Note d'application

Analysis of Chloroacetanilide and Acetamide Herbicide Degradates in Drinking Water by UPLC-MS/MS

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Abstract

This application note demonstrates the analysis of chloroacetanilide and acetamide herbicide degradates in drinking water by UPLC-MS/MS.

Introduction

Chloroacetanillide and acetamide herbicides are widely used in the U.S. for the control of broadleaf and annual weeds on crops such as corn and soybeans.[1] These herbicides are thought to be degraded by microbial action to the oxanilic acid (OA) and ethanesulfonic acid (ESA) metabolites of the parent compounds. The OA and ESA metabolites are more water soluble and therefore more mobile than the parent herbicides. Several studies by the United States Geological Survey (USGS) and others have reported higher occurrences of the OA and ESA metabolites than the parent herbicides in both ground and surface water.[2,3] The health effects of these metabolites is unknown and limits in drinking water have not been established.

U.S. EPA Method 535 was developed for the determination of OA and ESA metabolites of alachlor, acetochlor, metolachlor, propachlor, flufenacet and dimetheamid in drinking water.[4]

Six of the metabolites analyzed by EPA method 535 are among the 26 contaminants proposed to be regulated in second round of the Unregulated Contaminant Monitoring Rule (UCMR2). Alachlor OA, Alachlor ESA, Acetochlor OA, Acetochlor ESA, Metolachlor ESA and Metolachlor OA are on the screening survey list. Contaminants on the screening survey list will be monitored by approximately 1,100 public water systems (PWSs) during a 12 month period between July 2007 and June 2010. The results obtained from UCMR2 monitoring will be used to determine the occurrence of contaminants in drinking water, the population being exposed to these contaminants and the level of exposure. When the study is completed in 2010, this information can then be used by the EPA to determine if these contaminants should be regulated in the future.[5]

Experimental

U.S. EPA Method 535 Description

A 250 mL water sample is extracted using a graphitized carbon SPE cartridge, dried and made up to 1 mL with 5 mM ammonium acetate. The samples are injected on a C_{18} column at 65 °C and analyzed by LC-MS/MS in negative ion mode. The analyte concentrations are determined by use of a chromatographic internal standard.

The HPLC-tandem quadrupole MS method described in EPA method 535 has a 35 minute runtime and the HPLC-ion trap MS method has a 60 minute runtime. Two of the analytes, Alachlor ESA and Acetochlor ESA are structural isomers and are monitored using the same MRM transition (314 > 80).
Therefore, chromatographic separation is required. The method states that these analytes must be separated chromatographically with a resolution (Rs) of 1.0 or greater.

This note describes an UltraPerformance Liquid Chromatography (UPLC) and tandem mass spectrometry (MS/MS) method for the analysis. The advantages of using UPLC rather than HPLC include a 2.5 to 4x increase in throughput due to decreased runtimes, improved resolution of Alachlor ESA and Acetachlor ESA and increased sensitivity of 2 to 4x.

Waters ACQUITY UPLC and Quattro Micro API Mass Spectrometer.

LC Conditions

System:	Waters ACQUITY UPLC
Mobile phase A:	5 mM aqueous ammonium acetate*
Mobile phase B:	Methanol
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 150 mm, 1.7 µm (P/N 186002353)
Column temp:	65 °C
Flow rate:	0.45 mL/min

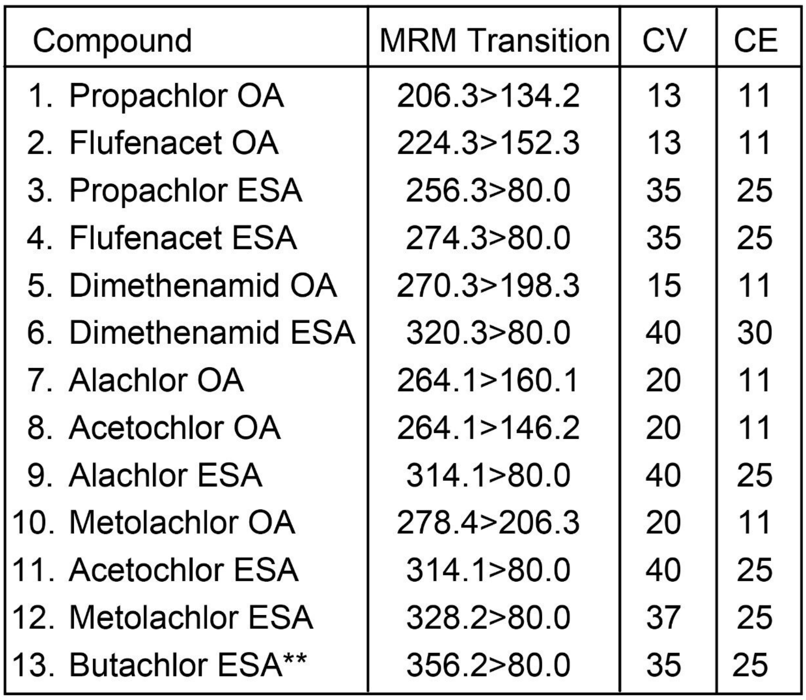
Gradient

Time	%A	%B
Initial	90	10
5.0	75	25
7.5	66	34
10.5	65	35
11.0	10	90
13.0	90	10

MS Conditions

System:	Waters Quattro micro API
Ion mode:	Electrospray negative

The MRM transitions, cone voltages (CV) and collision energies (CE) are listed in Table 1.



Compound	MRM Transition	CV	CE
1. Propachlor OA	206.3>134.2	13	11
2. Flufenacet OA	224.3>152.3	13	11
3. Propachlor ESA	256.3>80.0	35	25
4. Flufenacet ESA	274.3>80.0	35	25
5. Dimethenamid OA	270.3>198.3	15	11
6. Dimethenamid ESA	320.3>80.0	40	30
7. Alachlor OA	264.1>160.1	20	11
8. Acetochlor OA	264.1>146.2	20	11
9. Alachlor ESA	314.1>80.0	40	25
10. Metolachlor OA	278.4>206.3	20	11
11. Acetochlor ESA	314.1>80.0	40	25
12. Metolachlor ESA	328.2>80.0	37	25
13. Butachlor ESA**	356.2>80.0	35	25

Table 1. Optimized MRM transition parameters for EPA Method 535 analytes in ES-.

** 5 mM aqueous ammonium acetate should be replaced at least every 48 hours, preferably every 24 hours.*

*** Internal standard*

Software

Data was acquired with Waters MassLynx Software and processed with Waters QuanLynx Application Manager.

Results and Discussion

Figure 1 shows the chromatogram obtained using a traditional HPLC-MS/MS system and the gradient specified in the EPA 535 method. The first eluting compound is Propachlor OA with a retention time of 9 minutes. The internal standard, Butachlor ESA elutes last at 30 minutes. The total runtime for the HPLC method is 35 minutes and run times of up to 60 minutes using the ion trap MS instrument are reported in the

EPA method. Figure 2 illustrates the improved chromatography with respect to run time when the analysis is carried out by UPLC-MS/MS. The total runtime is shortened to 14 minutes.

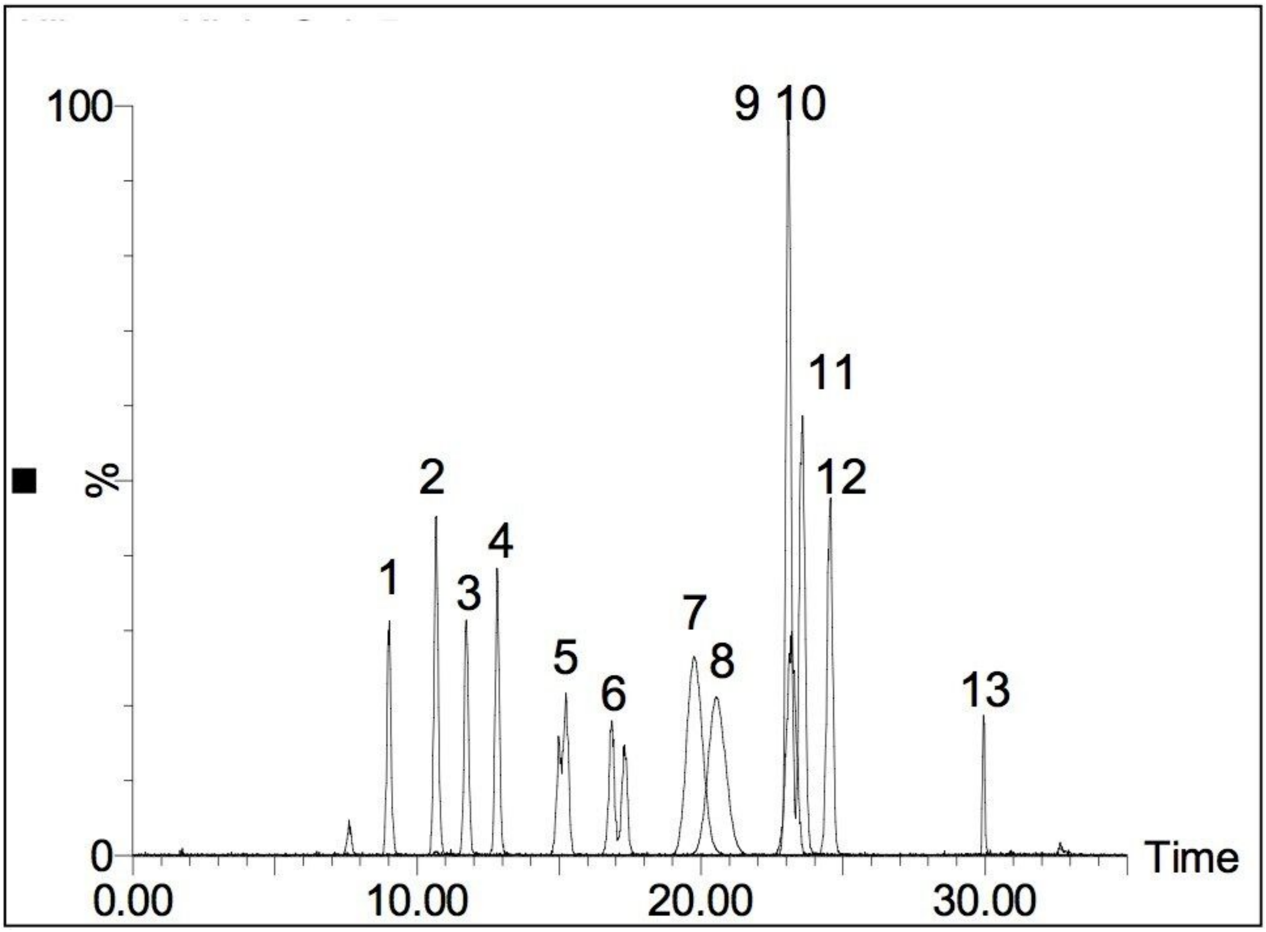


Figure 1. HPLC-MS/MS chromatogram for EPA Method 535 analytes.

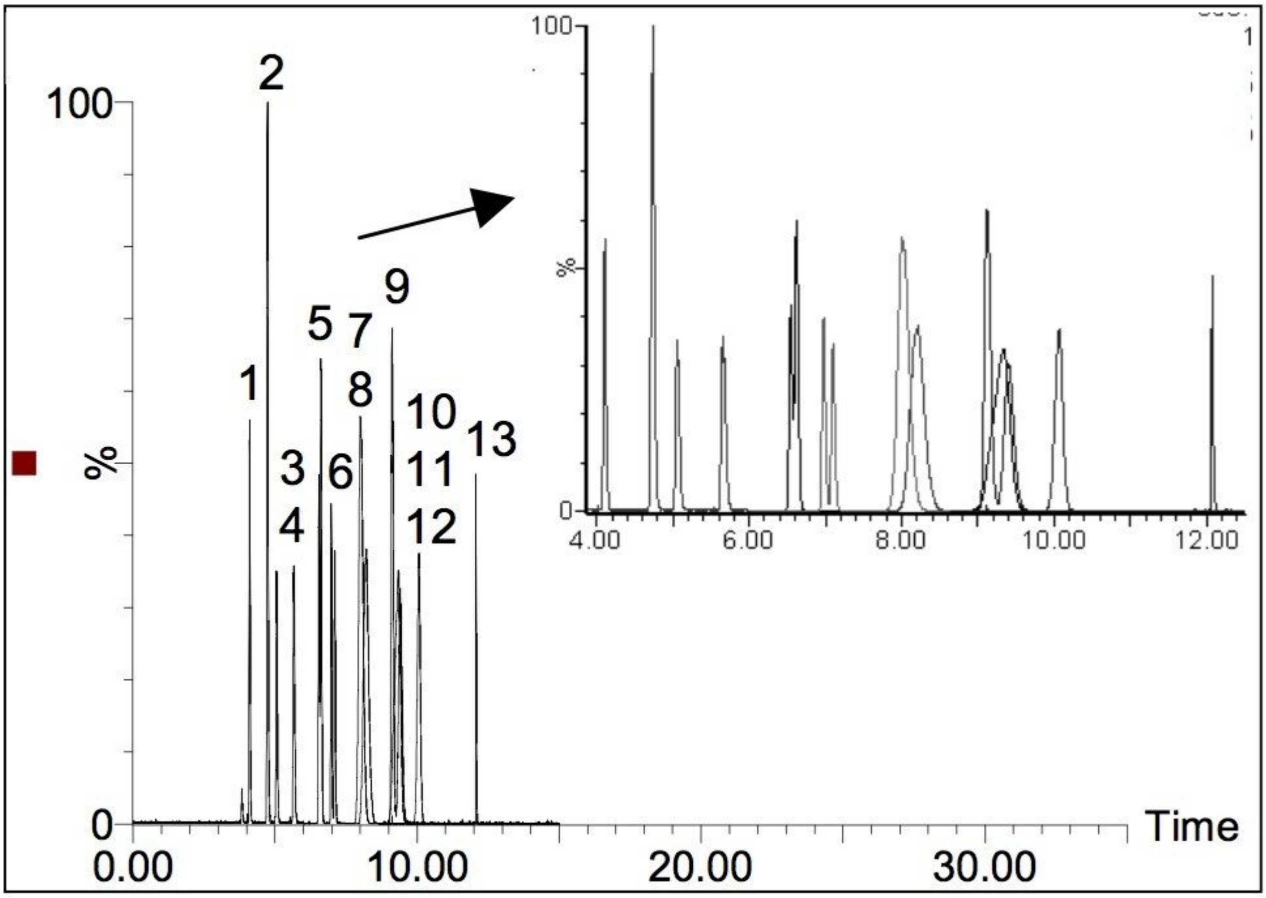


Figure 2. UPLC-MS/MS chromatogram for EPA Method 535 analytes.

Crucial to this method is the chromatographic separation of the structural isomers, Alachlor ESA and Acetochlor ESA. Figure 3 shows the spectra of these compounds. The two most intense product ions for both compounds are *m/z* 80 and *m/z* 121. Dissimilar product ions *m/z* 176 for Alachlor ESA and *m/z* 146 for Acetochlor ESA are approximately 15% the intensity of the product ion at *m/z* 80. Therefore, the use of these dissimilar product ions would greatly reduce the sensitivity if used for quantitation.

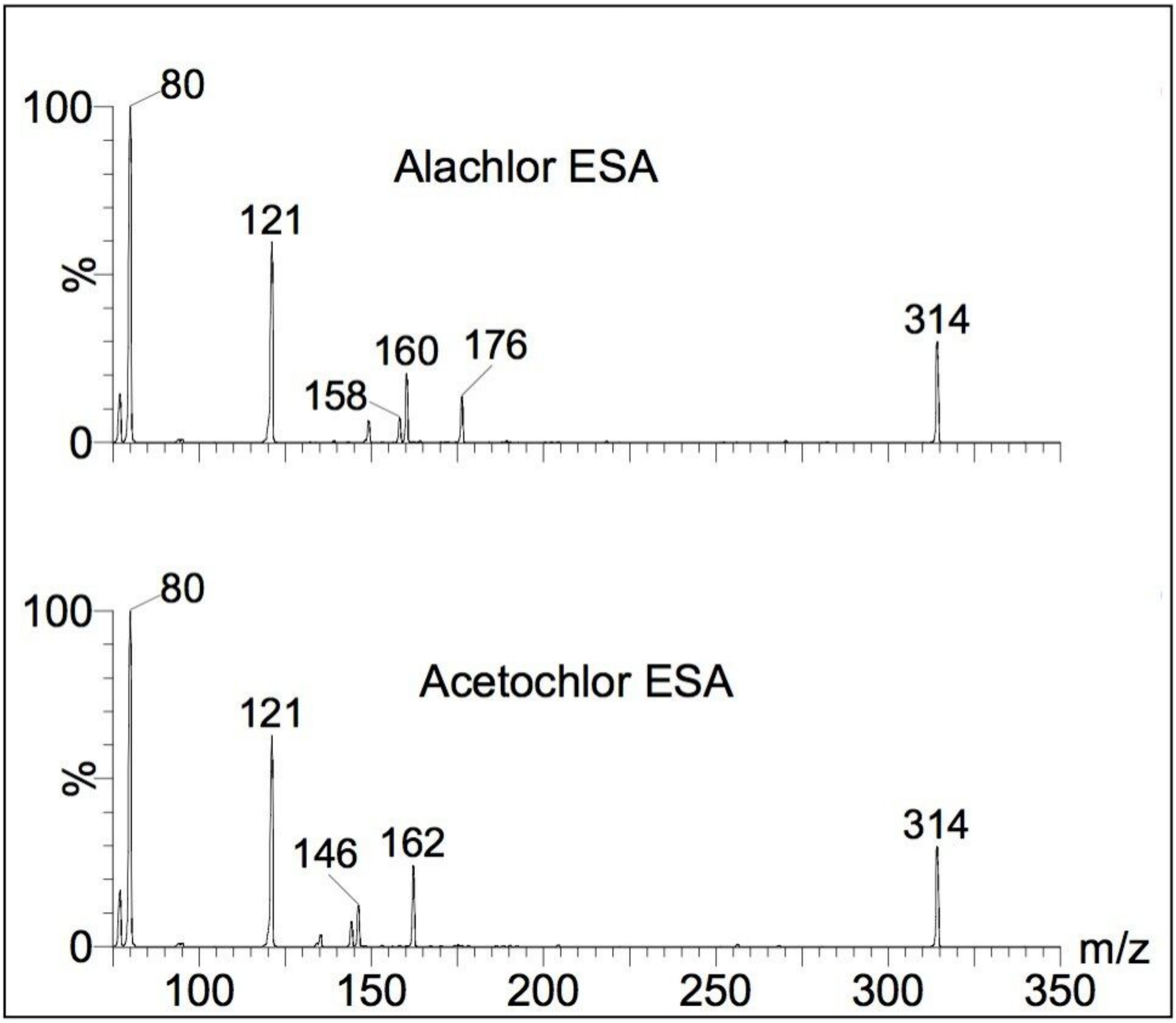


Figure 3. Product ion scan spectra of Alachlor ESA (above) and Acetochlor ESA (below).

Even though the UPLC-MS/MS method results in 2.5x increase in throughput, resolution is not compromised. In fact, the use of 1.7 µm particles in UPLC columns results in higher efficiencies. Figure 4 shows the resolution of Alachlor ESA and Acetochlor ESA on both UPLC-MS/MS and HPLC-MS/MS. The resolution obtained on the UPLC-MS/MS is superior to that obtained on the HPLC-MS/MS system and well surpasses the method requirement of Rs > 1 for these compounds.

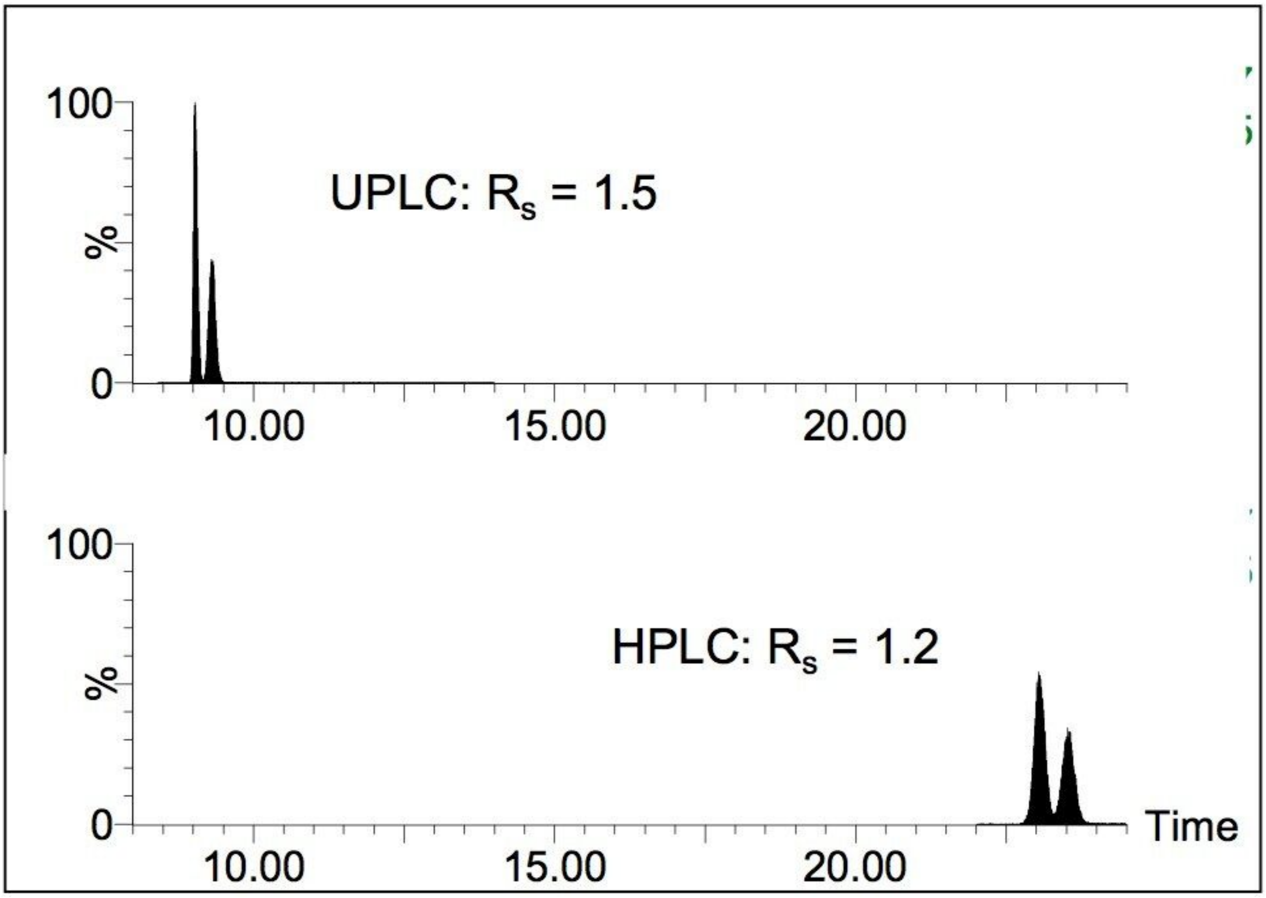
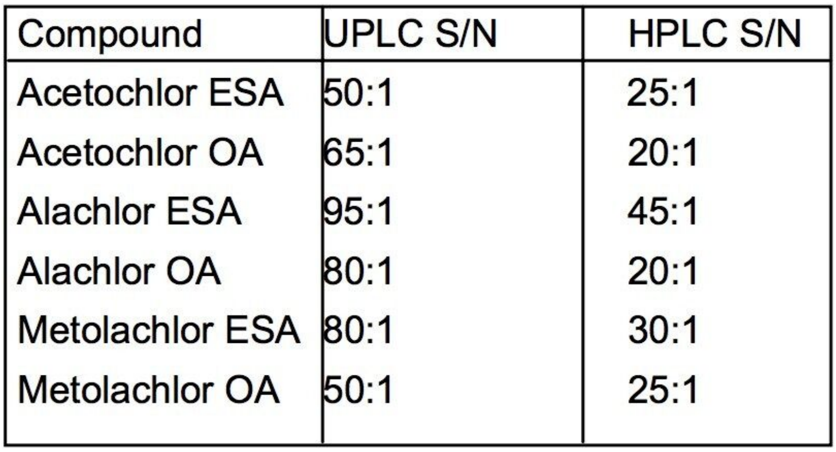


Figure 4. Comparison of resolution of Alachlor ESA and Acetochlor ESA obtained on UPLC (above) and HPLC (below).

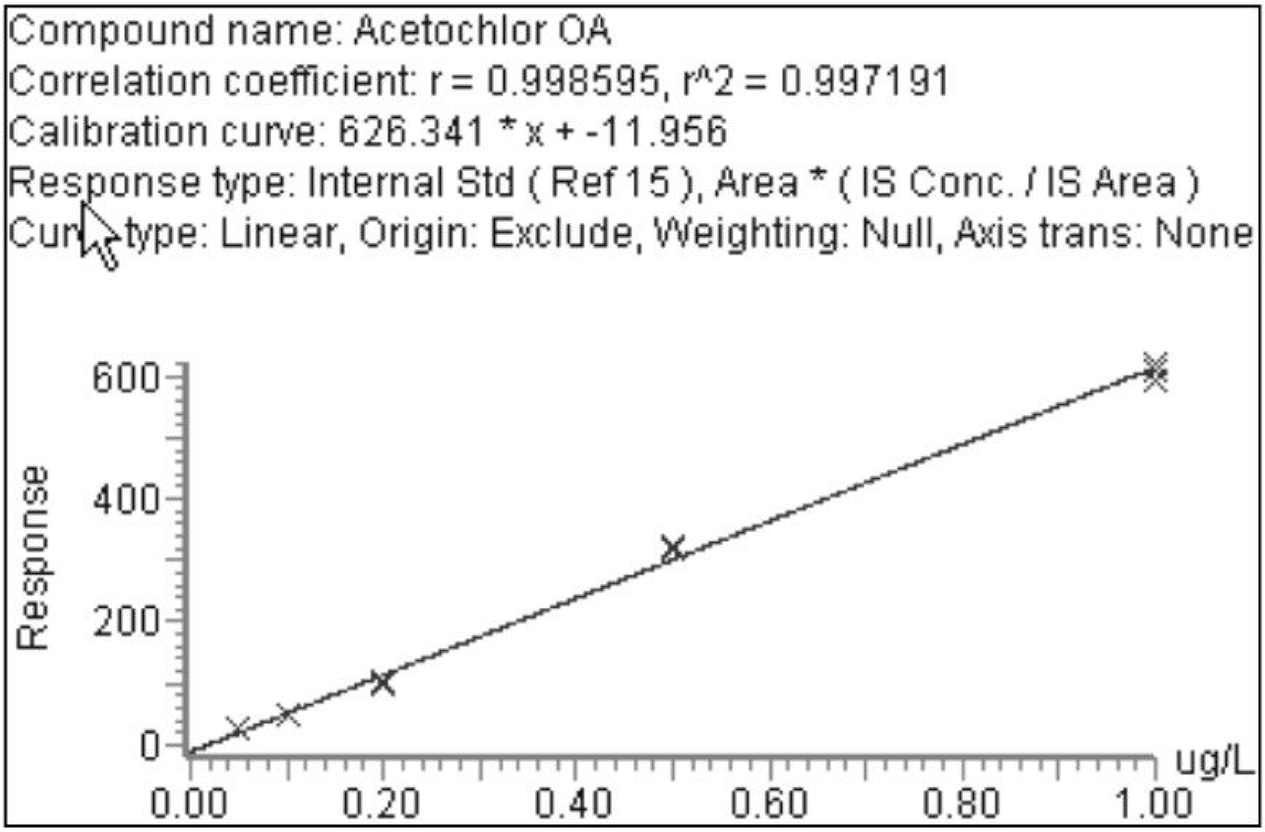
Improvements of 2 to 4x in sensitivity were achieved using UPLC versus HPLC. Table 2 lists the signal to noise values obtained for a 0.05 µg/L standard of the six herbicide metabolites proposed to be monitored under UCMR2.



Compound	UPLC S/N	HPLC S/N
Acetochlor ESA	50:1	25:1
Acetochlor OA	65:1	20:1
Alachlor ESA	95:1	45:1
Alachlor OA	80:1	20:1
Metolachlor ESA	80:1	30:1
Metolachlor OA	50:1	25:1

Table 2. Comparison of signal to noise values obtained for 0.05 µg/L standard on UPLC and HPLC.

The QuanLynx Application Manager was used to construct calibration curves using the internal standard technique. The calibration curves were linear with r^2 >0.995 in the range 0.05–1.0 µg/L. This satisfies the EPA method requirement of five calibration concentrations spanning a 20-fold concentration range. The calibration curve obtained for Acetochlor OA is shown in Figure 5.



Compound name: Acetochlor OA
Correlation coefficient: r = 0.998595, r^2 = 0.997191
Calibration curve: 626.341 * x + -11.956
Response type: Internal Std (Ref 15), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

Figure 5. Acetochlor OA calibration curve.

Conclusion

The use of UPLC-MS/MS for the analysis of the herbicide metabolites in EPA method 535 has many benefits over traditional HPLC-MS/MS. The advantages include a 2.5 to 4x reduction in total runtime, improved resolution for the structural isomers Acetochlor ESA and Alachlor ESA and a 2 to 4x increase in sensitivity.

References

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2. Phillips, P.J.; Eckhardt, D.A.; Rosenmann, L.; 2000, Pesticides and Their Metabolites in Three Public Water-

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3. Koplin, D.W.; Thurman, A; Goolsby, D.A.; *Environ. Sci. Technol*. 1996, 30, 335–340.

4. EPA Document #: EPA/600/R-05/053

5. http://www.epa.gov/safewater/ucmr/ucmr2/ <http://www.epa.gov/safewater/ucmr/ucmr2/>

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