

Analysis of Glyphosate by HPLC with Pre-column Derivatization

Glyphosate (*n*-(phosphonomethyl)-glycine) is an effective herbicide in killing a wide variety of plants. This note describes a determination method for glyphosate using an HPLC system.

In Japan, 2 mg/L is set as the target value and two determination methods are described in an ordinance from the Ministry of Health,

Labour, and Welfare concerning water quality standard for tap water. In conformity with one of them, 9-fluorenylmethyloxy-carbonyl chloride (FMOC) was used as a pre-column derivatization reagent in this note.

Chromatograms at one-hundredth of the target value

Good separation between FMOC-Glyphosate and FMOC-AMPA was achieved by adjusting acetonitrile concentration in mobile phase. After the derivatives were detected, the acetonitrile concentration was increased to elute other compounds, such as unreacted derivatization reagent, more rapidly from separation column. As a result, injection-to-injection cycle time was shortened to 30 min. **The CV value at one-hundredth concentration of the target value was 0.6 % ($n=5$) for each analyte, which shows excellent reproducibility of this method.**

Example of

the sample pretreatment

Sample water

10 mL

Derivatization

- Borate buffer 0.5 mL
- FMOC solution 2.6 mL
- Shaking for 5 min
- Wait for 30 min

Liquid-liquid extraction

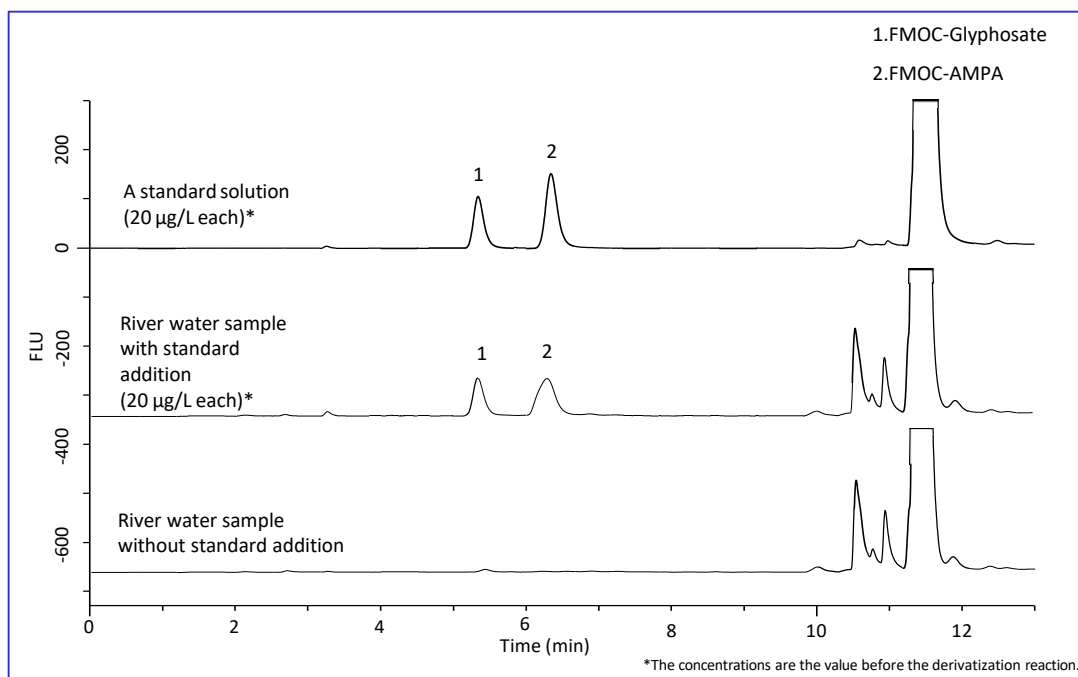
- Ethyl acetate 5 mL
- Shaking for 5 min
- Discard the organic layer

Inject the aqueous layer into the HPLC system

HPLC-FL

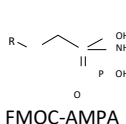
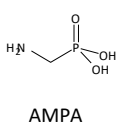
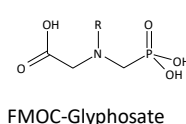
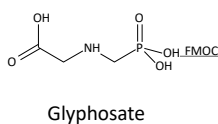
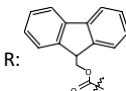
As well as glyphosate, aminomethylphosphonic acid (AMPA), which is its major metabolite, should be simultaneously determined because it is described in the ordinance that concentration of AMPA has to be added to that of glyphosate.

In the quality control of Japanese pesticide analysis, the coefficient of variation (CV) at one-hundredth concentration of the target value is required to be less than 20 %. In this note, CV values even at one-thousandth concentration of the target value were examined.



A scheme of derivatization reaction

Derivatization reagent FMOC: R-Cl



HPLC conditions

Column : Inertsil ODS-3
(4 µm, 150 x 4.6 mm I.D.)
Cat.No. 5020-04645

Eluent : A) CH₃CN
B) 50 mM phosphate buffer * A / B = 35 / 65 (8.5 min Hold), 70 / 30 (3.0 min Hold), (v/v)

Flow rate : 0.7 mL/min

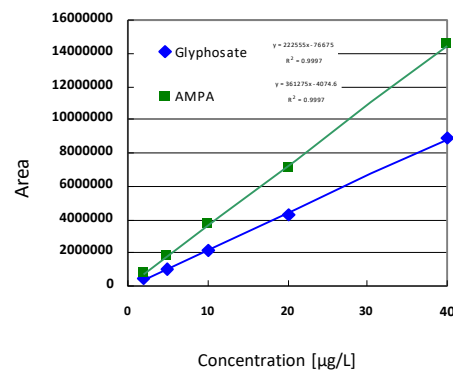
Col. Temp. : 40 °C

Detection : FL Ex. 270nm, Em. 315nm

Inj. Vol. : 10 µL

* 50 mM phosphate buffer :

6.8 g of potassium dihydrogen phosphate was dissolved in 1 L of water, and the pH value was adjusted to 2.5 by adding phosphoric

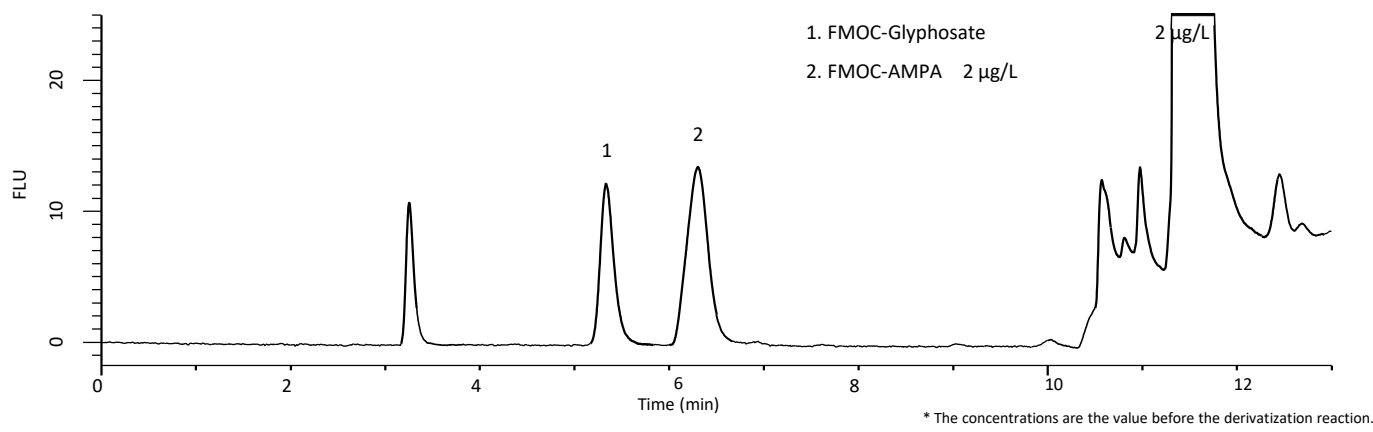


The calibration curves *

* The peak areas were obtained by derivatizing arbitrarily-diluted standard solutions.

A chromatogram at one-thousandth of the target value

V. values of peak area were obtained even with 10-fold lower concentration of standard solution than the concentration at which reproducibility should be examined in the quality control of Japanese pesticide analysis. As a result, both values (9.6 % for glyphosate, and 6.2 % for AMPA, respectively) were lower than 20 %, which represents good precision of this method even at the quite low concentration.



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