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 glyphsoate 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 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.

As well as glyphosate, aminomethylphosphonic acid（AMPA）, which is its major metabolite, should be simulatneously 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 (C.V.) at one-hundredth concentration of the target value is required to be less than 20%. In this note, C.V. values even at one-thousandth concentration of the target value were examined.

Chromatograms at one-hundredth of the target value

Good separation between FMOC-Glyphosate and FMOC-AMPA was achieved by adjusting acetonitrile conecentration in mobile phase. After the derivatives were detected, the acetontitrile 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 shorten to 30 min. The C.V. value at one-hunderedth concentration of the target value was 0.6% (n=5) for each analyte, which shows excellent reproducibility of this method.

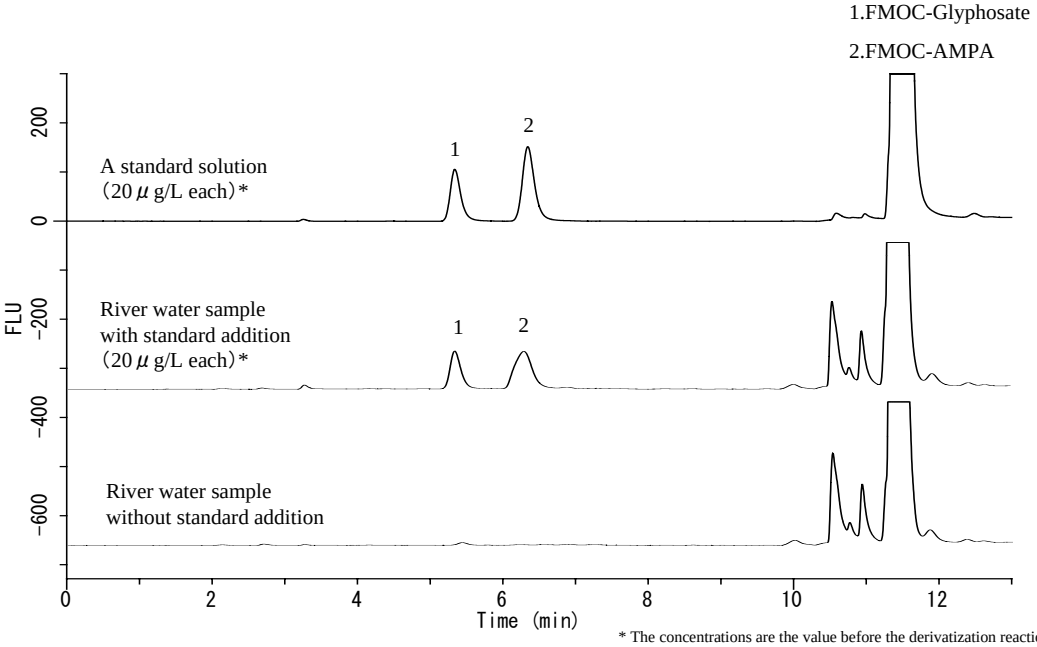
An example of the sample pretreatment

Sample water
- 10 mL

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

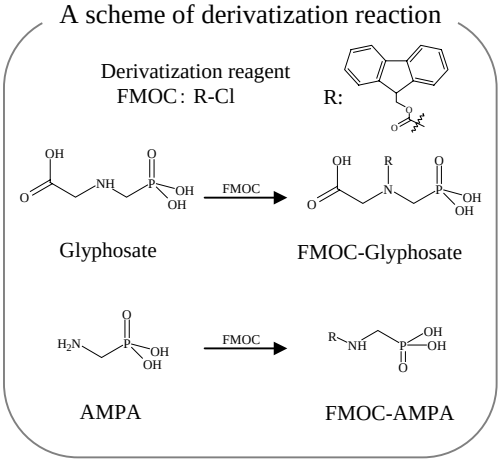
Liquid-liquid extraction
- Ethyl acetate 5 mL
- Shaking for 5 min
- Discard the organic layer
- Inject the aqueous layer into the HPLC system

HPLC-FL



* The concentrations are the value before the derivatization reaction.

A scheme of derivatization reaction



Structures are created using Chemistry 4-D Draw which is provided by ChemInnovayion Software, Inc.

HPLC conditions

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

Eluent : A) CH_3CN
B) 50 mM phosphate buffer *
A / B = 35 / 65（8.5 min Hold),
70 / 30（3.0 min Hold), (v/v)

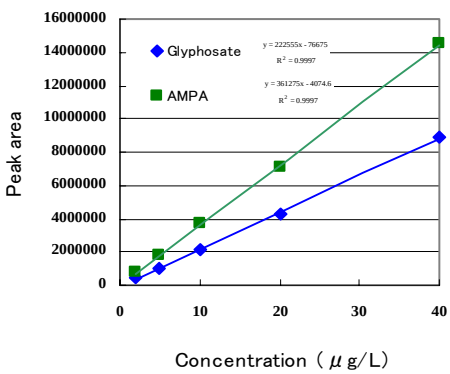
Flow rate : 0.7 mL/min

Col. Temp. : 40℃

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 acid.

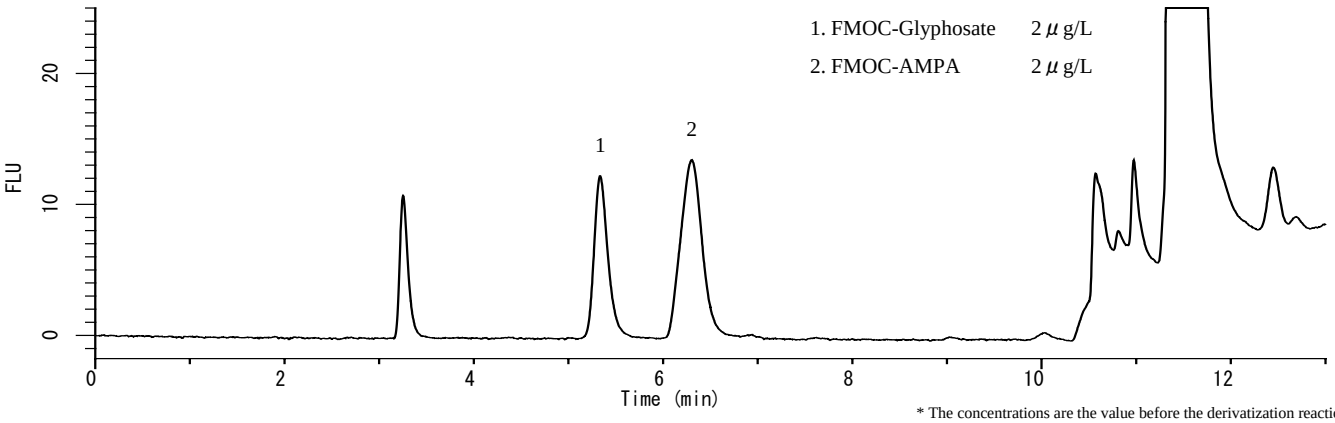


The calibration curves *

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

A chromatogram at one-thousandth of the target value

C.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 glysphosate, and 6.2% for AMPA, respectively) were lower than 20%, which represents good precision of this method even at the quite low concentration.



* The concentrations are the value before the derivatization reaction.

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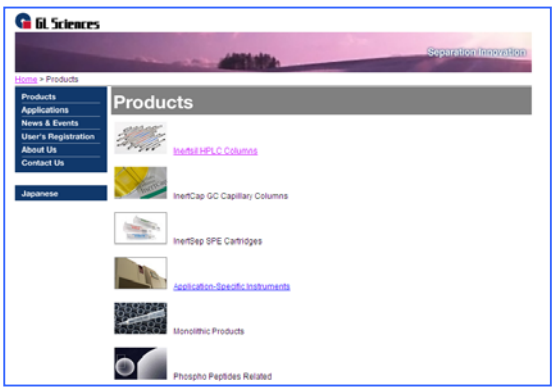

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