

One Stop Shop for EPA Method 535

Reliably Analyze Acetamide Herbicide Degradates by LC/MS/MS

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- Full package: reference standards, SPE cartridges, and HPLC columns.
- Chromatographic resolution of alachlor ESA and acetochlor ESA isomers.
- Meet method requirements, with superior sensitivity.

Acetamide herbicides are used in large quantities to suppress weed growth in corn and soybean fields. However, due to the polar nature of ethanesulfonic acid (ESA) and oxanilic acid (OA) degradation products, contamination of drinking water sources is a concern. EPA Method 535 is designed to monitor drinking water for ESA and OA breakdown products of these herbicides. Chromatographic analysis is extremely important for this method because two analytes, alachlor ESA and acetochlor ESA, are isomers that share the same mass spectral multiple reaction monitoring (MRM) transitions, and thus must be separated chromatographically.

Resolution of all Method 535 analytes, including alachlor ESA and acetochlor ESA isomers, can easily be accomplished using Restek's full line of Method 535 products, which includes reference standards, solid phase extraction (SPE) cartridges, and HPLC columns that meet method guidelines. In the procedure shown here, 6mL CarboPrep™ 90 SPE cartridges were used for sample preparation, both to help extend the lifetime of the analytical column as well as to prevent matrix enhancement or suppression. LC/MS/MS analysis was performed on an Ultra C18 column coupled to an Applied Biosystems API 3200™ LC/MS/MS system equipped with a TurboIonSpray® probe for the Turbo V™ source.

Figure 1 Easily resolve Method 535 herbicide degradates on an Ultra C18 HPLC column.

Sample: acetamide herbicides
 Inj.: 25µL
 Conc.: 50ng/mL
 Sample diluent: 5mM ammonium acetate in water

Column: Ultra C18
 Cat.#: 9174312
 Dimensions: 100mm x 2.1mm
 Particle size: 3µm
 Pore size: 100Å

Conditions:
 Mobile phase: A: 5mM ammonium acetate
 B: methanol

Time:	%B
0.0	20%
4.0	30%
10.0	30%
15.0	50%
17.0	85%
18.0	85%
18.1	20%
28.0	20%

Flow: 250µL/min.
 Temp.: ambient

Det.: API 3200™ LC/MS/MS system
 Ion Source: TurboIonSpray® probe for Turbo V™ source (negative)
 Mode: MRM
 Curtain gas: 25
 Collision gas: medium/5
 IonSpray voltage: -4,500
 Temperature: 500°C
 Ion source gas 1: 50
 Ion source gas 2: 50
 Interface heater: on
 Dwell time: 50
 Vertical probe position: 2

**Excellent response at low concentrations!
 Fully resolve alachlor ESA and acetochlor ESA isomers.**

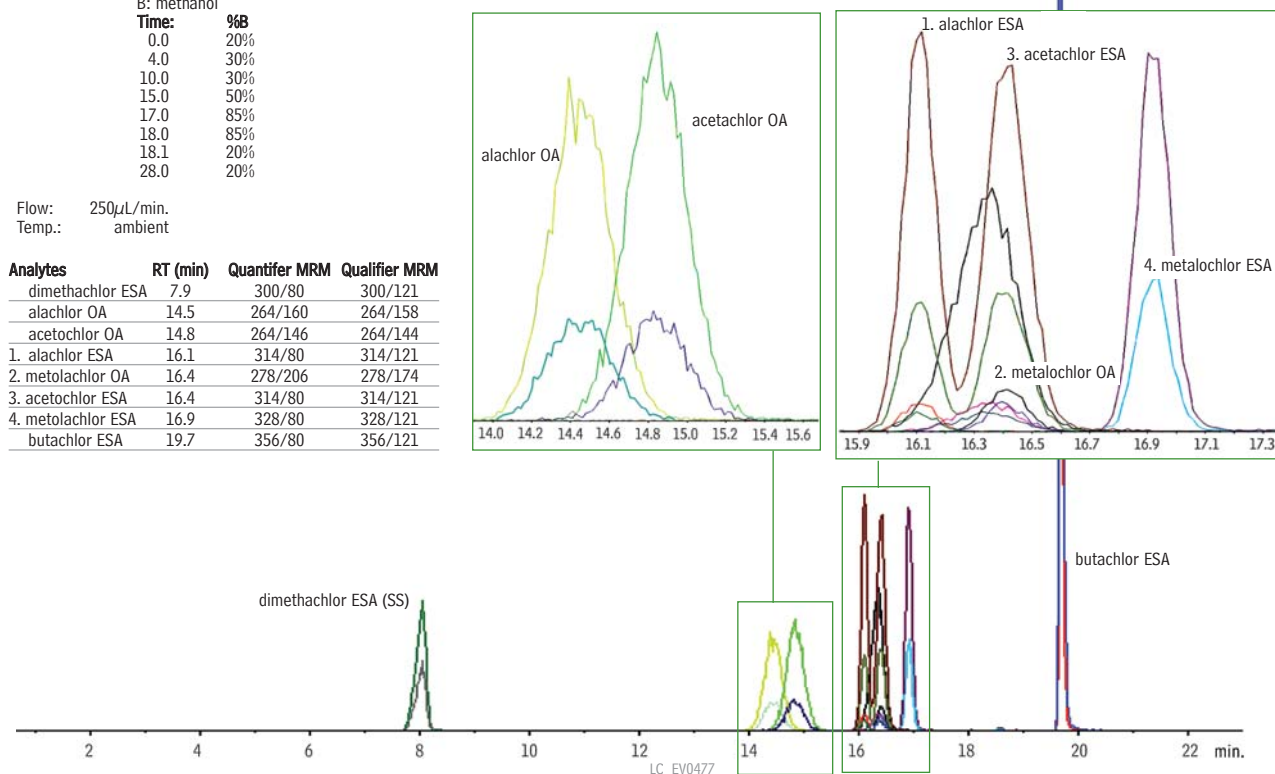


Table I Reliably achieve minimum detection limits of 0.004 $\mu\text{g/L}$ or less.

Analyte	LCMRL ($\mu\text{g/L}$)	Standard Deviation	Calculated Detection Limit in Matrix ($\mu\text{g/L}$)
alachlor OA	0.013	0.28	0.003
acetochlor OA	0.014	0.27	0.003
alachlor ESA	0.013	0.18	0.002
metolachlor OA	0.013	0.21	0.003
acetochlor ESA	0.012	0.29	0.004
metolachlor ESA	0.012	0.18	0.002

Seven matrix spikes prepared at 0.013 $\mu\text{g/L}$ (proposed MRL).

Table II Outstanding accuracy and precision using Ultra C18 HPLC columns.

Analytes	Average Recovery (%)	%RSD
dimethachlor ESA	100.1	9.2
metolachlor OA	95.0	8.5
metolachlor ESA	94.8	8.9
alachlor OA	96.6	8.5
acetochlor OA	97.0	8.9
alachlor ESA	92.5	8.6
acetochlor ESA	94.3	8.0

Four lab fortified blanks spiked at 0.2 $\mu\text{g/L}$.

Method requirements: average recovery $\pm 30\%$ of the true value, %RSD $\leq 20\%$.

CarboPrep™ SPE Cartridges Nonporous graphitized carbon

SPE Cartridge	Tube Volume, Bed Weight	qty.	cat#
CarboPrep 90	6mL, 500mg	30-pk.	26092

Ultra C18 Columns (USP L1) Excellent for a wide range of analyses

Physical Characteristics:

particle size: 3 μm , spherical
pore size: 100Å
carbon load: 20%

endcap: fully endcapped
pH range: 2.5 to 7.5
temperature limit: 80°C

3 μm Column, 2.1mm	cat. #
100mm	9174312

Method 535 Individual Compounds

Volume is 1mL/ampul. Concentration is $\mu\text{g/mL}$.

Compound	Solvent	Conc.	cat.#
acetochlor ESA sodium salt	M	100	33092
acetochlor OA	M	100	33094
alachlor ESA sodium salt	M	100	33096
alachlor OA	M	100	33099
metolachlor ESA sodium salt	M	100	33200
metolachlor OA	M	100	33201

M=methanol

Method 535 Internal Standard

butachlor ESA sodium salt
100 $\mu\text{g/mL}$ in methanol, 1mL/ampul

cat. # 33202

Method 535 Surrogate Standard

dimethachlor ESA sodium salt
100 $\mu\text{g/mL}$ in methanol, 1mL/ampul

cat. # 33203

Consistent chromatographic resolution of 3.5 or greater foralachlor ESA and acetochlor ESA was easily achieved as shown in Figure 1. Surrogate recoveries, matrix spikes, minimum detection limits, and internal standard recoveries produced

Resolution of all target analytes, includingalachlor ESA and acetochlor ESA isomers, can easily be achieved.

consistently acceptable results at low concentrations and showed no interferences from the drinking water matrix. The method reporting limits (MRL) listed in Table I are based on seven replicate fortified blanks prepared at the proposed MRL of 0.013 $\mu\text{g/L}$ in drinking water. An LCMRL of 0.012 to 0.014 $\mu\text{g/L}$ was established and validated with a calculated detection limit of 0.004 $\mu\text{g/L}$ or less. Precision and accuracy were demonstrated using four replicate fortified blanks at 0.2 $\mu\text{g/L}$; recovery and RSD values easily met method requirements (Table II). All analytes were detected in laboratory blanks at $\leq 1/3$ MRL values demonstrating low system background levels.

The optimized method developed here shows superior sensitivity for the ESA and OA degradates of chloroacetanilide herbicidesalachlor, acetochlor, and metolachlor, as well as reliable resolution between isomersalachlor ESA and acetochlor ESA. This method is simplified by Restek's suite of Method 535 products. All of the reference materials, sample preparation products, and HPLC columns needed are now available from a single source, to facilitate successful Method 535 analysis.

References

1. C. Borton. EPA Method 535; Detection of Degradates of Chloroacetanilides and other Acetamide Herbicides in Water by LC/MS/MS. Applied Biosystems, Foster City, CA, 2008.

