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Determination of Atrazine, Bromacil, Cyanazine, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Deethyl Atrazine (DEA), Deisopropyl Atrazine (ACET), and Diamino Chlorotriazine (DACT) in Well Water and River Water By Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry

Scope: This method is applicable to the analysis of Atrazine, Bromacil, Cyanazine, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Deethyl Atrazine (DEA), Deisopropyl Atrazine (ACET), and Diamino Chlorotriazine (DACT) in well water and river water using two different HPLC columns and LC/MS systems. Data obtained from these two columns and systems are presented in this method. The reporting limit for all chemicals is 0.05 ppb by APCI/LC/MS/MS.

Principle: Two conditioned Waters Oasis®MCX Cartridges connected in tandem are used to retain the analytes from well water and river water samples. The cartridges are placed under vacuum to eliminate any remaining water. The chemicals are eluted with 5% ammonium hydroxide in methanol. The eluant is then filtered, concentrated, reconstituted in 75/25 water / methanol and analyzed by APCI/LC/MS/MS. Two different HPLC columns have been validated to provide flexibility to the analyst, and to provide a means of confirmation for samples that have matrix interferences which are not present in the reference well water used for the validations.

Reagents, Equipment and Instruments:

Reagents:

1. Methanol, LCQ grade. Burdick & Jackson 230-4.
2. Distilled water, LCQ grade. Burdick & Jackson 365-4. Burdick & Jackson solvents are available from VWR and other suppliers.
3. Formic acid.
4. Ammonium for-mate.
5. Ammonium hydroxide.
   Note: The highest available purity reagents (1,2,3,4,5) should be specified when ordering.
6. Elution reagent: 5% ammonium hydroxide in methanol.
7. Reconstitution reagent: 75/25 water / methanol.
8. Mobil phase A: 95/5 (10 mM ammonium for-mate/methanol, 0.1% formic acid). For 500 mL mix 470 ± 2 mL Burdick & Jackson water, 25 ± 0.5 mL Burdick & Jackson methanol, 4.50 ± 0.25 mL 1 M ammonium for-mate, and 0.5 ± 0.05 mL formic acid. Double these quantities to prepare 1L.
9. Mobil phase B: 90/10 (methanol/O.1 M ammonium formate, 0.1% formic acid).
   For 500 mL, mix 450 ± 2 mL Burdick & Jackson methanol and 45 ± 0.5 mL Burdick & Jackson water with 4.5 ± 0.25 mL of 1 M ammonium for-mate and 0.5 ± 0.05 mL of formic acid. Double these quantities to prepare 1L.
10. Working standards in 75/25 water / methanol (diluted from stock standards).

Reagents: (cont.)

Chemicals	CAS *Registry Numbers*
Diamino Chlorotriazine (DACT)	3397-62-4
Deisopropyl Atrazine (ACET)	11007-28-g
Deethyl Atrazine (DEA)	6190-65-4
Metribuzin	2 1087-64-g
Bromacil	3 14-40-g
Atrazine	19 12-24-9
Norflurazon	27314-13-2
Cyanazine	21725-46-2
Simazine	122-34-g
Hexazinone	5 1235-04-2
Diuron	330-54-l
Prometon	1610-18-O
Prometryn	7287-19-6
Propazine (surrogate)	139-40-2

Equipment:

1. In-house vacuum manifold.
2. Solid phase extraction cartridges: Waters Oasis@ MCX 6 cc (150 mg), 60-micron particle size cartridge, Waters Division of Millipore Corporation.
3. Nylon Acrodisc®, 0.2 micron, Gelman Sciences.
4. Vat-Elut SPS 24, Varian Analytical.
5. N-EVAP, Meyers Organomation Associates Incorporated-Model 112.
6. Vibrating or vortex mixer.
7. Syringe and plunger for filtration, 10 mL.
8. Graduated test tube, 15 mL (calibrated at 0.5 mL with methanol).
9. Fiberglass filters, 1μm x 47 mm.
10. Sample filtration apparatus.

Instruments:

System A:

1. LCQ™DECA LC/MS^n System. ThermoQuest/Finnigan Corporation
2. Waters 2690 HPLC system with autosampler.

System B:

1. LCQ™LC/MS^n System. ThermoQuest/Finnigan Corporation
2. ThermoQuest/ThermoSeparation Products HPLC system, consisting of the SPC 1000 membrane degasser solvent module, the P4000 quatemary pump module, and the AS3000 autosampler.

Analysis:

Sample Extraction *for well water:*

1. Allow each sample to come to room temperature. Pour the sample from the 1 L amber glass bottle into a 1000 mL beaker. Record the sample weight in grams (g) by weighing the bottle before and after transfer. Sample weight should be close to 500.0 g. Adjust pH to -3 with 6N HCL. Add 0.1 µg propazine (100 µL of 1ng/µL spiking solution in methanol) as a surrogate to the sample. Note: The volume of methanol in spiking solution added to the sample should be 0.1% or less of the sample volume.
2. Two MCX cartridges are connected together in tandem and connected to the house vacuum using the manifold as shown in Diagram # 1.
3. Condition the cartridges at a flow rate of about 10 ml/minute with about 15 mL of methanol followed by about 15 mL of purified water by applying vacuum. ***Do*** not let ***the cartridges*** go ***to dryness.*** Turn off the vacuum when the purified water has just passed through the cartridges. Detach the cartridges from the vacuum line and fill up the cartridges with purified water.

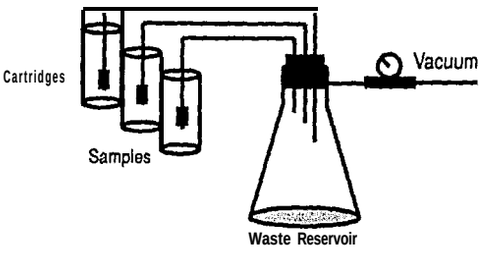

Diagram # 1

4. Reattach the conditioned cartridges to the vacuum line and transfer to the 1000 mL beaker containing the water sample. Allow the sample to pass through the conditioned cartridges by applying vacuum. Adjust the flow rate to about 10 mL/minute to 15 mL/minute.
5. After all of the water sample has passed through the cartridges, remove the cartridges and insert them into the inlets of the Vac-Elut SPS 24 at the "waste position". Turn the vacuum on (-5 psi) for 2 minutes to dry the cartridges. Turn the vacuum off and reverse the order of the cartridge positions. Add 5 mL of elution reagent to each cartridge. Switch the Vat-Elut SPS 24 to the "collect position" and turn the vacuum on. Elute all chemicals with 5 mL ± 0.5 mL of 5 % ammonium hydroxide in methanol at a flow rate of about 5 mL/minute Collect the eluant in a 15 mL graduated test tube.
6. Filter the eluant through a 0.2 µm Acrodisc into a 15 m Lgraduated test tube which has been calibrated at 0.5 mL using methanol with a 500 µL syringe. Concentrate the eluant to -0.2 mL in a 40 °C waterbath under a stream of nitrogen. Bring to a final volume of 0.5 mL with reconstitution reagent (75/25 water / methanol). Vortex for 30 seconds. Transfer the eluant into two autosampler vials with inserts. Analyze by APCI/LC/MS/MS.

Analysis: ***(con 't)***

Sample Extraction for river water:

1. Allow each sample to come to room temperature. Pour the sample from the 1L amber glass bottle into a 1000 mL beaker. Record the sample weight in grams (g) by weighing the bottle before and after transfer. Sample weight should be close to 500.0 g. Adjust pH to -3 with 6N HCl. Add 0.1 µg propazine (100 µL of 1ng/µL spiking solution in methanol) as a surrogate to the sample. Filter the sample through a 1µm x 47 mm fiberglass filter paper.
   Note: The volume of methanol in spiking solution added to the sample should be 0.1% or less of the sample volume.
2. Go to step # 2 -6 as described above.

Instrument Conditions for system A:

APCI Source Settings:

Vaporizer Temp (°C):	550
Sheath Gas Flow Rate (arb):	90
AUX Gas Flow Rate (arb):	15
Discharge Current (µA):	5
Discharge Voltage (kV):	5
Capillary Temp (°C):	210
Capillary Voltage (V):	5

HPLC Settings for SymmetryShield™C18 column:

Analytical column: Waters SymmetryShield™ 150 mm x 3.9 mm x 5 µm.
Guard column: RP-18, Cl8 7 µm x 15 mm x 3.2 mm.
Injection volume: 20 µL.
Pump program steps:

Time (min.)	*Flow rate (mL/min.)*	*%A*	*%B*
0.00	0.75	85	15
3.00	0.75	85	15
4.00	0.75	50	50
10.00	0.75	50	50
21.50	0.75	25	75
22.00.	0.75	5	95
26.00	0.75	5	95
26.50	0.75	85	15
30.00	0.75	85	15

MS Detector Settings for Waters SymmetryShield™C18: (con 't)

MS run time (min.): 22.5
Divert valve (min.): 0.00 to waste.
1.00 to source.
21.25 to waste.

Segment: 1
Duration time (min.): 2.00
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [146]⇒ [60-200]
MS/MS: Amp: 40% Q: 0.400 Time (msec.): 30 IsoW: 5.0

Segment: 2
Duration time (min.): 3.00
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [147]⇒ [60-200]
Ms/Ms: Amp: 40% Q: 0.400 Time (msec.): 30 IsoW: 5.0

Segment: 3
Duration time (min.): 4.50
Number of scan events: 2
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [175]⇒ [55-200]
Ms/Ms: Amp: 40% Q: 0.300 Time (msec.): 30 IsoW: 5.0
2. Pos [189]⇒ [60-210]
Ms/Ms: Amp: 30% Q: 0.300 Time (msec.): 30 IsoW: 4.0

Segment: 4
Duration time (min.): 1.6
Number of scan events: 3
Tune method: Study 182-l-500 Tune
Scan event: details:
1. Pos [253]⇒ [80-275]
Ms/Ms: Amp: 37% Q: 0.300 Time (msec.): 30 IsoW: 3.0
2. Pos [242]⇒ [75-275]
Ms/Ms: Amp: 37% Q: 0.300 Time (msec.): 30 IsoW: 5.0
3. Pos [215]⇒ [70-235]
Ms/Ms: Amp: 34% Q: 0.300 Time (msec.): 30 IsoW: 3.0

Segment: 5
Duration time (min.): 0.60
Number of scan events: 3
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [242]⇒ [75-275]
Ms/Ms: Amp: 37% Q: 0.300 Time (msec.): 30 IsoW: 5.0
2. Pos [215]⇒ [70-235]
Ms/Ms: Amp: 34% Q: 0.300 Time (msec.): 30 IsoW: 3.0
3. Pos [203]⇒ [65-225]
Ms/Ms: Amp: 36% Q: 0.300 Time (msec.): 30 IsoW: 5.0

Segment: 6
Duration time (min.): 0.70
Number of scan events: 3
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [203]⇒ [65-225]
Ms/Ms: Amp: 36% Q: 0.300 Time (msec.): 30 IsoW: 5.0
2. Pos [262]⇒ [100-280]
Ms/Ms: Amp: 34% Q: 0.350 Time (msec.): 30 IsoW: 5.0
3. Pos [215]⇒ [70-235]
Ms/Ms: Amp: 34% Q: 0.300 Time (msec.): 30 IsoW: 3.0

Segment: 7
Duration time (min.): 0.70
Number of scan events: 3
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [203]⇒ [65-260]
Ms/Ms: Amp:, 36% Q: 0.300 Time (msec.): 30 IsoW: 5.0
2. Pos [262]⇒ [100-280]
Ms/Ms: Amp: 34% Q: 0.350 Time (msec.): 30 IsoW: 5.0
3. Pos [226]⇒ [70-250]
Ms/Ms: Amp: 38.5% Q: 0.300 Time (msec.): 30 IsoW: 3.0

Segment: 8
Duration time (min.): 1.80
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [226]⇒ [70-250]
Ms/Ms: Amp: 38.5% Q: 0.300 Time (msec.): 30 IsoW: 3.0

Segment: 9
Duration time (min.): 2.10
Number of scan events: 2
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [217]⇒ [70-235]
Ms/Ms: Amp: 36% Q: 0.300 Time (msec.): 30 IsoW: 5.0
2. Pos [305]⇒ [100-350]
Ms/Ms: Amp: 39% Q: 0.350 Time (msec.): 30 IsoW: 5.0

Segment: 10
Duration time (min.): 0.50
Number of scan events: 2
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [235]⇒ [65-250]
Ms/Ms: Amp: 35% Q: 0.270 Time (msec.): 30 IsoW: 5.0
2. Pos [305]⇒ [100-350]
Ms/Ms: Amp: 39% Q: 0.350 Time (msec.): 30 IsoW: 5.0

Segment: 11
Duration time (min.): 0.50
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [235]⇒ [65-250]
Ms/Ms: Amp: 35% Q: 0.270 Time (msec.): 30 IsoW: 5.0

Segment: 12
Duration time (min.): 4.50
Number of scan events: 3
Tune method: Study 182- l-500 Tune
Scan event details:
1. Pos [235]⇒ [65-250]
Ms/Ms: Amp: 35% Q: 0.270 Time (msec.): 30 IsoW: 5.0
2. Pos [231]⇒ [75-250]
Ms/Ms: Amp: 40% Q: 0.300 Time (msec.): 30 IsoW: 5.0
3. Pos [242]⇒ [75-275]
Ms/Ms: Amp: 37.5% Q: 0.300 Time (msec.): 30 IsoW: 3.0

HPLC Settings for Phenyl-hexyl column:

Analytical column: Phenyl-hexyl 150 cm x 4.6 mm x 3 µm
Guard column: Phenyl-hexyl 4 mm x 2.00 mm
Injection volume: 25 µL.
Pump program steps:

Time (min.)	*Flow rate (mL/min.)*	%A	%B
0.00	0.75	85	15
3.00	0.75	85	15
4.00	0.75	50	50
9.00	0.75	50	50
25.50	0.75	25	75
26.00	0.75	5	95
30.00	0.75	5	95
31.00	0.75	85	15
35.00	0.75	85	15

MS Detector Settings for Phenyl-hexyl column.

MS run time (min.): 26.5
Divert valve (min.): 0.00 to waste
3.00 to source
26.5 to waste

Segment: 1
Duration time (min.): 3.00
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [147]⇒ [60-200]
Ms/Ms: Amp: 35% Q: 0.400 Time (msec.): 30 IsoW: 5.0

Segment: 2
Duration time (min.): 3.50
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [147]⇒ [60-200]
Ms/Ms: Amp: 40% Q: 0.400 Time (msec.): 30 IsoW: 5.0

Segment: 3
Duration time (min.): 3.00
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:
1. Pos [175]⇒ [55-200]
Ms/Ms: Amp: 40% Q: 0.300 Time (msec.): 30 IsoW: 5.0

MS Detector Settings for Phenyl-hexyl column: (con 't)

Segment: 4 .

Duration time (min.): 2.50
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:

1. Pos [189]⇒ [60-210]
   Ms/Ms: Amp: 38% Q: 0.30 Time (msec.): 30 IsoW: 3.0

Segment: 5

Duration time (min.): 3.50
Number of scan events: 2
Tune method: Study 182-l-500 Tune
Scan event details:

1. Pos [242]⇒ [75-275]
   Ms/Ms: Amp: 36% Q: 0.300 Time (msec.): 30 IsoW: 5.0
2. Pos [203]⇒ [65-225]
   Ms/Ms: Amp: 36.5% Q: 0.300 Time (msec.): 30 IsoW: 5.0

Segment: 6

Duration time (min.): 0.90
Number of scan events: 2
Tune method: Study 182-l-500 Tune
Scan event details:

1. Pos [215]⇒ [70-235]
   Ms/Ms: Amp: 34% Q: 0.300 Time (msec.): 30 IsoW: 3.0
2. Pos [262]⇒ [85-280]
   Ms/Ms: Amp: 32.5% Q: 0.350 Time (msec.): 30 IsoW: 5.0

Segment: 7

Duration time (min.): 0.70
Number'of scan events: 2
Tune method: Study 182-1-500 Tune
Scan event details:

1. 1. Pos [215]⇒ [70-235]
   Ms/Ms: Amp: 34% Q: 0.300 Time (msec.): 30 IsoW: 3.0
2. Pos [226]⇒ [70-250]
   Ms/Ms: Amp: 38% Q: 0.300 Time (msec.): 30 IsoW: 3.0

Segment: 8

Duration time (min.): 1.80
Number of scan events: 3
Tune method: Study 182-l -500 Tune
Scan event details:

1. Pos [217]⇒ [70-235]
   Ms/Ms: Amp: 36% Q: 0.300 Time (msec.): 30 IsoW: 5.0
2. Pos [253]⇒ [80-275]
   Ms/Ms: Amp: 35.5% Q: 0.300 Time (msec.): 30 IsoW: 3.0
3. Pos [226]⇒ [70-250]
   Ms/Ms: Amp: 38% Q: 0.300 Time (msec.): 30 IsoW: 3.0

MS Detector Settings for Phenyl-hexyl column: (con't)

Segment: 9

Duration time (min.): 1.10
Number of scan events: 1
Tune method: Study 182-l-500 Tune
Scan event details:

1. Pos [235]⇒ [65-250]
   Ms/Ms: Amp: 35% Q: 0.270 Time (msec.): 30 IsoW: 5.0

Segment: 10

Duration time (min.): 2.00
Number of scan events: 3
Tune method: Study 182-l-500 Tune
Scan event details:

1. Pos [235]⇒ [65-250]
   Ms/Ms: Amp: 35% Q: 0.270 Time (msec.): 30 IsoW: 5.0
2. Pos [305]⇒ [100-350]
   Ms/Ms: Amp: 39% Q: 0.350 Time (msec.): 30 IsoW: 5.0
3. Pos [231]⇒ [75-250]
   Ms/Ms: Amp: 39% Q: 0.300 Time (msec.): 30 IsoW: 5.0

Segment: 11

Duration time (min.): 4.75
Number of scan events: 3
Tune method: Study 182-l-500 Tune
Scan event details:

1. Pos [305]⇒ [100-350]
   Ms/Ms: Amp: 39% Q: 0.300 Time (msec.): 30 IsoW: 5.0
2. Pos [231]⇒ [75-250]
   Ms/Ms: Amp: 39% Q: 0.300 Time (msec.): 30 IsoW: 5.0
3. Pos [242]⇒ [75-275]
   Ms/Ms: Amp: 37% Q: 0.300 Time (msec.): 30 IsoW: 3.0

Instrument Conditions for system B:

APCI Source Settings:

Vaporizer Temp (°C):	450
Sheath Gas Flow Rate (arb):	72
Aux Gas Flow Rate (arb):	1
Discharge Current (μA):	5
Discharge 'Voltage (kV):	6
Capillary Temp (“C):	170
Capillary Voltage (V):	26

HPLC Settings for Phenyl-hexyl column: (con't)

Analytical column:. Phenyl-hexyl 150 cm x 4.6 mm x 3 µm
Guard column: Phenyl-hexyl 4 mm x 2.00 mm
Injection volume: 50 µL.
Pump program steps:

Time (min.)	*Flow rate (mL/min.)*	*%A*	*%B*
0.00	0.6	90	10
2.50	0.6	90	10
3.00	0.6	50	50
25.00	0.6	20	80
26.00	0.6	5	95
37.00	0.6	5	95
38.00	0.6	90	10
44.00	0.6	90	10

MS Detector Settings for Phenyl-hexyl column.

MS run time (min.): 30.75
Divert val-ve (min.): 0.00 to waste
4.50 to source
30.50 to waste

Segment: 1
Duration time (min.): 5.00
Number of scan events: 1
Tune method: 1-175 150 DACT
Scan event. details:
1. Pos [147]⇒ [60-200]
Ms/Ms: Amp: 34% Q: 0.400 Time (msec.): 30 IsoW: 4.0

Segment: 2
Duration time (min.): 3.50
Number of scan events: 1
Tune method: l-750 150 DACT
Scan event details:
1. Pos [147]⇒ [60-200]
Ms/Ms: Amp: 34% Q: 0.400 Time (msec.): 30 IsoW: 4.0

Segment: 3
Duration time (min.): 3.00
Number of scan events: 1
Tune method: 2-350 150
Scan event details:
1. Pos [175]⇒ [55-190]
Ms/Ms: Amp: 34.5% Q: 0.300 Time (msec.): 30 IsoW: 4.0

MS Detector Settings for Phenyl-hexyl column: (con't)

Segment: 4

Duration time (min.): 5.00
Number of scan events: 1
Tune method: 2-350 150
Scan event details:

1. Pos [189]⇒ [60-210]
   Ms/Ms: Amp: 32% Q: 0.30 Time (msec.): 30 IsoW: 4.0

Segment: 5

Duration time (min.): 3.15
Number of scan events: 2
Tune method: l-500 150
Scan event details:

1. Pos [242]⇒ [75-260]
   Ms/Ms: Amp: 30.2% Q: 0.300 Time (msec.): 30 IsoW: 4.0
2. Pos [203]⇒ [65-230]
   Ms/Ms: Amp: 35.2% Q: 0.300 Time (msec.): 30 IsoW: 4.0

Segment: 6

Duration time (min.): 1.20
Number of scan events: 2
Tune method: l-1000 150
Scan event details:

1. Pos [215]⇒ [70-230]
   Ms/Ms: Amp: 31% Q: 0.300 Time (msec.): 30 IsoW: 1.5
2. Pos [262]⇒ [85-285]
   Ms/Ms: Amp: 22% Q: 0.300 Time (msec.): 75 IsoW: 6.0

Segment: 7

Duration time (min.): 0.60
Number of scan events: 3
Tune method: l-500 150
Scan event details:

1. Pos [215]⇒ [70-230]
   Ms/Ms: Amp: 31% Q: 0.300 Time (msec.): 30 IsoW: 1.5
2. Pos [262]⇒ [85-285]
   Ms/Ms: Amp: 22% Q: 0.300 Time (msec.): 75 IsoW: 6.0
3. Pos [226]⇒ [70-240]
   Ms/Ms: Amp: 34.2% Q: 0.300 Time (msec.): 30 IsoW: 1.5

MS Detector Settings for Phenyl-hexyl column: (con't)

Segment:. 8
Duration time (min.): 2.35
Number of scan events: 3
Tune method: l-500 150
Scan event details:
1. POS [217]⇒ [70-230]
Ms/Ms: Amp: 34.2% Q: 0.300 Time (msec.): 30 IsoW: 4.0
2. Pos [253]⇒ [80-275]
Ms/Ms: Amp: 25% Q: 0.300 Time (msec.): 30 IsoW: 1.5
3. Pos [226]⇒ [70-240]
Ms/Ms: Amp: 34.2% Q: 0.300 Time (msec.): 30 IsoW: 1.5

Segment: 9
Duration time (min.): 1.60
Number of scan events: 1
Tune method: l-1000 150
Scan event details:
1. Pos [236]⇒ [65-250]
Ms/Ms: Amp: 31% Q: 0.265 Time (msec.): 30 IsoW: 3.5

Segment: 10
Duration time (min.): 0.70
Number of scan events: 2
Tune method: l-500 150
Scan event details:
1. Pos [236]⇒ [65-250]
Ms/Ms: Amp: 31% Q: 0.265 Time (msec.): 30 IsoW: 3.5
2. Pos [231]⇒ [75-250]
Ms/Ms: Amp: 36% Q: 0.300 Time (msec.): 30 IsoW: 4.0

Segment: 11
Duration time (min.): 1.60
Number of scan events: 2
Tune method: l-500 150
Scan event details:
1. Pos [305]⇒ [100-350]
Ms/Ms: Amp: 39% Q: 0.300 Time (msec.): 30 IsoW: 4.0
2. Pos [231]⇒ [75-250]
Ms/Ms: Amp: 36% Q: 0.300 Time (msec.): 30 IsoW: 4.0

MS Detector Settings for Phenyl-hexyl column: (con't)

Segment: 12

Duration time (min.): 3.05
Number of scan events: 2
Tune method: l-500 150
Scan event details:

1. Pos [242]⇒ [75-265]
   Ms/Ms: Amp: 33% Q: 0.300 Time (msec.): 30 IsoW: 1.5
2. Pos [231]⇒ [75-250]
   Ms/Ms: Amp: 39% Q: 0.300 Time (msec.): 30 IsoW: 4.0

Calculations:

The results are reported in µg/L:

$$\mu g/L = \frac{\mu g/mL \text{ (from standard curve) x final volume (mL)}}{\text{Sample weight (g)}} \times 1000 \text{ g/L}$$

Method Performance:

Quality Control.:

1. Sample storage: All field samples shall be kept refrigerated at 4 °C ± 2 until extracted.
2. Sample extraction: All extracts shall be kept refrigerated at 4 °C ± 2 until analyzed.
3. For each set of samples, at least one matrix blank and one matrix spike shall be included. Each set of samples shall not contain more than twelve samples.

Recovery data:

This analytical method was validated by preparing five sets of samples using the provided background river water and well water. Each set contained four different levels of spike and a matrix blank. Each set was processed through the entire analytical method on a different day. Each sample was injected twice on each column. The results are shown in Appendices I to V.

Method detection limit (MDL):

Method Detection Limit (MDL) refers to the lowest concentration of analytes that a method can detect reliably. To determine the MDL, 7 replicate background samples were spiked at 0.100 µg. The standard deviation from the spiked samples was used to calculate the MDL using the following equation:

$$MDL = tS$$

where:
t is the Student t value for the 99% confidence level with n-l degrees of freedom and S denotes the standard deviation obtained from n replicate analyses, For the n=7 replicates used to determine the MDL, t=3.143. See Appendix VI to X for recovery data from the determination of the Method Detection Limits.
The Reporting Limit (RL) refers to the level at which quantitative results may be obtained. By convention, the RL is chosen in a range l-5 times the MDL. The Reporting Limit for this method is 0.05 µg/L for all analytes.

Method Performance: .

Discussion:

This method has been modified recently for its instrument parameters. The new instrument parameters allow to quantify all analytes in a single injection.

Propazine is used as a surrogate. Add 0.1 μg of propazine to each sample and processed through the entire analytical method. This allows the extraction steps to be monitored. Control limits for propazine in well water are mean ± 2 σ, in river water are mean ± 3 σ. If the surrogate recovery is outside the ranges, the sample should be re-analyzed. If the recovery is still out of range, the sample should be re-extracted. See Appendix I, II, III, IV and V for recovery data of propazine in well and river water.

The segment durations in the mass spectrometer settings determine the retention time windows for each analyte. As the HPLC column performance may change over time because of irreversible contamination, phase stripping, etc., it may be necessary to adjust these windows before beginning a sequence for the observed retention times of the analytes. Installation of a new guard column or analytical column may also necessitate adjustments of window times. These retention time windows should be verified before each sequence, and adjusted as necessary.

A standard curve consisting of five levels was used for every twelve injections. At the analyst' discretion, a six-level calibration may be run. This has been found especially useful for the LCQ classic (system B), which may show deviations from linearity at the highest calibration level for some analytes. Each sample was injected twice back to back since the extracts are observed to degrade once the vial cap septum is punctured. The external standard technique with the average of all standard levels from the beginning of a sequence to the end of a sequence was used to quantify samples. The response of a same level standard before and after samples should not differ by more than 25% and 35% for well water and river water respectively. If the standard responses are not in acceptable range, and recoveries of the analytes are out of the acceptable ranges (determined by two sigmas for well water and three sigmas for river water),, a root analysis should be done to identify the causes,

After each sequence is completed, the column should be rinsed with high organic mobile phase (≥ 80%) solution for few hours and stored in that condition, Before starting a sequence, test standards should be run first to ensure that the column is fully equilibrated. Test standards will not be used in quantitation.

References:

SOP #EM 501.4
SOP # EM 501.5

Acknowledgment:

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Appendix I: Recovery data for method validation by SymmetryShield™ C18 column by system A for well water

Diamino Chlorotriazine (DACT):

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	97.0	2.0	119.3
0.1	80.5	2.0	88.8
0.1	70.0	2.0	83.4
0.1	82.0	2.0	74.6
0.1	90.0	2.0	87.5
0.5	120.0	6.0	103.5
0.5	88.1	6.0	105.3
0.5	97.6	6.0	91.7
0.5	81.0	6.0	84.7
0.5	84.8	6.0	88.5

Deisopropyl Atrazine (ACET):

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	96.0	2.0	114.6
0.1	84.5	2.0	86.6
0.1	84.5	2.0	90.0
0.1	100.0	2.0	83.8
0.1	102.0	2.0	98.4
0.5	100.3	6.0	98.6
0.5	83.8	6.0	100
0.5	94.8	6.0	99.2
0.5	82.6	6.0	81.7
0.5	80.0	6.0	83.4

Deethyl Atrazine (DEA): 4

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	86.0	2.0	96.8
0.1	75.5	2.0	77.8
0.1	79.0	2.0	88.8
0.1	88.0.	2.0	85.2
0.1	87.0	2.0	96.6
0.5	92.3	6.0	87.3
0.5	79.8	6.0	94.8
0.5	98.2	6.0	99.5
0.5	79.6	6.0	85.0
0.5	75.0	6.0	84.0

Appendix I: Recovery data for method validation by SymmetryShield™ Cl8 column by system A for well water

Bromacil:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	87.5	2.0	**99.2**
0.1	93.0	2.0	108.7
0.1	101.0	2.0	97.8
0.1	94.0	2.0	91.0
0.1	110.0	2.0	99.2
0.5	99.5	6.0	88.8
0.5	104.1	6.0	125.4
0.5	106.0	6.0	100.5
0.5	89.2	6.0	93.3
0.5	87.2	6.0	91.3

Cvanazine:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	82.0	2.0	**89.9**
0.1	79.0	2.0	83.7
0.1	82.0	2.0	86.8
0.1	87.0	2.0	83.0
0.1	86.0	2.0	93.0
0.5	89.5	6.0	89.0
0.5	85.0	6.0	90.0
0.5	90.6	6.0	92.5
0.5	77.2	6.0	87.5
0.5	77.2	6.0	88.2

Diuron:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	83.5	2.0	78.1
0.1	79.0	2.0	72.3
0.1	80.0	2.0	99.4
0.1	85.0	2.0	93.8
0.1	84.0	2.0	103.8
0.5	81.7	6.0	90.3
0.5	75.5	6.0	89.5
0.5	100.8	6.0	97.7
0.5	83.0	6.0	96.2
0.5	89.6	6.0	103.7

Appendix I: Recovery data for method validation by SymmetryShield™ C18 column by system A for well water

Hexazinone:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	73.0	2.0	91.2
0.1	71.0	2.0	79.2
0.1	64.0	2.0	83.6
0.1	74.0	2.0	76.4
0.1	74.0	2.0	81.6
0.5	84.6	6.0	94.2
0.5	74.3	6.0	86.4
0.5	81.2	6.0	88.3
0.5	67.4	6.0	82.5
0.5	69.4	6.0	80.2

Metribuzin:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	**79.0**	2.0	**83.9**
0.1	72.5	2.0	72.4
0.1	82.0	2.0	79.8
0.1	89.5	2.0	80.0
0.1	82.0	2.0	89.8
0.5	77.3	6.0	79.7
0.5	69.3	6.0	86.8
0.5	95.4	6.0	94.5
0.5	79.2	6.0	76.7
0.5	71.6	6.0	81.3

Norflurazon:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	89.0	2.0	**99.0**
0.1	78.0	2.0	87.2
0.1	82.0	2.0	96.8
0.1	91.0	2.0	87
0.1	88.0	2.0	96.0
0.5	97.5	6.0	96.8
0.5	78.9	6.0	94.5
0.5	99.8	6.0	98.2
0.5	80.0	6.0	90.8
0.5	84.6	6.0	91.2

Appendix I: Recovery data for method validation by SymmetryShield™ C18 column by system A for well water

Prometryn:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	82.5	2.0	**93.1**
0.1	74.0	2.0	75.5
0.1	73.0	2.0	85.0
0.1	87.0	2.0	80.4
0.1	85.0	2.0	90.0
0.5	86.3	6.0	86.8
0.5	74.6	6.0	91.0
0.5	91.2	6.0	93.8
0.5	76.6	6.0	82.8
0.5	70.4	6.0	81.0

Prometon:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	75.0	2.0	95.6
0.1	72.5	2.0	79.4
0.1	63.0	2.0	82.2
0.1	73.0	2.0	79.0
0.1	76.0	2.0	90.2
0.5	85.8	6.0	84.6
0.5	15.3	6.0	91.7
0.5	88.6	6.0	95.8
0.5	71.2	6.0	80.7
0.5	69.8	6.0	82.0

Simazine:

Spike level (µg/L)	**Recovery (%)**	Spike level (µg/L)	Recovery (%)
0.1	85.5	2.0	100.6
0.1	70.5	2.0	72.8
0.1	74.0	2.0	82.2
0.1	86.0	2.0	76.2
0.1	84.0	2.0	90.4
0.5	94.3	6.0	89.0
0.5	73.3	6.0	91.8
0.5	91.4	6.0	95.5
0.5	74.4	6.0	75.3
0.5	72.0	6.0	77.8

Appendix I: Recovery data for method validation by SymmetryShield™ C18 column by system A for well water

Atrazine:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	77.0	2.0	84.9
0.1	71.5	2.0	71.0
0.1	74.0	2.0	79.8
0.1	86.0	2.0	79.0
0.1	78.0	2.0	88.4
0.5	80.6	6.0	76.3
0.5	70.3	6.0	86.7
0.5	90.2	6.0	95.5
0.5	76.2	6.0	77.3
0.5	69.4	6.0	78.0

Propazine (surrogate):

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	84.3	2.0	83.8
0.1	**79.0**	2.0	72.3
0.1	85.0	2.0	79.5
0.1	95.5	2.0	78.5
0.1	87.0	2.0	88.0
0.5	83.3	6.0	78.3
0.5	75.5	6.0	89.5
0.5	100.0	6.0	91.5
‘0.5	83.0	6.0	75.5
0.5	**80.0**	6.0	78.0

Appendix II: Recovery data for method validation for Phenyl hexyl column by system A for well water

Diamino Chlorotriazine (DACT):

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	67.0	2.0	70.0
0.1	71.0	2.0	78.2
0.1	87.0	2.0	99.8
0.1	85.0	2.0	100.0
0.1	79.0	2.0	86.6
0.5	65.2	6.0	75.3
0.5	80.0	6.0	96.0
0.5	89.2	6.0	106.3
0.5	93.0	6.0	100.7
0.5	86.0	6.0	94.3

Deisopropyl Atrazine (ACET):

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	74.0	2.0	77.2
0.1	90.0	2.0	95.2
0.1	90.0	2.0	111.8
0.1	93.0	2.0	107.6
0.1	91.0	2.0	106.8
0.5	76.2	6.0	81.7
0.5	83.8	6.0	104.0
0.5	97.6	6.0	108.2
0.5	93.2	6.0	112.8
0.5	93.4	6.0	100.3

Deethyl Atrazine (DEA):

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	73.0	2.0	77.6
0.1	82.0	2.0	94.2
0.1	78.0	2.0	100.0
0.1	81.0	2.0	97.8
0.1	79.0	2.0	98.6
0.5	79.4	6.0	79.5
0.5	79.6	6.0	98.7
0.5	89.6	6.0	97.2
0.5	84.6	6.0	97.8
0.5	89.6	6.0	94.5

Appendix II: Recovery data for method validation for Phenyl-hexyl column by system A for well water

Bromacil:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	75.0	2.0	81.2
0.1	85.0	2.0	102.6
0.1	90.0	2.0	103.2
0.1	87.0	2.0	104.2
0.1	76.0	2.0	98.6
0.5	86.2	6.0	89.2
0.5	92.6	6.0	103.5
0.5	95.2	6.0	101.0
0.5	90.0	6.0	104.5
0.5	92.2	6.0	90.8

Cyanazine:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	70.0	2.0	77.4
0.1	79.0	2.0	87.8
0.1	79.0	2.0	93.2
0.1	81.0	2.0	87.8
0.1	80.0	2.0	91.2
0.5	76.6	6.0	80.2
0.5	81.6	6.0	98.5
0.5	83.8	6.0	93.2
0.5	79.6	6.0	95.2
0.5	85.2	6.0	99.5

Dim-on:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	81.0	2.0	86.2
0.1	89.0	2.0	88.4
0.1	77.0	2.0	84.4
0.1	81.0	2.0	92.2
0.1	73.0	2.0	95.2
0.5	78.0	6.0	94.2
0.5	86.6	6.0	93.8
0.5	76.4	6.0	87.0
0.5	75.0	6.0	90.3
0.5	85.4	6.0	89.0

Hexazinone:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	66.0	2.0	76.4
0.1	78.0	2.0	89.6
0.1	70.0	2.0	93.8
0.1	83.0	2.0	85.8
0.1	77.0	2.0	91.4
0.5	73.4	6.0	81.7
0.5	74.6	6.0	95.8
0.5	86.4	6.0	88.5
0.5	77.6	6.0	92.5
0.5	82.4	6.0	86.8

Metribuzin:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	73.0	2.0	77.4
0.1	73.0	2.0	83.6
0.1	69.0	2.0	88.2
0.1	74.0	2.0	91.6
0.1	73.0	2.0	92.6
0.5	74.6	6.0	73.5
0.5	72.8	6.0	93.8
0.5	82.8	6.0	92.5
0.5	78.8	6.0	100.2
0.5	88.4	6.0	89.0

Norflurazon:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	73.0	2.0	**79.4**
0.1	79.0	2.0	**96.4**
0.1	83.0	2.0	102.2
0.1	79.0	2.0	97.2
0.1	74.0	2.0	97.0
0.5	76.2	6.0	80.0
0.5	91.0	6.0	100.5
0.5	87.2	6.0	106.5
0.5	83.4	6.0	96.8
0.5	91.4	6.0	96.2

Appendix II: Recovery data for method validation for Phenyl-hexyl column by system A for well water

Prometryn:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	72.0	2.0	83.4
0.1	74.0	2.0	92.8
0.1	68.0	2.0	97.6
0.1	72.0	2.0	88.2
0.1	73.0	2.0	93.2
0.5	84.8	6.0	84.0
0.5	78.4	6.0	97.7
0.5	80.2	6.0	96.0
0.5	75.0	6.0	94.7
OS	88.8	6.0	95.8

Prometon:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	64.0	2.0	74.6
0.1	69.0	2.0	93.2
0.1	67.0	2.0	99.2
0.1	75.0	2.0	95.2
0.1	73.0	2.0	92.6
0.5	75.0	6.0	84.2
0.5	76.4	6.0	95.3
0.5	87.0	6.0	93.5
0.5	80.6	6.0	97.0
0.5	82.8	6.0	87.3

Simazine:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	68.0	2.0	74.2
0.1	77.0	2.0	85.6
0.1	74.0	2.0	99.4
0.1	78.0	2.0	84.2
0.1	78.0	2.0	89.2
0.5	76.2	6.0	75.7
0.5	71.2	6.0	95.3
0.5	89.4	6.0	98.7
0.5	81.6	6.0	93.7
0.5	90.0	6.0	87.7

Appendix II: Recovery data for method validation for Phenyl-hexyl column by system A for well water

Atrazine:

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	78.0	2.0	73.6
0.1	80.0	2.0	87.4
0.1	76.0	2.0	90.2
0.1	82.0	2.0	93.4
0.1	79.0	2.0	87.0
0.5	69.6	6.0	76.8
0.5	72.2	6.0	88.8
0.5	84.6	6.0	90.7
0.5	77.6	6.0	88.7
0.5	84.2	6.0	80.8

Propazine (surrogate):

Spike level (µg/L)	Recovery (%)	Spike level (µg/L)	Recovery (%)
0.1	80.0	2.0	80.0
0.1	86.5	2.0	99.0
0.1	83.5	2.0	98.0
0.1	53.5	2.0	90.0
0.1	77.5	2.0	93.5
0.5	85.5	6.0	83.5
0.5	84.0	6.0	96.0
0.5	86.5	6.0	101.5
0.5	90.0	6.0	101.5
0.5	94.0	6.0	94.0