SHIMADZU
Excellence in Science

Application News

No. C162

Liquid Chromatograph Mass Spectrometry

Quantitative Analysis of Highly Polar Pesticides in Food Using SFC/MS

Since achieving sufficient retention and favorable separation in normal batch analysis of highly polar pesticides has proved difficult due to their chemical characteristics, a number of individual analysis methods are employed for LC/MS/MS analysis. To rectify this situation, EURL-SRM (Stuttgart, Germany), an EU Reference Laboratories member in charge of individual analysis method development, is developing a batch analysis method called "QuPPe (Quick Polar Pesticides)" for highly polar pesticides that are difficult to analyze using pretreatment with the QuEChERS method as well as normal batch analysis methods. This method proposes multiple methods to suit each sample and target chemical compound (M. Anastassiades et al; QuPPe of EURL-SRM (Version 9.1; 2016)).

Until now, analysis of highly polar pesticides using LC/MS/MS has used a variety of separation methods including HILIC mode, mixed mode, normal phase, and reversed phase. However, all of these methods have restrictions on the chemical compounds that can be analyzed together and this remains a problem. On the contrary, supercritical fluid chromatography (SFC) has the advantage of being able to separate a wide array of chemical compounds at once due to the characteristics of the mobile phase that is used. In addition, since the separation behavior with SFC differs from that with LC even when using a column of the same separation mode, SFC may be effective for the analyses of chemical compounds for which retention and separation are difficult in LC. This article introduces an example of batch analysis of highly polar pesticides using SFC.

Y.Fujito, D. Baker, A. Barnes, C. Titman, J. Horner, N. Loftus

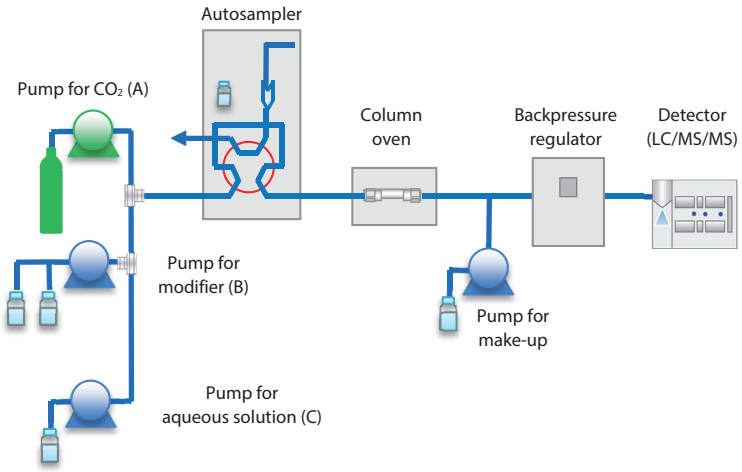


Fig. 1 SFC/MS System Configuration Diagram

In this experiment, an examination of adding a small amount of water to a modifier was performed for the purpose of eluting and separating highly polar pesticides.
In order to simplify this examination, a low-pressure gradient pump (LPGE) was used as pump B and the modifier was automatically prepared by mobile phase blending.

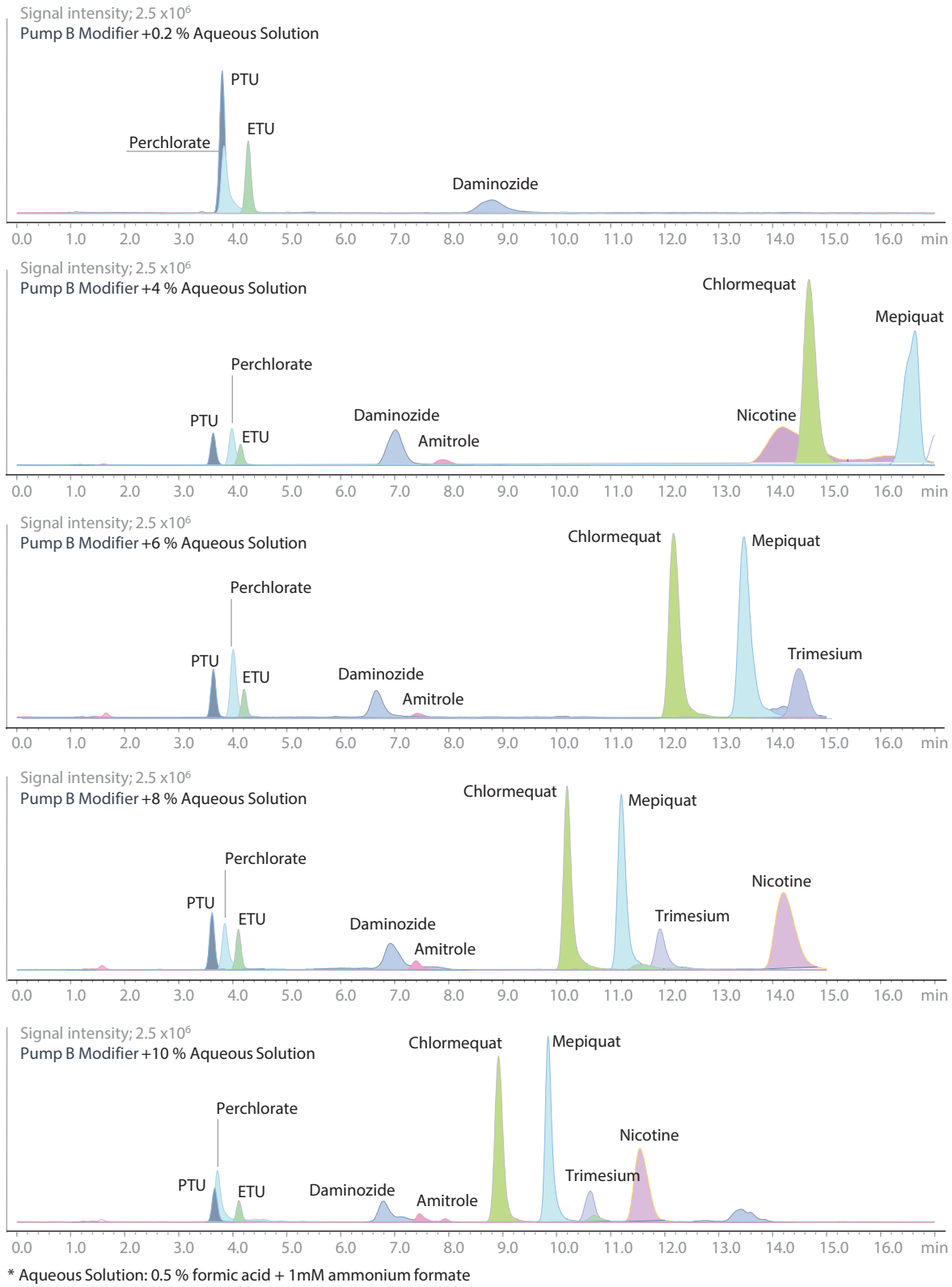
Table 1 SFC/MS Analysis Conditions

Supercritical fluid chromatography		Mass spectrometry	
SFC	Nexera UC system	**LC-MS/MS**	LCMS-8060
Analytical column	Restek Ultra Silica (150 × 2.1 mm 3 µm)	**Ionisation mode**	Heated ESI
Column temperature	50 °C	**Scan speed**	15,000 u/sec
Flow rate	0.8 mL/min (0.6 mL/min 13-22 min)	**MRM Dwell time**	3 msec
Pump A	CO_2	**Pause time**	1 msec
Pump B (modifier solvent)	Acetonitrile + 0.5 % formic acid + 10 mM ammonium formate	**Interface temp.**	300 °C
Pump C (modifier solvent)	Water + 0.5 % formic acid + 10 mM ammonium formate	**Heating block**	350 °C
Pump D (make up solvent)	Methanol	**Desolvation line**	250 °C
Makeup solvent flow rate	0.2 mL/min		

Examination of SFC Separation Conditions

Normally, SFC performs gradient separation using supercritical carbon dioxide and an organic solvent (such as methanol and acetonitrile), which is referred to as a modifier. However, some highly polar chemical compounds exhibit strong retention in columns resulting in cases where separation and elution is insufficient even with 100 % organic solvent. In this experiment, since a number of highly polar pesticides could not be eluted with 100 % organic solvent, separation was examined by adding a small amount of water to the modifier.

Supercritical carbon dioxide has low polarity and low miscibility with water. This means that only a limited amount of water can be added to the modifier (normally about 0.1 to 10 %). We therefore examined separation behavior by adding water by the amount equivalent to 0.2, 4, 6, 8, and 10 % to the modifier. Through examination based on the peak profiles and separation patterns of the eluted components, we adopted a water content of 6 %. However, there were chemical compounds that could not be eluted even with this condition.



* Aqueous Solution: 0.5 % formic acid + 1mM ammonium formate

Fig. 2 Effect of Water on Separation Behavior of Highly Polar Pesticides in SFC/MS

Optimization of SFC Separation Conditions

When we examined addition of water to the modifier, we were able to confirm elution of most chemical compounds with the 6 % aqueous solution. However, nicotine and kasugamycine, which both exhibit strong retention, could not be eluted. Any further addition of aqueous solution in the presence of carbon dioxide adversely affects gradient accuracy and may impair the stability of the analysis method. For this reason, aqueous solution was added using a separate pump (pump C) after the modifier reached 100 % (Fig. 4).

This allowed elution of the remaining highly polar pesticides and enabled batch separation of the highly polar pesticides from logP-3.47 to 1.96.

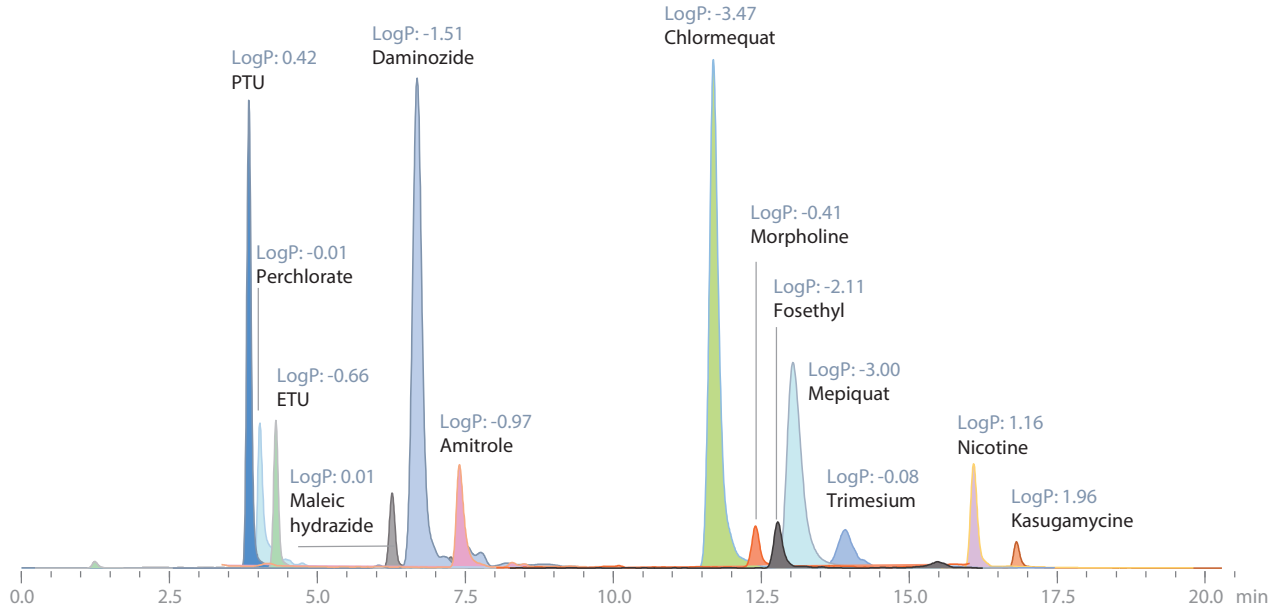
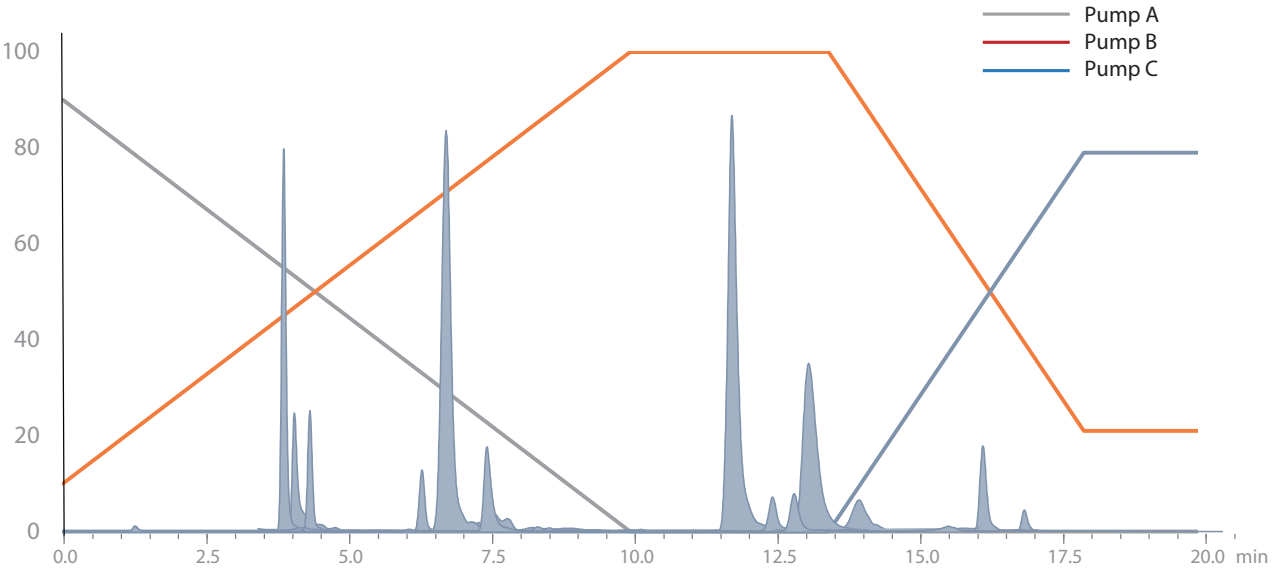


Fig. 3 MRM Chromatogram of Highly Polar Pesticides Using SFC-MS
(Addition of 200 ppb Pesticide Standard Solution into Flaxseed Extract Using QuPPe)



The initial SFC/MS conditions;
Pump A 90 % : Carbon Dioxide
Pump B 10 % : 6 % Water in Acetonitrile containing 0.5 % formic acid and 10 mM ammonium formate
Pump C 0 % : Aqueous solution containing 0.5 % formic acid + 10 mM ammonium formate

Fig. 4 Ternary Gradient Program

Sample Preparation and Analysis

Flaxseed and lemon were used as food samples and extraction was performed using a method compliant with QuPPe. (The extracts were provided by Concept Life Sciences, a contract analytical laboratory located in the U.K.) Standard solution of highly polar pesticides was added to these matrix solutions, which were then directly injected into the SFC-MS/MS.

Quantitative Analysis of Highly Polar Pesticides

In order to verify the quantitative performance of the developed SFC/MS analysis method, matrix calibration curves were created using each food extract to which standard solution of the highly polar pesticides was added. The calibration curve range was 10 to 200 ppb and accuracy was verified using the internal standard method regarding components for which an internal standard substance labeled with a stable isotope was obtained.

The calibration curve created for each sample showed favorable linearity for all chemical compounds regardless of the sample matrix.

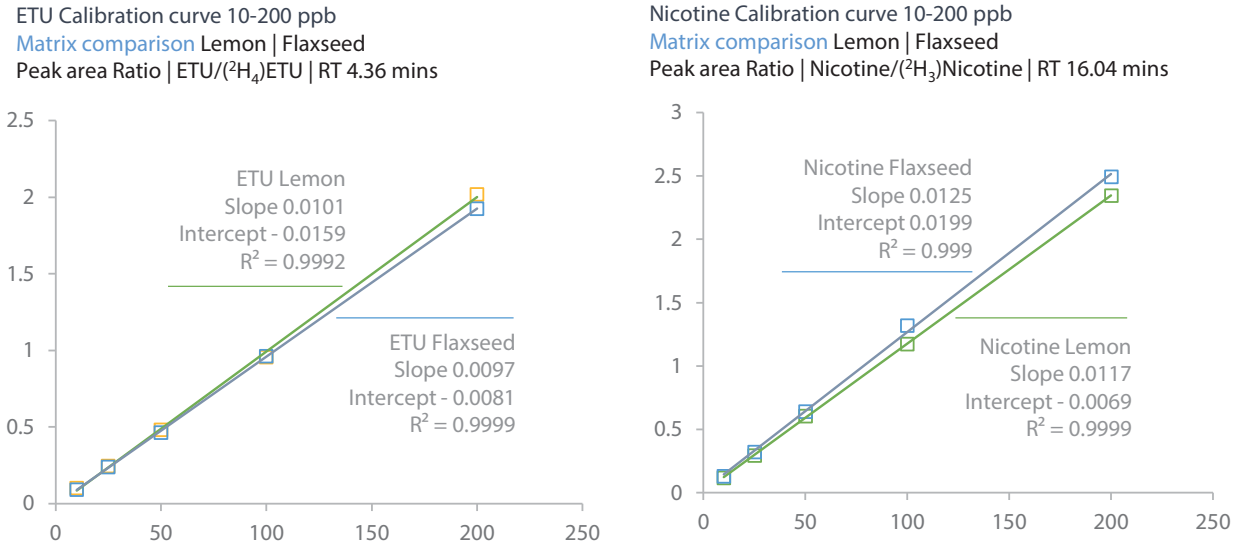


Fig. 5 Matrix Calibration Curves of Representative Highly Polar Pesticides
(ETU: fast eluting compound, Nicotine: slow eluting compound, Samples: lemon, flaxseed)

Table 2 Calibration Curve Linearity and Repeatability at 100 ppb of Eight Highly Polar Pesticide Components

Compound	RT (min)	Internal Standard	IS RT (min)	Quan MRM	%RSD 100ppb	R^2
Perchlorate	3.95	$^{18}O_4$ Perchlorate	3.91	99.00 > 82.90	4.98	0.968
ETU	4.36	$^{2}H_4$ ETU	4.26	103.10 > 44.05	4.84	0.999
Maleic hydrazide	6.28	$^{2}H_2$ Maleic hydrazide	6.28	113.00 > 67.10	6.81	0.997
Chlormequat	11.58	$^{2}H_4$ Chlormequat	11.54	121.90 > 58.10	1.75	1.000
Fosethyl	12.50	$^{2}H_{15}$ Fosethyl	12.50	109.00 > 80.95	6.78	0.999
Morpholine	12.19	$^{2}H_8$ Morpholine	12.23	87.90 > 70.05	10.74	0.996
Mepiquat	12.72	$^{2}H_3$ Mepiquat	12.69	114.30 > 98.10	7.66	0.998
Nicotine	16.06	$^{2}H_3$ Nicotine	16.03	163.00 > 130.00	2.31	0.999

First Edition: Nov. 2017

Shimadzu Corporation
www.shimadzu.com/an/