

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

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Determination of Atrazine, Simazine, Diuron, Prometon, Bromacil, Prometryn, Hexazinone, Cyanazine, Metribuzin in River Water

Scope: This method is for the determination of atrazine, simazine, diuron, prometon, bromacil, prometryn, hexazinone, cyanazine, metribuzin in river water. The reporting limits for this method are: 0.05 ppb for atrazine, simazine, diuron, prometon, bromacil, prometryn, and 0.2 ppb for hexazinone, cyanazine, metribuzin.

Principal: Atrazine, simazine, diuron, prometon, bromacil, prometryn, hexazinone, cyanazine, metribuzin in river water are extracted with methylene chloride. The extract is evaporated to almost dryness, exchanged to methanol and passed through a conditioned C 18 sep-pak for HPLC-UV and GC-NPD analyses.

Reagents and Equipments:

Reagents:

1. Solvents: Acetonitrile, methanol, water (**HPLC** Grade)
Methylene chloride (Pesticide quality or equivalent)
2. Sodium sulfate- (**ACS**) Granular, anhydrous
3. Individual stock standard solutions (1 **mg/mL**): Obtain standards from Standards Repository, California Department of Food and Agriculture, Center for Analytical Chemistry, 3292 Meadowview Rd. Sacramento, CA 95832

Equipments:

1. Rotary Evaporator
2. Nitrogen evaporator, Organomation Model # 112
3. Boiling flask - 500-mL, with standard taper to fit rotary evaporator
4. Separator-y funnel - 1000-mL, with TFE stopcock
5. Graduated test tube - 15-mL
6. Syringe - **10-mL**
7. Graduated cylinders - **1000-mL, 250-mL**
8. **Acrodisc®**, 0.2 μm filter. **Gelman** Sciences
9. Balance - Analytical
10. C 18 sep-pak

Analysis:

Sample Extraction:

1. Remove **sample from** the refrigerator and bring it to room temperature.
2. Mix the sample well, weigh 500.0 **g** of the sample and transfer into a **1000-mL** separator-y **funnel**.

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Analysis:

Sample Extraction:(cont.)

3. Add 75 mL of methylene chloride to the separatory and gently shake for two minutes *with periodic venting to release excess pressure*. Allow the organic layer to separate from water layer. If the emulsion interface between layers occurs, the analyst must employ a mechanical technique such as stirring using a glass rod to complete the phase separation. Drain the bottom organic layer through a 75-cm funnel which contains glasswool and 40 g of sodium sulfate into a 500-mL boiling flask.
4. Repeat step # 3 two more times.
5. Evaporate the extract to just about dryness using a rotary evaporator set at 40 °C, and a vacuum of 20 inches Hg.
6. Transfer the residue from the flask into a 15-mL graduated test tube using 10 mL of methanol.
7. Condition a C18 sep-pak with 5 mL of methanol, pass the 10 mL extract through the conditioned C 18 sep-pak connected with a 0.2 µm HPLC filter into a 15-mL graduated test tube.
8. Concentrate the extract from 10 mL to 1 mL using a Nitrogen evaporator set at 40 °C.
9. Mix well and transfer the extract into two microvials. One is for HPLC- UV analysis and the other for GC-NPD analysis.

Instrument Condition:

HPLC-UV Parameter for atrazine, simazine, bromacil, diuron:

Instrument: HPLC HP-1050 with a UV Variable Wavelength Detector.

Detector: UV Variable Wavelength.

Wavelength: 280 nm.

Time table:	Wavelength
6.20 min.	238 nm
13.80 min.	280 nm

Column: Ultrasphere ODS 5 µm 4.6 mm x 25 cm.

Guard column: Ultrasphere ODS 5 µm 4.6 mm x 5 cm.

Mobile phase: Isocratic 40% ACN, 60% Water.

Flow rate: 1 mL per minute.

Injected volume: 20 µL.

Retention time:	Bromacil: 5.80 min.
	Simazine: 6.60 min.
	Atrazine: 10.30 min.
	Diuron : 11.20 min.

Stop time: 20 min.

HPLC-UV Parameter for hexazinone, cyanazine, metribuzin:

Instrument: HPLC HP-1050.

Detector: UV Variable Wavelength.

Wavelength: 238 nm.

Column: Ultrasphere ODS 5 µm 4.6 mm x 25 cm.

Guard column: Ultrasphere ODS 5 µm 4.6 mm x 5 cm.

Determination of Atrazine, Simazine, Diuron, Prometon, Bromscil, Prometryn, Hexazinone, Cyanazine and Metribuzin in River Water

Analysis:

Instrument Condition:

HPLC-UV Parameter for hexazinone, cyanazine, metribuzin:(cont.)

Mobile phase: Isocratic 30% ACN, 70% Water.

Flow rate: 1 mL per min.

Injected volume: 20 µL.

Retention time: Hexazinone: 8.68 min.

Cyanazine: **12.21 min.**

Metribuzin: **13.54 min.**

Stop time: 20 min.

GC-NPD parameter for atrazine, simazine, prometon, prometryn:

Instrument: GC HP- 6890.

Column: HP-35 35% Phenyl Methyl Siloxane 30 m x 0.53 mm x 1.0 µm

Oven temperature; Initial temp: 70 °C

Initial time : 1.00 min

Ramps: 10 °C per min.

Final temp: 280 °C

Final time: 5 min.

Run time: 27 min.

Detector: NP Detector

Temperature: 300 °C

Hydrogen flow: 3.0 mL/min.

Air flow: 60.0 mL/min.

Mode: Constant column + make up (helium) = 30.0 mL/min.

Adjust offset: 50.00

Injector: Splitless

Temperature: 250 °C

Pressure: 4.1 psi

Injected volume: 3 µL.

Retention time: Prometon: 15.87 min..

Atrazine: 16.21 min.

Simazine: 16.31 min.

Prometryn: 17.76 min.

Calculations:

The results to be reported in part per billion (ppb) :

$$\text{ppb (ng/g)} = \frac{\text{ng/}\mu\text{L (from standard curve)} \times \text{final volume (}\mu\text{L)}}{\text{Sample weight(g)}}$$

Method performance:

Quality Control :

1. Sample storage: All field samples shall be kept **refrigerated** at 4 °C until extracted.
2. Sample extraction: All extracts shall be kept **frozen** at -10 °C until analyzed.
3. Freezer, **refrigerator** and oven temperatures shall be monitored and recorded daily.

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Method performance:

Quality Control: (cont.)

4. A 3-point or more calibration curve shall be obtained at the beginning and the end of each set of samples.
5. For each set of samples, one matrix blank, one distilled water blank, and one matrix spike shall be included, and each set of samples shall not contain more than twelve samples. Each sample shall be injected two times to determine reproducibility of the analysis.

Recovery data:

The analytical method was validated by preparing five sets of sample. Each set contained four different levels of spike, a distilled water blank, and a matrix blank. Each set was processed through the entire analytical method at a different time and the following results were tabulated:

For Atrazine:

<u>Spiked levels</u> (ng/g)	<u>Results</u> (ng/g)	<u>Recovery</u> (%)
0.100	0.099	99.0
0.100	0.098	98.0
0.100	0.103	103
0.100	0.096	96.0
0.100	0.096	96.0
0.500	0.400	80.0
0.500	0.458	91.6
0.500	0.472	94.4
0.500	0.504	101
0.500	0.476	95.2
2.000	2.168	108
2.000	1.860	93.0
2.000	2.133	107
2.000	1.890	94.5
2.000	1.975	98.8
6.000	6.340	106
6.000	6.420	107
6.000	6.440	107
6.000	6.400	107
6.000	6.474	108

For Simazine:

0.100	0.108	108
0.100	0.098	98.0
0.100	0.099	99.0
0.100	0.099	99.0
0.100	0.125	125
0.500	0.447	89.4
0.500	0.468	93.6
0.500	0.503	101
0.500	0.529	106

Determination of Atrazine, Simazine, Diuron, Prometon, Bromacil, Prometryn, Hexazinone, Cyanazine and Metribuzin in River Water

Method performance:

Recovery data:

For Simazine:(cont.)

<u>Suiked levels</u>	<u>Results</u>	<u>Recovery</u>
(ng/g)	(ng/g)	(%)
0.500	0.487	97.4
2:000	1.881	94.1
2.000	1.845	92.3
2.000	2.126	106
2.000	2.063	103
2.000	2.027	101
6.000	6.294	105
6.000	6.000	100
6 . 0 0 0	6.440	111
6.000	6.400	107
6.000	6.372	106

For Diuron:

0.100	0.090	90.0
0.100	0.102	102
0.100	0.102	102
0.100	0.071	71.0
0.100	0.082	82.0
0.500	0.418	83.6
0.500	0.422	84.4
0.500	0.45 1	90.2
0.500	0.466	93.2
0.500	0.479	95.8
2.000	1.694	84.7
2.000	1.731	86.6
2.000	2.042	102
2.000	1.689	84.5
2.000	1.910	95.5
6.000	5.714	95.2
6.000	5.420	90.3
6.000	6.230	104
6.000	5.618	93.6
6 . 0 0 0	5.934	98.9

For Prometon:

0.100	0.096	96.0
0.100	0.085	85.0
0.100	0.089	89.0
0.100	0.095	95.0
0.100	0.083	83.0
0.500	0.464	92.8
0.500	0.415	83.0
0.500	0.464	91.8

Determination of Atrazine, Simazine, Diuron, Prometon, Bromacil, Prometryn, Hexaxinone, Cyanazine and Metribuzin in River Water

Method performance:

Recovery data:

For Prometon:(cont.)

<u>Soiked levels</u> (ng/g)	<u>Results</u> (ng/g)	<u>Recovery</u> (%)
0.500	0.428	85.6
0.500	0.409	81.8
2.000	1.950	97.5
2.000	1.820	91.0
2.000	2.157	108
2.000	1.765	88.3
2.000	1.684	84.2
6.000	5.604	93.4
6.000	4.956	82.6
6.000'	5.894	98.2
6.000	5.110	85.2
6.000	4.958	82.6

For Prometryn:

0.100	0.104	104
0.100	0.091	91.0
0.100	0.099	99.0
0.100	0.086	86.0
0.100	0.086	86.0
0.500	0.492	98.4
0.500	0.441	88.2
0.500	0.511	102
0.500	0.436	87.2
0.500	0.433	86.6
2.000	1.992	99.6
2.000	1.921	96.1
2.000	2.119	106
2.000	1.807	90.4
2.000	1.665	83.3
6.000	6.000	100
6.000	5.336	88.9
6.000	6.190	103
6.000	5.350	89.2
6.000	5.228	87.1

For Bromacil:

0.100	0.099	99.0
0.100	0.098	98.0
0.100	0.087	87.0
0.100	0.094	94.0
0.100	0.089	89.0
0.500	0.458	91.6
0.500	0.475	95.0

Determination of **Atrazine, Simazine, Diuron, Prometon, Bromacil, Prometryn, Hexazinone, Cyanazine** and **Metribuzin** in River Water

Method performance:

Recovery data:

For Bromacil:(cont.)

<u>Spiked levels</u> (ng/g)	<u>Results</u> (ng/g)	<u>Recovery</u> (%)
0.500	0.506	101
0.500	0.466	93.2
0.500	0.498	99.6
2.000	1.861	93.1
2.000	1.867	93.4
2.000	2.079	104
2.000	1.991	99.6
2.000	2.142	107
6.000	6.086	101
6.000	5.852	97.5
6.000	6.408	107
6.000	6.212	104
6.000	5.972	99.5

For Hexazinone:

0.300	0.298	99.3
0.300	0.299	99.7
0.300	0.298	99.3
0.300	0.307	102
0.300	0.365	122
0.500	0.428	85.6
0.500	0.453	90.6
0.500	0.524	105
0.500	0.444	88.8
0.500	0.534	107
2.000	1.927	96.4
2.000	1.951	97.6
2.000	2.009	101
2.000	2.066	103
2.000	2.100	105
6.000	5.984	99.7
6.000	5.530	92.2
6.000	5.980	99.7
6.000	5.900	98.3
6.000	6.244	104

For Cyanazine:

0.300	0.330	110
0.300	0.293	97.8
0.300	0.279	93.0
0.300	0.295	98.3
0.300	0.295	98.3
0.500	0.445	89.0

Determination of **Atrazine, Simazine, Diuron, Prometon, Bromacil, Prometryn, Hexazinone, Cyanazine and Metribuzin** in River Water

Method performance:

Recovery data:

For Cyanazine:(cont.)

0.500	0.475	95.0
0.500	0.455	91.0
0.500	0.467	93.4
0.500	0.465	-93.0
2.000	2.191	110
2.000	2.137	107
2.000	2.058	103
2.000	2.115	106
2.000	2.063	103
6.000	6.080	101
6.000	6.282	105
6.000	6.526	109
6.000	6.348	106
6.000	6.310	105

For Metribuzin:

0.300	0.296	98.7
0.300	0.289	96.3
0.300	0.287	95.7
0.300	0.259	86.3
0.300	0.271	90.3
0.500	0.434	86.8
0.500	0.458	91.6
0.500	0.462	92.4
0.500	0.438	87.6
0.500	0.452	90.4
2.000	1.863	93.2
2.000	1.859	93.0
2.000	1.827	91.4
2.000	1.937	96.9
2.000	1.777	88.9
6.000	5.400	90.0
6.000	5.862	97.7
6.000	5.836	97.3
6.000	5.358	89.3
6.000	5.984	99.7

Method detection limit:

Method Detection Limit (**MDL**) refers to the lowest concentration of analytes that a method can detect reliably. To determine the MDL, 7 replicated background samples were spiked at 0.050 μg (for atrazine, simazine, diuron, prometon, prometryn), and 0.200 μg (for hexazinone, cyanazine, **metribuzin**). The standard deviations derived from the spiked samples were used to calculate the MDL using the following equation:

Determination of Atrazine, Simazine, Diuron, Prometon, Bromacil, Prometryn, Hexazinone, Cyanazine and **Metribuzin** in River Water

Method performance:

Method detection limit:(cont.)

$$MDL = tS$$

where:

t is the Student t value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - α = **0.99**) which is 3.143, n represents the number of replicates which is 7. S denotes the standard deviation obtained **from replicate** analyses.

The MDL and RL were tabulated as follow:

<u>Chemical</u>	<u>Method detection limit (ppb)</u>	<u>*Reporting limit (ppb)</u>
Atrazine	0.026	0.050
Simazine	0.014	0.050
Diuron	0.031	0.050
Prometon	0.026	0.050
Bromacil	0.025	0.050
Prometryn	0.023	0.050
Hexazinone	0.048	0.200
Cyanazine	0.040	0.200
Metribuzin	0.062	0.200

*Reporting limit (**RL**) refers to the level which quantitative results may be obtained usually **1-5** times the MDL

Dicussion:

Standards for quantitation of prometon, atrazine, **simazine**, and prometryn by **GC/NPD** must be made from the matrix blank extracts to compensate for the matrix enhanced response.

Confirmations

All positive samples at reporting limits or above will be confirmed by **APCI-LC/MS/MS**.

WRITTEN BY: Duc Tran

APPROVED BY: Catherine Cooper



TITLE: Agricultural Chemist II



TITLE: Agricultural Chemist III

Determination of Atrazine, Simazine, Diuron, Prometon, Bromacil, Prometryn, **Hexazinone**, Cyanazine and **Metribuzin** in River Water

Appendix I: Recovery data for determination of method detection limits.

For Atrazine:

<u>Soiked level</u> (μg)	<u>Results</u> (μg)	<u>Recovery</u> (%)
0.050	0.048	96.0
0.050	0.046	92.0
0.050	0.047	94.0
0.050	0.056	112
0.050	0.046	92.0
0.050	0.042	84.0
0.050	0.048	96.0

For Simazine:

0.050	0 . 0 5 1	102
0.050	0.053	106
0.050	0.049	98.0
0.050	0.056	112
0.050	0.052	104
0.050	0.051	102
0.050	0.051	102

For Diuron:

0.050	0.045	90.0
0.050	0.045	90.0
0.050	0.046	92.0
0.050	0.055	110
0.050	0.053	106
0.050	0.041	82.0
0.050	0.047	94.0

For Bromacil :

0.050	0.040	80.0
0.050	0.044	88.0
0.050	0.040	80.0
0.050	0.048	96.0
0.050	0.042	84.0
0.050	0.039	78.0
0.050	0.049	98.0

For Prometon:

0.050	0.045	90.0
0.050	0.050	100
0.050	0.050	100
0.050	0.058	116
0.050	0.048	96.0
0.050	0.049	98.0
0.050	0.053	106.

Determination of **Atrazine, Simazine, Diuron, Prometon, Bromacil, Promettyn, Hexazinone, Cyanazine** and **Metribuzin** in River Water

Appendix I: *Recovery data for determination of method detection limits (cont.)*

For Prometryn:

<u>Spiked level</u> (μg)	<u>Results</u> (μg)	<u>Recovery</u> (%)
0.050	0.047	94.0
0.050	0.042	84.0
0.050	0.039	78.0
0.050	0.048	96.0
0.050	0.038	76.0
0.050	0.042	84.0
0.020	0.042	84.0

For Hexazinone:

0.200	0.187	93.5
0.200	0.196	98.0
0.200	0.191	95.5
0.200	0.180	90.0
0.200	0.184	92.0
0.200	0.184	92.0
0.200	0.201	101

For Cyanazine:

0.200	0.219	110
0.200	0.216	108
0.200	0.227,	114
0.200	0.228	114
0.200	0.220	110
0.200	0.215	108
0.200	0.231	116

For Metribuzin:

0.200	0.204	102
0.200	0.195	90.0
0.200	0.196	98.0
0.200	0.205	103
0.200	0.216	108
0.200	0.185	92.5
0.200	0.197	98.5