

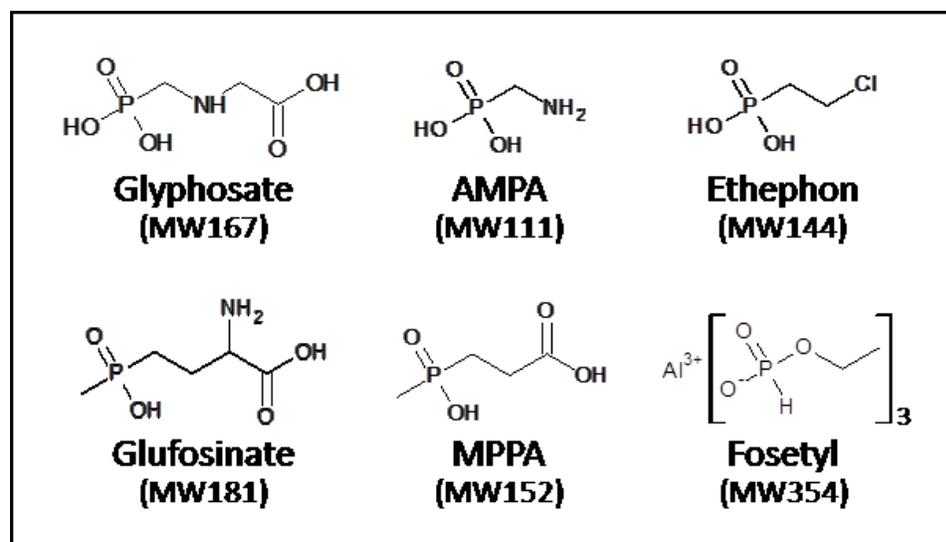
LC/MS Analysis of Glyphosate and Its Related Compounds Using Shodex™ HILICpak™ VT-50 2D (1)

Introduction

Organophosphate herbicides such as glyphosate and glufosinate are globally used as a non-selective weed killer, effective for most weeds. Many countries have set regulations for organophosphate herbicides use due to concerns on their toxicity to humans. Therefore, monitoring their concentrations in environment and foods are important.

HPLC has become the preferred analysis method due to the high hydrophilicity of the compounds. Unfortunately, the samples are hardly retained under reversed phase mode. To solve the problem, methods using pre-column derivatization or addition of ion- pair reagent to the eluent have been typically applied. Using these methods have created significant drawbacks including complicated time taking processes of the derivatization and increase of background level from the ion-pair reagent residues on the column and the flow-lines.

The purpose of this work was to develop an LC/MS/MS method for high- sensitivity simultaneous analysis of six organophosphate herbicide related compounds including glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), and glufosinate and its metabolites, 3-methylphosphinicopropionic acid (MPPA), ethephon, and fosetyl. The Shodex™ HILICpak™ VT-50 2D column has been packed with materials modified with quaternary ammonium as functional group. In order for the method to be an effective quantification tool for measuring the above listed compounds, we aimed to measure their concentrations in 1 ng/mL levels, the required regulation levels set by most countries.



Experimental

A mixture of commercially available standards was dissolved in ultrapure water and used to test following conditions. Isocratic method was used throughout.

Instrument: Shimadzu Nexera / LCMS-8030 Plus

Column: Shodex™ HILICpak™ VT-50 2D (2.0 mm I.D. x 150 mm, 5 μm)

Eluent: (a) Acidic condition, pH about 2.4; 50 mM HCOOH aq. / CH₃CN = 90 / 10

(b) Neutral condition, pH about 6.8; 50 mM HCOONH₄ aq. / CH₃CN = 80 / 20

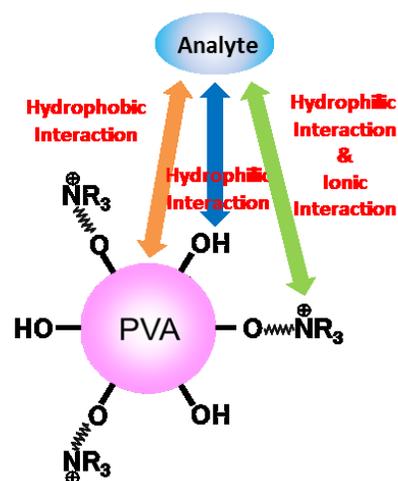
(c) Alkaline condition, pH about 8.5; 50 mM HNH₄CO₃ aq. / CH₃CN = 50 / 50 Flow rate: 0.3 mL/min

Detector: ESI-MS SIM(-) or MRM(-) Column temp.: 40 °C

The organophosphate herbicides easily form metal complexes, and thus those compounds are easily adsorbed on the metallic flow-lines and results in tailing. PEEK housing was used for all flow-line except a part in autosampler (SUS) to avoid adsorption of metal complexes inside the column.

Shodex™ HILICpak™ VT-50 2D

- Polyvinyl alcohol base material modified with quaternary ammonium
- PEEK housing prevents the adsorption of metal ligands
- Any ratio of acetonitrile or methanol to water mixtures can be used as an eluent
- Retains anions without derivatization nor presence of ion-pair reagents in the eluent
- Works under reversed phase and ion exchange modes in low organic solvent condition and works under HILIC and anion exchange modes in high organic solvent condition
- Usable in wide pH range of 2-12
- 2 mm column I.D. allows high sensitivity analysis with LC/MS (normal flow rate about 0.2 mL/min)



Results

1. The effects of eluent pH on separation

Figure 1 shows the chromatograms obtained by injecting 5 μ L of 1 μ g/mL standard mixture analyzed under SIM(-) mode.

(a) Acidic condition: AMPA was barely retained, glyphosate tailing, long elution time of ethephon (60 min), and fosetyl being not detected were problems.

(b) Neutral condition: The analysis time was shortened to 10 min. However, AMPA and glufosinate were still barely retained and AMPA and glyphosate were tailing.

(c) Alkaline condition: Demonstrated the best analysis result as even the earliest eluting compound, fosetyl, had retention factor of over 2, provided sharp symmetrical peaks for all compounds, and completed the analysis within 20 min.

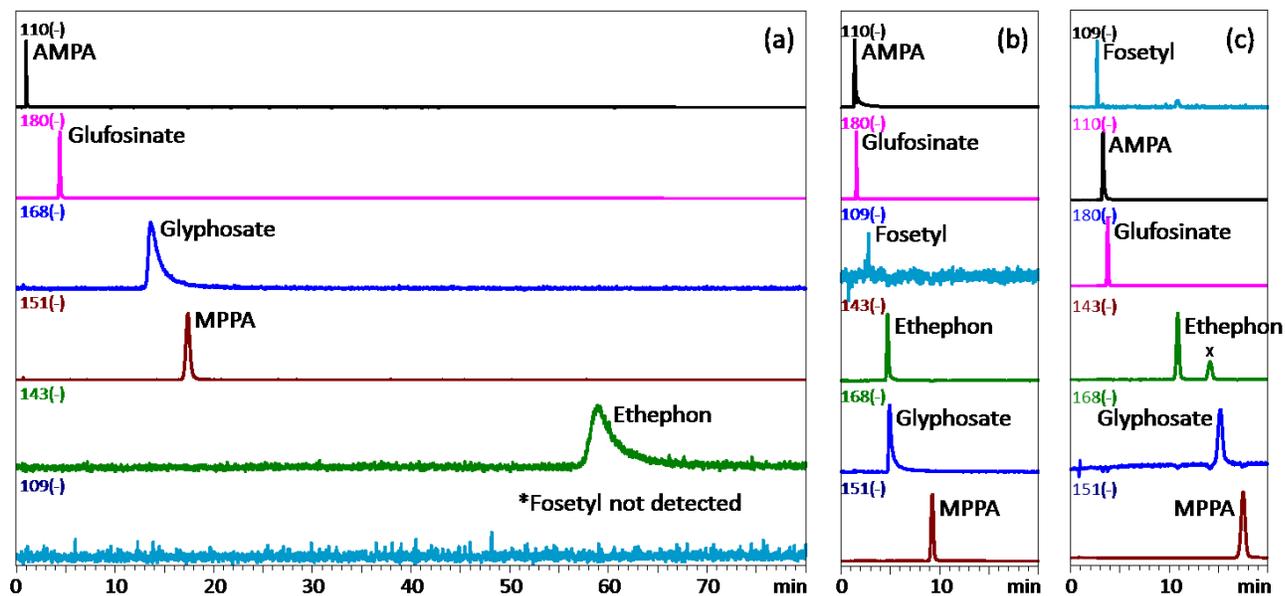


Fig. 1. SIM chromatograms obtained using three different pH eluents: (a) acidic, (b) neutral, and (c) alkaline * x = derived from other compounds except ethephon

2. High sensitivity analysis using alkaline eluent and MRM mode

Figure 2 shows the chromatograms obtained by injecting 10 μ L of 10 ng/mL standard mixture analyzed under MRM(-) mode.

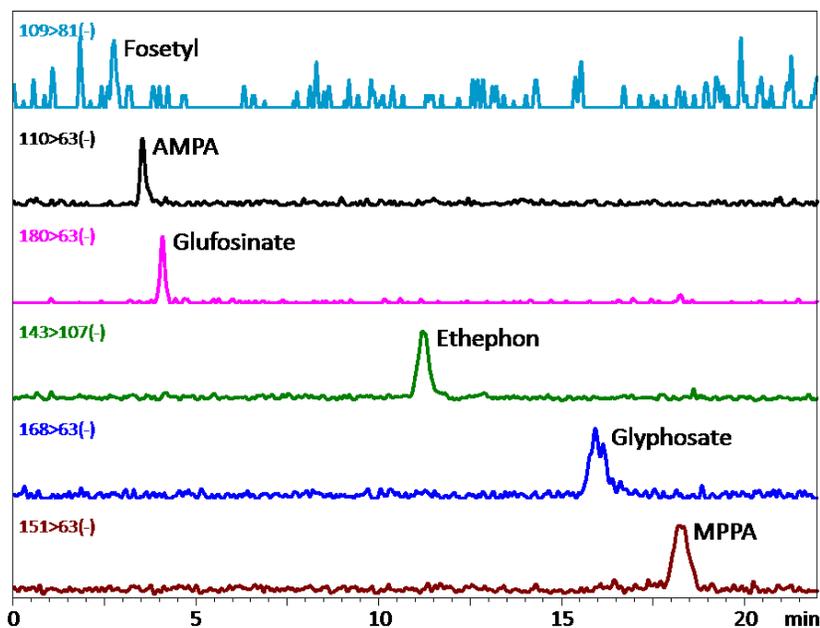


Fig.2 MRM chromatograms obtained by injecting 10 μ L of 10 ng/mL standard mixture. The same standard mixture was injected in 10, 50, 100, and 250 ng/mL. Figure 3 shows calibration curves of each compound. Good linearity was observed for all compounds except MPPA showing a quadratic curve.

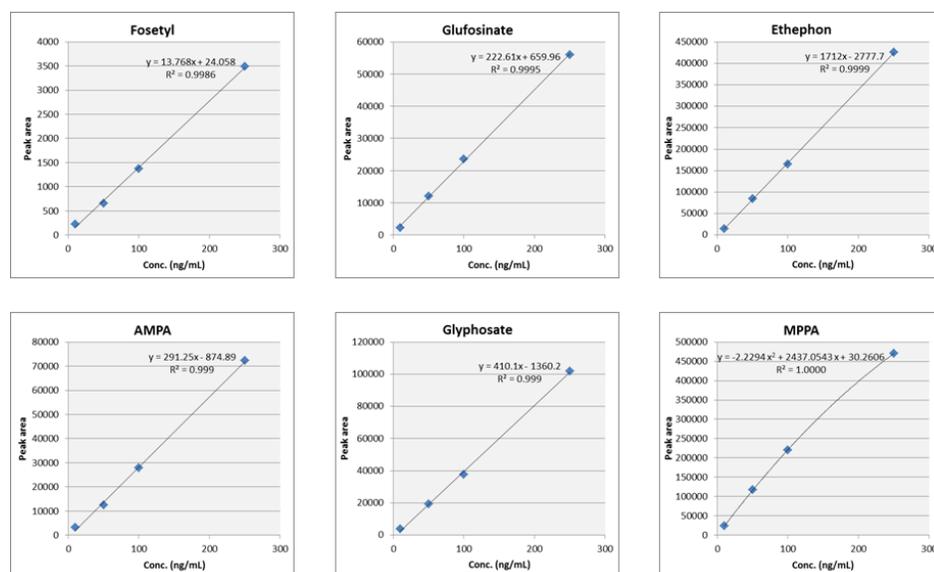


Fig.3 Calibration curves of six compounds

Quantification of the target analytes except for fosetyl in 1 ng/mL level was achieved by injecting 50 μ L of the standard mixture (Figure 4), while their detection was difficult with injection volume of 10 μ L.

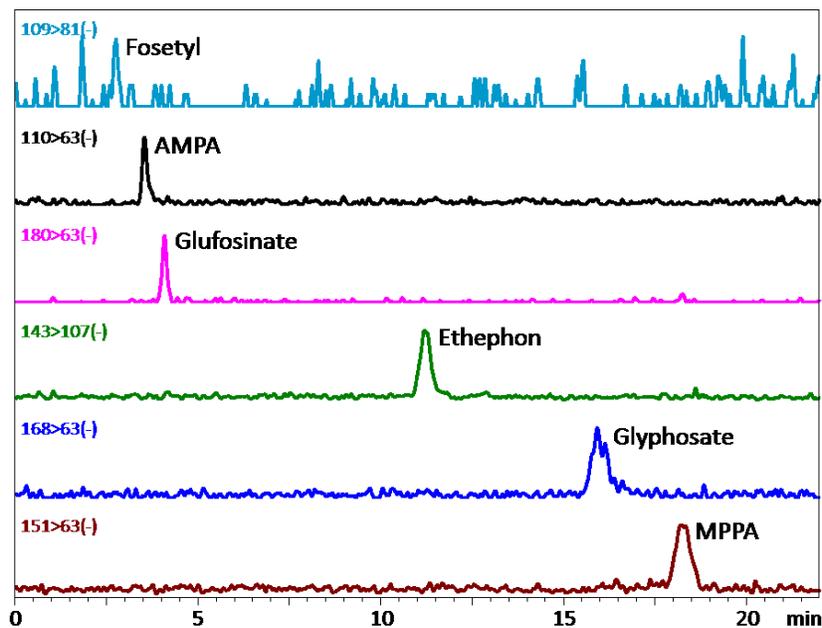


Fig.4 MRM chromatograms obtained by injecting 50 μ L of 1 ng/mL standard mixture

3. Recovery test for glyphosate in tap water

Figure 5 shows MRM chromatograms obtained by injecting 50 μ L of 10 ng/mL glyphosate standard dissolved in ultrapure and tap water. Relatively good recovery rate (84%) was achieved even when glyphosate was dissolved in tap water.

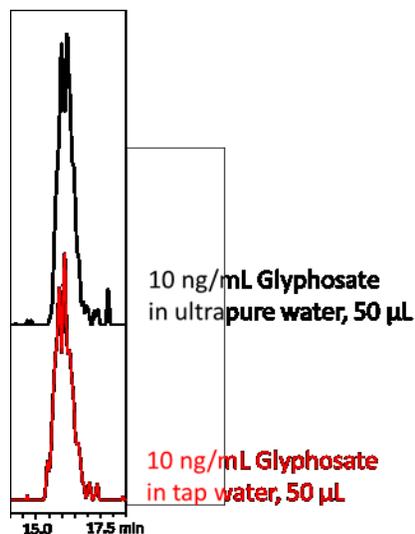


Fig.5 Chromatographs of glyphosate dissolved in ultrapure and tap water

Conclusions

This application demonstrated the effectiveness of Shodex™ HILICpak™ VT-50 2D under alkaline eluent containing ammonium bicarbonate for the simultaneous analysis of several organophosphate herbicides including glyphosate and its related compounds. The analysis success was achieved due to the column with polymer-based packing material that allowed the use of alkaline eluent. The column's PEEK housing also prevented tailing. The developed method showed a fast and stable analysis of organophosphate herbicide and related compounds without the use of pre-column derivatization, ion-pair reagents, nor gradient elution.