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Determination of Residues of Alachlor and Metolachlor and Selected Metabolites in Surface Water by Liquid Chromatography-Mass Spectrometry

Scope: This method is for the determination of the residues of Alachlor and Metolachlor and selected metabolites in surface water. These metabolites are ((2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl)amino) oxo-acetic acid, ((2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl)amino) 2-oxo-ethanesulfonic acid, ((2,6 diethylphenyl) (methoxymethy)amino) oxo-acetic acid, and ((2,6-diethyl phenyl) (methoxymethyl)amino) 2-oxo-ethanesulfonic acid. These six compounds are analyzed by liquid chromatography with a C-8 reverse phase column with ion trap mass spectrometry in MS/MS mode The reporting limit is 0.05 µg/L for all compounds. The lowest validated spiking level is 0.1 µg/L for all compounds in surface water. The lowest amount standard injected is 0.5 ng, 50 µL of 0.01 ng/µL, for all compounds.

Principle: A 150 mL aliquot of filtered surface water is passed through a C-18 solid phase extraction columns (1 g). The analytes and the adsorbed water are eluted with methanol. The methanol is evaporated at 45 °C with a gentle stream of nitrogen to just below 0.4 mL. A 0.1 mL acetonitrile is added and the final extract volume is adjusted to 0.5 mL with water. The extract is analyzed by LC/MS/MS using a C-8 column and acidified mobile phase. All metabolites are analyzed using ESI negative ion mode. The residues of Alachlor and Metolachlor are analyzed using APCI positive ion mode.

Definitions not in Glossary:

Reagents:

Use residue grade solvents for sample extraction and ultra pure grade solvents (Burdick & Jackson or equivalent) and reagents for HPLC elution and Mass Spectrometry detection.

1. Alachlor, CAS # 015972-60-8, 1.0 mg/mL in methanol, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture.
2. Metolachlor, CAS #051218-45-2 , 1.0 mg/mL in methanol, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture

3. Metolachlor OXA, CAS #152019-73-3, 1.0 mg/mL in water, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture, its chemical name is ((2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl)amino) oxo-acetic acid.
4. Metolachlor ESA, CAS # not known, 1.0 mg/mL in water, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture, its chemical name is ((2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl)amino) 2-oxo-ethanesulfonic acid
5. Alachlor ESA, CAS # not known, 1.0 mg/mL in water, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture, its chemical name is ((2,6-diethyl phenyl) (methoxymethyl)amino) 2-oxo-ethanesulfonic acid
6. Alachlor OXA, CAS # not known, 1.0 mg/mL in water, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture its chemical name is (2,6 diethylphenyl) (methoxymethy)amino) oxo-acetic acid
7. Methanol, ultra pure grade from Burdick & Jackson, Cat #230-4 or equivalent
8. Acetonitrile, ultra pure grade from Burdick & Jackson, Cat #o18-04 or equivalent
9. Water, ultra pure grade, Burdick & Jackson, Cat #365-4 or equivalent
10. Acetic acid, HPLC grade Fisher Cat #A35-500 or equivalent
11. Acrodisc® 0.2 µm, Gelman Laboratory, Cat # 09730191.
12. C-18 Solid phase extraction cartridge (1g), Waters Sep-Pak Vac 6 cc, Part #36905 or equivalent
13. Glass fiber filter, Gilman, 47 mm, capable retain particles larger than 1 micron

Safety:

No known carcinogens are used in this method. All general laboratory safety procedures must be followed (e.g. wear safety glasses, gloves, use ventilation hood, etc…)

Equipment:

1. Vacuum manifold, in-house system with multi-channels, a 1 liter glass filtration device attached to each channel.
2. Vacuum manifold, Supelco 24 port model, Cat # 913-0445
3. Larger Volume Sampler, Supelco, Cat #57275
4. Vacuum pump or in-house vacuum, at least 25 inches vacuum
5. Balance, analytical
6. Graduated cylinders
7. Nylon Acrodisc, 0.2 um, Gelman, Part #4436
8. Graduated conical test tube, 15 mL, calibrated for 0.5 mL
9. Nitrogen evaporator, Organomation, Model 112
10. Vortex mixer, Fisher Scientific, Model Vortex-Genie 2
11. Autosampler vial, Waters total recovery vial, 12X32mm and cap with preslit PTFE/Silicon septa, Part #186000385

Instrument: (see detail in operating parameters)

1. HPLC with autosampler and column oven
2. Mass spectrometer

3. Computer

Interference:

The MS/MS detection of all these analytes is specific. Multiple factors are used to eliminate possible interferences. The factors are parent mass $(M-H)^-$, or $(M+H)^+$ and specific daughter ions:

	Parent mass m/z	Parent ion	Daughter ions m/z
Alachlor OXA	264	(M-H)-	192, 160
Alachlor ESA	314	(M-H)-	121
Metolachlor OXA	278	(M-H)-	206
Metolachlor ESA	328	(M-H)-	121, 192
Alachlor	270	$(M+H)^+$	238
Metolachlor	284	$(M+H)^+$	252

Standard Preparation:

The individual stock standards of 1.0 mg/mL are obtained from the Standards Repository, CAC, CDFA. They obtained the neat standards from either the manufactures or from commericial supplers of standards. The individual stock solution of alachlor and metolachlor are prepared in methanol. The individual stock solutions of the four metabolites are prepared in water. They are sealed in ampules and are stored in a refrigerator (less than 5 °C). The working standards of the four metabolites and the two parent compounds are combined and prepared by mixing equal amount of stock solutions, then diluted with a mixtutre of water and acetonitrile (80/20) to the following concentrations: 0.5, 0.2, 0.1, 0.05, 0.02 and 0.01 ng/μL The lowest standard required for the standard curve is 0.017 ng/uL. This concentration equals to the reporting limit of 0.05 ppb.

Sample Preservation and storage:

Check and record sample temperature upon arrival. Store all samples in a locked designated area in the walk-in refrigerator (less than 5 °C). Return samples to the refrigerator immediately after subsample is taken.

Sample Extraction:

1. Measure 200±0.1 gram surface water into a 500 mL beaker. Do sample spike at this step, if required (such as for MDL, method validation, and continuing QC).
2. Set up a multi-channel vacuum manifold with one liter glass filtration device attached to each channel. Use 47 mm Gelman type A/E glass fiber filter for filtration.
3. Filter the 200 mL sample through the glass fiber filter.
4. Measure 150±0.1 gram of the filtered subsample.
5. Set up a Supelco 24 channels manifold extraction device.

6. Connect a C-18 SPE columns (1 gram) to each channel. Turn off the unused channels of the manifold. Pre-condition the SPE columns by passing 10 mL of methanol followed by 20 mL of D.I. water. Do not allow the columns to go dry.
7. Apply the sample at the rate of 5-10 mL per minute by adjusting the vacuum. The typical operating pressure is about 10-15 inch Hg. Maintain at least 1 cm water level in the column until all sample has passed through the cartridge.
8. As soon as the sample has passed through the column, rinse the beaker with 10 mL of D.I. water and continue the extraction until all the rinsate has passed through the columns. Make sure all the columns are properly labeled before disconnecting them.
9. Remove the sampling tube. Apply a 25 inches vacuum for 5 minutes to allow excess water to be removed.
10. Elute the columns with 10 mL methanol and collecte into a 15 mL graduated conical centrifuge tube. Filter the solution through a 0.2 micron Acrodisc and rinse the tube with 2 mL methanol. Pass the rinsate through the same Acrodisc filter and combine the filtrates
11. Evaporate the eluant in a water bath at 45 °C with a gentle stream of nitrogen. Continue the evaporation to just 0.4 mL. Further evaporation will result in a significant low recovery of Alachlor.
12. Add 0.1 mL of acetonitrile and vortex for 20 seconds. Add water to a final volume of 0.50 mL and vortex for 15 seconds.
13. Transfer the entire content to a Waters total recovery autosampler vial.

Equipment Conditions:

1. 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: Zorbax SB-C8 4.6 x 150mm 3.5 Micron (part number: Agilent 863953-906)
Precolumn: Phenomenex C-18 4 mm L x 2.0 mm ID cartridge (part number: AJO-4286)
Column Temperature: 40 °C
Solvent: Isocratic: 65% solvent A and 35 % solvent B,
Solvent A: 0.1% acetic acid in methanol (Burdick & Jackson or equivalent)
Solvent B: 0.1% acetic acid in ultra pure water (Burdick & Jackson or equivalent)
Note: A gradient mobile phase profile with the same column and the same mobile phase also works, and the retention times change accordingly, although the separations are not significantly improved. However, the retention time consistency is improved. The parameters of the modification are listed in the table 7 and the verification spike/recovery data of the modified method is listed in the table 8.
Flow rate: 0.6 mL/ min
Injection volume: 50 µL

Retention time:		
	Alachlor OXA:	8.5 min
	Alachlor ESA:	8.8 min
	Metolachlor OXA:	9.6 min
	Metolachlor ESA:	9.23 min

Alachlor	11.50 min
Metolachlor	12.50 min

Note: An alternative C-8 column or other reversed phase column will probably work. The retention times may be different.
The retention times listed above are for reference only. The retention times of alachlor and metolachlor are consistent. But the retention times of the metabolites are not very consistent. It probably due to the high polarity of the metabolites and the large volume injection.

2. Mass Spectrometry System and Operating Parameters:

Instrumentation:

Finnigan LCQ Deca, ion trap mass spectrometer with ESI ion in negative ion mode for the analysis of metabolites and with APCI ion source in positive ion mode for the analysis of Alachlor and Metolachlor.
Instrument control and data handling: Gateway computer model E-4200 with 10 MB hard disk.
Software: Xcalibur Version 1 SR1.

Tune Methods:
Table 3 for ESI tune methods
Table 5 for APCI tune method

Instrument Method:
Table 4 for ESI instrument methods
Table 6 for APCI instrument method

MS Detector Settings : ESI ion source and negative mode for the analysis of the metabolites. APCI ion source and positive mode for the analysis of the parent compounds.

Instrument Calibration:

A 6 level standard curve is run before and after each sample set. The concentration of working standards are 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 ng/μL and the injection volume is 50 μL.

Analysis:

Build a sequence table and inject the first standard at least twice to condition the instrument. Input the correct dilution factors. The typical sequence order is standards, blank, spikes, 10 samples and standards, then repeat the order for the second injection .

Calculations:

Calculate the concentration of chemical(s) of a sample as follows:

$$\mu g/L = \frac{\text{(peak area. sample) (std. conc.) (std. vol. injected) (final vol. sample, mL)}}{\text{(peak area. std.) (sample vol. injected) (sample vol., mL)}} \times \text{dilution Factor}$$

The LCQuan software in Xcalibur is used for calculations.
In general, std vol. injected = sample vol. injected.
final volume =0.50 mL
sample vol.= 150 mL
The ions used for calculation are listed in the following table

Analytes	Ions used for calculation
Alachlor	238
Metolachlor	252
Alachlor OXA	192,160
Alachlor ESA	121,160
Metolachlor OXA	206
MetolachlorESA	121,192

Method Performance:

Method Detection Limit:

Method Detection Limit (MDL) refers to the lowest concentration of analytes that a method can detect reliably in either a sample or blank. To determine the MDL, each of the 7 samples containing 200 mL of background American River water supplied by DPR (matrix blank) were spiked separately with 0.1 µg/L (15 ng) of Alachlor OXA, Alachlor ESA, Metolachlor OXA and Metolachlor ESA, Alachlor and Metolachlor. These spiked samples along with a blank were analyzed using the described method. The standard deviation derived from the analytical results of the 7 spiked samples was used to calculate the MDL using the following equation:

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

where:

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

S denotes the standard deviation obtained from replicate analyses.

Reporting Limit:

Report Limit (RL) refers to the level above which quantitative results may be obtained usually 1-5 times the MDL. In this case, the reporting limit is 0.05 μg/L for all six compounds.

Spiking solution and spiking volume:

MDL, method validation and QC spikes are made by spiking 200.0 mL of background surface water obtained for this study , which is from the American River.

The concentration of mixed standard for spiking is 1.0 ng/μL and 30.0 ng/μL for all six compounds. The volumes spiked are as in the following table.

	Sample Size (mL)	Volume Added (μL)		Analyte Spiked (ng)	Equivalent to (μg/L)
Spiking Solution		1.0 ng/μL	30 ng/μL		
MDL	200	20		20	0.1
Validation level 1	200	20		20	0.1
Validation level 2	200	40		40	0.2
Validation level 3	200	100		100	0.5
Validation level 4	200		6.66	200	1.0
Validation level 5	200		13.3	400	2.0
Set QC	200	60		60	0.3

MDL Data:

Table 1

Method Validation Data:

Table 2

Acceptance Criteria:

1. The standard curves at the beginning and end of each sample set should not have a percent change greater than 20%. The % change in response is calculated as follows:
   % Change in response = absolute value of [slope of (STD curve before - STD curve after)/ STD curve before] x 10
2. The sample results are calculated based on the average of two adjacent calibration curves using Xcalibur software.
3. The R^2 of each calibration shall be larger than 0.990
4. The recoveries of the spike recovery shall be within the control limit
5. When the above criteria meet, the chemist may report the average of the two injections.

Discussion:

In the beginning, we developed the parameters of analysis for these metabolites with a used C-18 column. As we changed to an identical new column to run the analysis, the method did not work. The reason is unknown. A renewed effort to use a C-8 column and an isocratic mobile phase, as described in this method, provide us with acceptable results.

We presented two instrument methods for determination of the metabolites. One method does not always meet the acceptance criteria for all analytes. When this happens we run the second method.

It was a difficult task to develop a method to analyze these highly water soluble acidic compounds. In order to get an acceptable chromatogram, addition of acid into the mobile phase is necessary, but too much acid reduces the negative ion ionization. We found that 0.1 % acetic acid in mobile phase gives good chromatograms and the required sensitivity.

The evaporation step, in the sample preparation section (step 9), to reduce the volume to 0.4 mL is critical. We experienced a significant low recovery of Alachlor and slightly low recovery of Metolachlor, if the evaporation continues.

We chose to use isocratic elution in the HPLC operation, which provides us a wide, but symmetrical bell shape peak and stable response. It also provides us more data points across each peak and reproducible results. Later, we found the retention time shift significantly between standards and samples. In order to reduce the retention shifting problem, I tried to use gradient with large amount aqueous in the beginning of sample introduction and return to isocratic in 4 minutes. The change has been verified. (See Tables 7 and 8)

In order to achieve sensitivity and stable response, we have to analyze Alachlor and Metolachlor with APCI ion source and their metabolites with ESI ion source.

This method provides acceptable results, as measured by the average recovery at all spiking level for all six analytes. No residues or interferences are found in background water

Reference:

1. Method of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group-Update and Additions to the Determination of Chloroacetanilide Herbicide Degradation Compounds in Water Using High-Performance Liquid Chromatography/Mass Spectrometry. By E.A. Lee, J.L. Kish, L.R. Zimmerman, and E.M. Thurman
   U.S. Department of the Interior, U.S. Geological Survey.
   Open-File report 01-10
2. Determination of Metolachlor (CGA-24705)and CGA-77102, and their Degradates CGA-50212, CGA-354743, CGA-380168, CGA-37735, CGA-67125, and CGA-41638 in Water by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Validation Data.

Determination of Residues of Alachlor and Metolachlor and Selected Metabolites in Surface water by Liquid Chromatography-Mass Spectrometry

Method Number: AG-682 of Novartis Crop Protection, Inc. Enviromental Safety Department, Enviromental Residue Studies.

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Table 1 data derived for MDL determination

Replicates	Spiked Amount (ppb)	Alachlor	Metolachlor	Alachlor OXA	Alachlor ESA	Metolachlor OXA	Metolachlor ESA
1	0.1	0.070	0.077	0.073	0.074	0.061	0.076
2	0.1	0.074	0.089	0.081	0.087	0.076	0.102
3	0.1	0.078	0.093	0.084	0.101	0.069	0.091
4	0.1	0.074	0.089	0.077	0.084	0.065	0.108
5	0.1	0.074	0.093	0.076	0.094	0.071	0.098
6	0.1	0.062	0.079	0.084	0.092	0.066	0.069
7	0.1	0.077	0.093	0.081	0.102	0.079	0.081
Average		0.071	0.093	0.079	0.098	0.075	0.090
STDEV		0.0054	0.0066	0.0034	0.0105	0.0075	0.0138
MDL		0.0169	0.0207	0.0108	0.0331	0.0235	0.0434
RL		0.05	0.05	0.05	0.05	0.05	0.05

Table 2. Surface Water Spike Recovery Data

Spiked (0.1ppb)	Alachlor		Metolachlor		Alachlor OXA		Alachlor ESA	
	Found (ppb)	Recovery	Found (ppb)	Recovery	Found (ppb)	Recovery	Found (ppb)	Recovery
0.10	0.074	74.0%	0.087	87.0%	0.089	89.0%	0.096	96.0%
	0.085	85.0%	0.088	88.0%	0.087	87.0%	0.097	97.0%
	0.074	74.0%	0.082	82.0%	0.090	90.0%	0.092	92.0%
	0.087	87.0%	0.099	99.0%	0.079	79.0%	0.088	88.0%
	0.084	84.0%	0.111	111%	0.078	78%	0.097	97%
0.20	0.175	87.5%	0.199	99.5%	0.181	90.5%	0.201	100.5%
	0.152	76.0%	0.179	89.5%	0.179	89.5%	0.202	101.0%
	0.149	74.5%	0.162	81.0%	0.161	80.5%	0.183	91.5%
	0.160	80.0%	0.184	92.0%	0.160	80.0%	0.184	92.0%
	0.152	76.0%	0.186	93.0%	0.171	85.5%	0.181	90.5%
0.50	0.513	102.6%	0.545	109.00%	0.54	108.00%	0.555	111.00%
	0.45	90.0%	0.502	100.40%	0.531	106.20%	0.557	111.40%
	0.455	91.0%	0.472	94.40%	0.518	103.60%	0.554	110.80%
	0.496	99.2%	0.524	104.80%	0.469	93.80%	0.465	93.00%
	0.503	100.6%	0.524	104.80%	0.466	93.20%	0.503	100.60%
1.00	0.832	83.2%	0.866	86.6%	1.004	100.4%	1.046	104.6%
	0.874	87.4%	0.941	94.1%	0.989	98.9%	1.051	105.1%
	0.902	90.2%	0.920	92.0%	1.000	100.0%	0.938	93.8%
	1.013	101%	1.062	106.2%	0.971	97.1%	0.854	85.4%
	0.943	94.3%	1.091	109.1%	1.003	100.3%	1.018	101.8%
2.0	1.621	81.1%	1.708	85.4%	1.826	91.3%	1.881	94.1%
	1.655	82.8%	1.839	92.0%	1.797	89.9%	1.890	94.5%
	1.720	86.0%	1.751	87.6%	1.896	94.8%	1.818	90.9%
	1.961	98.1%	2.052	102.6%	1.912	95.6%	1.906	95.3%
	1.780	89.0%	1.894	94.7%	1.786	89.3%	1.872	93.6%
Average		87.0%		95.4%		92.5%		97.3%
STDEV		8.8%		8.8%		8.2%		7.1%
Control Range		60.5%-113.4%		69.1%-121.7%		67.7%-117.2%		75.9%-118.6%
Warning Range		69.4%-104.6%		77.9%-113%		76%-108.9%		83.0%-113.5%

Table 2 Continued

Spiked (0.1ppb)	Metolachlor OXA		Metolachlor ESA	
	Found (ppb)	Recovery	Found (ppb)	Recovery
0.10	0.081	81.0%	0.115	115.0%
	0.086	86.0%	0.119	119.0%
	0.076	76.0%	0.108	108.0%
	0.074	74.0%	0.089	89.0%
	0.069	69%	0.065	65%
0.20	0.118	59.0%	0.199	99.5%
	0.126	63.0%	0.206	103.0%
	0.148	74.0%	0.167	83.5%
	0.144	72.0%	0.165	82.5%
	0.139	69.5%	0.189	94.5%
0.50	0.507	101.40%	0.55	110.00%
	0.539	107.80%	0.469	93.80%
	0.499	99.80%	0.461	92.20%
	0.408	81.60%	0.498	99.60%
	0.432	86.40%	0.497	99.40%
1.00	0.904	90.4%	1.031	103.1%
	0.961	96.1%	1.067	106.7%
	0.818	81.8%	0.920	92.0%
	0.899	89.9%	1.049	104.9%
	0.887	88.7%	1.052	105.2%
2.0	1.780	89.0%	2.003	100.2%
	1.892	94.6%	2.072	103.6%
	1.662	83.1%	1.870	93.5%
	1.725	86.3%	1.636	81.8%
	1.668	83.4%	1.882	94.1%
Average		83.4%		97.6%
STDEV		12.0%		11.6%
Control Range		47.5%-119.2%		65.6%-132.5%
Warning Range		59.4%-107.3%		74.3%-120.9%

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Table 3 Tune methods

	Tune method 1		Tune method 2
Capillary Temp (C):	275		225
APCI Vaporizer Temp	0		0
Ion Time (ms):	5		5
Sheath Gas Flow ():	75		29
Aux Gas Flow ():	26		54
Source Type:	ESI		ESI
Injection Waveforms:	Type 1		Type 2
AGC:	On		On
POSITIVE POLARITY			
Source Voltage (kV):	5		4.5
Source Current (uA):	80		80
Capillary Voltage (V):	10		11.5
Tube Lens Offset (V):	-5		93
Octapole RF Amplifie	735		735
Octapole 1 Offset (V)	-6.5		-7.6
Octapole 2 Offset (V)	-8.5		-9.5
Entrance Lens (V):	-20		-12
InterOctapole Lens V	-64		-38
Trap DC Offset Voltag	-10		-10
Zoom Micro Scans:	5		5
Zoom AGC Target:	10000000		10000000
Zoom Max Ion Time (	50		50
Full Micro Scans:	3		3
Full AGC Target:	50000000		50000000
Full Max Ion Time (m	50		50
SIM Micro Scans:	5		5
SIM AGC Target:	20000000		20000000
SIM Max Ion Time (m	200		200
MSn Micro Scans:	- 2		2
MSn AGC Target:	20000000		20000000
MSn Max Ion Time (r	400		400
NEGATIVE POLARITY			
Source Voltage (kV):	5		6
Source Current (uA):	4.5		4.5
Capillary Voltage (V):	-7		-7
Tube Lens Offset (V):	-60		-60
Octapole RF Amplifie	370		370
Octapole 1 Offset (V)	7.75		7.75
Octapole 2 Offset (V)	10.5		12.5
InterOctapole Lens V	20		34
Entrance Lens (V):	40		36
Trap DC Offset Voltag	10		10
Zoom Micro Scans:	5		5
Zoom AGC Target:	10000000		10000000
Zoom Max Ion Time (	0		0
Full Micro Scans:	3		3
Full AGC Target:	20000000		20000000
Full Max Ion Time (m	50		50
SIM Micro Scans:	5		5
SIM AGC Target:	20000000		20000000
SIM Max Ion Time (m	200		200
MSn Micro Scans:	2		2
MSn AGC Target:	20000000		20000000
MSn Max Ion Time (r	400		400

Determination of Residues of Alachlor and Metolachlor and Selected Metabolites in Surface water by Liquid Chromatography-Mass Spectrometry

Table 4. Instrument Methods

Instrument Method 1		Instrument Method 2	
Instrument Method:	D:\A P LEE\1 year2001\Ala&Meto\8-20-01 try out.meth	Instrument Method:	D:\A P LEE\1 year2001\Ala&Meto\metabolites0-1%acid2-22-01-65-35.meth
MS Run Time (min): 13.50		MS Run Time (min): 15.00	
Divert Valve: in use during run		Divert Valve: in use during run	
	Valve State		Valve State
	==========		==========
0	To Waste	0	To Waste
8.07	To Source	7.85	To Source
13.41	To Waste	14.77	To Waste
Contact Closure: not used during run		Contact Closure: not used during run	
MS Detector Settings:		MS Detector Settings:	
Acquisition Start Delay (min): 7.50		Acquisition Start Delay (min): 8.00	
Real-time modifications to method disabled		Real-time modifications to method disabled	
Segment 1 Information		Segment 1 Information	
Duration (min): 13.50		Duration (min): 15.00	
Number of Scan Events: 4		Number of Scan Events: 4	
Tune Method: ESI 8-20-01		Tune Method:	ESI negative high flow tune10-10-00acidwaveform2
Scan Event Details:		Scan Event Details:	
1: Neg (278.0)->o(75.0-350.0)		1: Neg (278.0)->o(75.0-350.0)	
MS/MS: CE 34.0% IsoW 2.0		MS/MS: CE 20.0% IsoW 2.0	
2: Neg (328.0)->o(90.0-350.0)		2: Neg (328.0)->o(90.0-350.0)	
MS/MS: CE 44.0% IsoW 2.0		MS/MS: CE 35.0% IsoW 2.0	
3: Neg (264.0)->o(70.0-350.0)		3: Neg (264.0)->o(70.0-350.0)	
MS/MS: CE 32.0% IsoW 2.0		MS/MS: CE 31.0% IsoW 2.0	
4: Neg (314.0)->o(85.0-350.0)		4: Neg (314.0)->o(85.0-350.0)	
MS/MS: CE 33.0% IsoW 2.0		MS/MS: CE 35.0% IsoW 2.0	
Custom Dependent Data Settings:		Custom Dependent Data Settings:	
Not enabled		Not enabled	

Determination of Residues of Alachlor and Metolachlor and Selected Metabolites in Surface water by Liquid Chromatography-Mass Spectrometry

Table 5. APCI Tune Method	
Capillary Temp (C):	150
APCI Vaporizer Temp (C):	500
Ion Time (ms):	5
Sheath Gas Flow ():	89
Aux Gas Flow ():	3
Source Type:	APCI
Injection Waveforms:	Off
AGC:	On
POSITIVE POLARITY	
Source Voltage (kV):	6
Source Current (uA):	5
Capillary Voltage (V):	44
Tube Lens Offset (V):	55
Octapole RF Amplifier (Vp-p):	400
Octapole 1 Offset (V):	-4.75
Octapole 2 Offset (V):	-8
Entrance Lens (V):	-16
InterOctapole Lens Voltage (V)	-52
Trap DC Offset Voltage (V):	-10
Zoom Micro Scans:	5
Zoom AGC Target:	20000000
Zoom Max Ion Time (ms):	50
Full Micro Scans:	1
Full AGC Target:	50000000
Full Max Ion Time (ms):	300
SIM Micro Scans:	3
SIM AGC Target:	20000000
SIM Max Ion Time (ms):	100
MSn Micro Scans:	1
MSn AGC Target:	20000000
MSn Max Ion Time (ms):	1000
NEGATIVE POLARITY	
Source Voltage (kV):	5
Source Current (uA):	80
Capillary Voltage (V):	-47
Tube Lens Offset (V):	-35
Octapole RF Amplifier (Vp-p):	120
Octapole 1 Offset (V):	6.25
Octapole 2 Offset (V):	12
InterOctapole Lens Voltage (V)	68
Entrance Lens (V):	52
Trap DC Offset Voltage (V):	10
Zoom Micro Scans:	5
Zoom AGC Target:	20000000
Zoom Max Ion Time (ms):	0
Full Micro Scans:	3
Full AGC Target:	50000000
Full Max Ion Time (ms):	150
SIM Micro Scans:	5
SIM AGC Target:	20000000
SIM Max Ion Time (ms):	200
MSn Micro Scans:	2
MSn AGC Target:	20000000
MSn Max Ion Time (ms):	800

Tabl 6 APCI Instrument Method

Instrument Method: D:\A P LEE\1 year2001\Ala&Meto\parent apci 4-15-01.meth

MS Run Time (min): 15.00

Divert Valve: in use during run

Divert Time (min)	Valve State
0	To Waste
5	To Source

Contact Closure: not used during run

MS Detector Settings:

Acquisition Start Delay (min): 8.00
Real-time modifications to method disabled

Segment 1 Information

Duration (min): 15.00
Number of Scan Events: 2
Tune Method: apci high flow 238 tune

Scan Event Details:
1: Pos ·(284.0)->o(75.0-290.0)
MS/MS: CE 25.0% IsoW 3.0

2: Pos ·(270.0)->o(150.0-290.0)
MS/MS: CE 25.0% IsoW 3.0

Custom Dependent Data Settings:
Not enabled

Table 7 Method Modification Table

		Original method	Modified method		
HPLC	Mode	Isocratic 65% solution A and 35% solution B	Gradient 0 min 2 min 4 min 16 min 18 min 20 min	 10%A 10%A 65%A 65%A 10%A 10%A	 90%B 90%B 35%B 35%B 90%B 90%B
	Injection volume	50 µL	Unchanged		
MS	Parameters		Unchanged		

Determination of Residues of Alachlor and Metolachlor and Selected Metabolites in Surface water by Liquid Chromatography-Mass Spectrometry

Table 8 Method Modification Verification Spike/recovery Data

Verification #1

Date: 9-13-2002

		Alachlor OXA				Alachlor ESA			
	injection #	Calibrateded by linear and Forced 0		Calibrateded by linear and included 0		Calibrateded by linear and Forced 0		Calibrateded by linear and included 0	
Spiked (ppb)		Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)
0.1	1	0.083	83.0%	0.088	88.0%	0.075	75.0%	0.086	86.0%
	2	0.088	88.0%	0.101	101.0%	0.071	71.0%	0.084	84.0%
0.2	1	0.149	74.5%	0.158	79.0%	0.150	75.0%	0.160	80.0%
	2	0.171	85.5%	0.183	91.5%	0.157	78.5%	0.168	84.0%
0.5	1	0.458	91.6%	0.466	93.2%	0.475	95.0%	0.481	96.2%
	2	0.502	100%	0.511	102%	0.438	87.6%	0.447	89.4%
1	1	0.805	80.5%	0.796	79.6%	0.965	96.5%	0.978	97.8%
	2	0.916	91.6%	0.932	93.2%	0.92	92.0%	0.937	93.7%
2	1	1.667	83.4%	1.674	83.7%	1.776	88.8%	1.804	90.2%
	2	1.786	89.3%	1.819	91.0%	1.765	88.3%	1.799	90.0%

		Metolachlor OXA				Metolachlor ESA			
	injection #	Calibrateded by linear and Forced 0		Calibrateded by linear and included 0		Calibrateded by linear and Forced 0		Calibrateded by linear and included 0	
Spiked (ppb)		Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)
0.1	1	0.078	78.0%	0.084	84.0%	0.070	70.0%	0.093	93.0%
	2	0.086	86.0%	0.094	94.0%	0.060	60.0%	0.102	102%
0.2	1	0.163	81.5%	0.168	84.0%	0.154	77.0%	0.176	88.0%
	2	0.16	80.0%	0.168	84.0%	0.165	82.5%	0.202	101%
0.5	1	0.427	85.4%	0.43	86.0%	0.453	90.6%	0.468	93.6%
	2	0.438	87.6%	0.443	88.6%	0.421	84.2%	0.448	89.6%
1	1	0.8	80.0%	0.808	80.8%	0.955	95.5%	0.983	98.3%
	2	0.869	86.9%	0.88	88.0%	0.915	91.5%	0.968	96.8%
2	1	1.598	79.9%	1.611	80.6%	1.912	95.6%	1.969	98.5%
	2	1.825	91.3%	1.839	92.0%	1.797	89.9%	1.904	95.2%

Verification #2

Date 9-18-2002

		Alachlor OXA				Alachlor ESA			
	injection #	Calibrateded by linear and Forced 0		Calibrateded by linear and included 0		Calibrateded by linear and Forced 0		Calibrateded by linear and included 0	
Spiked (ppb)		Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)
0.1	1	0.086	86.0%	0.107	107.0%	0.068	68.0%	0.086	86.0%
	2	0.077	77.0%	0.099	99.0%	0.071	71.0%	0.088	88.0%
0.2	1	0.171	85.5%	0.19	95.0%	0.172	86.0%	0.188	94.0%
	2	0.158	79.0%	0.178	89.0%	0.157	78.5%	0.173	86.5%
0.5	1	0.454	90.8%	0.467	93.4%	0.405	81.0%	0.417	83.4%
	2	0.391	78.2%	0.406	81.2%	0.397	79.4%	0.409	81.8%
1	1	0.788	78.8%	0.818	81.8%	0.859	85.9%	0.882	88.2%
	2	0.805	80.5%	0.834	83.4%	0.832	83.2%	0.856	85.6%
2	1	1.486	74.3%	1.547	77.4%	1.74	87.0%	1.786	89.3%
	2	1.619	81.0%	1.678	83.9%	1.788	89.4%	1.834	91.7%

		Metolachlor OXA				Metolachlor ESA			
	injection #	Calibrateded by linear and Forced 0		Calibrateded by linear and included 0		Calibrateded by linear and Forced 0		Calibrateded by linear and included 0	
Spiked (ppb)		Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)	Found (ppb)	Recovery (%)
0.1	1	0.092	92.0%	0.094	94.0%	0.067	67.0%	0.081	81.0%
	2	0.078	78.0%	0.081	81.0%	0.064	64.0%	0.078	78.0%
0.2	1	0.175	87.5%	0.177	88.5%	0.153	76.5%	0.166	83.0%
	2	0.171	85.5%	0.174	87.0%	0.151	75.5%	0.164	82.0%
0.5	1	0.401	80.2%	0.403	80.6%	0.408	81.6%	0.417	83.4%
	2	0.381	76.2%	0.383	76.6%	0.397	79.4%	0.406	81.2%
1	1	0.835	83.5%	0.839	83.9%	0.889	88.9%	0.907	90.7%
	2	0.839	83.9%	0.843	84.3%	0.837	83.7%	0.855	85.5%
2	1	1.739	87.0%	1.747	87.4%	1.888	94.4%	1.922	96.1%
	2	1.857	92.9%	1.864	93.2%	1.805	90.3%	1.841	92.1%