

Application News

No.L497

Supercritical Fluid Extraction / Chromatography

Using the Nexera UC Online SFE-SFC-MS System to Analyze Residual Pesticides in Agricultural Products

The Nexera UC online SFE-SFC-MS system combines supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) in one online system, so that the entire process from extraction of target components to acquisition of data can be performed completely automatically. Furthermore, the system can add polar organic solvents (modifiers) to the supercritical carbon dioxide fluid during SFE and SFC, so that the system can be used to extract and analyze components with a wide range of polarities.

Meanwhile, ever since the positive list system was enacted in 2006 in Japan for residual pesticides in foods, which applies to more than 800 types of pesticides, there has been increasing demand for a system able to simultaneously analyze multiple pesticides with a wide range of properties, including pretreating samples.

This article describes an example of using the Nexera UC online SFE-SFC-MS system to analyze residual pesticides in agricultural products.

Online SFE-SFC-MS System

The operating principle of the Nexera UC online SFE-SFC-MS system is shown in Fig. 1. The extraction vessel filled with the sample is placed in the SFE unit and heated to an internal temperature of 40 °C (Fig. 1A). Then supercritical carbon dioxide fluid is pumped into the extraction vessel. After filling the vessel, the flow is stopped to allow static extraction of target components (Fig. 1B). After static extraction, the fluid is pumped through the extraction vessel for dynamic extraction (Fig. 1C). During dynamic extraction, extracted substances flow from the extraction vessel and into the analytical column. However, due to the high level of contaminant components in agricultural products, passing all the extract substances through the analytical column or mass spectrometer could damage the column or contaminate the mass spectrometer. Therefore, the Nexera UC online SFE-SFC-MS system splits the flow to send only a portion of the substances extracted from dynamic extraction through the analytical column. After dynamic extraction, fluid is only sent through the analytical flow line, where the analytical column is used for gradient separation and the mass spectrometer for detecting the target components (Fig. 1D).

Sample Preparation

The QuEChERS is a well-known method that prioritizes simplicity and speed and is commonly used to pretreat agricultural products for residual pesticide analysis. However, the method involves many steps, such as adding reagents, solvent extraction, purification by dispersive solid phase extraction, and centrifugal separation. In contrast, the online SFE-SFC-MS system requires only mixing 1 g of agricultural product crushed with 1 g of a dehydrating agent* and placing the mixture in the extraction vessel, as shown in Fig. 2. Consequently, the system improves analytical productivity, reduces the environmental impact, and also avoids human errors involved in the pretreatment steps. Using a dedicated rack changer, the system can continuously extract and analyze up to 48 samples at a time.

* "Miyazaki Hydro-Protect" Patent No. 3645552

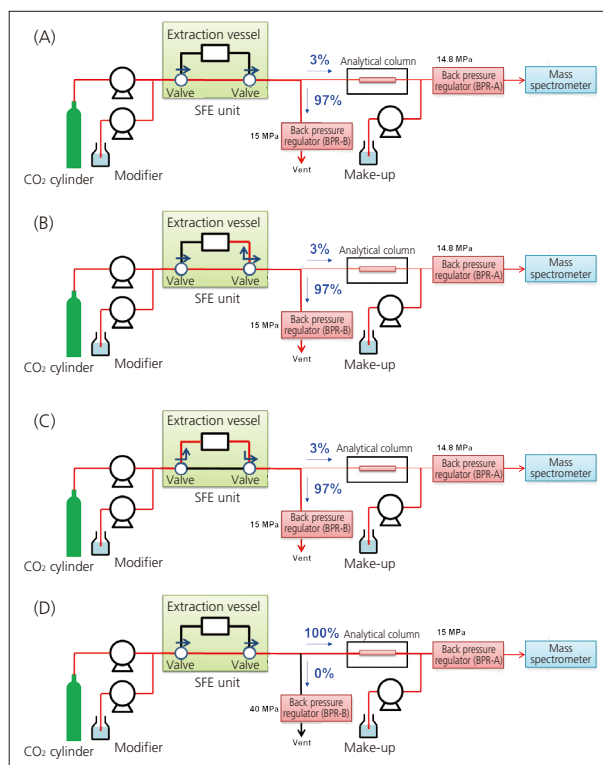


Fig. 1 Analysis Flow by Online SFE-SFC-MS

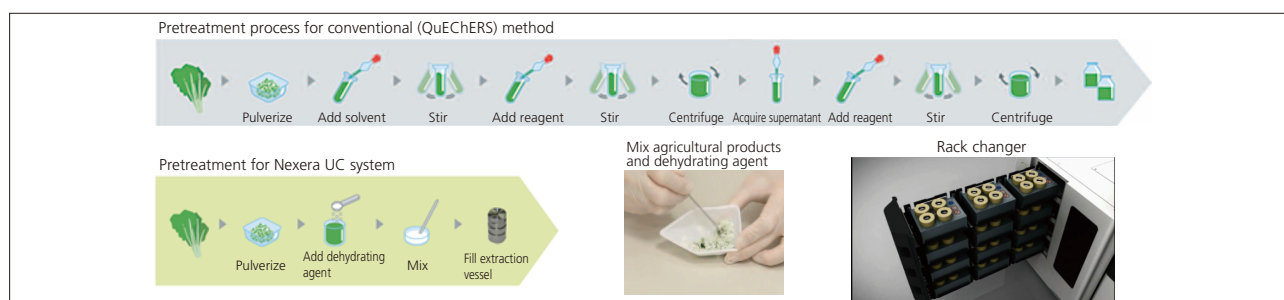


Fig. 2 Sample Preparation

Table 1 Analytical Conditions

[SFE]	[SFC]
Solvent : A) Super critical fluid of CO ₂ B) 0.1 % Ammonium formate in methanol	Column : Shim-pack UC-RP (250 mm L. × 4.6 mm I.D., 5 μm)
Flowrate : 5 mL/min	Mobile Phase : A) Super critical fluid of CO ₂ B) 0.1 % Ammonium formate in methanol
Extraction : 0-3 min. Static mode (B.Conc. 5 %) 3-6 min. Dynamic mode (B.Conc. 5 %)	Time Program : B.Conc. 0 % (0 min.) → 10 % (11 min.) → 30 % (14 min.) → 40 % (14.01-17 min.)
Extraction Vessel Temp. : 40 °C	Flowrate : 3 mL/min
BPR Pressure : A) 14.8 MPa, B) 15 MPa (split rate: 3 %)	Make-up : 0.1 % Ammonium formate in methanol (0.1 mL/min.)
Make-up : 0.1 % Ammonium formate in methanol (0.4 mL/min.)	Column Temp. : 40 °C
	BPR Pressure : A) 15 MPa, B) 40 MPa
	Detector : LCMS-8050 MRM mode

■ Analysis of Standard Mixture of Pesticides

The standard mixture sample of 510 pesticide components were mixed with a dehydrating agent and analyzed using the analytical conditions indicated in Table 1. Fig. 3 shows the results. Using the system, we were able to accomplish the entire process, from extraction to data acquisition, in about 45 minutes per analysis. For 327 components, we obtained good repeatability for the concentration range from 1 to 100 ng/g (less than 30 %RSD for relative standard deviation for peak area at respective concentrations) and good linearity (contribution ratio of at least R² = 0.99). Table 2 also shows how pesticides with a wide range of polarities were analyzed with good repeatability and linearity.

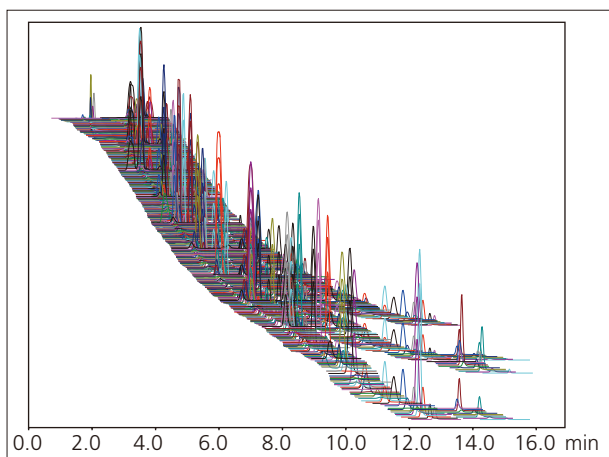


Fig. 3 Mass Chromatogram of Standard Pesticide Mixture Solution

Table 2 Repeatability and Linearity for Representative Pesticides

Compounds	LogPow	Repeatability (%RSD, n=5)	Range (ng/g)	R ²
Ethofenprox	6.9	6.1	1-100	0.9991
Hexaflumuron	5.68	6.8	1-100	0.9992
Benzofenap	4.69	1.4	2-200	0.9990
Mepronil	3.66	4.6	1-100	0.9993
Prometryn	3.34	2.7	1-100	0.9994
Fenamidone	2.8	3.0	2-200	0.9991
Ethylchlozate	2.5	3.0	1-100	0.9996
Imazosulfuron	1.6	6.2	1-100	0.9998
Bensulfuron methyl	0.79	8.1	1-100	0.9996
Primisulfuron methyl	0.2	5.5	1-100	0.9994
Halosulfuron methyl	-0.02	5.5	1-100	0.9996
Azimsulfuron	-1.4	4.2	1-100	0.9998

<Acknowledgments>

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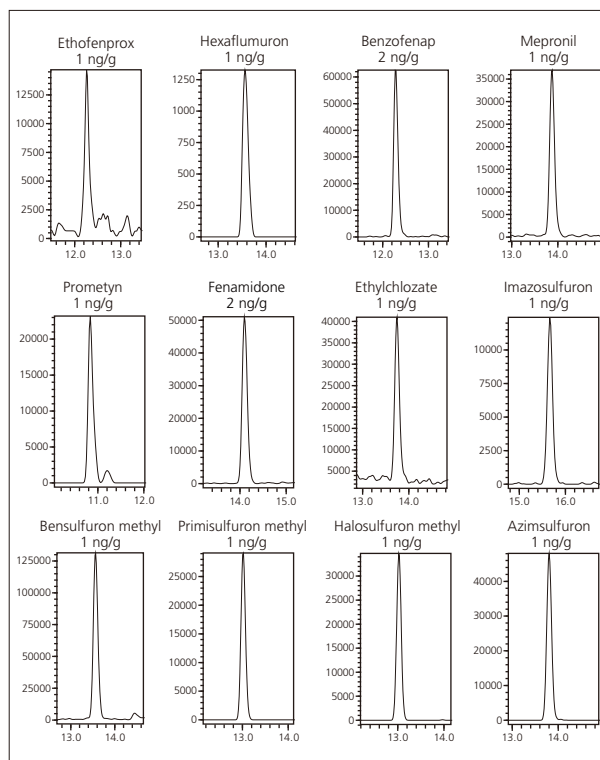


Fig. 4 MRM Chromatograms of Representative Pesticides

■ Analysis of a Tomato

Analysis of 10 ng/g of 510 pesticide components added to a tomato resulted in good repeatability (less than 20 %RSD for the relative standard deviation of the peak area) and a good recovery rate (70 to 120 %) for 248 components. Plots of LogPow and recovery rate results are shown in Fig. 5. It shows that pesticides with a wide range of polarities were analyzed with good recovery.

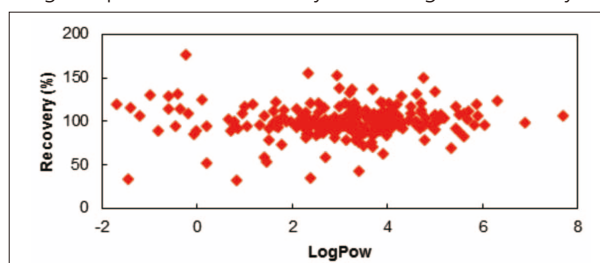


Fig. 5 LogPow vs. Recovery Rate for Tomato Analysis