

Improving Identification and Quantification of Polar Herbicides by CESI-MS

Achieving better differentiation of glyphosate, fosetyl aluminum and their degradation products

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Overview

Who should read this: Senior scientists, lab directors

Focus: Demonstrate the advantages of CESI-MS for separating, identifying and quantifying the polar herbicides glyphosate and fosetyl aluminum as well as their degradation products.

Goals: Develop an effective CESI-MS method for separating, identifying and quantifying polar herbicides and compare the selectivity, accuracy and reproducibility of that method to those of an approved, currently used LC-MS method.

Problem: Concerns about the safety of glyphosate-based herbicides (GBHs) have made it essential to be able to detect glyphosate in foods (especially fruits and nuts) and distinguish it from other alternative herbicides such as fosetyl aluminum. Current LC-MS methods have significant limitations, including ion suppression, retention time instability and problems in distinguishing between degradation products of these herbicides (Figure 1). Both glyphosate and fosetyl aluminum are regulated, but false positive identification (ID) and inaccurate quantification of their degradation products, phosphate and phosphonate, is possible using current LC-MS methods. Therefore, a method is needed that provides accurate ID and quantification of these degradation products.

Results: The developed CESI-MS method demonstrated an excellent ability to distinguish between glyphosate and its degradants, and between similar degradation products of another widely used herbicide, fosetyl aluminum. It also demonstrated better migration/retention time stability and quantitative linearity than the LC-MS method.



The CESI 8000 Plus System

Key challenges:

- Separation of highly polar molecules by LC requires either time-consuming analyte derivatization prior to reverse-phase LC, or reliance on less reliable LC techniques, such as HILIC or anion exchange chromatography
- LC-MS methods suffer from a variety of issues, including derivatization selectivity, ion suppression due to matrix effects and retention time instability
- LC-MS methods frequently have difficulty resolving phosphate and phosphonate, the final degradation products of glyphosate and fosetyl aluminum, respectively

Key features:

- Capillary electrophoresis is well suited to the separation of polar herbicides
- The CESI-MS method provided excellent specificity, easily resolving and identifying glyphosate, fosetyl aluminum and many of their degradation products
- The CESI-MS method demonstrated very good migration time stability over more than 160 runs
- The CESI-MS method exhibited excellent quantitative linearity when analyzing phosphonate, the degradation product of fosetyl aluminum, in matrices

Differentiating glyphosate, other herbicides and their degradation products

Glyphosate is a common, broad-spectrum, systemic herbicide widely used to kill weeds that compete with crops. Concerns about the safety of glyphosate^{1,2} have led to increasing restrictions on glyphosate-based herbicides (GBHs), most recently in the European Union (EU).³ As such, it is increasingly important to develop robust analytical methods with the sensitivity and selectivity to identify and quantify glyphosate and its degradation products in foods and differentiate them from other herbicides, such as fosetyl aluminum.

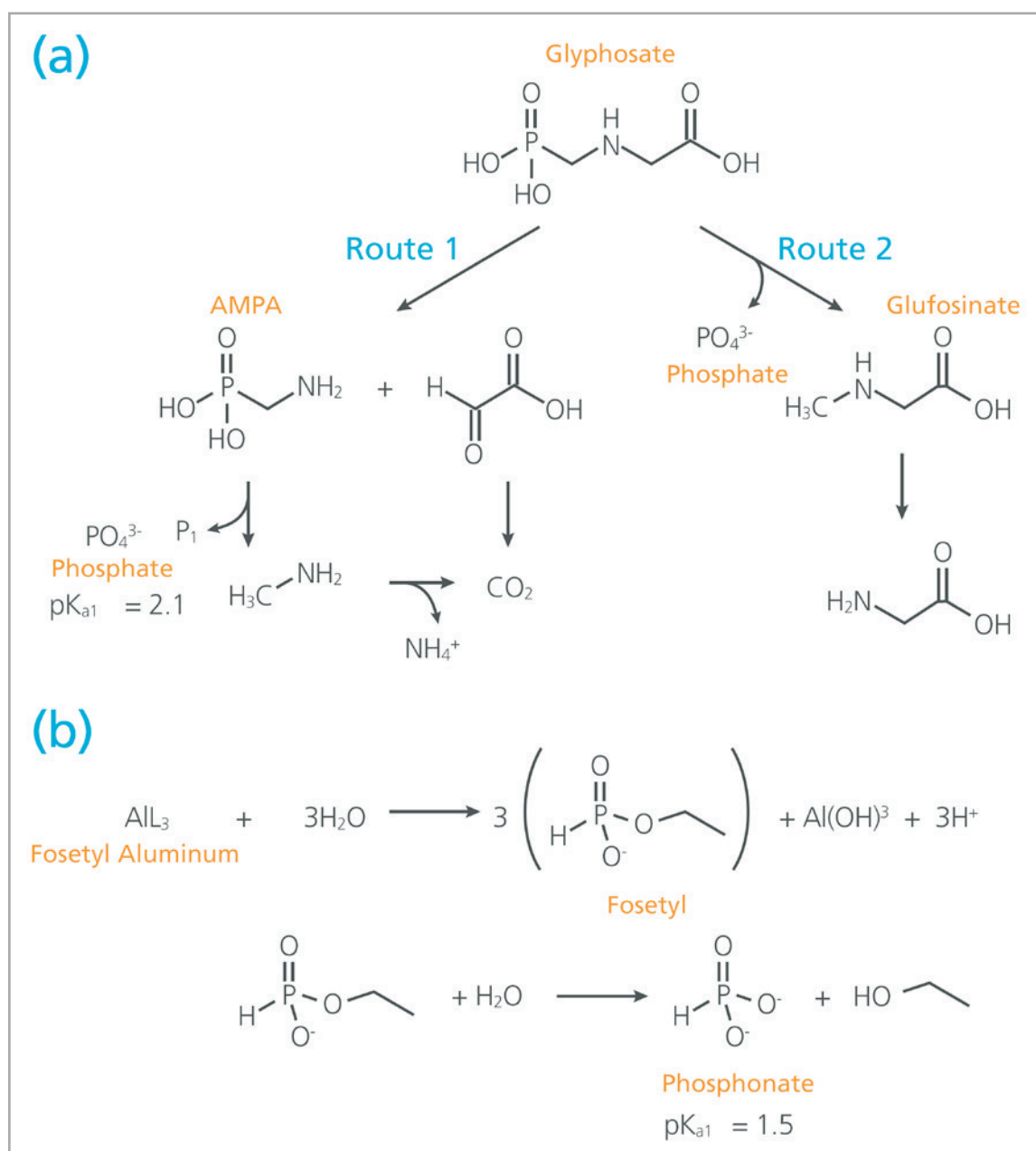


Figure 1. Degradation pathways for (a) glyphosate and (b) fosetyl aluminum.

Limitations of LC-MS methods

Several LC-MS-based methods are currently used to analyze glyphosate and its degradation products, with many listed in the Quick Polar Pesticides (QuPPE) Method document created by the EU Reference Laboratories for Residues of Pesticides.⁴ The methods use anion-exchange, porous-graphitized carbon or HILIC liquid chromatography coupled with mass spectrometry. Reverse-phase LC methods have also been used but require analyte derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) before sample analysis.⁵ While LC-MS methods generally can differentiate glyphosate and fosetyl aluminum (Figure 1), these methods can suffer from derivatization selectivity, matrix effects, ion suppression and poor retention time reproducibility. Additionally, LC-MS methods are generally not suitable for resolving phosphate and phosphonate, the final degradation products of glyphosate and fosetyl aluminum, especially in real-world matrices.⁴

Advantages of CESI-MS

Capillary electrophoresis (CE) is well suited to the analysis of polar ions and has already proven useful in the analysis of pesticides.⁶ The mechanism of separation is by differences in pKa and hydrodynamic radii. In the case of phosphate and phosphonate, they differ in pKa by 0.6 units (Figure 1). Integration of capillary electrophoresis and electrospray ionization (CESI) into a single dynamic process facilitates the mass spectrometric use of CE detection and analysis. The developed CESI-MS method readily differentiated glyphosate, fosetyl aluminum and their degradation products (Figure 2). It demonstrated far better migration/retention time stability than a corresponding LC-MS method (Figure 3). Finally, quantitative CESI-MS/MS analysis of phosphonate in real-world nut extracts showed exceptional linearity while the corresponding anion-exchange LC-MS/MS method exhibited significant ion suppression due to matrix effects (Figure 4).

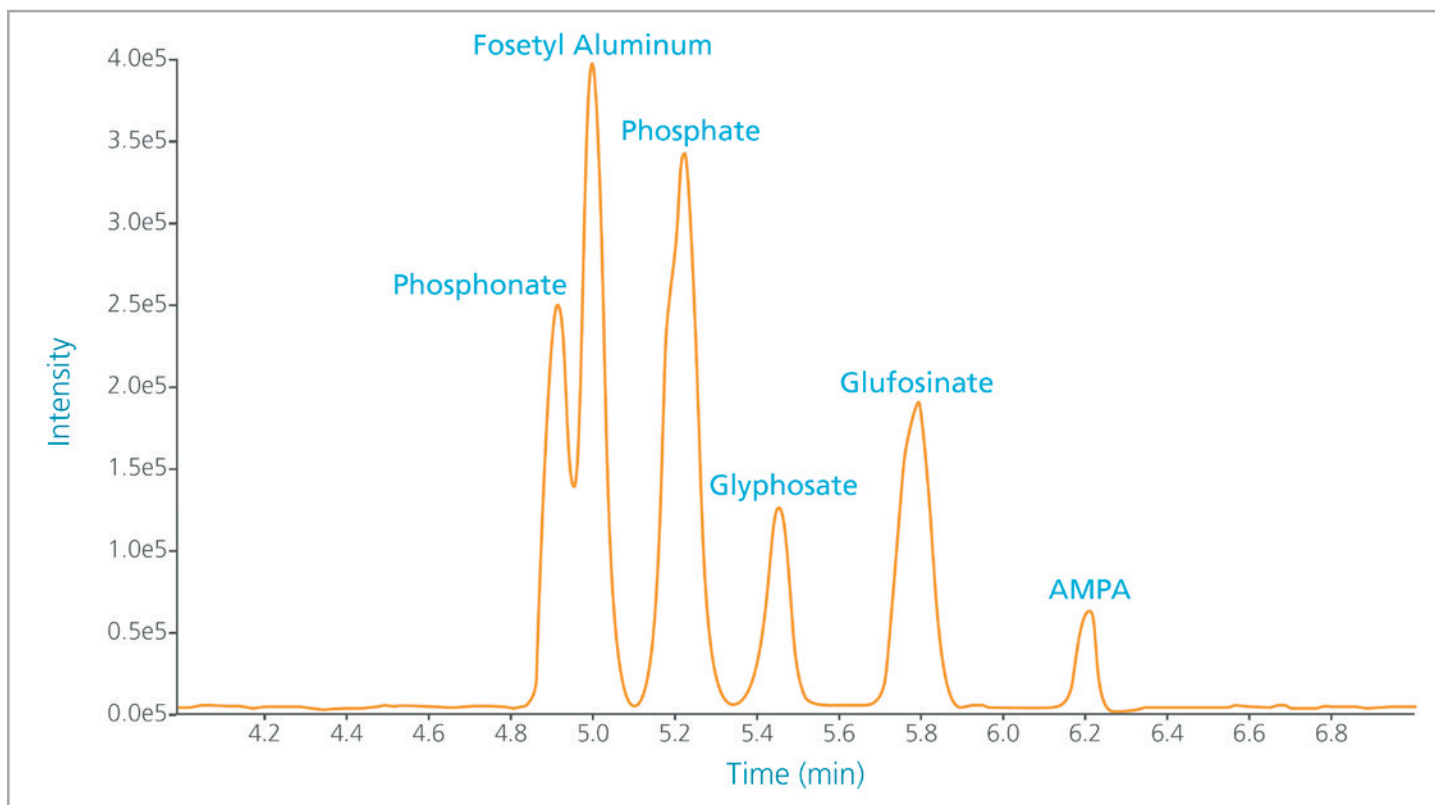


Figure 2. CESI-MS provides clear separation and detection of glyphosate and 3 of its degradation products (glufosinate, AMPA and phosphate) along with fosetyl aluminum and 1 of its degradation products (phosphonate).

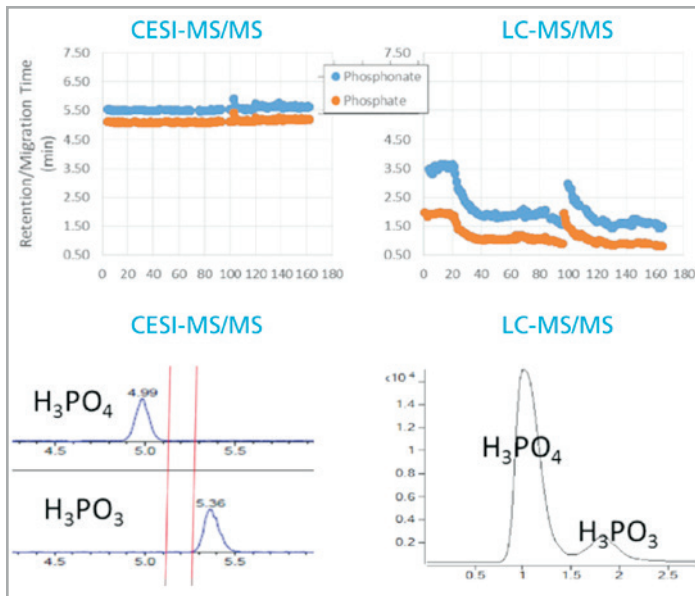


Figure 3. Migration/retention times for phosphonate and phosphate across more than 160 analyses, with sample electropherograms (CESI-MS/MS) and chromatograms (LC-MS/MS). CESI-MS proved far more stable over time and baseline separation was achieved only in the CESI-MS analysis.

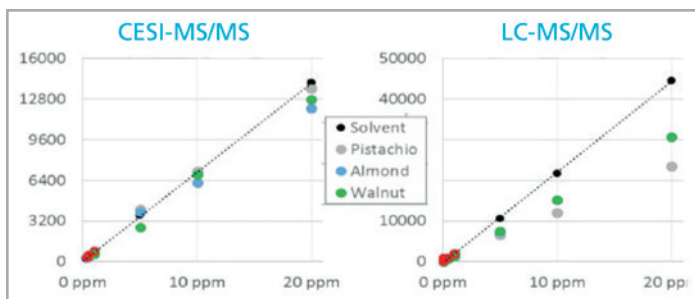


Figure 4. CESI-MS/MS and anion-exchange LC-MS/MS quantification of phosphonate spiked into 0.1% formic acid (solvent) and nut extracts. Significant ion suppression due to matrix effects is clearly visible in the LC-MS/MS results, but not in the CESI-MS/MS results. Fit lines are for the solvent curves only.

References

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