EPA Method 535:

Detection of Degradates of Chloroacetanilides and other Acetamide Herbicides in Water by LC/MS/MS

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Overview

Described here is the analysis of Chloroacetanilide and Acetamide degradates in drinking water using Liquid Chromatography Tandem Mass Spectrometry, LC/MS/MS. This analysis follows U.S. Environmental Protection Agencies' Method 535 guidelines for sample preparation and analysis. The method used two Multiple Reaction Monitoring (MRM) transitions per analyte for both quantitation and confirmation. Monitoring a second MRM transition for each target compound adds an additional order of confidence when looking at dirty matrices, therefore, the possibility of false positive detection is reduced. Detection limits in drinking water were determined to be 0.002 to 0.004 µg/L using established guidelines.

Introduction

The EPA has established Method 535 for the analysis of ethanesulfonic acid (ESA) and oxanilic acid (OA) degradates of chloroacetanilide herbicides in drinking water and surface water. Chloroacetanilide herbicides are extensively used for weed control on crops throughout the US. In this method degradates of Alachlor, Acetochlor and Metolachlor are monitored and a Lowest Concentration Minimum Reporting Level (LCMRL) of 0.012 to 0.014 µg/L was validated for all compounds. Chromatography has also been set up to include the degradates of Dimethenamid, Flufenacet, and Propachlor in future work. The surrogate and internal standard used for this method were Dimethachlor ESA and Butachlor ESA respectively.

Despite using a specific MS/MS scan, Alachlor ESA and Acetochlor ESA are structural isomers (Figure 1) that have similar product ions of m/z 80 and 121. For this reason, consistent chromatographic resolution is necessary and was achieved using a shallow stepping gradient on a Restek Ultra C_{18} column (Figure 2).

Results showed consistent performance for both standards and samples over several days of work. From the product ion spectra in Figure 1, there are several unique product ions for both Alachlor ESA, m/z 158, 160, 176, and Acetochlor ESA, m/z 144, 146, 162. These product ions are not as sensitive as m/z 80 and 121, therefore they were not used. As an alternative to the method described below, using the unique product ions for both Alachlor ESA and Acetochlor ESA would eliminate the need for baseline chromatographic separation. It is important to note that using product ions other than m/z 80 and 121 will result in loss of sensitivity.

Both matrix effects, such as ion enhancement and ion suppression, due to high total organic carbon (TOC) were a real concern during method development. Surrogate recoveries, matrix spikes, MDLs, and internal standard recoveries were monitored but did not indicate any interference using the method below with drinking water.

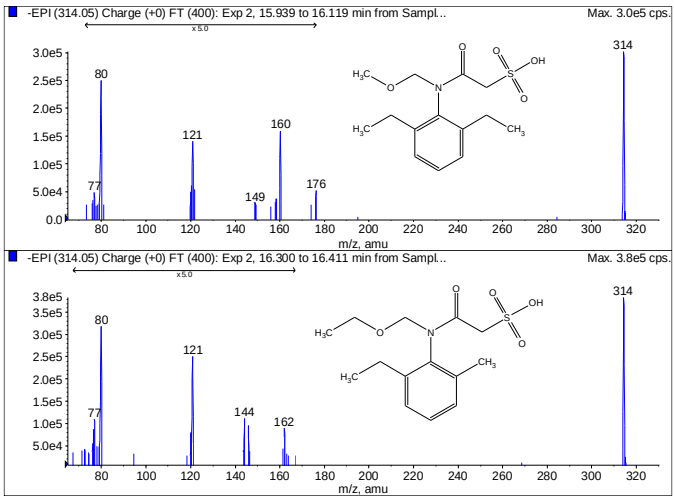

Figure 1. Product Ion spectra of structural isomers Alachlor ESA (top) and Acetochlor ESA (bottom)

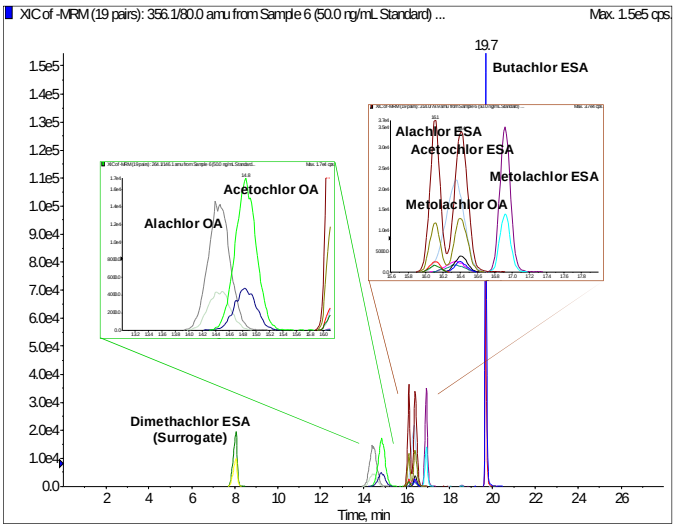

Figure 2. Reproducible chromatography was achieved using a gradient on a Restek Ultra C18 3 µm 100x2.1 mm column. A 50 ng/mL initial calibration point is shown. Sufficient baseline separation was achieved for structural isomers Alachlor ESA and Acetochlor ESA with a consistent resolution factor of 3.5 or greater.

Experimental

The method uses an AB SCIEX API 3200™ LC/MS/MS system equipped with Turbo V™ source and Electrospray Ionization probe. All compounds were detected using negative ionization in Multiple Reaction Monitoring (MRM) mode using two MRM transitions for each target compound and surrogate. The following mass spectrometer conditions were used: Curtain Gas™ interface: 25 psi, IS voltage: -4500 V, Gas1: 50 psi. Gas2: 50 psi, Ion source temperature: 500°C, Collision gas: Medium, Interface heater: On, Vertical probe position: 2, Horizontal probe position: 5, Dwell time: 50 ms.

An Agilent 1200 HPLC system was used consisting of a binary pump, autosampler with thermal unit, and column oven. Chromatographic separation was achieved on a Restek Ultra C18 3 µm 100x2.1 mm column using mobile phases, A: 5 mM ammonium acetate, B: methanol with the gradient in Table 1. A 25 µL injection volume was used.

Table 1. LC gradient

Time (min)	Flow rate (µl/min)	A (%)	B (%)
0.00	250	80	20
4.00	250	70	30
10.0	250	70	30
15.0	250	50	50
17.0	250	15	85
18.0	250	15	85
18.1	250	80	20
28.0	250	80	20

Sample preparation was performed using Solid Phase Extraction (SPE). Restek Carbora 90, 6.0 mL tube size, SPE cartridges were used. Cartridges were conditioned with 20 mL of 10 mM ammonium acetate/methanol and then rinsed with 30 mL reagent water. Cartridges were not allowed to go dry at any time during the sample loading process. After conditioning, 250 mL of sample was prepared by adding 25-30 mg ammonium chloride and spiked with 5 µL of a 12 µg/mL surrogate standard, mixed, and then loaded using a vacuum manifold at a flow rate of 10-15 mL/min. After loading, each cartridge was rinsed with 5 mL reagent water and then allowed to dry using nitrogen. Cartridge elution was performed using 15 mL of 10 mM ammonium acetate/methanol at gravity flow. The extracts were concentrated to dryness using a gentle stream of nitrogen in a heated water bath, 60-70°C. Finally, 1 mL of 5 mM ammonium acetate/reagent water and 10 µL of a 5 µg/mL internal standard solution were added and transferred to an autosampler vial.

Six calibration points (0.5, 1.0, 10.0, 50.0, 100.0, and 125.0 ng/mL) prepared in 5 mM ammonium acetate/reagent water, were used for the initial calibration curve. A linear fit was used with 1/x weighting and a correlation coefficient, r, of 0.995 or greater was achieved. All stock and primary dilution standards were prepared in methanol and stored at 4°C.

Results and Discussion

Method development was performed using the automated Quantitative Optimization feature of the Analyst® Software. Each target compound, surrogate, and internal standard was infused into the mass spectrometer at a low flow rate of 10 µL/min. Quantitative Optimization identified the precursor ion, the most sensitive product ions for each compound, and optimized all compound dependant parameters automatically. Results are shown in Table 2.

Table 2. Detection in Multiple Reaction Monitoring (MRM). Two MRM transitions were monitored for all target analytes and the surrogate. Only 1 MRM was used to monitor the internal standard (IS).

Analyte	Quantifier MRM	Qualifier MRM	MRM Ratio Range (±20%)
Alachlor OA	264 / 160	264 / 158	0.24 – 0.36
Acetochlor OA	264 / 146	264 / 144	0.23 – 0.35
Alachlor ESA	314 / 80	314 / 121	0.28 – 0.42
Metolachlor OA	278 / 206	278 / 174	0.10 – 0.15
Acetochlor ESA	314 / 80	314 / 121	0.33 – 0.50
Metolachlor ESA	328 / 80	328 / 121	0.32 – 0.48
Dimethachlor ESA (Surrogate)	300 / 80	300 / 121	0.39 – 0.59
Butachlor (IS)	356 / 80	–	–

MRM area ratios were used for confirming detection. Each ratio, displayed in Table 2, was calculated by determining the MRM ratio of each calibration standard and then taking the average of all standards. A ±20% range was then applied to each unknown sample. The Analyst Reporter automatically flagged any unknown sample with a calculated MRM ratio outside the established 20% range.

Once method development was completed an Initial Demonstration of Capability (IDC) was performed. First an initial demonstration of low system background was run by preparing a Laboratory Reagent Blank (LRB). For each analyte, detection in the prepared LRB needed to be < 1/3 of the MRL detection (Figure 3).

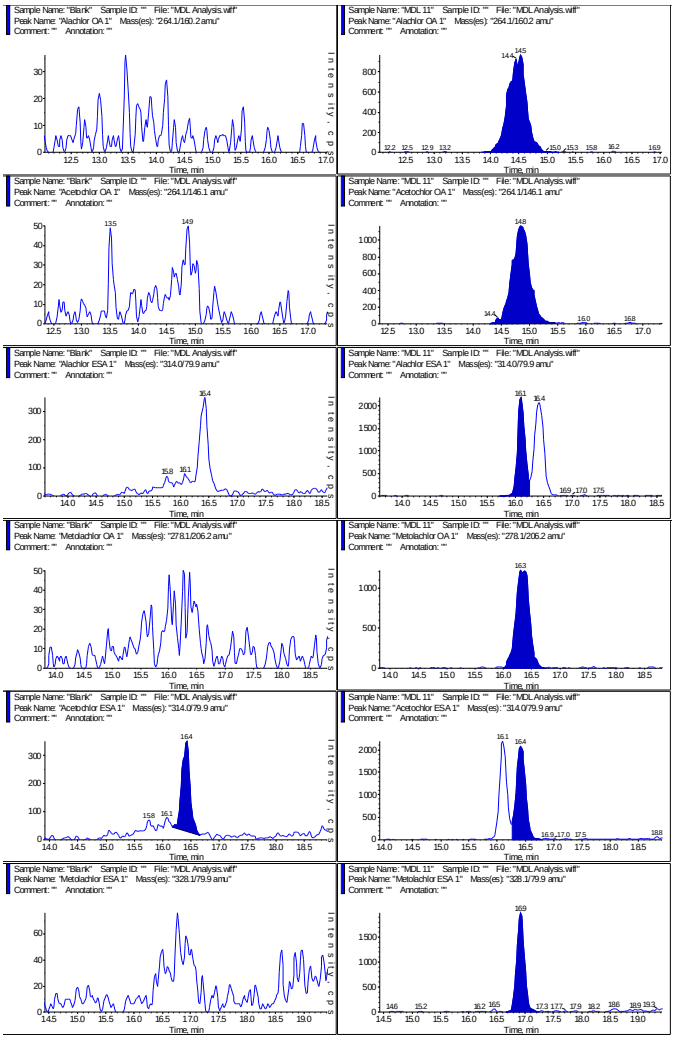

Figure 3. Extracted Ion Chromatograms (XIC) for the Laboratory Reagent Blank (LRB) for all target analytes are displayed in the left column and the XIC of the proposed Method Reporting Limit (MRL) are in the right column.

To validate the proposed MRL, seven replicate LRBs were spiked at a concentration of 0.013 µg/L and processed through the sample preparation procedure above. All chromatographic peaks for both quantifier and qualifier MRM transitions required a signal to noise ratio of at least 3:1. Using the proposed procedure for calculating an LCMRL in EPA Method 535 a calculated detection limit of 0.004 µg/L or less was determined for all analytes (Table 3).

Table 3. Minimum reporting limit confirmation

Analyte	LCMRL	Standard Deviation	HR_{PIR}	Lower PIR	Upper PIR	Extract LOD (µg/L)	Sample LOD (µg/L)	On Column LOD (fg)
Alachlor OA	0.013	0.28	1.1	72.4	142.3	0.868	0.003	86.8
Acetochlor OA	0.014	0.27	1.1	74.0	141.9	0.843	0.003	84.3
Alachlor ESA	0.013	0.18	0.7	82.1	127.9	0.569	0.002	56.9
Metolachlor OA	0.013	0.21	0.8	75.7	128.2	0.651	0.003	65.1
Acetochlor ESA	0.012	0.29	1.1	62.4	134.7	0.897	0.004	89.7
Metolachlor ESA	0.012	0.18	0.7	76.1	122.5	0.576	0.002	57.6

After the MRL was confirmed an Initial Demonstration of Precision on Accuracy was performed. Four replicate LRBs were fortified at a concentration of 0.2 µg/L. The Percent Relative Standard Deviation (%RSD) for all analytes was ≤20% and the average recovery was within ±30% of the true value. Therefore the method satisfied the precision and accuracy requirements (Table 4).

Table 4. Initial demonstration of precision and accuracy

Analyte	Average Recovery (%)	% RSD
Alachlor OA	96.6	8.5
Acetochlor OA	97.0	8.9
Alachlor ESA	92.5	8.6
Metolachlor OA	95.0	8.5
Acetochlor ESA	94.3	8.0
Metolachlor ESA	94.8	8.9
Dimethachlor ESA (Surrogate)	100.1	9.2

Finally the recoveries of the internal standard and surrogate were monitored over a period of 48 hours. Samples were analyzed consecutively over this time and the recovery and %RSD of Dimethachlor ESA, surrogate, and Butachlor ESA, internal standard, were calculated. The results, shown in Table 5, for the surrogate indicated that the sample preparation efficiency is acceptable. In addition, internal standard recoveries show that the mass spectrometer is maintaining consistent sensitivity over long analysis times. Most importantly, results of both QC analytes indicate that there is no ion suppression or enhancement taking place that may affect the results of the target analytes.

Table 5. Surrogate and internal standard recoveries were within acceptable limits of 70-130% and 50-150% respectively.

Analyte	Spike Level (µg/L)	Average Recovery (%)	% RSD
Dimethachlor (ESA (Surrogate)	0.24	97.3	12.7
Butachlor ESA	0.20	87.4	23.4

Conclusion

A method following US EPA guidelines for Method 535 has been presented. This method shows superior sensitivity for ethanesulfonic acid and oxanilic acid degradates of chloroacetanilide herbicides Alachlor, Acetochlor, and Metolachlor using SPE and LC/MS/MS with an API 3200™ system. A Lowest Concentration Minimum Reporting Level (LCMRL) of 0.012 to 0.014 µg/L was verified with a calculated detection limit of 0.004 µg/L or less. Two MRM transitions were used for both quantitation and confirmation of target analytes. Surrogate and internal standard recoveries indicate that there is no matrix interference. This method is easily transferable to any AB SCIEX LC/MS/MS system and is available upon request.

Acknowledgements

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Reference

1 U.S. Environmental Protection Agency Method 535: “Measurement of Chloroacetanilide and other Acetamide Herbicide Degradates in Drinking Water by Solid Phase Extraction and Liquid Chromatography / Tandem Mass Spectrometry (LC/MS/MS)” Version 1.1 (April 2005) J.A. Shoemaker, M.V. Bassett

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