Analysis of Glyphosate and Aminomethyl Phosphonic Acid by Liquid Chromatography/Mass Spectrometry

Application

Environmental

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Abstract

A liquid chromatography/mass spectrometry method using an electrospray ionization source in positive ion mode was developed for the analysis of glyphosate (N-phosphonomethyl glycine) and its metabolite, aminomethyl phosphonic acid in water. Both glyphosate and its metabolite were derivatized using 9-fluorenylmethyl chloroformate in buffer solution prior to reversed phase high performance liquid chromatography separation. The method cleanly resolved both target molecules with excellent sensitivity in both positive and negative ion modes.

Background

Glyphosate (N-phosphonomethyl glycine), $(HO)_2P(O)\text{-}CH_2\text{-}NH\text{-}CH_2CO_2H$, is a global herbicide widely used in forest management, agricultural applications, and urban landscape management. Glyphosate is recognized as a benign, environmentally friendly herbicide with low toxicity [1]. However, there are health and environmental concerns over its toxicity, which are documented in a recent review [2].

Judging from the scope and quantity of glyphosate in use worldwide, it is important that a rugged, routine method be available for the accurate determination of both glyphosate and its metabolite, aminomethyl phosphonic acid (AMPA), $(HO)_2P(O)\text{-}CH_2\text{-}NH_2$, in environmental matrices. Any analytical method must analyze for both, since an absence of glyphosate may have been caused by its conversion to AMPA.

Because of the highly polar nature of these compounds, organic solvents cannot extract them from environmental matrices. This has made their determination a difficult challenge. Many analytical methods for these molecules were reported by Stalikas and Konidari [3] listing diverse techniques including gas chromatography (GC), high performance liquid chromatography (HPLC), ion chromatography (IC), enzyme-linked immunosorbent assays, and capillary electrophoresis (CE). Although, gas chromatography/mass spectrometry (GC/MS) based methods are economical and have good selectivity and sensitivity, they use complicated derivatization processes and require highly specialized personnel. HPLC methods suffer from the relatively poor response of these molecules to ultraviolet (UV) detection.

The present method, offering a liquid chromatography/mass spectrometry (LC/MS) approach with pre-column derivatization, combines simplicity and sensitivity, adequate for a 10–50 ppb determination, given a 100-mL water sample.

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Method

Sample Preparation

1. Prepare reagent solution: 10 mg/mL 9-fluorenylmethyl chloroformate (FMOC) in acetonitrile.
2. Prepare buffer solution: 5% sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10\ H_2O$), pH = 9.1
3. To 50 µL of sample extract, add 50 µL of buffer solution and mix.
4. Add 50 µL of reagent solution.
5. Let stand 4 hrs.

Instrument: Agilent 1100 LC/MS with electrospray ionization (ESI) in Positive Ion Mode

- Drying gas: 12 L/min, 350 °C
- Nebulizer gas: 60 psi
- V_{cap}: 3500 V
- Fragmentor: 100 V for both scan and SIM runs
- SIM ions: 334 and 392 *m/z*
- Scan range: 120 to 1000 *m/z*

LC conditions

- Column: ZORBAX XDB-C8, 4.6 mm id × 50 mm long, 5 µm particles, 40 °C
- Precolumn pump: Agilent 1100 binary
- Mobile phase A: 50 mM ammonium acetate, aqueous
- Mobile phase B: acetonitrile, 0 to 95% in 5 min, hold 3 min
- Pre-column flow rate: 0.7 mL/min
- Sample size injected: 1 µL
- Post-column pump: Agilent 1100 isocratic. Flow: 0.3 mL/min of 0.6% formic acid
- Binary pump with diode array detector (DAD) and well-plate sampler

Results

Figure 1 shows molecular structures for the starting compounds and their derivatized products. It is these derivatives that are analyzed and depicted in subsequent figures.

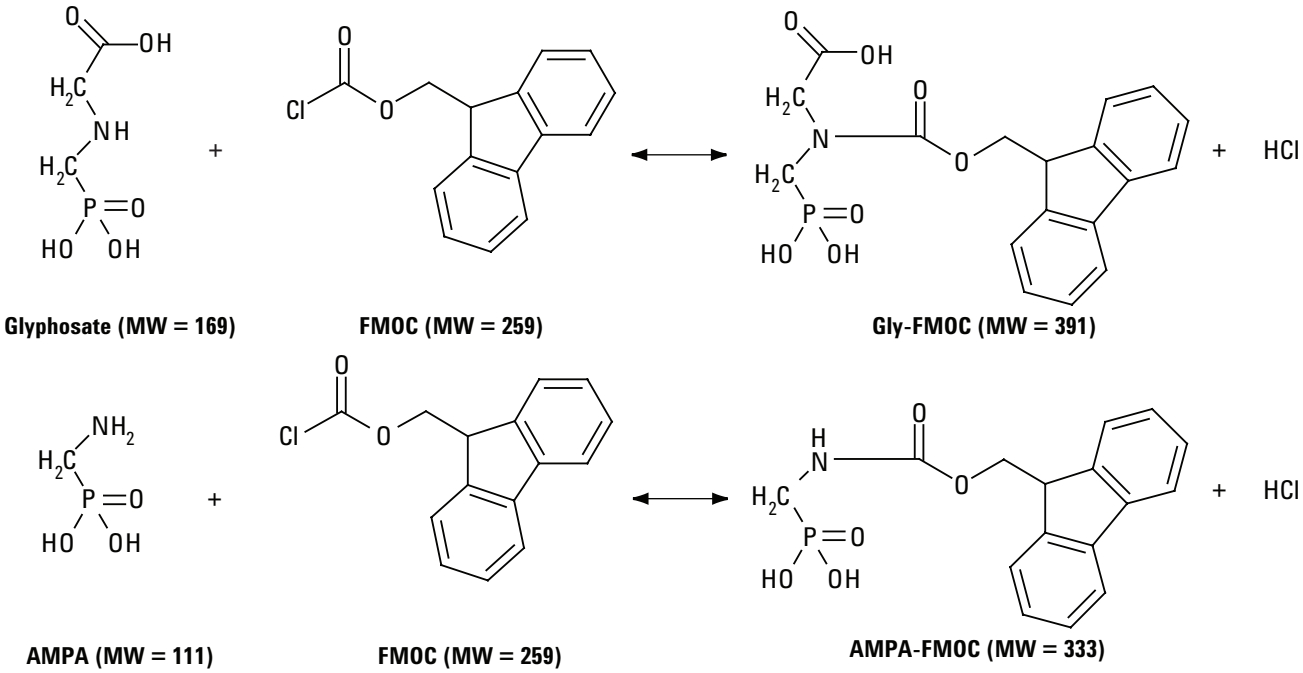

Figure 1. Derivatization reactions for glyphosate and AMPA with FMOC.

Figure 2 displays the mass spectra of both derivatized molecules. Positive ions 392 and 334, representing glyphosate and AMPA respectively, were chosen for further analysis.

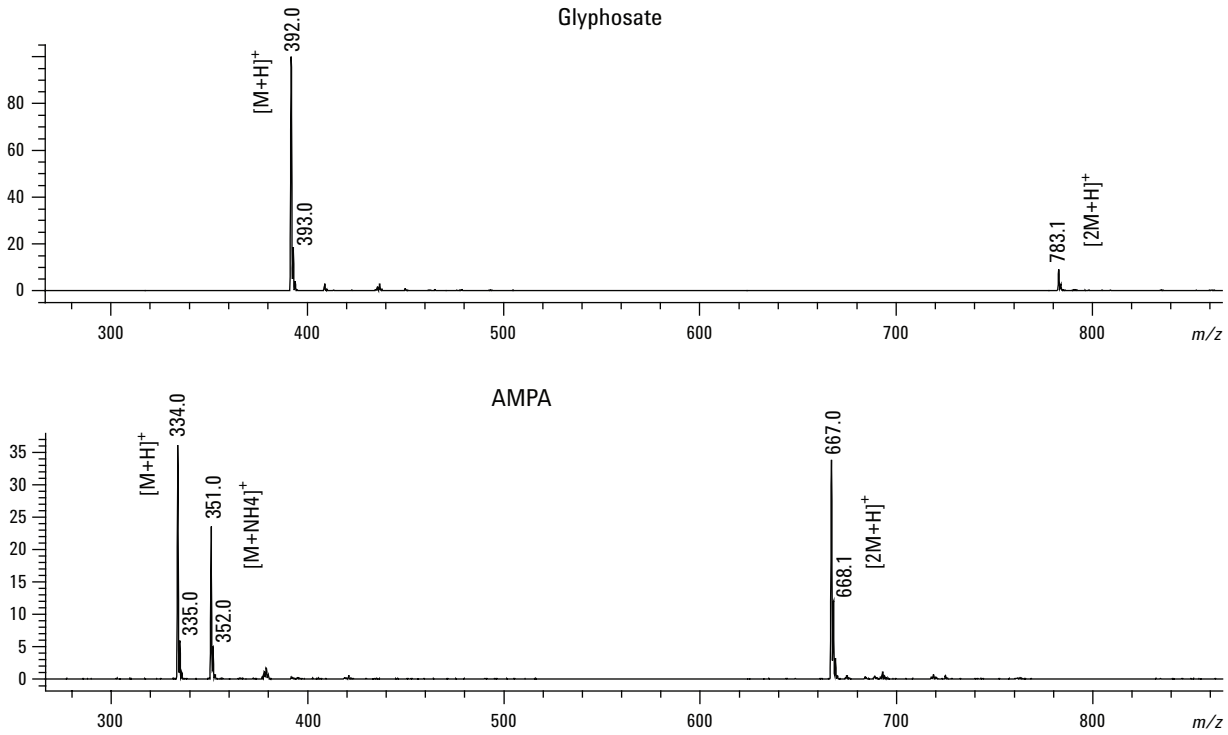

Figure 2. Mass spectra of target molecules.

Figure 3 is a stacked extracted ion chromatogram of the derivatized target molecules at low concentration.

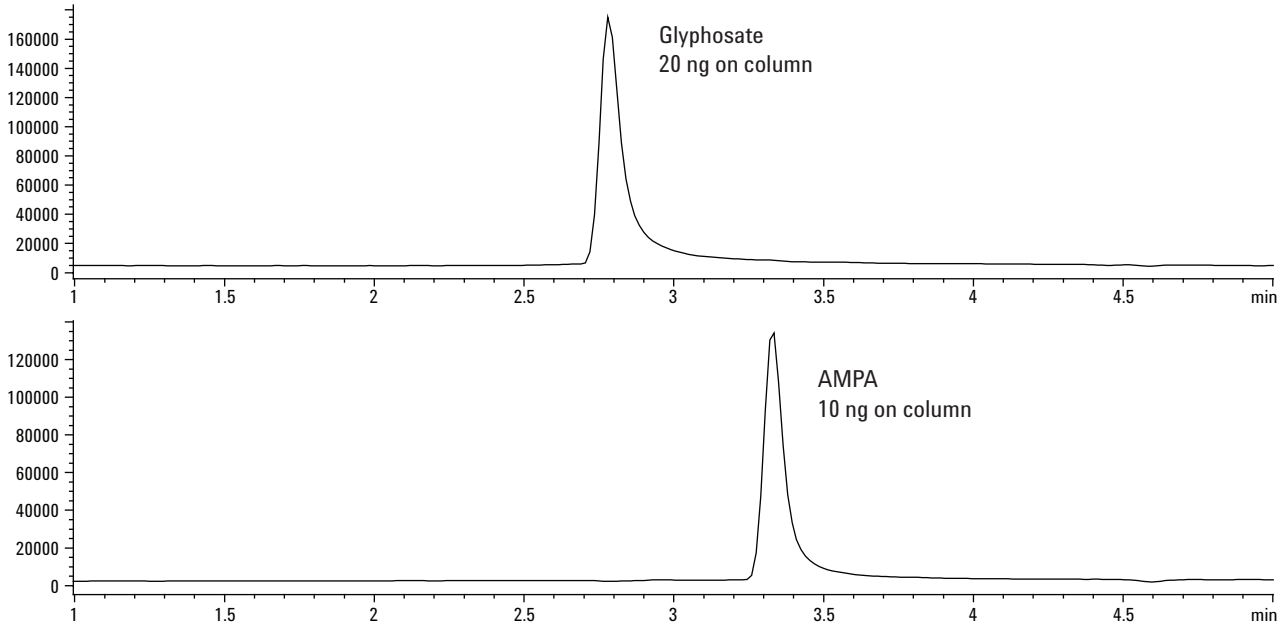

Figure 3. Low level extracted ion chromatograms for both target molecules.

Figure 4 is a similar stacked plot, but at a higher concentration.

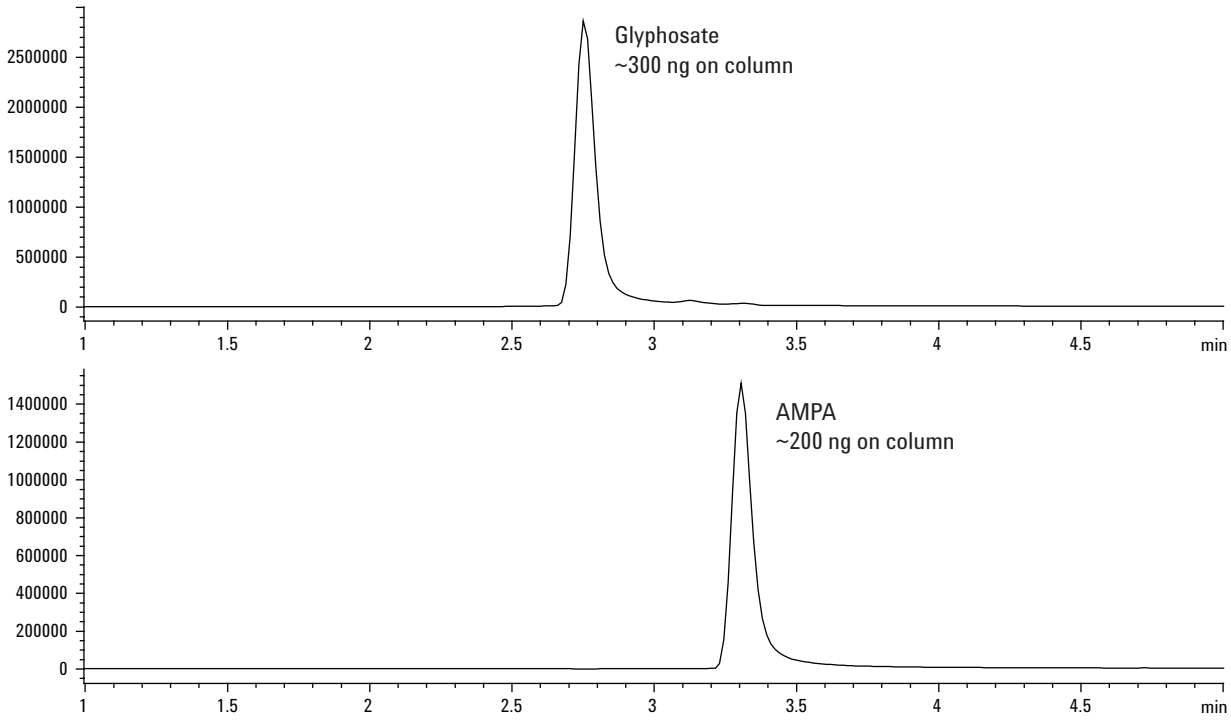

Figure 4. Higher level extracted ion chromatograms for both target molecules.

Figures 5 and 6 are abundance vs. concentration plots for the derivatized target molecules, as the positive ions for glyphosate and AMPA, respectively.

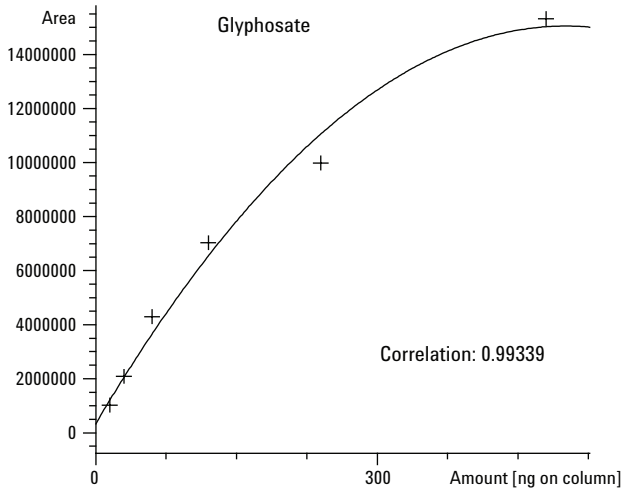

Figure 5. Abundance vs. concentration for derivatized glyphosate, detected as $[M+H]^+$, 392 *m/z*.]

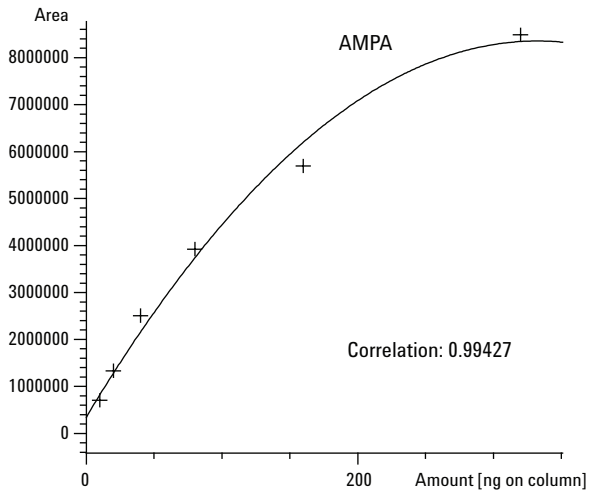

Figure 6. Abundance vs. concentration for derivatized AMPA, detected as $[M+H]^+$, 334 *m/z*.]

The non-linearity of the positive ion experiments at the higher concentrations is believed due to insufficient FMOC used in this initial experiment. This belief is strengthened by the work of Yang, et al [4] where linearity was achieved over a comparable concentration range with an optimized derivatization, also using FMOC, but analyzed in negative ion mode.

Figures 7 and 8 show abundance vs. concentration data, per Yang [4], for the derivatized target molecules as the negative ions, 390 and 332 *m/z* for glyphosate and AMPA, respectively.

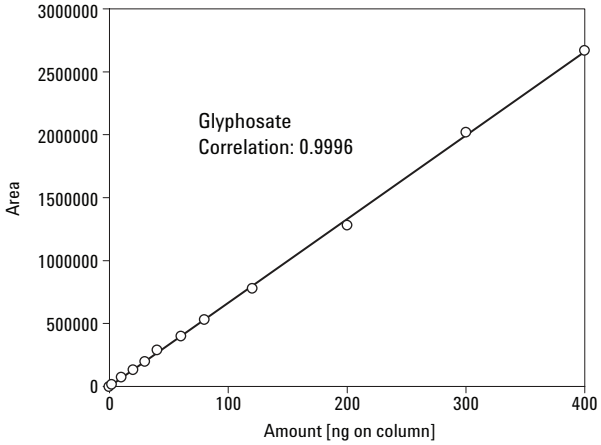

Figure 7. Abundance vs. concentration for derivatized glyphosate, detected as [M-H]⁻, 390 *m/z*.

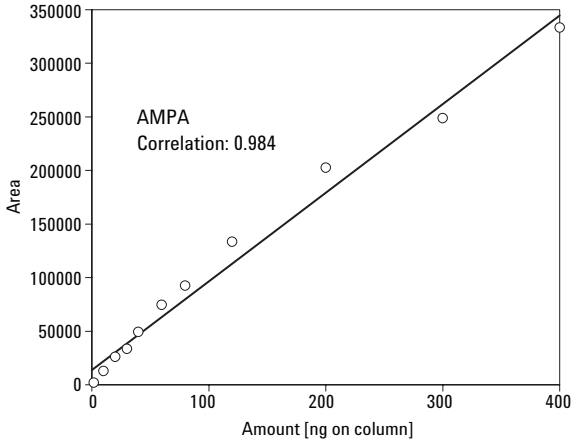

Figure 8. Abundance vs. concentration for derivatized AMPA, detected as [M-H]⁻, 332 *m/z*.

Conclusions

- Both glyphosate and AMPA, as FMOC derivatives, can be readily and sensitively detected using LC/MS with electrospray ion source in positive or negative ion mode.
- Chromatography is excellent with good separation between parent and breakdown product.
- Instrument can easily measure 10–20 ng on column.
- Sensitivity is adequate for a 10–50 ppb determination given a 100 mL water sample size.
- In positive ion mode, linearity is good below 200 ng on column. Non-linearity at higher concentrations is caused by depletion of FMOC. A drop in FMOC's UV response at higher target concentrations supports this, even though absorbencies were well within the UV detector linear range.
- The present positive ion work can be a starting point for further method development to increase both linear range and applicable matrices.
- The negative ion method shows what a fully engineered method can accomplish.
- Full sample extraction procedures can be found in Reference 4.
- This work represents an example of how derivatization can enhance the power of LC/MS. Generally, derivatization is not thought of as an LC/MS option.
- Other derivatization strategies can be studied for similar compounds.

References

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3. Stalikas, C. D. and C. N. Konidari, Analytical methods to determine phosphonic and amino acid group-containing pesticides. *J. Chromatography*, 2001 A 907 1-19.
4. L. Grey, B. Nguyen, and P. Yang "High Performance/Electrospray Ionization/Isotopic Dilution Mass Spectrometry Analysis of N-phosphonomethyl glycine and mass spectrometry analysis of aminomethyl phosphonic acid in environmental water and vegetation matrices", *J. AOAC*, 2001, 84(6), 1770-1780.

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