

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Determination of Pesticides
with Post-Column Derivatization

Pesticide residues contained in foods and pesticides in environmental water and drink water have become a grave social problem and there has arisen an active movement for controlling such contaminants. For the analyses of such pesticides, the GCMS, GC, and HPLC are often applied.

When analyzing pesticides by the HPLC, since generally detection is often carried out by the absorption detector, depending on samples, it may

occur that quantitation of the target components is hindered by peaks of foreign substances. As increase in sensitivity and selectivity can be expected by using a fluorescence detector, for the substances for which fluorescence detection can be applied by derivatization, the merit of that method is greater.

Introduced here are examples of analyses for several kinds of agricultural pesticides by a post-column derivatization-fluorescence detection method.

■ Determination of N-methylcarbamate Pesticides

N-methylcarbamate pesticides are characteristic of the structure as shown in Figure 1. These compounds, when heated in an alkaline solution, undergo hydrolysis, generating methylamine (reaction ①). Methylamine, which is a primary amine, can be derivatized into a fluorescent substance by using o-phthalaldehyde (OPA) reagent (reaction ②).

Figure 2 shows the flow diagram of a system designed for the analysis for N-methylcarbamate pesticides by making use of such a reaction. After separation of compounds by reversed phase chromatography, the above-mentioned two reactions are let to occur in turn so that fluorescence detection may be possible.

Figure 3 shows a chromatogram of a mixture solution of typical three compounds, and shown in Figure 4 is the result of chromatography of 1g of Japanese tea leaves spiked with 10 μg of Ethiofencarb and was extracted with acetonitrile. The chromatogram of Figure 4, which was obtained by sensitivity 8 times as high as that of Figure 3, allows determination of Ethiofencarb with almost no interference in spite of special pretreatment like cleanup.

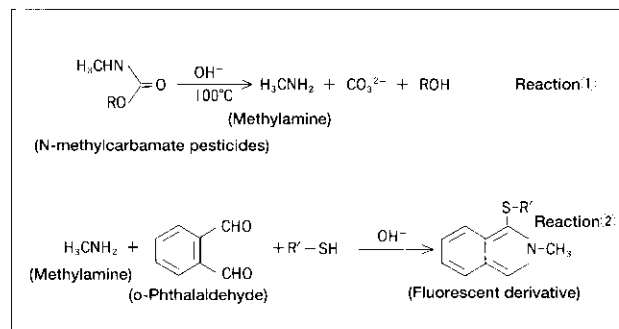


Fig.1 Derivatization Reaction Sequence for N-Methylcarbamate Pesticides

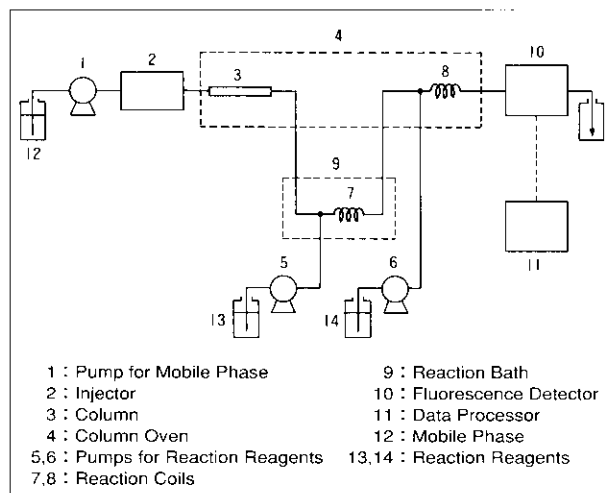


Fig.2 Flow Diagram

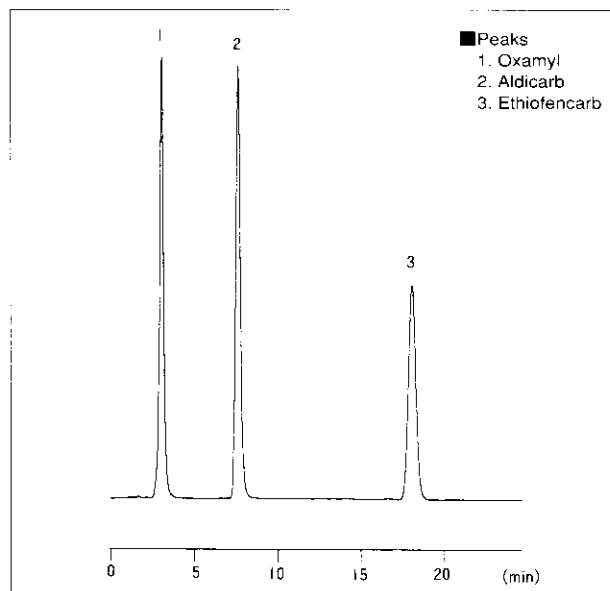


Fig.3 Chromatogram of Standard Mixture

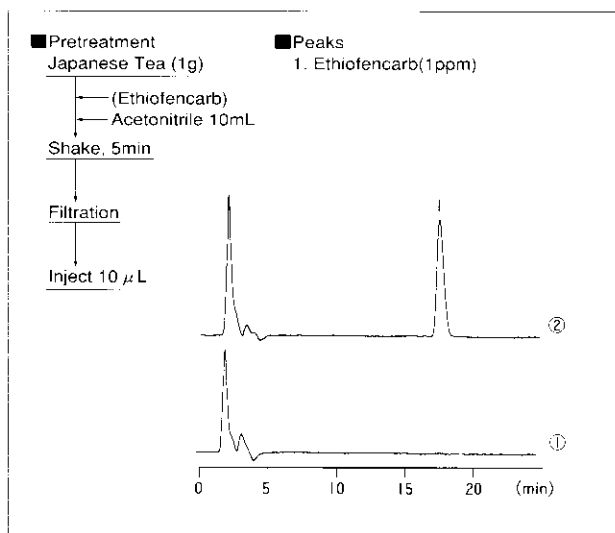


Fig.4 Chromatogram of Japanese Tea
 ① Unspiked
 ② Spiked with 10 µg of Ethiofencarb

Determination of Glyphosate [N-(Phosphonomethyl) glycine]

Glyphosate, which is a water-soluble pesticide, reacts with hypochlorite under ambient temperature and generates glycine. Then, when mixed with an OPA reagent, it becomes detectable by a fluorescence detection method (Figure 5). Here, Glyphosate was separated by the anion exchange chromatography and was derivatized, and detected by fluorescence detection. The construction of the system was the same as that for Figure 2, but is different in the composition of reaction reagent 1 and the temperature used.

Figure 6 shows a chromatogram of a standard mixture. Here, Glufosinate and Glyphosate were simultaneously analyzed; in this analysis, with respect to Glufosinate, which is a primary amine, only combination with OPA which corresponds to reaction ② of Figure 5 occurred.

Table 2 Analytical Conditions for Glyphosate

Colum	: Shim-pack IC-A1 (4.6mm I.D. × 100mm L.)
Mobile Phase	: 3mM (Sodium) Phosphate Buffer (pH 6.9)
Flow Rate	: 1.0mL/min
Temperature	: 45°C
Reagent 1	: OCl ⁻ Reagent ¹⁾
Flow Rate	: 0.3mL/min
Temperature	: 45°C
Reagent 2	: OPA Reagent ²⁾
Flow Rate	: 0.3mL/min
Temperature	: 45°C
Detector	: RF-10A (Ex=345nm, Em=450nm)

* 1 OCl⁻ Reagent... A/B=500/0.2

- A : 0.05M (Sodium) Borate Buffer (pH 11.6)
- B : Sodium Hypochlorite Solution
(Available Chlorine : ca. 5%)

* 2 OPA Reagent... Same as in Table 1

Table 1 Analytical Conditions for N-Methylcarbamate Pesticides

Colum	: STR ODS-II (4.6mm I.D. - 150mm L.)
Mobile Phase	: Acetonitrile/Water=3/7
Flow Rate	: 1.0mL/min
Temperature	: 45°C
Reagent 1	: 0.05M NaOH
Flow Rate	: 0.3mL/min
Temperature	: 100°C
Reagent 2	: OPA Reagent ¹⁾
Flow Rate	: 0.3mL/min
Temperature	: 45°C
Detector	: RF-10A (Ex=345nm, Em=450nm)

* 1 OPA Reagent... A/B/C=500/7/1

- A : 0.05M (Sodium) Borate Buffer (pH 9.2)
- B : 400mg O-phthalaldehyde in 7mL Ethanol
- C : 2-mercaptoethanol

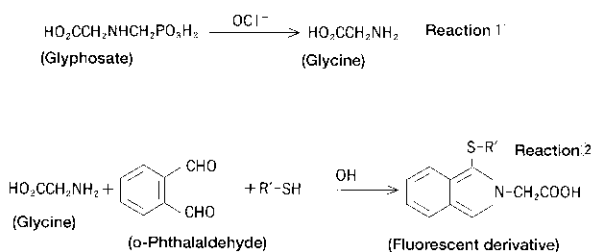


Fig.5 Derivatization Reaction for Glyphosate

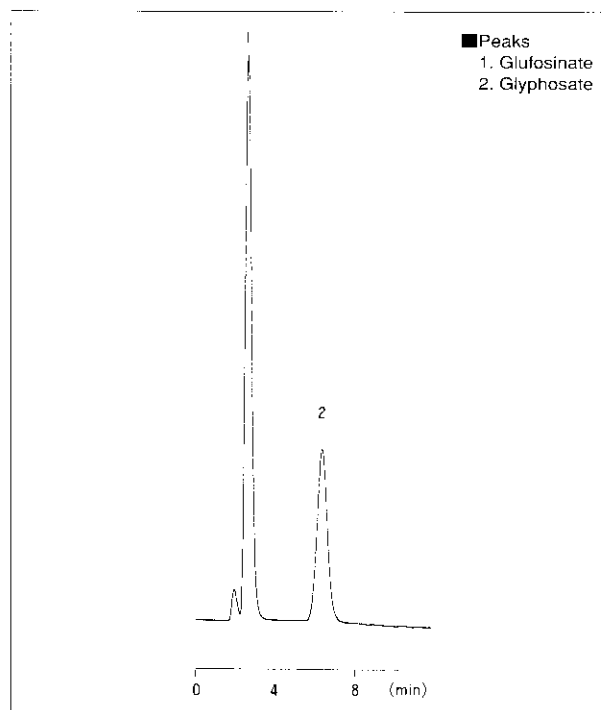


Fig.6 Chromatogram of Standard Mixture

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