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

Determination of Propanil in Surface Water using Liquid Chromatography with Ultra Violet Detector and Positive Ion Electrospray Ionisation Mass Spectrometry

Scope: This method is for the determination of Propanil in surface water. The reporting limit of this method is 0.05 ppb.

Principle: Propanil in water is extracted with methylene chloride. After evaporating the methylene chloride, the extracted residues are redissolved in methanol and analyzed by LC/UV. The extracted residues can also be analyzed using ESI/LC/MS/MS.

Reagents:

1. Propanil 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 and LCQ grade. Burdick & Jackson 230-4
4. Water, HPLC grade and LCQ grade. Burdick & Jackson 365-4
5. Acetonitrile, HPLC grade
6. Sodium sulfate, anhydrous, granular, ACS 10-60 mesh
7. Acetic Acid, HPLC grade (Fisher #A35-500 or equivalent)

Safety:

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

Equipment:

1. Separatory funnels, 1 L
2. Boiling flasks, flat-bottomed, 24/40 joints, 500 mL
3. Rotary evaporator, Büchi-Brinkmann, Model R 110
4. Nitrogen evaporator, Organomation, Model 12
5. Conical test tube with glass stopper, 15 mL, graduated
6. Vortex mixer, Thermolyne, Model 37600
7. Acrodisc®, Gelman, 25 mm x 0.2 µm, disposable filter

Instrument:

1. LC/UV: Hewlett-Packard 1050 with the ChemStation and UV Detector
2. ESI/LC/MS/MS: Waters 2690 LC system connected to a Finnigan Deca LCQ MS

Interference:

There are no interferences for Propanil in the background water provided.

Standard Preparation:

1. The 1mg/mL standards are diluted to 10 μg/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 them.
2. Sign the chain of custody and obtain the EMON number from supervisor.
3. Store all samples waiting for analysis in the walk-in-refrigerator.

Procedure:

1. Remove samples from refrigerated storage and allow them to come to room temperature (± 5 °C).
2. Shake each sample and weigh out 500 grams by difference. Place this aliquot into a separatory funnel.
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 lower methylene chloride layer through 25 ~ 30 g of anhydrous sodium sulfate, into a 500 mL boiling flask.
5. Repeat steps 3 and 4 two more times with 80 mL of methylene chloride each time.
6. After draining the final extraction, 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 in a conical test tube.
9. Rinse the flask two times with 2 mL of methylene chloride each. Filter through the same Acrodisc® and collect the rinse in the same test tube.
10. Place extract in a nitrogen evaporator with water bath set at 35 °C and evaporate to dryness under a gentle stream of nitrogen.
11. Pipet 1.0 mL of methanol and mix contents by vortexing for about 15 seconds.
12. Transfer the contents into an autosampler vial for analysis.

Instrument Conditions:

LC/UV

Instrument:	HPLC, Hewlett-Packard Model 1050, controlled by Chemstation
Column:	Beckman Ultrasphere C18 4.6 mm x 25 cm x 5 μm
Mobile phase:	Isocratic 40 % water and 60 % acetonitrile
Flow:	1.0 mL/min.
Injection volume:	20 μL
UV detector:	250 ηm
Retention Time:	5.66

Instrument Conditions continued:

ESI/LC/MS/MS

HPLC System and Operating Parameters

Instrument: Waters model 2690 HPLC, gradient pump, autosampler, column heater with remote control through the Finnigan Xcalibur system

Detector: Finnigan LCQ Deca Mass Spectrometer

Column: Phenomenex C18 Luna 3μ 50 mm x 3 mm x 3 μ (part number: 00B-4251-Y0)

Precolumn: Phenomenex C18 4 mm L x 2.0 mm ID cartridge (part number: AJ0-4286)

Column Temp: 40 °C

Mobile phase: Gradient Program

Solvents A: 0.1% acetic acid in methanol

B: 0.1 % acetic acid in water

Time (min)	Flow (mL/min.)	A (%)	B (%)
0.0	0.50	20.0	80.0
1.0	0.50	20.0	80.0
2.0	0.50	20.0	80.0
7.0	0.50	90.0	10.0
10.0	0.50	90.0	10.0
12.0	0.50	20.0	80.0
14.0	0.50	20.0	80.0

Injection volume: 25 µL

Retention Time: 8.27

Mass Spectrometry System and Operating Parameters

Instrumentation: Finnigan LCQ Deca, ion trap mass spectrometer with ESI ion source

Instrument control and data handling: Gateway computer model E-4200

Software: Xcalibur Version 1 SR1

MS run time: 11.00 min

Divert value: in use during run

Divert Time (min)	Valve State
0.00	To Waste
6.87	To Source
10.49	To Waste

Contact Closure: not used during run

MS Detector Settings:

Acquistion Start Delay (min): 7.00

Segment 1Information

Duration (min): 11.00

Number of Scan Events: 1

Tune Method: ESIFLOWACIDPROPANIL218

Scan Event Details

1: Pos (218)->(60.0-250) Parent ion 218

MS/MS: CE 28.0% IsoW 1.0 Daughter ion 162

Instrument Conditions continued:

Tune method: ESIFLOWACIDPROPANIL218

ESI Source settings:

Sheath Gas Flow Rate	29
Aux Gas Flow Rate	54
I Spray Voltage	4.50
Capillary Temp (°C)	225.00
Capillary Voltage (V)	3.00
Tube Lens Offset (V)	50.00

Optics Settings:

Octapole 1 Offset (V)	-7.50
Lens Voltage (V)	-18.00
Octapole 2 Offset (V)	-8.50
Octapole RF Amplitude (V p-p)	735.00
Entrance Lens (V)	-34.00

Automatic Gain Control: on

Full Mass Target	5 x e7
SIM	2 x e7
MSn Target	2 x e7
Zoom Target	2 x e7
Inject Waveform	off

Analysis:

Quality Control

1. A 5-point calibration curve of 0.025, 0.05, 0.1, 0.5 and 1.0ηg/μL was obtained at the beginning and the end of each set of samples for the response factor calculating.
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 together with standards twice more. A sample set is 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, 7 samples each containing 500 ± 1g of background water were spiked at 0.1 ppb and process each through the entire method along with a blank. The standard deviation computed from the 7 results (ppb) was used to calculate the MDL using the following equation.

$$MDL = S\,t$$

Analysis: continued

where:
t is the student's "t" value for the 99% confidence level with n-1 degrees of freedom (n-1,1-α = 0.99). n represents the number of replicates.
S denotes the standard deviation obtained from replicate analyses.

The results for the standard deviations and MDL are shown below.

Propanil MDL results for water using the LC/UV and ESI/LC/MS/MS

Spike #	Propanil on LC/UV	Propanil on ESI/LC/MS/MS
1	0.0887	0.078
2	0.0869	0.068
3	0.0856	0.070
4	0.0914	0.070
5	0.0706	0.068
6	0.0917	0.072
7	0.0952	0.066
S=	0.00740	0.003904
MDL = 3.143 x S	0.0233	0.0123

Reporting Limit (RL):

Reporting Limit (RL) refers to level above which quantitative results may be obtained. In this method the RL is set at 0.05 ppb.

Recovery Data:

Method validation was performed, by spiking background water with four different levels (0.1, 0.5, 1.0, and 10 ppb) of propanil for five replicates. The background water (American River water) was obtained from Department of Pesticide Regulation. Each set was processed through the entire analytical method. Recovery for the propanil is shown below.

Analysis: continued

Propanil Method Validation Results and Recovery using the LC/UV and ESI/LC/MS/MS

	LC/UV		ESI/LC/MS/MS	
Spike Level (ppb)	Results (ppb)	Recovery (%)	Results (ppb)	Recovery (%)
0.1	0.0914	91.4	0.086	86.0
	0.0965	96.5	0.097	97.0
	0.0862	86.2	0.078	78.0
	0.124	124	0.060	60.0
	0.091	90.6	0.085	85.0
0.5	0.448	89.6	0.381	76.2
	0.476	95.2	0.447	89.4
	0.485	97.0	0.420	84.0
	0.468	93.6	0.419	83.8
	0.474	94.7	0.404	80.8
1.0	0.963	96.3	0.976	97.6
	0.747	74.7	0.884	88.4
	0.966	96.6	1.03	103
	0.997	99.7	0.929	92.9
	0.930	93.0	0.754	75.4
10	9.59	95.9	10.1	101
	9.33	93.3	12.7	127
	9.02	90.2	10.8	108
	9.46	94.6	8.39	83.9
	9.37	93.7	10.6	106

Calculations:

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

$$\text{where: response factor } (\eta g) = \frac{\Sigma[\,(\text{std. conc.}_n, \eta g/\mu L)\,(\text{std. vol. injected, } \mu L)/\,(\text{std. peak ht})]}{n}$$

n = number of standards

Discussion:

This method was validated using both LC/UV and ESI/LC/MS/MS. The ESI/LC/MS/MS results for the validation covered a wider range than the LC/UV results. The LC/UV will be used as the primary instrument with the ESI/LC/MS/MS used for confirmation.

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WRITTEN BY: Jane White

Jane White

TITLE: Agricultural Chemist II

REVIEWED BY: Catherine Cooper

Catherine Cooper

TITLE: Agricultural Chemist III
Supervisor