

Analysis of Pesticides Residues by HPLC

The standards for pesticide residues contained in foods, specified in the Japanese Food Sanitation Law, have been revised twelve times since their establishment in October 1992. As of April 2003, residue standards are set for 229 pesticides.

These pesticides are analyzed using HPLC, GC, GCMS and LCMS, and analysis methods are specified

for each pesticide.

Application News L267 introduced an example of analyzing N-methyl carbamate pesticides using the post-column fluorescence derivatization method. This Application News introduces examples of analyzing several standard pesticides prescribed to be analyzed by HPLC.

■ Analysis of Fenpyroximate

The fenpyroximate content is expressed as the sum of forms E and Z. Table 1 shows the analytical conditions, and Fig. 1 shows the chromatogram for a standard fenpyroximate sample.

Table 1 Analytical Conditions

Column	: Shim-pack VP-ODS (250mmL, ×4.6mmI.D.)
Mobile Phase	: Water/Acetonitrile=1/4(v/v)
Flow Rate	: 1.0mL/min
Temperature	: 40°C
Detection	: SPD-10AV _{VP} at 254nm

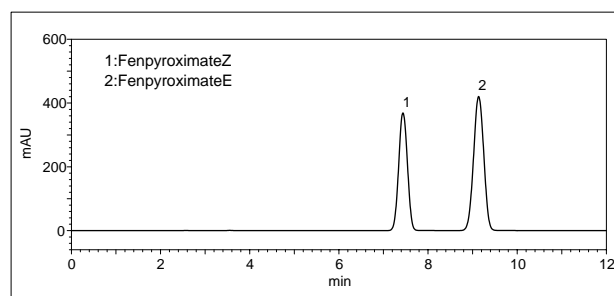


Fig.1 Chromatogram of Fenpyroximate (44µg/mL E-form, 36µg/mL Z-form, 50µL injected)

■ Analysis of Cyromazine

Cyromazine has high polarity and is not sufficiently retained by an ODS column. Therefore it is analyzed using an aminopropyl column. Table 2 shows the analytical conditions and Fig. 2 shows the chromatogram for a standard cyromazine sample.

Table 2 Analytical Conditions

Column	: Shodex Asahipak NH2P-50 4E (250mmL, ×4.6mmI.D.)
Mobile Phase	: Water/Acetonitrile=7/93(v/v)
Flow Rate	: 0.8mL/min
Temperature	: 40°C
Detection	: SPD-10AV _{VP} at 215nm

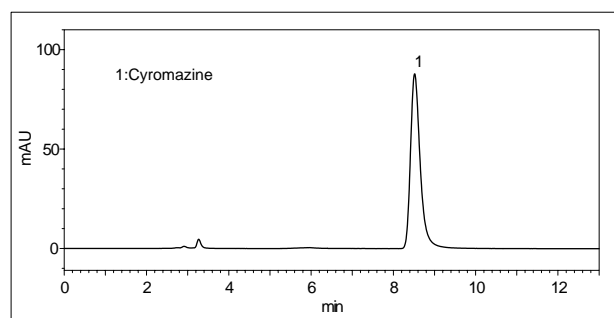


Fig.2 Chromatogram of Cyromazine (2µg/mL, 50µL injected)

■ Analysis of Nitenpyram

To determine the content of nitenpyram, nitenpyram is analyzed by HPLC, and its metabolite CPF is analyzed by GC. The official analysis method prescribes the use of acetone as the sample solvent. However, this may cause peak distortion. To avoid this problem, this example used the mobile phase as the sample solvent. Table 3 shows the analytical conditions and Fig. 3 shows the chromatogram for a standard nitenpyram sample.

Table 3 Analytical Conditions

Column	: Shim-pack VP-ODS (150mmL.×4.6mmI.D.)
Mobile Phase	: 50mM KH ₂ PO ₄ /Methanol=17/3(v/v)
Flow Rate	: 0.8mL/min
Temperature	: 40°C
Detection	: SPD-10AVVP at 270nm

■ Analysis of Chlorfluazuron

Chlorfluazuron and other six pesticides can be analyzed simultaneously. Table 4 shows the analytical conditions and Fig. 4 shows the chromatogram for the standard samples.

Table 4 Analytical Conditions

Column	: Shim-pack VP-ODS (250mmL.×4.6mmI.D.)
Mobile Phase	: Water/Acetonitrile=3/7(v/v)
Flow Rate	: 0.8mL/min
Temperature	: 40°C
Detection	: SPD-10AVVP at 250nm

■ Analysis of Amitrole

Amitrole can be analyzed by pre-column derivatization and fluorescence detection, after the pretreatment of amitrole-fluorescamine reaction. Fig. 5 shows the pre-treatment procedures, Table 5 the analytical conditions, and Fig. 6 the chromatogram for a standard amitrole sample.

Table 5 Analytical Conditions

Column	: Shim-pack VP-ODS (150mmL.×4.6mmI.D.)
Mobile Phase	: Phosphate (Na) buffer*/Acetonitrile=7/3(v/v)
Flow Rate	: 0.6mL/min
Temperature	: 40°C
Detection	: RF-10AXL Ex. at 380nm, Em at 484nm

*Solution Adjusted to pH=3 by adding 10% Phosphoric Acid to 50mmol/L NaH₂PO₄

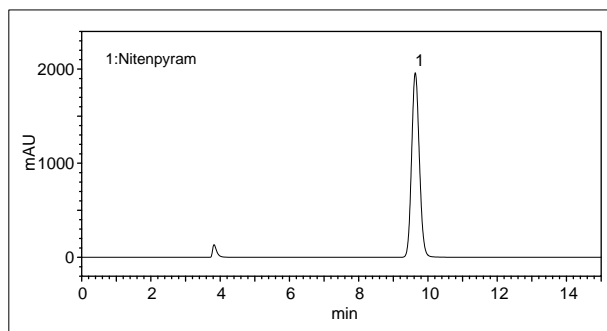


Fig.3 Chromatogram of Nitenpyram (220µg/mL, 50µL injected)

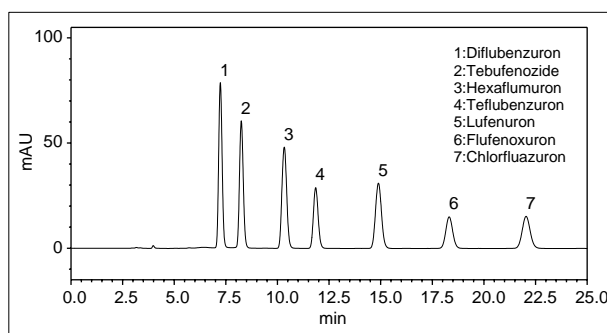


Fig.4 Chromatogram of 7 Pesticides (11µg/mL Diflubenzuron, 15µg/mL Tebufenozide, 12µg/mL Hexaflumuron, 7.1µg/mL Teflubenzuron, 11µg/mL Lufenuron, 6.0µg/mL Flufenoxuron, 5.7µg/mL Chlorfluazuron, 20µL injected)

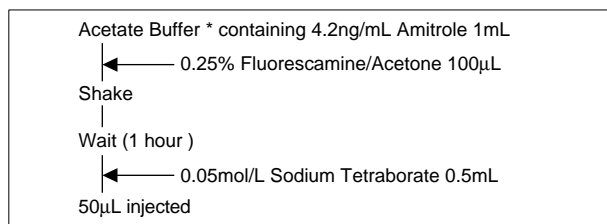


Fig.5 Derivatization Procedure

*50mmol/L Acetic Acid 800mL + 50mmol/L Sodium Acetate 200mL

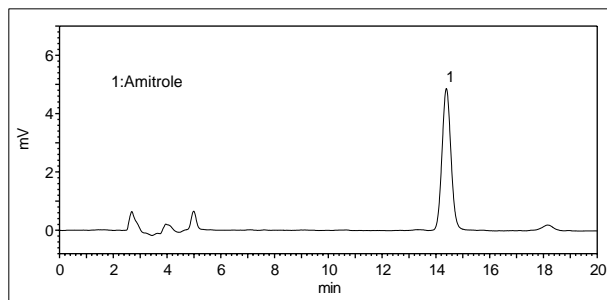


Fig.6 Chromatogram of Amitrole (4.2ng/mL, 50µL injected)