Application Note: 491

Analysis of Glyphosate and AMPA in Environmental Water by Ion Chromatography Electrospray Tandem Mass Spectrometry (IC-ESI-MS/MS)

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Key Words

- TSQ Quantum Access
- Ion chromatography
- EPA
- Herbicides
- Water analysis

Introduction

Glyphosate [N-(phosphonomethyl) glycine] is a nonselective herbicide that inhibits the shikimic acid pathway in plants. Glyphosate is the most commonly used agricultural pesticide and the second most used pesticide around homes and gardens.[1] It is applied to control woody and herbaceous weeds in forestry, cropped, and non-cropped sites. Although the bacteria in soil break down glyphosates into aminomethylphosphonic acid (AMPA), wastewater discharge samples and drinking water samples in the United States and Europe have tested positive for glyphosate.[2-4] Studies have raised global health and environmental concerns about the usage of glyphosate.[5] In 2006, the US EPA set the minimum contaminant level (MCL) for glyphosate at 0.7 mg/L.[6] Long-term exposure to glyphosate at levels above the MCL may cause kidney damage and reproductive defects in human biological systems.

The U.S. EPA established Method 547 for the determination of glyphosate in drinking water by direct aqueous injection high pressure liquid chromatography (HPLC), post-column derivatization, and fluorescence detection. Other methods for the quantitation of glyphosate typically use preliminary derivatization or solid-phase extraction (SPE) followed by post-column derivatization. Silica-based reversed-phase C18 columns, which use cation-exchange mechanisms, experience difficulty with the retention of such polar compounds. Here, we present a two-dimensional technique that separates glyphosate and AMPA by using anion-exchange columns coupled to a triple stage quadrupole mass spectrometer. This system eliminates the need for derivatization and preparation of complex mobile phases.

Goal

To develop an ion chromatography-mass spectrometry (IC-MS/MS) method to separate and quantitate glyphosate and AMPA without derivatization or preparation of complex mobile phases.

Experimental Conditions

Ion Chromatography

IC analysis was performed on a Dionex ICS 3000 ion chromatography system (Dionex Corporation, Sunnyvale, CA). Samples were directly injected and no sample pre-treatment was required. The IC conditions used are as follows:

First Dimension

Column set:	IonPac® AG19 (2.1 × 50 mm) / AS19 (2.1 × 250 mm); guard and separator columns (Dionex) IonPac UTAC (3 × 50 mm) Ultratrace anion concentrator column (Dionex)
Suppressor:	ASRS® 300, 2 mm; operated at 30 mA (Dionex)
Column temperature:	30 °C
Injection volume:	200 µL
Mobile phase:	Potassium hydroxide, electrolytically generated with an EGC-KOH cartridge
Gradient:	0–12 min: 8 mM KOH 12–16 min: 8–40 mM KOH 16–21 min: 40 mM KOH
Flow rate:	300 µL/min

Second Dimension

Column set:	IonPac AG21 (2.1 × 50 mm) / AS21 (2.1 × 250 mm); guard and separator columns (Dionex) Suppressor: ASRS 300, 2 mm; operated at 48 mA (Dionex)
Column temperature:	35 °C
Mobile phase:	Potassium hydroxide, electrolytically generated with an EGC-KOH cartridge
Gradient:	0–20 min: 1 mM KOH 20–30 min: 1–40 mM KOH 30–35 min: 40 mM KOH
Flow rate:	300 µL/min

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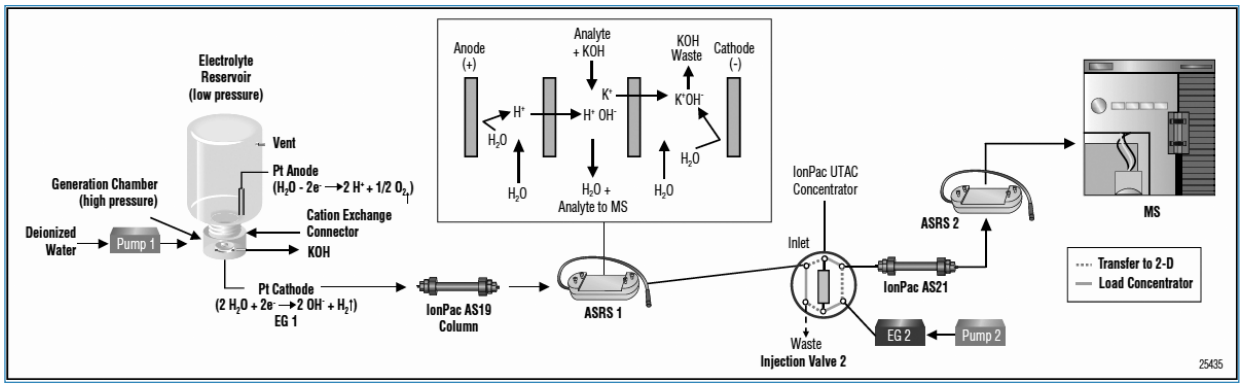

Figure 1. The flow schematic for a two-dimensional IC-MS/MS application. The first dimension separates the analytes of interest from a majority of the matrix ions. The second dimension improves peak shape and keeps the source of the MS clean.

The high-purity IC eluent is automatically produced in situ (Figure 1). The pump delivers water to an eluent generator cartridge (EGC) that converts the water into the selected concentration of potassium hydroxide eluent using electrolysis. After separation on the column, the eluent enters the anion self-regenerating suppressor (ASRS) that produces hydronium ions to exchange with potassium in the eluent. This makes the mobile phase compatible with the mass spectrometer liquid inlet system.

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific TSQ Quantum Access triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) source. The MS conditions were as follows:

Ion source polarity:	Negative ion mode
Spray voltage:	3000 V
Sheath gas pressure:	40 arbitrary units
Ion sweep gas pressure:	1 arbitrary unit
Auxiliary gas pressure:	2 arbitrary units
Capillary temperature:	400 °C
Collision gas pressure:	1.5 bar
Scan conditions:	Table 1

Table 1. MS Scan Conditions

Compound	Mass	Scan Width	Scan Time (s)	Collision Energy	Tube Lens
AMPA	110.17 / 63.3	0.01	0.5	19	60
AMPA	110.17 / 79.2	0.01	0.5	35	60
Glyphosate	168.09 / 150.1	0.01	0.5	13	51
Glyphosate	168.09 / 79.4	0.01	0.5	40	51

Results and Discussion

The major matrix peaks of chloride, nitrate, carbonate, and sulfate were well-resolved. The separation of all compounds occurred in both dimensions in 30 minutes. The calibration curves showed excellent linearity using only external quantitative measurements without internal standards.

For quantitation, samples were run in the MS/MS selective reaction monitoring (SRM) mode on the TSQ Quantum Access™ triple stage quadrupole instrument. The calibration range was 0.1–50 ppb for AMPA and 0.05–50 ppb for glyphosate. The correlation coefficients (R^2) of the 110 → 63 and 110 → 79 SRM transitions of AMPA were both 0.9997 (Figures 2 and 3). The 168 → 150 transition of glyphosate had an R^2 of 0.9997 and the 168 → 79 transition yielded an R^2 of 0.996 (Figures 4 and 5, respectively).

The minimum detection limit (MDL) in matrix was calculated by seven replicate injections of 5 ppb in a simulated matrix with high concentrations of chloride, carbonate, nitrate, and sulfate (250 ppm chloride and sulfate, 150 ppm sodium bicarbonate, 20 ppm nitrate). The MDLs for AMPA and glyphosate were calculated by using the equation MDL= $t_{99\%} \times S_{(n-1)}$, where t equals the Student's t test at 99% confidence intervals ($t_{99\%, (6)}$ = 3.143) and S is the standard deviation. The calculated MDL for AMPA was 0.313 ppb and 0.252 ppb for glyphosate. This is well below the current MDLs of 6 ppb for glyphosate in reagent water and 8.99 ppb in ground water specified by the EPA guidelines found in Method 547.

Using ion chromatography to quantitate AMPA and glyphosate accurately at this level without sample pretreatment requires the use of a mass spectrometer. However, the source of the instrument can be subject to fouling from routine analysis of samples of high-ionic strength. The use of multi-dimensional chromatography significantly reduces the introduction of matrix ions to the mass spectrometer, increasing the method robustness in challenging sample matrices. The recoveries for AMPA and glyphosate were 97.2% and 82.1%, respectively, for 5 ppb spiked into high-ionic strength samples. The relative standard deviations were less than 5% for both compounds, even without an internal standard (Figure 6).

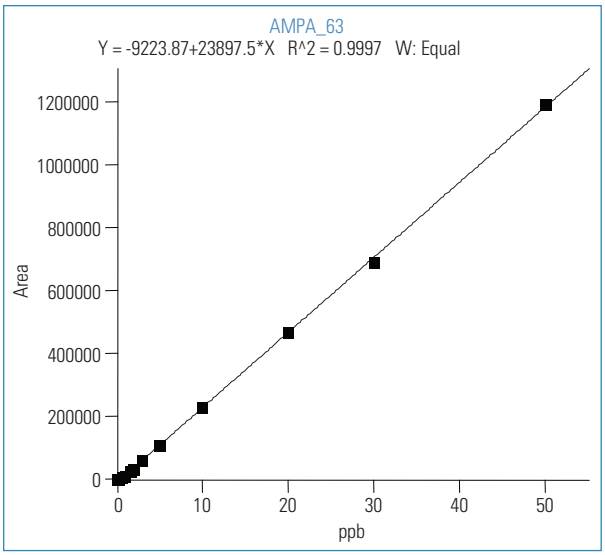

Figure 2. Calibration curve of the 110 → 63 SRM transition for AMPA.

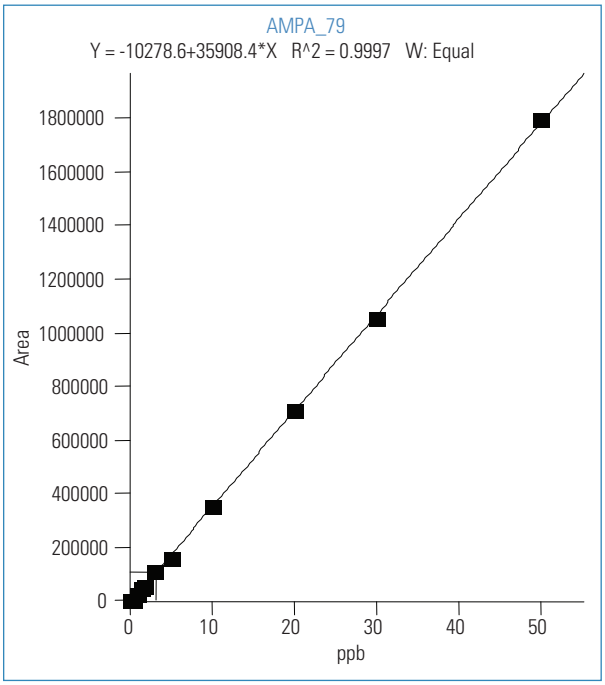

Figure 3. The calibration curve of the SRM 110 → 79 for AMPA.

The response of the standards decreased over time. However, when using freshly prepared standards, the response remained constant; this suggests there may be temperature stability issues with the samples. Although excellent short-term (30 hour) stability yielded standard deviations less than 5%, using a refrigerated autosampler and an isotopically-labeled internal standard will help minimize systematic sample degradation and response variation.

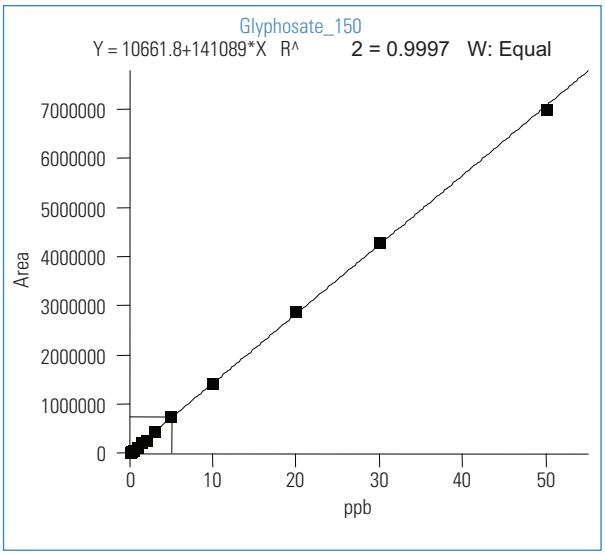

Figure 4. The calibration curve of the SRM 168 → 150 for glyphosate.

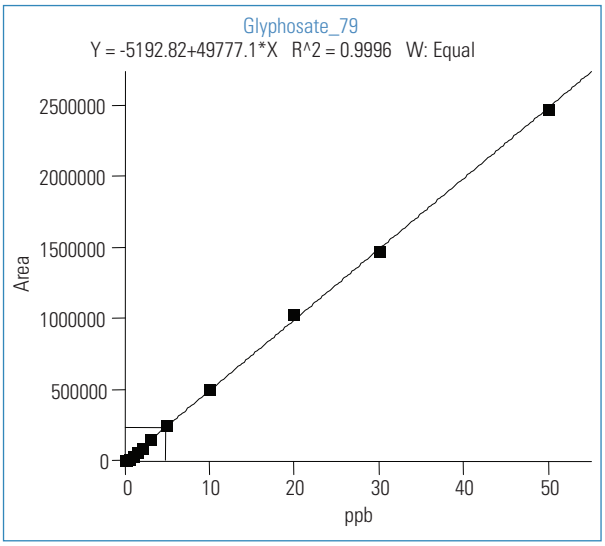

Figure 5. The calibration curve of the SRM 168 → 79 for glyphosate.

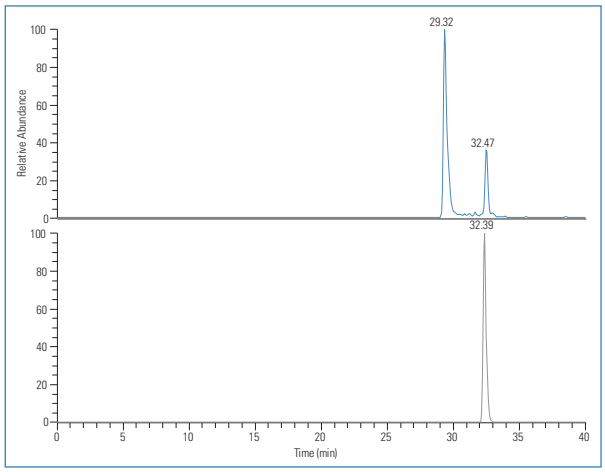

Figure 6. The total ion chromatogram (TIC) of 5 ppb of glyphosate and AMPA spiked into a matrix of chloride, nitrate, carbonate and sulfate.

Conclusion

The advantage of the IC-MS/MS methodology described here is the elimination of derivatization and acidification steps required by EPA Method 547 and other techniques. The analysis requires no sample preparation. Separation of both compounds in both dimensions occurs in approximately 30 minutes. Calibration levels of 0.05 to 50 ppb for glyphosate show that this method can be used to quantitate low (ppb) levels of glyphosate in high-ionic strength matrices. Using stable-labeled internal standards will help compensate for the effects of ion suppression in the source.

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