

# Analysis of Residual Pesticides in Agricultural Products by using on-line SFE-SFC-MS and off-line SFE/GC-MS

Takanari Hattori<sup>1</sup>, Takato Uchikata<sup>1</sup>, Hidetoshi Terada<sup>1</sup>, Chigusa Ichikawa<sup>1</sup>, Yasuhiro Funada<sup>1</sup>, Yayoi Ichiki<sup>2</sup>, Miho Sakai<sup>3</sup>, Takashi Ando<sup>3</sup>, Yoshihiro Izumi<sup>4, 5</sup>, Eiichiro Fukusaki<sup>5</sup>, Takeshi Bamba<sup>4, 5</sup>

1 Shimadzu Corporation, Kyoto, Japan. 2 Miyazaki Enterprise Promotion Organization, Miyazaki, Japan. 3 Miyazaki Agricultural Research Institute, Miyazaki, Japan. 4 Kushu University, Fukuoka, Japan. 5 Osaka University, Suita, Japan.

## 1. Introduction

Analysis of pesticides is crucial to assess the safety of agricultural products. Extraction of pesticides in agricultural products is commonly performed by QuEChERS method. However, it is time-consuming, and a large amount of organic solvent is consumed. Off-line supercritical fluid extraction (SFE) is one of the traditional techniques for sample preparation of residual pesticides especially for hydrophobic ones. However, hydrophilic pesticides can be extracted from sample as well as hydrophobic ones by adding a modifier in the SFE process.

Supercritical fluid chromatography (SFC) is one of the separation techniques and it can separate numerous pesticides that have widely ranged different polarities simultaneously.

We have established a high throughput analytical system for residual pesticides in agricultural products consisting of on-line SFE-SFC-MS and off-line SFE/GC-MS. The former affords fully automated extraction and screening. The latter affords accurate determination.

## 2. Materials and Methods

### 2-1. Sample Preparation

Sample-preparation procedures of agricultural products for on-line SFE-SFC-MS and off-line SFE/GC-MS analysis were quite simple. Figure 1 shows the procedures. 1 g of homogenized sample and 1 g of absorbent were mixed then the mixture was enclosed into an extraction vessel.



Figure 1 Sample-preparation procedures

### 2-2. On-line SFE-SFC-MS and off-line SFE/GC-MS

On-line SFE-SFC-MS analysis was carried out using Nexera UC system coupled with a LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Japan). SFE was performed both in static and dynamic extraction modes using carbon dioxide and methanol containing 0.1% of ammonium formate as a modifier. Separation was achieved using a C18 reversed phase column.

Off-line SFE was carried out using Nexera UC system (Shimadzu Corporation, Japan). SFE was performed both in static and dynamic extraction modes using carbon dioxide and methanol as a modifier. The extract was trapped using a C18 reversed phase column and eluted using mixture of acetone and hexane. The eluate was corrected using a fraction collector, added mixture of acetone and hexane to make 2 mL, and analyzed using GCMS-TQ8040 (Shimadzu Corporation, Japan).

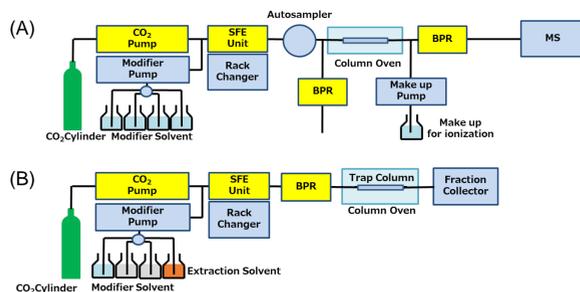


Figure 2 On-line SFE-SFC-MS system (A) and off-line SFE system (B)

Table 1 Analytical conditions for on-line SFE-SFC-MS

SFE conditions (Nexera UC system, Shimadzu)	
Solvent	: A CO <sub>2</sub> : B 0.1% w/v (16 mM) ammonium formate in methanol
Flow rate	: 5 mL / min.
Extraction	: 6 min (Static mode → Dynamic mode)
Extraction vessel temp.	: 40°C
BPR	: A 14.8 MPa, B 15 MPa (split rate: 3%)
Make-up	: 0.1% w/v (16 mM) ammonium formate in methanol (0.4 mL / min.)
SFC conditions (Nexera UC system, Shimadzu)	
Column	: Shim-pack UC-RP 5 μm (250 mm x 4.6 mm I.D.)
Mobile phase	: A CO <sub>2</sub> : B 0.1% w/v (16 mM) ammonium formate in methanol
Gradient program	: 0% B (0 min.) → 10% B (11 min.) → 30% B (14 min.) → 40% B (14.01-17 min.)
Flow rate	: 3 mL / min.
Make-up	: 0.1% w/v (16 mM) ammonium formate in methanol (0.1 mL / min.)
Column temp.	: 40°C
BPR	: A 15 MPa, B 40 MPa
Detector	: LCMS-8050 MRM mode

Table 2 Analytical conditions for off-line SFE/GC-MS

SFE conditions (Nexera UC system, Shimadzu)	
Solvent	: A CO <sub>2</sub> : B Methanol
Flow rate	: 5 mL / min.
Extraction	: 8 min (Static mode → Dynamic mode)
Extraction vessel temp.	: 40°C
BPR	: 15 MPa
Trap column	: Shim-pack VP-ODS 5 μm (50 mm x 4.6 mm I.D.)
Eluent solvent	: Acetone / Hexane = 50 / 50 (2 mL / min, 2 min)
GC-MS conditions (GCMS-TQ8040, Shimadzu)	
Column	: Rxi®-5Sil MS 30 m x 0.25 mm I.D., df = 0.25 μm
Column temp.	: 50°C (1 min) → (25°C/min) → 125°C → (10°C/min) → 300°C (15 min)
Carrier gas	: He (Constant linear velocity mode)
Linear velocity	: 47.2 cm/sec
Injection mode	: Splitless (Sampling time 1.00 min)
High press inj.	: 250 kPa (1.5 min)
Injection volume	: 1 μL
Interface temp.	: 250°C
Ion source temp.	: 200°C
MS mode	: MRM mode
Loop time	: 0.3 sec

## 3. Result

### 3-1. Analysis of Pesticide Standards using on-line SFE-SFC-MS

In this study, we evaluated 510 of pesticides that were applied to the on-line SFE-SFC-MS analysis. As a result of analysis for absorbent that was spiked with standard pesticides, 327 pesticides were reliably analyzed (Peak area RSD <30%) and showed good linearity ( $r^2 > 0.99$ ) within a range of 1-100 ng/g.

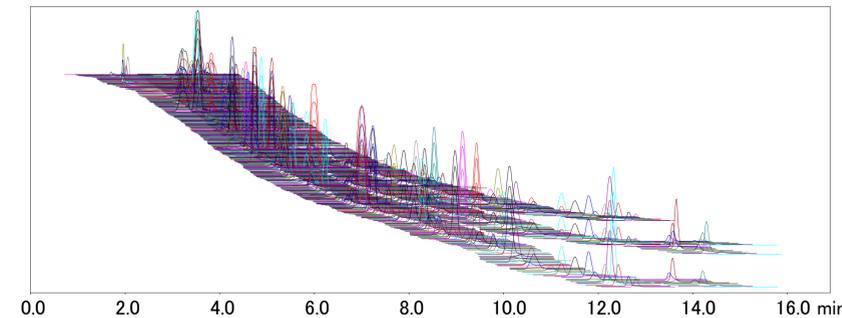


Figure 3 Simultaneous analysis of 510 pesticides

Table 3 Repeatability and linearity of typical 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
Prometryn	3.34	2.7	1-100	0.9994
Ethylchlorzate	2.5	3.0	1-100	0.9996
Imazosulfuron	1.6	6.2	1-100	0.9998
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

### 3-2. Analysis of Pesticides in Agricultural Products using on-line SFE-SFC-MS

As a result of analyses for tomato that were spiked with 10 ng/g of standard pesticides, 248 pesticides were reliably analyzed (Peak area RSD <20%) and were recovered properly within a range of 70-120%.

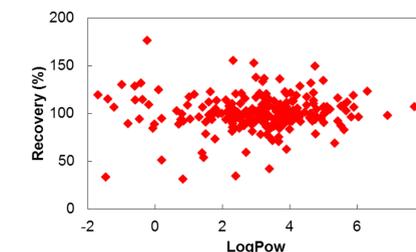


Figure 4 LogPow - recovery for tomato analysis

Table 4 Repeatability and recovery for representative pesticides

compounds	repeatability (%RSD, n=6)	recovery(%)
Dimethoate	5.1	89
EPTC	7.8	100
Folpet	9.7	96
Mepanipyrim	6.6	102
Myclobutanil	10.0	102

### 3-3. Analysis of Pesticides in Agricultural Products using off-line SFE/GC-MS

354 pesticides for GC/MS were analyzed using off-line SFE/GC-MS system. Brown rice that was spiked with 100 ng/g of standard pesticides was used as a test sample. Figure 5 shows the MRM chromatogram of eluate of brown rice obtained using GC-MS. As a result, 301 pesticides were reliably analyzed (Peak area RSD <10%) and were recovered properly within a range of 70-120%.

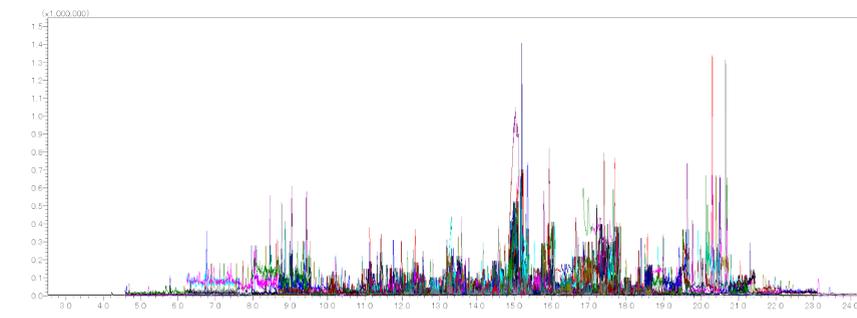


Figure 5 MRM chromatogram of eluate of brown rice

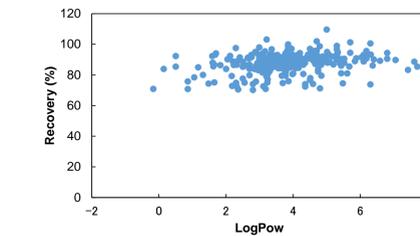


Figure 6 LogPow - recovery for brown rice analysis

Table 5 Repeatability and recovery for representative pesticides

compounds	repeatability (%RSD, n=6)	recovery(%)
Cyhalofop-butyl	4.2	93
Etofenprox	3.8	90
Iprodione	2.5	93
Malathion	3.2	93
Piperonyl butoxide	3.8	89

## 4. Conclusions

- The sample preparation for analysis of residual pesticides in agricultural products by using on-line SFE-SFC-MS and off-line SFE/GC-MS was simple and did not require complex pretreatments.
- On-line SFE-SFC-MS was full-automatically able to extract and analyze numerous pesticides that have widely ranged different polarities.
- Off-line SFE/GC-MS was accurately able to determine numerous pesticides for GC/MS.

## Acknowledgements

This work was partially supported by the Development of Systems and Technology for Advanced Measurement and Analysis Project by the Japan Science and Technology Agency.