting Limit may become higher.

References:

1. Lee, Paul, *HPLC Determination of Surflan in Water,* 3-24-92, Environmental Monitoring Methods, California Department of Food and Agriculture.

2. Hsu, Jean, *Oryzalin and Napropamide in well water, 2-7-97,* Environmental Monitoring Methods, California Department of Food and Agriculture.
3. *Determination if Nitrogen and Phosphorus Containing Pesticides In Water By Gas Chromatography With A Nitrogen Phosphorus Detector*, EPA Manual of Analytical Methods 500 Series. Revision 2.0, 1989.

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