

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 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 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.

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

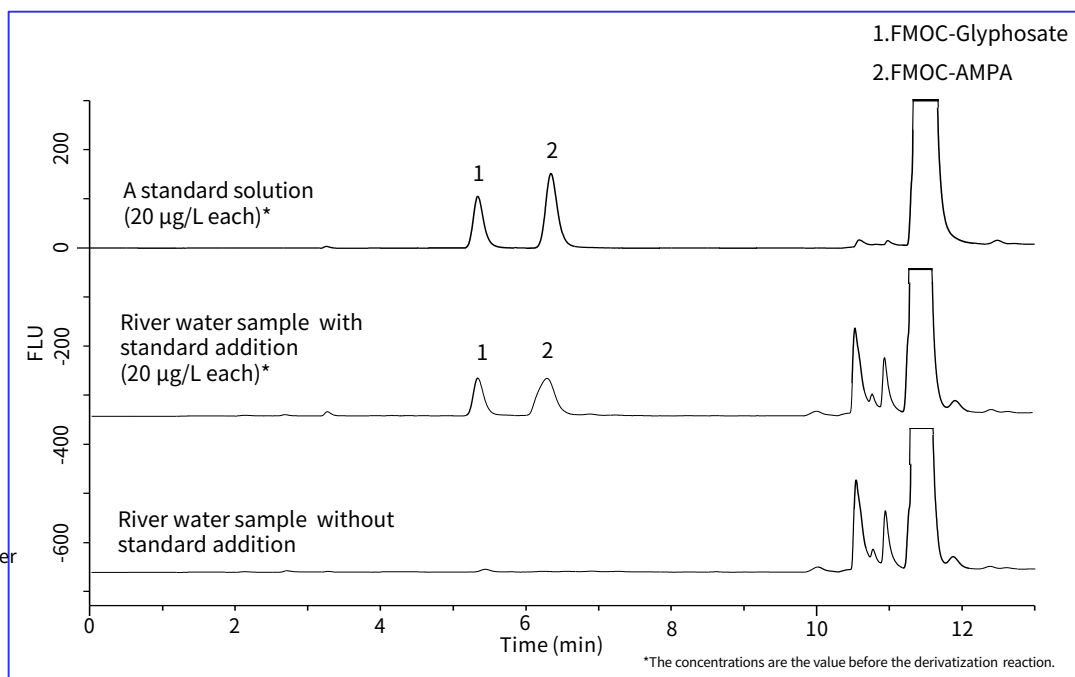
- Borate buffer 0.5 mL
- FMOC solution 2.6 mL
- Shaking for 5 min
- Wait for 30 min

Liquid-liquid extraction

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

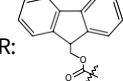
Inject the aqueous layer into the HPLC system

HPLC-FL



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 /
70 / 30 (8.5 min Hold),
70 / 30 (3.0 min Hold), (v/v)

Flow rate : 0.7 mL/min

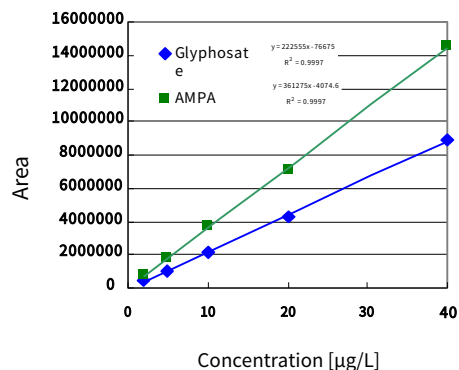
Col. Temp. : 40 °C

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

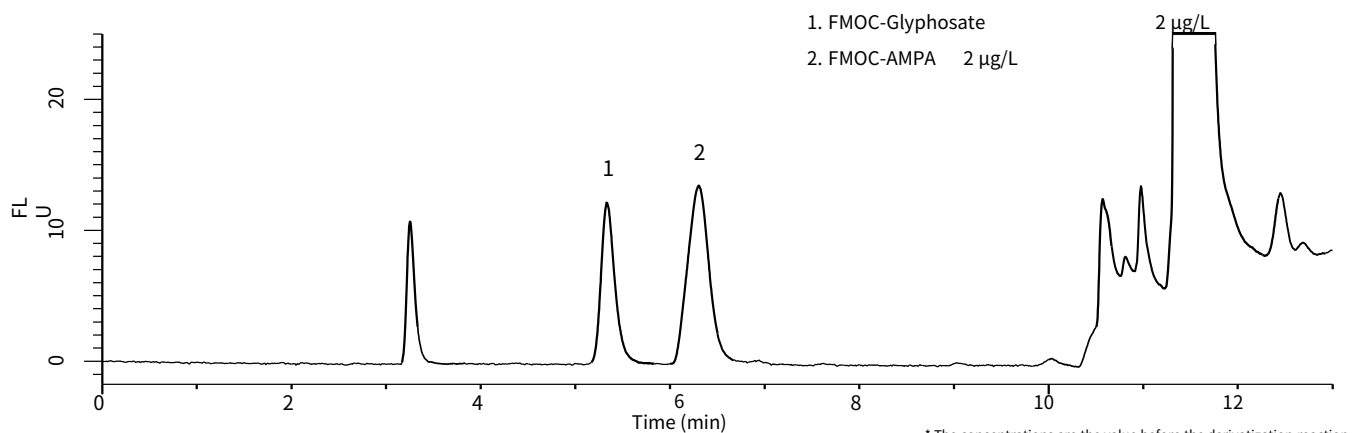


The calibration curves *

* 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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GL Sciences Inc. Japan

22-1 Nishishinjuku 6-chome
Shinjuku-ku, Tokyo
163-1130, Japan

Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@glsc.co.jp
Web: www.glsciences.com

GL Sciences Inc. USA

4733 Torrance Blvd. Suite 255
Torrance, CA 90503
USA

Phone: +1-310-265-4424
Fax: +1-310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

GL Sciences B.V.

Dillenburgstraat 7C
5652AM, Eindhoven
The Netherlands

Phone: +31-40-254-9531
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (Shanghai) Limited

Tower B, Room 2003
Far East International Plaza
No.317 Xianxia Road, Changning District
Shanghai, China 200051

Phone: +86-21-62782272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn



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