

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

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INTRODUCTION

Glyphosate [N-(phosphonomethyl glycine)] is a nonselective herbicide which inhibits the shikimic acid pathway in plants. Glyphosate is the most commonly used agricultural pesticide and second most commonly used pesticide around homes and gardens.¹ It is used to control woody and herbaceous weeds in forestry, cropped, and non-cropped sites. Although the bacteria in soil break down glyphosate into the amino-methyl phosphonic acid (AMPA), wastewater discharge samples and drinking water samples in United States and Europe have tested positive for glyphosate.^{2,3} Studies have raised global health and environmental concerns about the usage of glyphosate.⁴

In 2006, the US EPA set the maximum contaminant level (MCL) for glyphosate at 0.7 mg/L.⁵ Long-term exposure to glyphosate at levels above the MCL may cause kidney damage and reproductive defects in human biological systems.

Typical methods for quantitation of glyphosate use preliminary derivatization or solid-phase extraction (SPE) followed by postcolumn 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 using anion-exchange columns coupled to a triple quadrupole mass spectrometer which eliminates the need for derivatization and preparation of complex mobile phases.

EXPERIMENTAL

Hardware

Chromatography System

Dionex ICS-3000 Ion Chromatography System

DP- Dual Pump

EG- Eluent Generator

DC- Detector Compartment with conductivity detection

AS- Autosampler

Columns and Accessories

IonPac[®] AG19 (2.1 × 50 mm) / AS19 (2.1 × 250 mm);

First dimension guard and separator columns

IonPac UTAC (3 × 50 mm) Ultra Trace Anion Concentrator column;
for trapping heart-cut regions from the first dimension

IonPac AG21 (2.1 × 50 mm) and AS21 (2.1 × 250 mm)

Second dimension guard and separator columns

EGC-KOH Potassium Hydroxide eluent generator cartridge

ASRS[®] 300 (2 mm) Used to exchange the salts in the mobile phase to a form that is compatible with mass spectrometry

Mass Spectrometers

MSQ Plus[™] Single Quadrupole Mass Spectrometer

TSQ Quantum Access[™] Triple Quadrupole Mass Spectrometer

Software

Chromeleon[®] 6.8SR5 Chromatography Data System—Operating system for front-end chromatography and MSQ operation

Xcalibur[®] 2.0.7—Operating system for TSQ

DCMS^{Link™} 2.5—Dionex Chromatography Mass Spectrometer
Software link for TSQ Xcalibur control of front-end chromatography hardware

Solutions and Standards

Ammonium Nitrate, EM Science

Sodium Chloride, JT Baker

Sodium Sulfate, EM Science

Sodium Carbonate, EM Science

Glyphosate, Supelco 44690-U

AMPA, Sigma

LC Conditions

First Dimension

Mobile Phase: Potassium Hydroxide (electrolytically generated)
Gradient: 0–12 min 8 mM KOH
12–16 min 8–40 mM KOH
16–21 min 40 mM KOH
Column Temp.: 30 °C
Flow Rate: 300 µL/min
Suppressor: ASRS 300 operated at 30 mA
Inj. Volume: 200 µL

Second Dimension

Mobile Phase: Potassium Hydroxide (electrolytically generated)
Gradient: 0–20 min 1 mM KOH
20–30 min 1–40 mM KOH
30–35 min 40 mM KOH
Column Temp.: 35 °C
Flow Rate: 300 µL/min
Suppressor: ASRS 300 operated at 48 mA

MS Conditions

MSQ Plus

ESI Source: Negative ionization mode
Source Temp.: 400 °C
Gas Pressure: 5 bar
Needle Voltage: 3 kV
Scan Conditions: Table 1

Table 1				
Compound	Mass	Span	Dwell	Cone Voltage
AMPA	110.0	0.3	0.3	70
Glyphosate	168.00	0.5	0.3	45

TSQ Quantum Access

ESI Source: Negative ionization mode
Source Voltage: 3000 V
Sheath Gas: 40 units
Ion Sweep Gas Pressure: 1 unit
Auxiliary Gas Pressure: 2 units
Capillary Temperature: 400 °C
Collision Gas Pressure: 1.5 bar
Scan Conditions: Table 2

Table 2					
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

ANALYSIS

Eluent generation technology allows automatic in-situ production of high-purity IC eluent (Figure 1). The pump delivers water to an eluent generator cartridge (EGC) which converts the water into the selected concentration of potassium hydroxide eluent using electrolysis. After separation on the column, the eluent enters the ASRS suppressor, which produces hydronium ions to exchange with potassium in the eluent, making the mobile phase compatible with a mass spectrometer liquid inlet system.

In this method, the first chromatographic dimension separates matrix ions from the AMPA and glyphosate using a high-capacity IonPac AS19 column. The AMPA and glyphosate are heart-cut and trapped onto an anion concentrator column. This concentrator column is back-flushed onto the IonPac AS21, the second analytical column. This low-capacity column significantly improves the peak shape of glyphosate and AMPA, and reduces the introduction of matrix ions into the mass spectrometer.

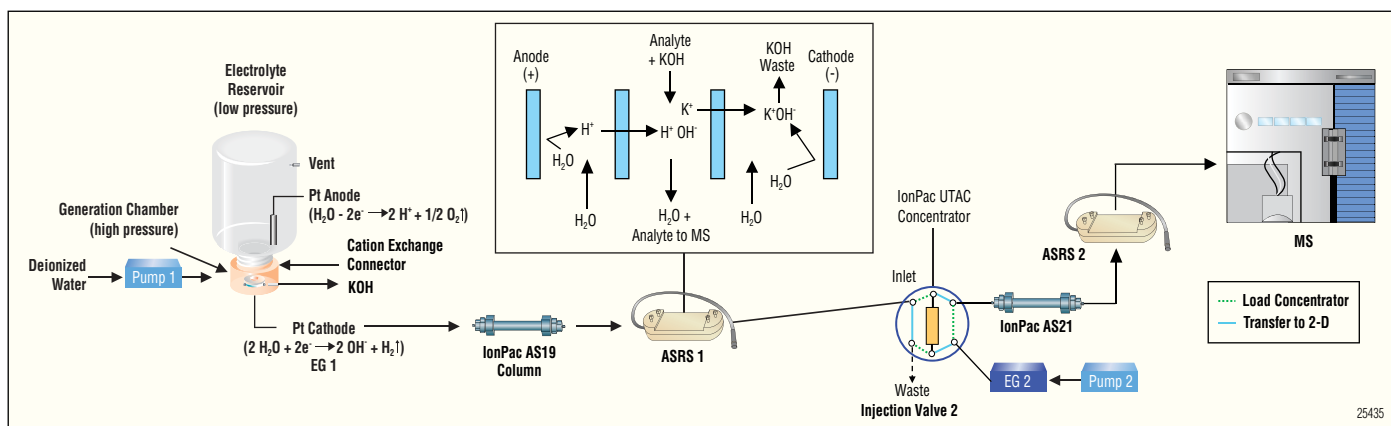


Figure 1. The flow schematic for a two-dimensional IC-MS/MS application. The first dimension separates the analytes of interest from a majority of the matrix ions. The second dimension improves peak shape and keeps the source of the MS clean.

RESULTS

The chemistry of the IonPac AS19 column provided resolution between the major matrix peaks of chloride, nitrate, carbonate, and sulfate. Separation of all compounds occurred in both dimensions in 30 min. Calibration curves generated on both the single and triple quadrupole instruments showed excellent linearity using only external quantitative measurements without internal standards. The single quad MSQ Plus, used for the preliminary examination of AMPA and glyphosate, yielded a detection range for both compounds of 0.5–50 ppb with r^2 values of 0.9991 for AMPA and 0.9995 for glyphosate (Figure 2).

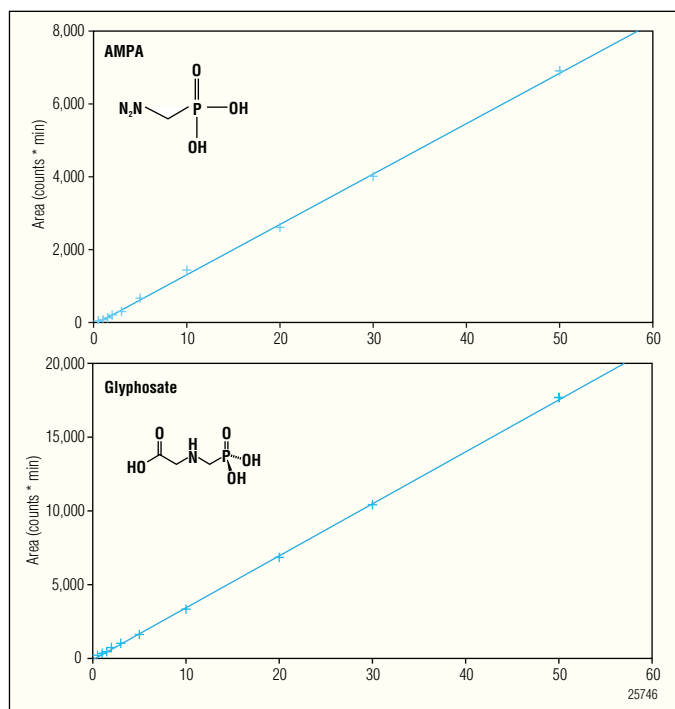


Figure 2. Calibration curve for preliminary data collected from a single quadrupole MSQ Plus instrument. Linearity is observed for both AMPA (top curve) and glyphosate (bottom curve).

For final quantitation, samples were run in MS/MS SRM mode on the TSQ Quantum Access triple quadrupole instrument. The calibration range was 0.1–50 ppb for AMPA and 0.05–50 ppb for glyphosate. The squared correlation coefficient of the $110 \rightarrow 63$ and $110 \rightarrow 79$ transitions of AMPA were both 0.9997 (Figure 3). The $168 \rightarrow 150$ transition of glyphosate had a correlation coefficient of 0.9997, while the $168 \rightarrow 79$ transition yielded an r^2 of 0.996 (Figure 4).

The minimum detection limit (MDL) in matrix was calculated individually for both instruments 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). 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 MDLs for both compounds were calculated. For the MSQ Plus, the estimated MDL for AMPA in matrix was 0.877 ppb, and the MDL for glyphosate was 0.542 ppb. In comparison, using MS/MS detection on the TSQ, the calculated MDL for AMPA was 0.313 ppb and 0.252 ppb for glyphosate.

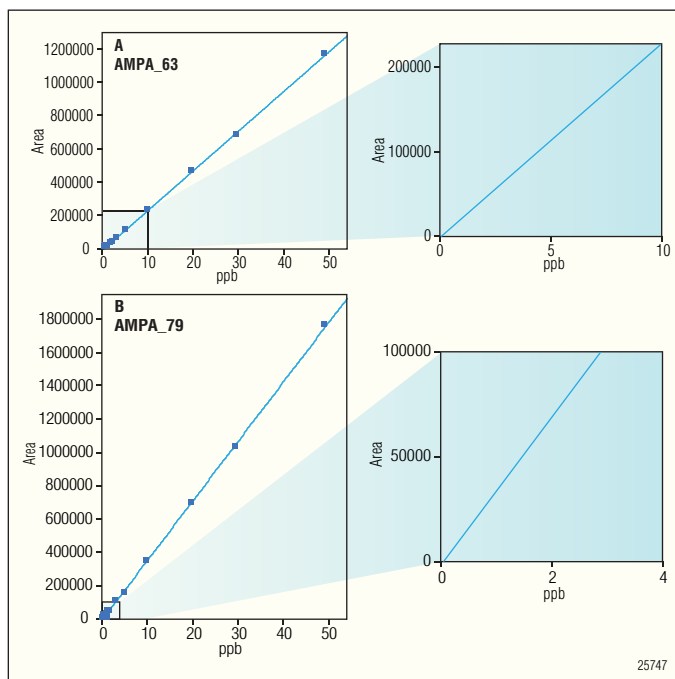


Figure 3. (A) Calibration curve of the SRM 110 \rightarrow 63 transition for AMPA in the TSQ Quantum access. (B) Calibration curve of the SRM 110 \rightarrow 79 transition for AMPA in the TSQ Quantum access. The calibration range was 0.1–50 ppb for both transitions.

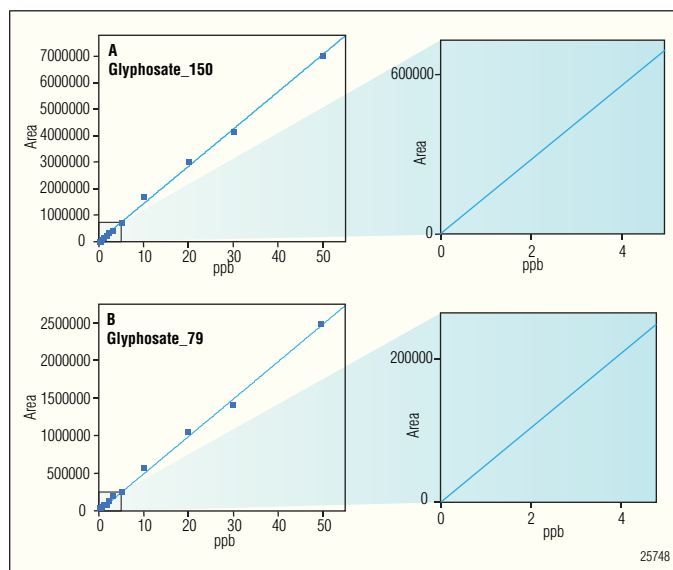


Figure 4. (A) Calibration curve of the SRM 168 \rightarrow 150 transition for glyphosate in the TSQ Quantum access. (B) Calibration curve of the SRM 168 \rightarrow 79 transition for glyphosate in the TSQ Quantum access. The calibration range was 0.05–50 ppb for both transitions.

DISCUSSION

Current US EPA guidelines found in Method 547 specify MDLs for glyphosate in reagent water of 6 ppb and 8.99 ppb in ground water.⁶ Using ion chromatography to quantitate these compounds accurately at this level without sample pretreatment requires the use of a mass spectrometer (Figure 5). However, the sources of these intruments 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. Using the TSQ, recoveries for AMPA and glyphosate were 97.2% and 82.1% respectively for 5 ppb spiked into high-strength samples. The relative standard deviations were less than 5% for both compounds, even without an internal standard (Figure 6).

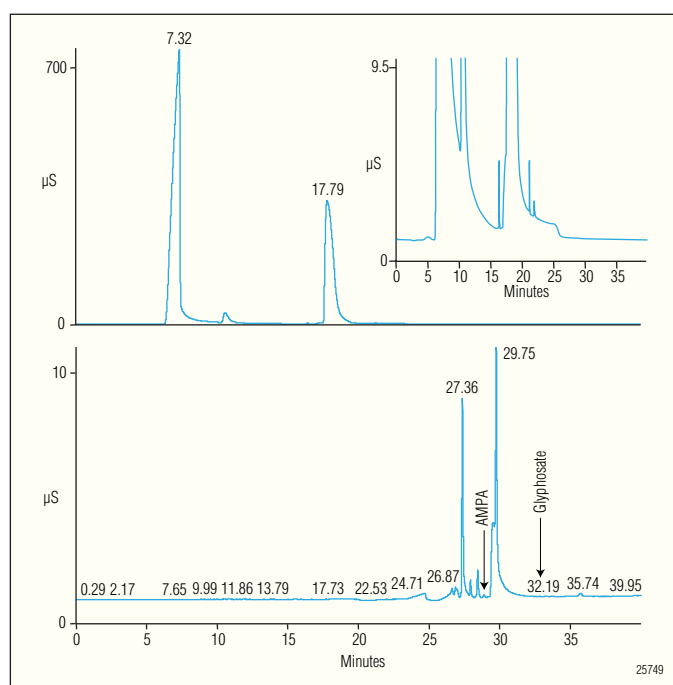


Figure 5. The data collected from conductivity detectors in both dimensions shows low-level quantitation by conductivity in matrix is not possible due to the high concentration matrix. Top trace: First dimension using the IonPac AS19 data with inlay of first dimension amplified. Bottom Trace: Second dimension using the IonPac AS21.

The first chromatographic dimension separated the AMPA and glyphosate from the majority of the matrix ions using the IonPac AS19 column. However, poor peak shape was observed when the samples were introduced into the mass spectrometer. Trapping a heart-cut region of AMPA and glyphosate onto the anion concentrator column and eluting the analytes of interest onto a lower capacity, second dimension column yields improved peak shape and the added benefit of detecting the analytes in higher ionic strength samples.

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Careful attention was given to the time allotted for the heart-cut step of this analysis. When diverting to the concentrator column for extended periods of time, recovery yields for glyphosate were poor. Although not tested, it is hypothesized that larger injection volumes or samples with higher concentrations may show improved recoveries using a 4 mm first dimension column due to the increased capacity of the larger column format.

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

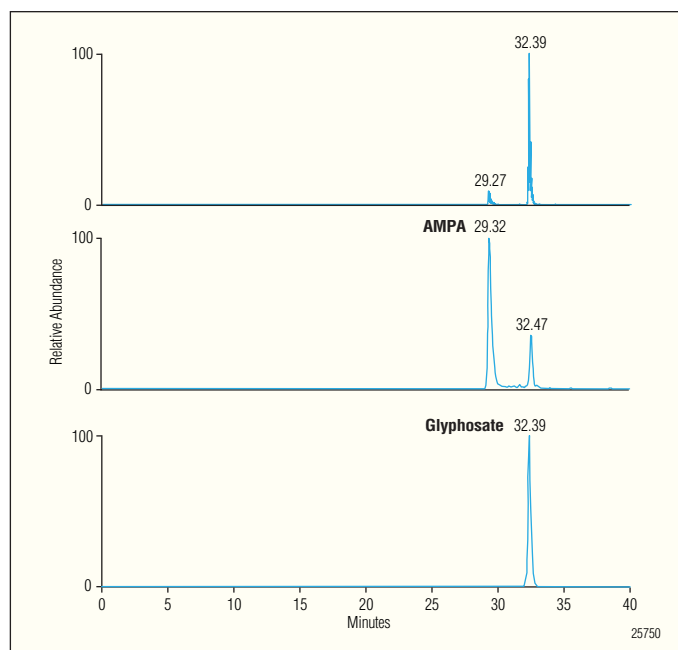


Figure 6. The top trace shows the TIC of 5 ppb glyphosate and AMPA spiked into a matrix of chloride, nitrate, carbonate and sulfate. The middle trace is the SRM for AMPA (110 → 79) and the bottom is glyphosate (168 → 150). These data show the recoveries of 97.2% for AMPA and 82.1% for glyphosate.

CONCLUSION

The advantage of this methodology is the elimination of derivatization and acidification steps required by other techniques. The analysis requires no sample preparation. Separation of both compounds in both dimensions occurs in approximately 30 minutes. Calibration levels of

0.05–50 ppb for glyphosate show this method can be used to quantitate low (ppb) levels of glyphosate in high-ionic strength matrices. Quantitation in samples should be investigated using stable-labeled internal standards, which may help compensate for the effects of ion suppression in the source.

FURTHER INVESTIGATION

The goal of future analyses will be to simplify the method to achieve detection and quantitation of these compounds using a single dimension. Working on a single, high-capacity column—such as the IonPac AS24—to separate the glyphosate and AMPA may remove some of the added complexity of the current method.

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