Application Note: 403

# Analysis of Chloroacetanilide and Other Acetamide Herbicide Degradates In Drinking Water (EPA Method 535) by LC-MS/MS

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**Key Words**

- TSQ Quantum™
- Drinking Water Analysis
- EPA Methods
- Pesticides

## Introduction

Recently, the U.S. Environmental Protection Agency (EPA) promulgated Method 535 entitled "Measurement of Chloroacetanilide and Other Acetamide Herbicide Degradates in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)."[1] This method outlines a technique for the analysis of the fate of chloroacetanilide herbicide compounds in drinking water, specifically ethanesulfonic acid (ESA) and oxanillic acid (OA) degradates. These compounds may be found in water supplies near agricultural production areas and are used on various crops including corn and soybeans. Therefore, monitoring of these compounds is of interest to various regulatory agencies around the world.

The degradates of chloroacetanilide herbicide compounds are polar, making them ideal compounds for analysis by negative electrospray ionization mass spectrometry. This application note illustrates the direct analysis of drinking water spiked with chloroacetanilide herbicide compounds. Detection limits for these compounds are below the lowest required calibration levels outlined in the EPA's method, eliminating the need for time consuming sample preparation. Excellent linearity and reproducibility are demonstrated over the entire calibration range.

## Experimental Conditions

### *Samples*

The following chloroacetanilide herbicide compounds were purchased from Chem Service, Inc. (West Chester, PA). Acetochlor ESA, Alachlor ESA, Dimethenamid ESA, Flufenacet ESA, Metolachlor ESA, Propachlor ESA, Propachlor OA, Butachlor ESA, Dimethachlor ESA. While additional compounds are outlined in the EPA's method, not all of the compounds were commercially available. Stock solutions were prepared in methanol. Drinking water, purchased commercially, was spiked with these compounds prior to analysis and was used for all subsequent dilutions from stock solutions. Butachlor ESA was used as the internal standard for the analysis, added to all samples at a concentration of 116 ng/mL, and Dimethachlor ESA, the surrogate, was added to all samples at a level of 90 ng/mL. Calibrators were prepared over five levels, and the values are shown in Table 1.

| Compound | Cal 01 | Cal 02 | Cal 03 | Cal 04 | Cal 05 |
|---|---|---|---|---|---|
| Acetochlor ESA | 180 | 90 | 18 | 9 | 3.6 |
| Alachlor ESA | 150 | 75 | 15 | 7.5 | 3 |
| Dimethenamid ESA | 90 | 45 | 9 | 4.5 | 1.8 |
| Flufenacet ESA | 158 | 79 | 15.8 | 7.9 | 3.16 |
| Metolachlor ESA | 112 | 56 | 11.2 | 5.6 | 2.24 |
| Propachlor ESA | 286 | 143 | 28.6 | 14.3 | 5.72 |
| Propachlor OA | 56 | 28 | 5.6 | 2.8 | 1.12 |
| Butachlor ESA ISTD | 116 | 116 | 116 | 116 | 116 |
| Dimethachlor ESA SURR | 90 | 90 | 90 | 90 | 90 |

Table 1: Actual concentrations for all analytes in the analysis. All concentrations are given in ng/mL (ppb).

### *LC Conditions*

The LC gradient used in this method followed the method given in EPA Method 535. The gradient is shown in Table 2.

Instrument: Accela™ Pump
Autosampler: Accela Autosampler
Column: Hypersil GOLD™ 50 x 2.1 mm 3 µm particle size
Column Temperature: 65 °C
Flow Rate: 0.25 mL/min
Injection Volume: 25 µL – Full Loop Mode
Mobile Phase A: 5 mM Ammonium Acetate in Water
Mobile Phase B: Methanol

| Time | % A | % B |
|---|---|---|
| 0.0 | 90 | 10 |
| 7.0 | 80 | 20 |
| 10.0 | 75 | 25 |
| 18.0 | 75 | 25 |
| 20.0 | 20 | 80 |
| 25.0 | 20 | 80 |
| 25.1 | 90 | 10 |
| 40.0 | 90 | 10 |

Table 2: Gradient program used for the analysis. The flow rate was 0.25 mL/min using a Hypersil GOLD 50 x 2.1 mm 3 µm column heated to 65 °C.

### *MS Conditions*

MS: TSQ Quantum Discovery MAX™
Source: Heated-Electrospray (H-ESI)
Ionization: Negative ESI
ESI Voltage: 3500 V
Sheath Gas: 30 units
Auxiliary Gas: 15 units at 300 °C
Capillary Temp: 300 °C

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Precursor and product ions were optimized via direct syringe infusion. The optimized conditions for each compound are given in Table 3.

| Compound | Precursor Mass | Product Mass | CE |
|---|---|---|---|
| Acetochlor ESA | 314 | 80 | 31 |
| Alachlor ESA | 314 | 80 | 27 |
| Dimethenamid ESA | 320 | 121 | 25 |
| Flufenacet ESA | 274 | 121 | 22 |
| Metolachlor ESA | 328 | 121 | 26 |
| Propachlor ESA | 256 | 121 | 21 |
| Propachlor OA | 206 | 134 | 13 |
| Butachlor ESA ISTD | 356 | 80 | 31 |
| Dimethachlor ESA SURR | 320 | 121 | 25 |

Table 3. Optimized MS transitions for the compounds analyzed.

## Results and Discussion

An example chromatogram for the lowest calibration level (Cal 05, Table 1) is shown in Figure 1. Two of the compounds, Acetochlor ESA and Alachlor ESA elute very closely together and are not baseline separated. Therefore, instead of using peak area to construct the calibration curve, peak height was used. Additionally, Dimethachlor, the surrogate compound for the experiment exhibited peak splitting. This is noted in the EPA method notes. The EPA suggests that both of the peak's areas should be summed together for quantitation, and that suggestion was followed.

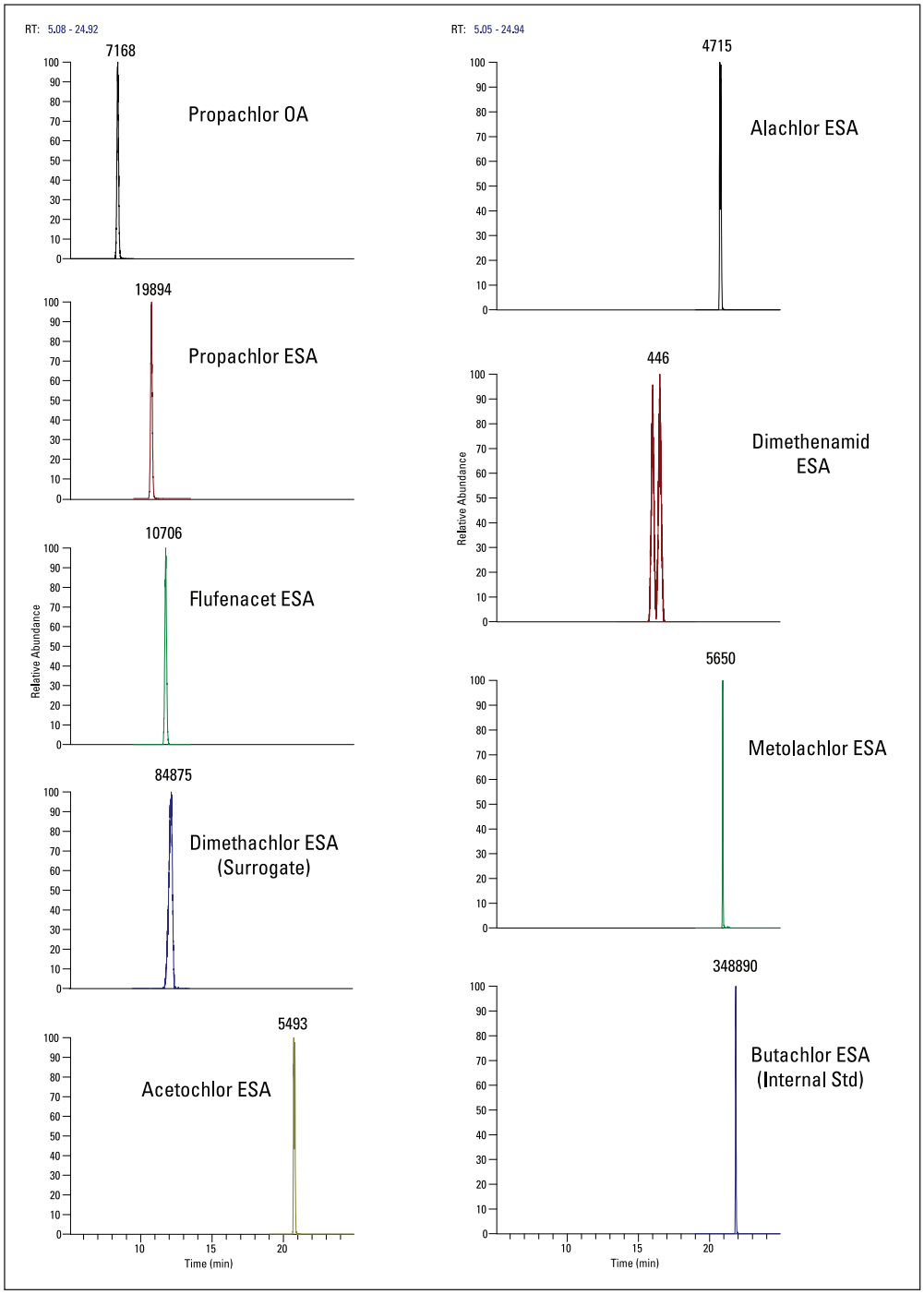


Figure 1: Chromatogram of the compounds at the lowest calibration level (Cal 05). The concentrations are given in Table 1.

All of the compounds in this analysis were eluted in 22 minutes. Due to the clean nature of the samples, the chromatographic run time could be shortened to less than 40 minutes, including the time required to equilibrated the column after the gradient. Furthermore, the Fast-HPLC capability of the Accela pump could be utilized along with a 1.9 µm particle size column to reduce the run time even more, while still providing adequate separation.

Excellent sensitivity was achieved for these analytes, even without any off line sample preconcentration. Comparing the levels of the lowest calibration level in this analysis to the EPA's published detection limit, this experiment achieved signal-to-noise ratios at factors of 4.8 - 20.8 times lower than the EPA's method. A comparison of the two methods is shown in Table 4.

| Analyte | EPA's Detection Limit (ng/mL~ppb~µg/L) | Lowest Calibrator on Discovery MAX (ppb) | Factor |
|---|---|---|---|
| Acetochlor ESA | 27.5 | 3.6 | 7.6 |
| Alachlor ESA | 30 | 3 | 10 |
| Dimethenamid ESA | 23.25 | 1.8 | 12.9 |
| Flufenacet ESA | 24.75 | 3.16 | 7.8 |
| Metolachlor ESA | 27.5 | 2.24 | 12.3 |
| Propachlor ESA | 27.5 | 5.72 | 4.8 |
| Propachlor OA | 23.25 | 1.12 | 20.8 |

Table 4: Comparison between the EPA's published detection limit and the Lowest Calibration Standard in this application note.

This table takes into account the difference between the two methods including the preconcentration step due to solid phase extraction and difference in injection volume. There is a 250x difference between the concentration of the extracts and the non extracted calibrators.

The surrogate and internal standard compounds were used to illustrate the precision of the method. The %RSD for the surrogate compound, Dimethachlor ESA, was 2.0% for 25 injections, while Butachlor ESA's %RSD was 2.3%. Excellent linearity for all of the compounds was observed, the lowest $R^2$ factor was 0.9972 (Alachlor ESA), and the highest was 0.9999 (Metolachlor ESA).

## Conclusion

This application note compared a slightly modified method to the EPA's method 535. Significant time savings were achieved by bypassing offline solid phase extraction and preconcentration. The sensitivity of the assay was also higher than the EPA method, and there is the potential for even higher sensitivity and time savings in this method if the EQuan™ online preconcentration system was used in conjunction with the Accela Fast HPLC chromatographic system.

## Reference

1. Shoemaker, J. A. and Bassett, M.V. EPA 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.

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