

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

Rolf Maurer,¹ Stacy Henday,² Leo Wang,² and Bill Schnute,² ¹Dionex Corporation, Olten, Switzerland, ²Dionex Corporation, Sunnyvale, CA USA

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 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 aminomethyl 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. Standard silica-based reversed-phase C18 columns, which use cation-exchange mechanisms, experience difficulty with the retention of such polar compounds. Here we present the separation of glyphosate and AMPA using anion-exchange columns coupled to a single quadrupole mass spectrometer. This analytical combination negates the need for derivatization and eliminates the preparation of complex mobile phases.

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC

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

Two-Dimensional Analysis

IonPac[®] AG19 (2.1 × 50 mm) and IonPac AS19 (2.1 × 250 mm);
First dimension guard and separation column

IonPac Ultra Trace Anion Concentrator (3 × 50 mm), Column trapping
heart-cut regions from the first dimension

IonPac AG21 (2.1 × 50 mm) and IonPac AS21 (2.1 × 250 mm);
Separation in the second dimension

One-Dimensional Analysis

IonPac AG24 (2.1 × 50 mm) and IonPac AS24 (2.1 × 250 mm)

Accessories

EGC-KOH Potassium Hydroxide eluent generator cartridge to
electrolytically produce hydroxide gradients for separation

ASRS3000 (2 mm) exchanges the salts of the eluent to a form
compatible with mass spectrometry

Mass Spectrometer

MSQ Plus™ Single Quadrupole Mass Spectrometer

Software

Chromeleon[®] 6.8 SR6 Chromatography Data System

Solutions and Standards

Ammonium Nitrate, EM Science
 Sodium Chloride, JT Baker
 Sodium Sulfate, EM Science
 Sodium Carbonate, EM Science
 Glyphosate, Supelco
 AMPA, Sigma

LC Conditions - Two Dimensional Approach

First Dimension

Mobile Phase: Potassium Hydroxide
 Gradient: 0–12 min 8 mM KOH
 12–16 min 8–40 mM KOH
 16–21 min 40 mM KOH
 Column: IonPac AG19 and AS19
 Temperature: 30 °C
 Flow Rate: 300 µL/min
 Suppressor: ASRS 300 (30 mA)

Second Dimension

Mobile Phase: Potassium Hydroxide
 Gradient: 0–20 min 8 mM KOH
 12–16 min 8–40 mM KOH
 16–21 min 40 mM KOH
 Column: IonPac AG21 and AS24
 Temperature: 30 °C
 Flow Rate: 300 µL/min
 Suppressor: ASRS 300 (48 mA)

LC Conditions - One Dimensional Approach

Mobile Phase: Potassium Hydroxide (electrolytically generated)
 Gradient: 0–1 min 7 mM KOH
 1–20 min 7–18 mM KOH
 20–27 min 18–38 mM KOH
 27–30 min 60 mM KOH
 30–35 min 7 mM KOH
 Column: IonPac AG24 and AS24
 Temperature: 15 °C
 Flow Rate: 250 µL/min
 Suppressor: ASRS® 300 operated at 38 mA
 Injection Volume: 50 µL

MS Conditions - Both Approaches

MSQ Plus

ESI negative ionization mode
 Source temperature: 400 °C
 Gas pressure: 5 bar
 Needle voltage: 3kV

Name	Mass	Span	Dwell	Cone Voltage
AMPA	110.0	0.3	0.5	70
Glyphosate	168.0	0.3	0.5	55

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 a 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 and neutralize the hydroxide, making the mobile phase compatible with a mass spectrometer liquid inlet system.

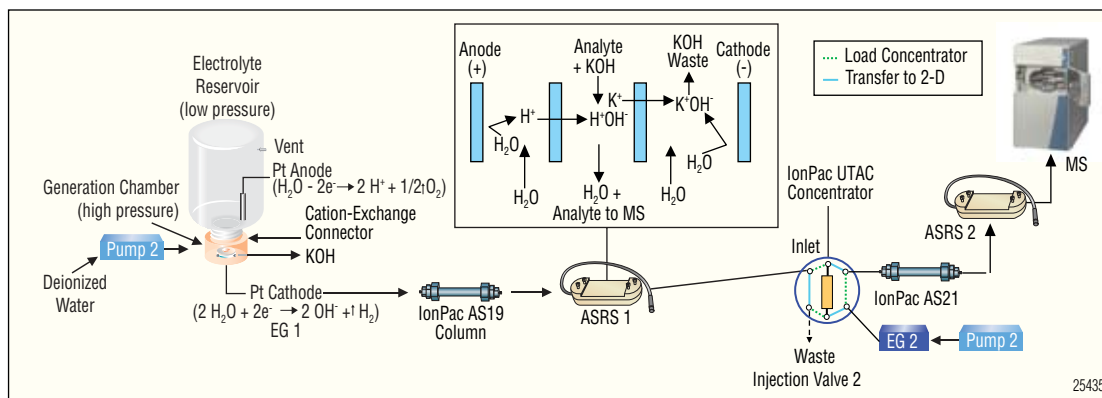


Figure 1. The flow schematic for a two dimensional IC-MS application. The first dimension separates the analytes of interest from a majority of the matrix ions. The second dimension improves peak shape and keeps the source of the MS clean.

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

Previously, the separation of glyphosate and AMPA was performed on an AS19 column alone. However, poor peak shape was noted with the AMPA using this single column method. Using a two dimensional approach, the first chromatographic dimension separates matrix ions from the AMPA and glyphosate using the 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 second analytical column and IonPac AS21. The addition of the AS21 improved the asymmetry of the AMPA peak, reduced the introduction of matrix ions into the mass spectrometer, and afforded lower detection limits, but added complexity to the analysis. To improve the two dimensional approach, a single dimension approach, using a different column, was examined. Separation on the high capacity IonPac AS24 provided excellent resolution of AMPA and glyphosate from the high ionic strength matrix, while maintaining the peak shape of AMPA.

RESULTS

In the two dimensional method, 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 the MSQ Plus™ showed excellent linearity using only external quantitative measurements without internal standard correction. The MSQ Plus 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).

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). Using the equation $MDL = t_{99\%} \times S(n-1)$, where t equals the Students 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 two dimensional method, the estimated MDL for AMPA in matrix was 0.877 ppb, and the MDL for glyphosate was 0.542 ppb.

When the single dimension IonPac AS24 analysis was run on the MSQ Plus, the linearity of the standards was calculated from 0.75 ppb–100 ppb for AMPA, and 0.50 ppb–100 ppb for glyphosate (Figure 3). The reproducibility of ten injections of 10 ppb standard spiked into the laboratory simulated matrix was 5.05% for AMPA and 3.36% for glyphosate

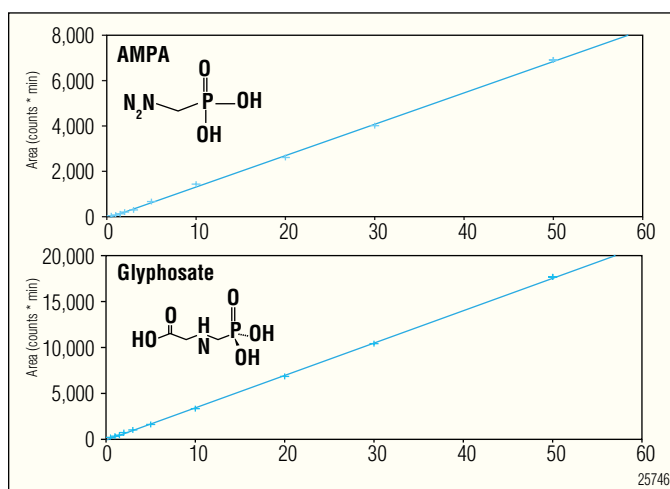


Figure 2. Calibration curve for two dimensional method. Linearity is observed for both AMPA (top curve) and glyphosate (bottom curve).

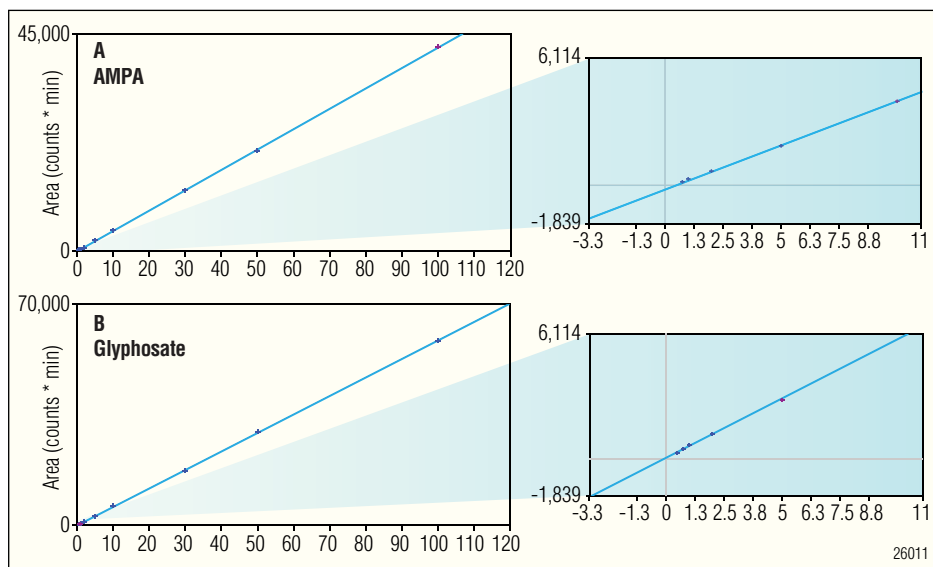


Figure 3. In the single dimension method, AMPA was calibrated with a range of 0.75 ppb–100 ppb. The correlation coefficient was calculated at 0.9999 (top trace). The calibration curve of glyphosate ranged from 0.50 ppb–100 ppb with an r^2 0.9999 (bottom trace).

(Figure 4). Using seven replicate injections and the aforementioned formula, the single dimension analysis yielded a calculated MDL for AMPA of 0.640 ppb, and the MDL for glyphosate in matrix was 0.820.

Using the single dimension approach, standards of AMPA and glyphosate were spiked into tap water from San Jose, CA. Recoveries of 93% were found for AMPA, and 95% for glyphosate, without the compensation of an internal standard. Although the concentration of matrix ions was less in the tap water spikes than the laboratory simulated matrix, this demonstrates that the method is compatible with real world samples.

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 4). However, the MS instrument source can be subject to fouling from routine analysis of samples of high-ionic strength. The use of multi-dimensional chromatography or a high capacity single dimension with matrix diversion significantly reduces the introduction of matrix ions to the mass spectrometer, increasing the method robustness in challenging sample matrices. The relative standard deviations for both separation methods were 5% or less for both compounds, without internal standard correction.

In the two dimensional analysis, the first chromatographic dimension separated the AMPA and glyphosate from the majority of the matrix ions on an IonPac AS19 column. However, poor peak shape was observed once 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, higher-efficiency second dimension column yielded improved peak shape and the added benefit of detecting the analytes in higher concentrations of matrix.

Careful attention was given to the time allotted for the heart-cut regions onto the concentrator column. When diverting to the concentrator column for extended periods, recovery yields for glyphosate were poor. We postulate that low glyphosate yields are due to excess salts from the sample prematurely eluting the glyphosate off the trap column.

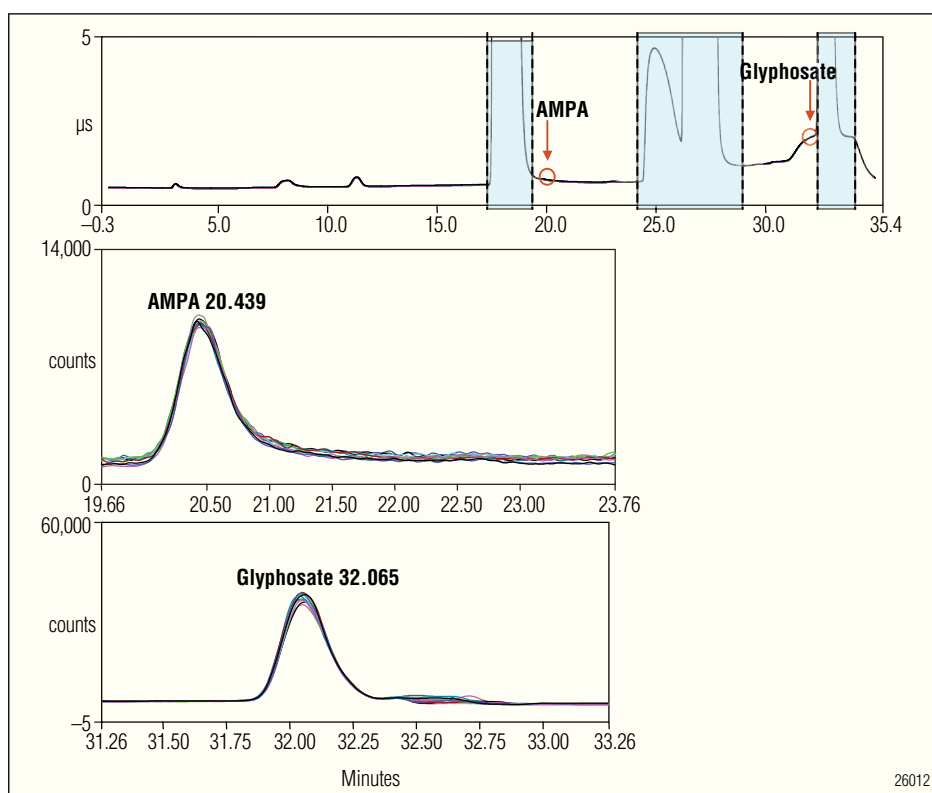


Figure 4. Reproducibility of ten injections of 10 ppb AMPA and glyphosate spiked into a simulated lab matrix sample. The top trace shows conductivity detection. The shaded areas of this chromatogram indicate where the matrix was diverted to waste. Due to high matrix interference, integration of the AMPA and glyphosate by conductivity alone is not possible. Using the MSQ Plus and examining the SIM scan for AMPA (middle trace) shows the reproducibility of AMPA with 5.05% RSD in this complex matrix. The bottom trace shows the reproducibility of the SIM data for glyphosate in the complex matrix has a 3% RSD.

In an attempt to simplify this analysis, a single dimension method was evaluated with a new column. The AS24, a high capacity column, provided excellent resolution between the peaks of AMPA and glyphosate from the matrix analytes. Regions of high matrix concentrations were diverted from the source of the mass spectrometer to minimize maintenance due to source fouling. The peak shape of AMPA was not sacrificed in order to achieve a single dimension method.

Over extended periods of time, the response of the standards decreased in both methods. However, with freshly prepared standards, diluted from refrigerated stock 1 ppm solution, the response returned; this suggests there may be temperature stability issues with the samples. While excellent short-term (over 30 h) stability yielded standard deviations less than 5%, it is assumed the use of a refrigerated autosampler and an isotopically-labeled internal standard would aid in minimizing systematic sample degradation and response variation.

Investigation on an MS/MS instrument validates current single quadrupole methodology. For further information, please refer to Dionex LPN 2177-02.

CONCLUSION

The advantage of these analytical methodologies for quantitation of glyphosate is the elimination of derivatization and acidification steps required by other techniques. Drinking water analysis requires no sample pretreatment. Separation in both methods occurs in approximately 30 minutes. Calibration levels of 0.05–100 ppb for glyphosate show these methods to be capable of quantitating low levels of glyphosate in high concentration matrix samples. The combination of IC/MS meets the needs specified by regulatory agencies for the quantification of glyphosate and AMPA in environmental matrices. It is recommended that quantitation in real matrix samples be investigated with utilization of stable labeled internal standards, which will aid in compensating for the possible effects of ion suppression in the ESI source.

REFERENCES

1. Tu, M., Hurd, C. & J.M. Randall. 2001. *Weed Control Methods Handbook*, The Nature Conservancy, <http://tncweeds.ucdavis.edu>, version: April 2001
2. Gilles-Eric Seralini, Sophie Richard, Safa Moslemi, Herbert Sipahutar, Nora Benachour, 24 February 2005. *Environmental health perspective Differential effects of glyphosate and Roundup on human placental cells and aromatase*
3. http://www.epa.gov/OGWDW/contaminants/dw_contamfs/glyphosa.html (Accessed January 5, 2008)
4. http://www.accustandard.com/asi/pdfs/epa_methods/547.pdf (Accessed January 5, 2008)

MSQ Plus is a trademark of Thermo Fisher Scientific, Inc. Chromeleon, IonPac, and ASRS are registered trademarks of Dionex Corporation.

Passion. Power. Productivity.



Dionex Corporation

1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America

U.S./Canada (847) 295-7500

South America

Brazil (55) 11 3731 5140

Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.dionex.com



LPN 2260-01 05/09
©2009 Dionex Corporation