

Analysis of Glyphosate and Aminomethyl Phosphonic Acid by Pre-column Derivatization-HPLC

Introduction

The analytical method for glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), is specified in Appendix 12 of the “Methods for Testing Water Quality Management Target Parameters” (Notification No.1010001, October 10, 2003, Final Revision March 24, 2023). This method uses pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl), followed by high-performance liquid chromatography (HPLC) with fluorescence detection.

For water quality testing, the quantification limit is required to be one-hundredth of the target concentration, with a coefficient of variation of $\leq 20\%$. For glyphosate and AMPA; however, the specified quantification limit is 0.0005 mg/L, despite a target concentration of 2 mg/L.

This application note describes the analysis of glyphosate and AMPA using an Unifinepak C18 ODS column in accordance with the specified pre-column derivatization–HPLC method.

Keywords

Glyphosate, aminomethyl phosphate, aminomethyl phosphonic acid, AMPA, Unifinepak C18, water quality standards, water quality testing, water quality management target parameters, HPLC, 9-fluorenylmethyl chloroformate, FMOC-Cl, pre-column derivatization, fluorescence detector, fluorescence detection

Experimental

Instruments

Pump: PU-4180*
Autosampler: AS-4050
Column oven: CO-4060
Detector: FP-4025

* with option units

LC Conditions

Column: Unifinepak C18
(4.6 mm I.D. x 250 mm L, 5 μ m)
Guard column: Guard cartridge column
(4.0 mm I.D. x 10 mm L, 5 μ m)
Eluent A: 0.05 M phosphate buffer (pH 2.5) /acetonitrile (75/25)
Eluent B: 0.05 M phosphate buffer (pH 2.5) /acetonitrile (35/65)
Gradient: (A/B), 100/0 (0 min) $\rightarrow$ 0/100 (12 min)
 $\rightarrow$ 100/0 (22.1 min); 1 cycle: 37 min
Flow rate: 1.2 mL/min (0 to 12.1 min, 22.1 to 37 min),
1.5 mL/min (12.1 to 22.1 min)
Column temperature: 40 $^{\circ}$ C
Injection volume: 5 μ L
Wavelength: Ex. 270 nm, Em. 315 nm, Gain x100
Standard: Mixed standard solutions of glyphosate and AMPA
(0.0005, 0.002, 0.005, 0.002, 0.05, 0.1 mg/L each)

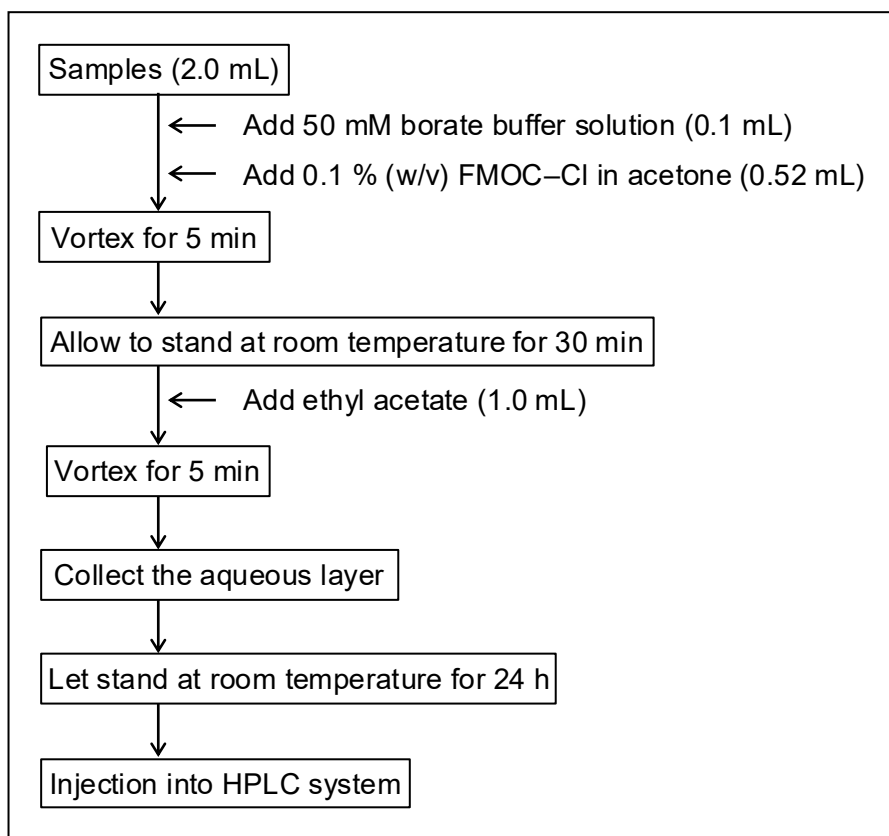


Fig. 1 Procedure for derivatization of glyphosate and AMPA

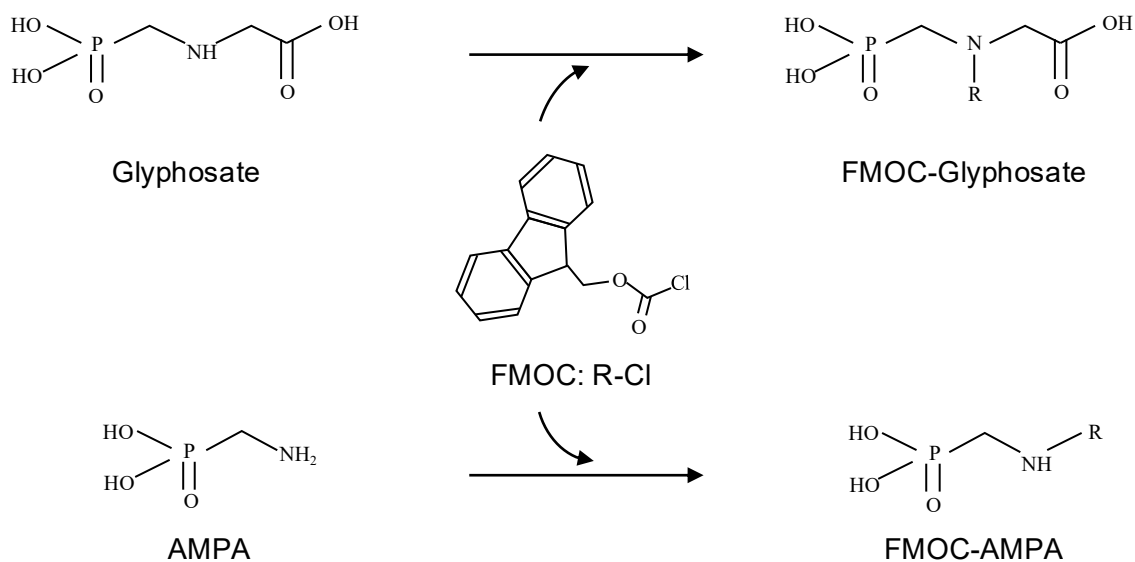


Fig. 2 Reaction formulas for glyphosate and AMPA with FMOC-Cl

Results

Figure 3-1 shows chromatograms of derivatized glyphosate and AMPA mixed standard solutions at concentrations ranging from 0.0005 to 0.1 mg/L. The calibration curves exhibited good linearity over this range, with correlation coefficients (r) of ≥ 0.9993 for both analytes (Figure 3-2).

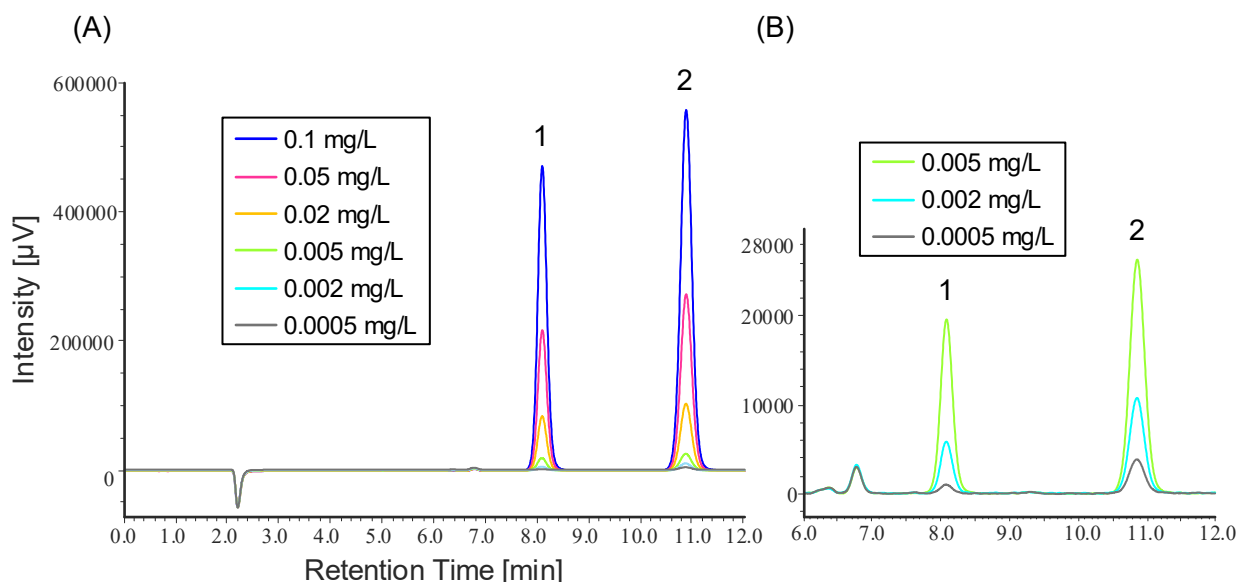


Fig. 3-1 Chromatograms of derivatized glyphosate and AMPA mixed standard solutions (0.0005, 0.002, 0.005, 0.02, 0.05, and 0.1 mg/L)

(A): Full chromatograms, (B): Expanded view of the 6.0 to 12.0 min retention time range for the low-concentration solutions (0.0005, 0.002, and 0.005 mg/L)

1: Derivatized glyphosate, 2: Derivatized AMPA

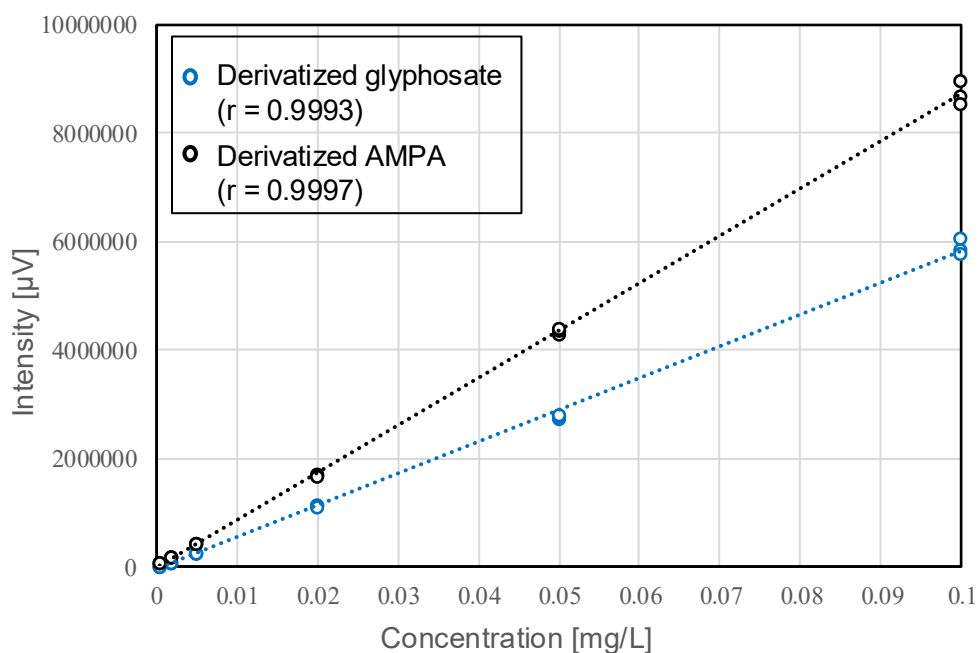


Fig. 3-2 Calibration curves for derivatized glyphosate and AMPA

Fig. 4 shows chromatograms obtained from six replicate measurements of a derivatized glyphosate and AMPA mixed standard solution containing 0.0005 mg/L of each analyte. Table 1 summarizes the repeatability of retention time and peak area. Both analytes exhibited good repeatability, with relative standard deviations (RSDs) of $\leq 0.1\%$ for retention time and $\leq 7.3\%$ for peak area.

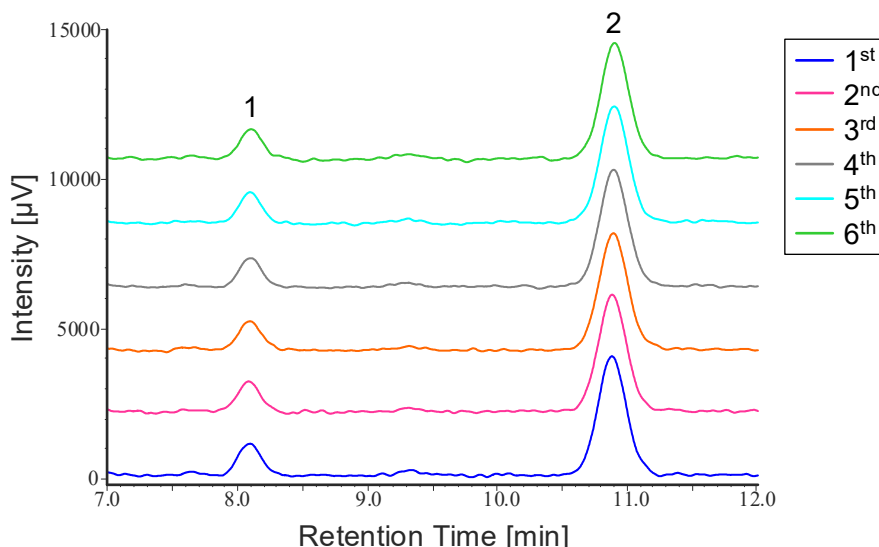


Fig. 4 Chromatograms from six replicate measurements of derivatized glyphosate and AMPA (0.0005 mg/L each, $n = 6$)
1: Derivatized glyphosate, 2: Derivatized AMPA

Table 1. Retention time and peak area repeatability for derivatized glyphosate and AMPA
(0.0005 mg/L each, $n = 6$)

Injection Number	Retention Time [min]		Peak Area	
	Glyphosate	AMPA	Glyphosate	AMPA
1	8.097	10.88	14,879	67,943
2	8.083	10.88	15,263	66,601
3	8.097	10.89	14,240	63,023
4	8.103	10.89	12,371	66,186
5	8.097	10.90	13,678	63,647
6	8.103	10.90	14,332	67,469
Average	8.097	10.89	14,127	65,811
SD	0.007	0.008	1,019	2,026
RSD [%]	0.09	0.08	7.22	3.08

Conclusion

Glyphosate and AMPA were successfully analyzed at the specified quantification limit of 0.0005 mg/L using pre-column derivatization–HPLC with fluorescence detection in accordance with the method for water quality management target parameters. Both analytes exhibited good calibration linearity over the concentration range of 0.0005 to 0.1 mg/L, with correlation coefficients (r) of ≥ 0.9993 . Six replicate measurements at 0.0005 mg/L demonstrated good repeatability, with relative standard deviations of $\leq 0.1\%$ for retention time and $\leq 7.3\%$ for peak area. These results demonstrate the method's suitability for determining glyphosate and AMPA at the specified quantification limit required by the Water Supply Law.

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