



Simultaneous LC/MS Analysis of Glyphosate and Its Related Compound

Introduction

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

Those compounds are highly hydrophilic, and thus HPLC is a preferred analysis method. However, they are hardly retained under reversed phase mode. To overcome the problem, pre-column derivatization or ion-pair reagents are often used. However, the use of derivatization adds additional steps in the analysis while the use of ion-pair reagent increases background level from the ion-pair reagent residues on the column and the flow-lines.

The purpose of this work was to develop a highly-sensitive simultaneous LC/MS/MS analysis of six organophosphate herbicide related compounds. They were glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), and glufosinate and its metabolites, 3-methylphosphinopropionic acid (MPPA), ethephon, and fosetyl. Figure 1 shows the structures of the compounds analyzed in this study.

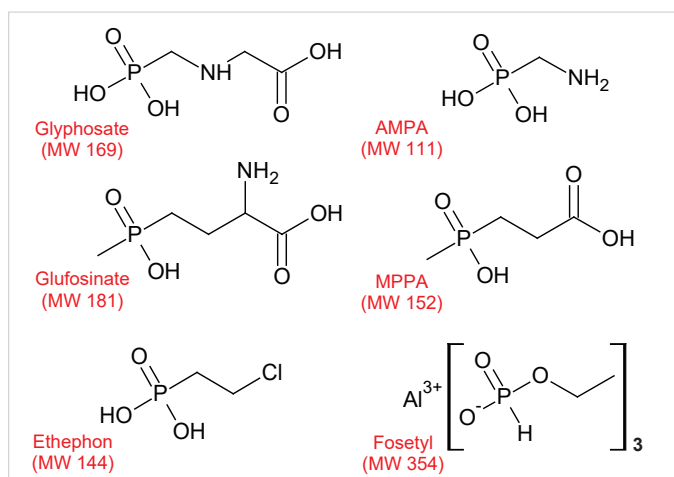


Fig. 1. Structures of organophosphate herbicide related compounds analyzed in this study.

Shodex HILICpak VT-50 2D was used for the separation. Figure 2 summarizes the features of the VT-50 2D column. To make the method an effective quantification tool for measuring the above listed compounds, we aimed to measure them in 1-ng/mL levels, which is the required regulation levels set by most countries.

Shodex HILICpak VT-50 2D

- Polyvinyl alcohol base material modified with quaternary ammonium
- PEEK housing prevents the absorption of metal ligand
- 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 (2 – 12)
- 2-mm column I.D. allows high sensitivity analysis with LC/MS (normal flow rate about 0.2 mL/min)

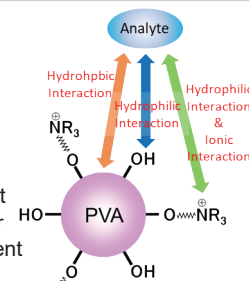


Fig. 2. Summary of Shodex™ HILICpak™ VT-50 2D column

Experimental

The LC/MS system used was Shimadzu Nexera/LCMS-8030 Plus. For the optimization, a mixture of commercially available standards were used by dissolving them in ultrapure water. The analytical conditions mentioned in figure 3 was used throughout.

The organophosphate herbicides easily form metal complexes, and thus those compounds are easily adsorbed on the metallic flow-lines and results in tailing. Therefore, we used PEEK for all flow-line except a part in autosampler (SUS). The PEEK column housing of the VT-50 2D also prevents the adsorption of metal complexes inside the column.

Results and Discussion

1. The effects of eluent pH

The effects of the eluent pH on the separation of target compounds were studied. Figure 3 shows the chromatograms obtained by injecting 5 μ L of 1- μ g/mL standard mixture analyzed under SIM(-) mode. Below is the summary of the observations.

- (a) Acidic condition: AMPA was barely retained. Tailing of glyphosate, long elution time of ethephon (60 min), and fosetyl being not detected were other 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. The retention factor of fosetyl, the earliest eluting compound was over 2, and all compounds achieved sharp symmetrical peaks. The analysis was completed within 20 min.

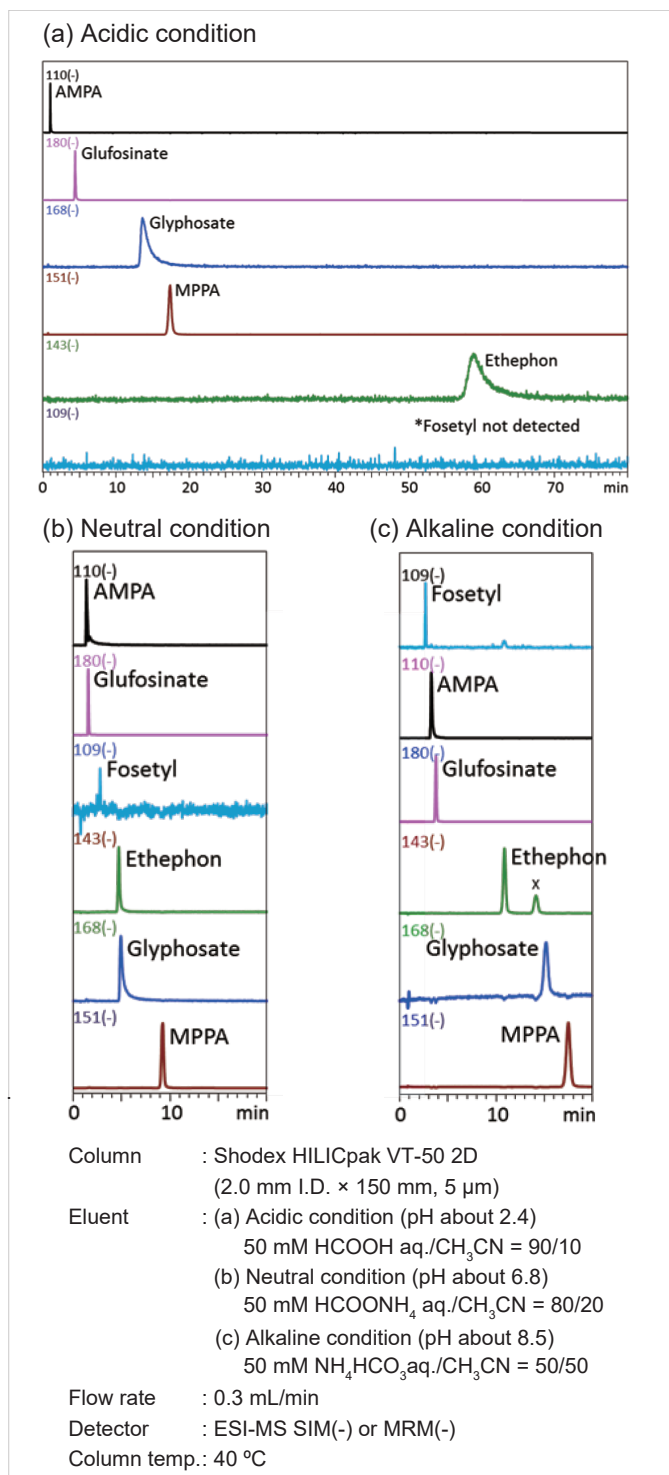


Fig. 3. SIM chromatograms obtained using three different pH eluents: (a) acidic, (b) neutral, and (c) alkaline.

x = derived from a compound other than ethephon

2. High sensitivity analysis using alkaline eluent and MRM mode

The standard mixture of different concentrations were injected (10, 50, 100, and 250 ng/mL). Figure 4 shows the chromatograms obtained by injecting 10 μL of 10-ng/mL standard mixture analyzed under MRM(-) mode. Good linearities were observed for the calibration curves of all compounds, except for MPPA which showed a quadratic curve.

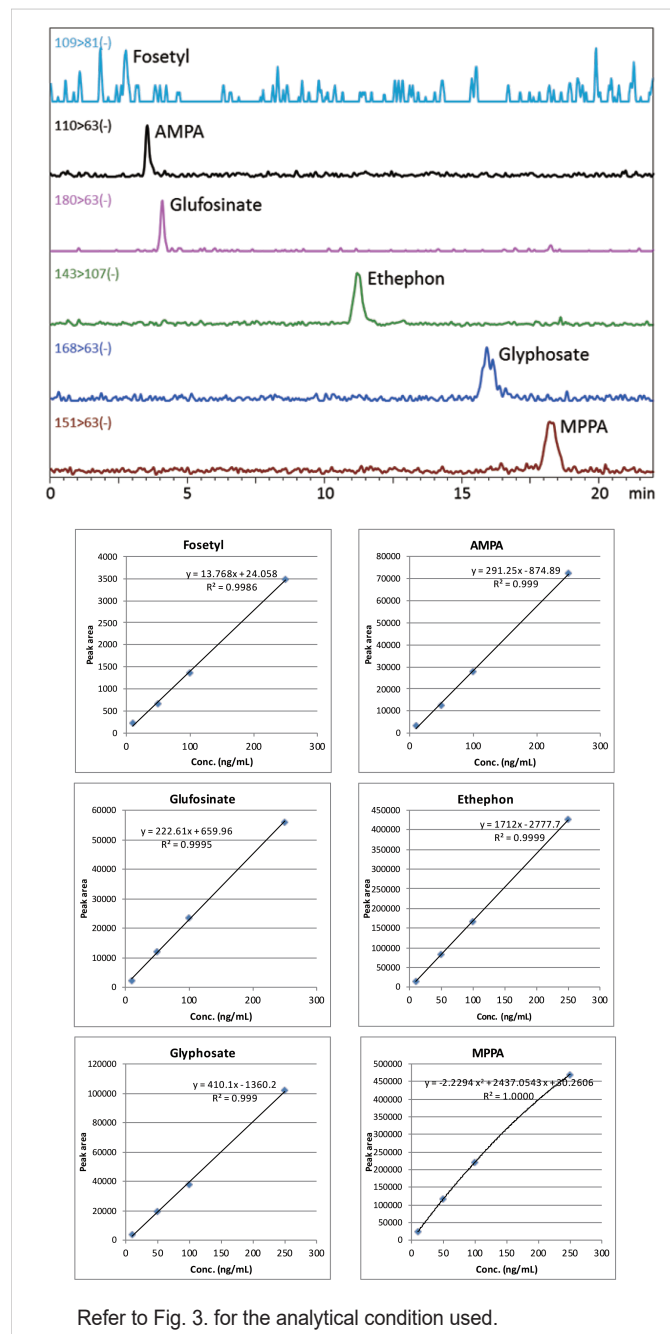


Fig. 4. MRM chromatograms obtained by injecting 10 μL of 10-ng/mL standard mixture

Quantification of target analytes, except for fosetyl in 1-ng/mL level, was achieved by injecting 50 μ L of the standard mixture (Fig. 5). However, their detection was difficult when using an injection volume of 10 μ L.

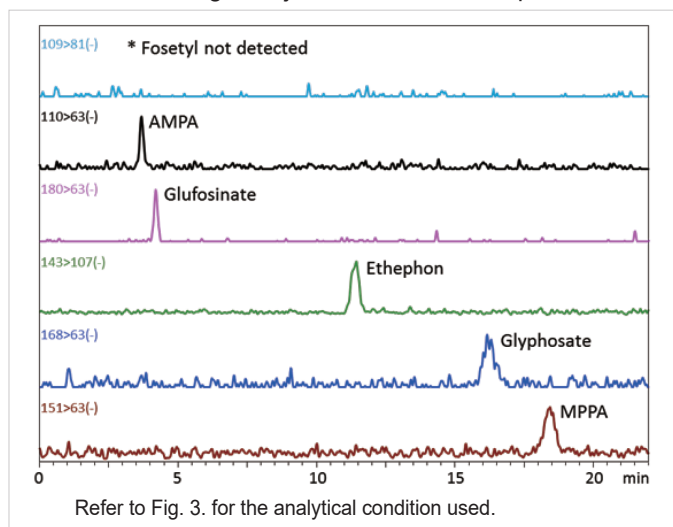


Fig. 5. Chromatograms of 1-ng/mL standards (50 μ L injection)

3. Recovery test of glyphosate in tap water

Figure 6 shows MRM chromatograms obtained by injecting 50 μ L of 10-ng/mL glyphosate standard dissolved in ultrapure or tap water. Relatively good recovery rate (84 %) was achieved even when glyphosate was dissolved in tap water.

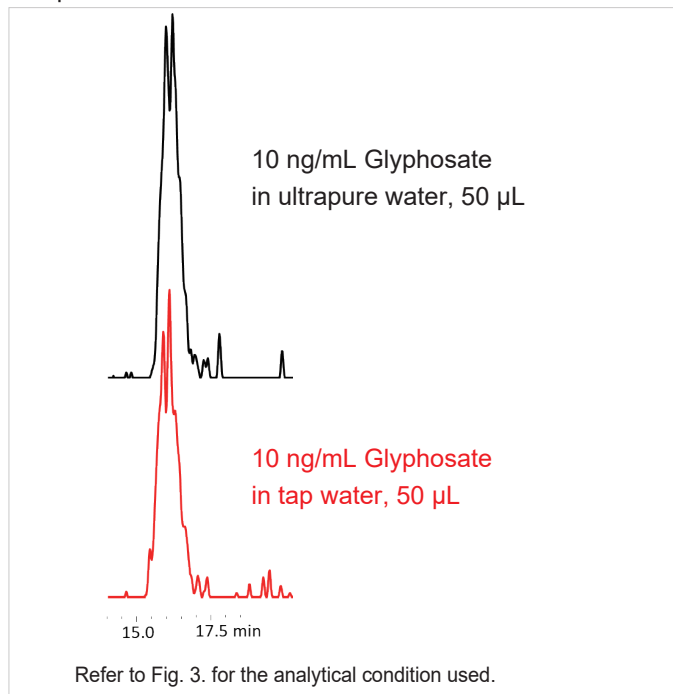


Fig. 6. Chromatograms of glyphosate standard dissolved in ultrapure or tap water

Conclusions

This application demonstrated the effectiveness of Shodex HILICpak VT-50 2D when used with an alkaline eluent containing ammonium bicarbonate for the simultaneous analysis of glyphosate, glufosinate, AMPA, MPPA, ethephon, and fosetyl. The key factor of the analysis was the column's alkaline durability, due to its polymer based packing materials. Its PEEK housing also helps to prevent the tailing problems. 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.

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