SHIMADZU
Excellence in Science

PO-CON1658E

Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

ASMS 2016 TP-233

Natsuyo Asano[1], David Baker[2], Yuki Uno[1],
Atsuhiko Toyama[1], Jun Watanabe[1]
[1] Shimadzu Corporation, Kyoto, Japan;
[2] Shimadzu Corporation, Manchester, United Kingdom

Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

Introduction

More than a thousand different types of pesticides are being used worldwide. In recent years, due to increased awareness of food safety, the amount of pesticide residues in food are controlled by local regulatory agencies that define the maximum residue levels (MRLs) and enforce compliance by regular inspection. LC-MS/MS is widely employed as an analytical method for pesticide residues in the food safety because it features excellent selectivity and sensitivity even in the matrix-matched samples. As for the separation with LC, ODS has long been the first choice column chemistry but biphenyl columns are emerging as an alternative. Here we performed simultaneous LC-MS/MS analysis of 167 pesticides using both ODS and biphenyl columns for comparison.

Methods

A mixture of 167 pesticides was diluted to working concentrations (80 ng/mL) in water/acetonitrile (60/40, v/v). Separation was achieved within 10 min using Kinetex XB-C18 (100 mmL x 2.1 mmID., 2.6 µm) and Kinetex Biphenyl (100 mmL x 2.1 mmID., 2.6 µm) maintained at 35 °C on a UHPLC system (NexeraX2, Shimadzu corporation, Kyoto, Japan). Data acquisition was performed on triple quadrupole mass spectrometer LCMS-8060 (Shimadzu Corporation, Kyoto, Japan). All samples were analyzed by multiple reaction monitoring (MRM) with fast polarity switching (5 msec) used for simultaneous detection of positively and negatively charged analytes. The dwell time was set to 4 msec for target ion and 1 msec for reference ion, and the pause time was 1 msec.

Table 1 Analytical condition.

UHPLC (Nexera X2)	
Mobile Phase A	: 2 mmol/L Ammonium formate + 0.002% Formic acid – Water
Mobile Phase B	: 2 mmol/L Ammonium formate + 0.002% Formic acid – Methanol
Gradient Program	: 3%B (0 min) – 10%B (1 min) – 55%B (3 min) – 100%B (10.5 – 12 min) – 3%B (12.01 -15 min)
Flow Rate	: 0.4 mL/min
Column Temperature	: 35 °C
Injection Volume	: 2 µL
MS (LCMS-8060)	
Ionization	: ESI positive and negative
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 10 L/min
Heating Gas Flow	: 10 L/min
Interface Temperature	: 350 °C
DL Temperature	: 150 °C
HB Temperature	: 300 °C
Pause Time	: 1 msec
Dwell Time	: 5 msec

Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

Figure 1 LCMS-8060 Triple Quadrupole Mass Spectrometer

Result

Pesticides encompass a wide range compound classes and chemical properties and consequently it is necessary to employ both positive and negative electrospray ionization for complete analysis in a single run. All of the compounds could be detected in 10 minutes by LC-MS/MS with fast polarity switching (5 msec) using both the ODS column and the biphenyl column.

a) ODS column

b) Biphenyl column

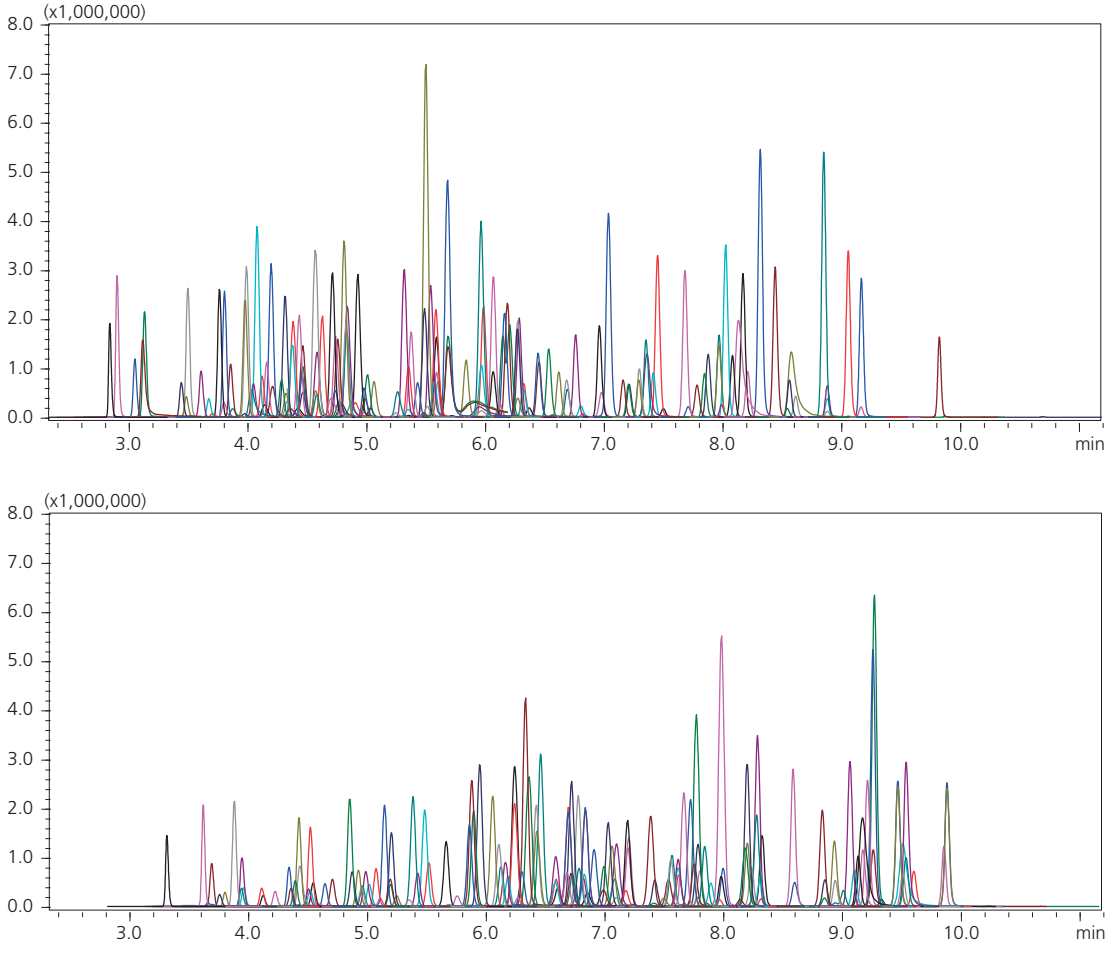

Figure 2 MRM chromatograms for 167 pesticides.

Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

While both columns efficiently separated 167 compounds by the same gradient program, the overall distribution of retention times was slightly shorter for the biphenyl column than for the ODS column. The resulting overlap of MRM acquisition was 42 events at maximum that included both positive and negative mode.

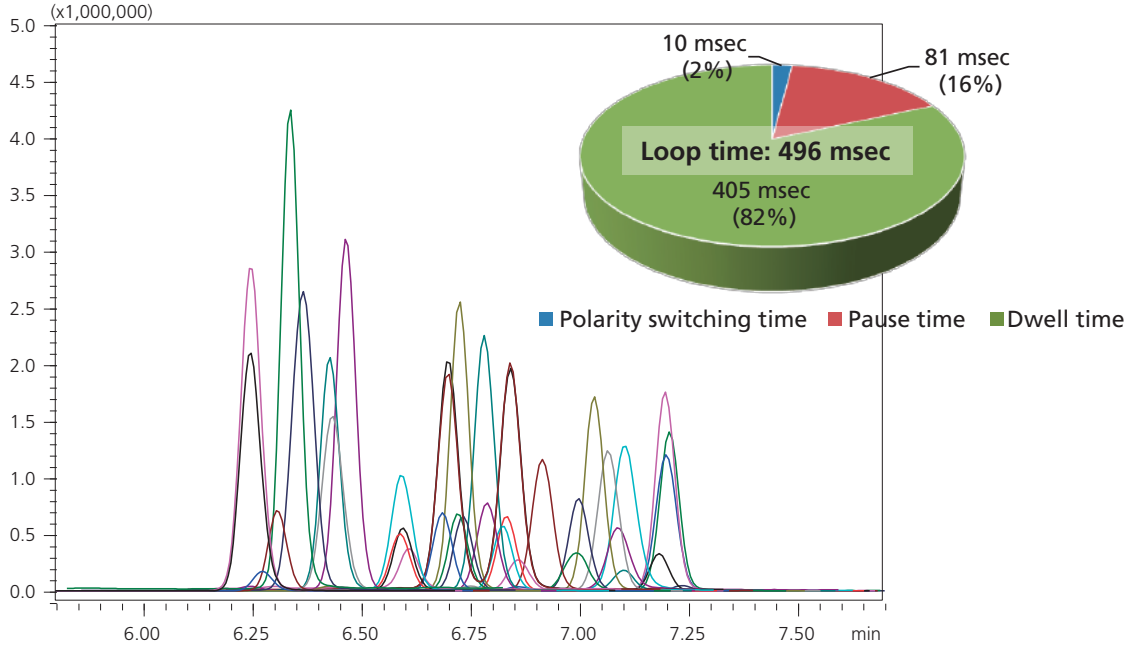

Figure 3 MRM chromatograms for 42 pesticides detected with retention times of 6.720 to 6.723 min (One/Two MRM events per compound, one polarity switching) using the biphenyl column and ratio of dwell time, pause time and polarity switching time in 496 msec of loop time.

Figure 3 shows the MRM chromatograms of 42 pesticides detected in retention time 6.20 – 7.30 min using the biphenyl column, and the pie chart that shows the breakdown of acquisition loop time during the period. Less than 20% of the total loop time of 496 msec was needed for polarity switching and pause time that may be considered as instrumental inefficiency associated with fast detection. The LCMS-8060 ensures the collection of sufficient data points (20 points per peak for peak width of 10 sec) without compromising ion loss.

The biphenyl column is characterized by its high retention capability and separation of aromatic compounds. In this experiment, the retention times of nearly half of all compounds increased by 1 min on average with the biphenyl column than with the ODS. Figure 4 shows MRM chromatograms of early-eluting 8 pesticides in the case of using ODS column. The retention times of all 8 compounds increased and the result indicates the high retention capability of a biphenyl column.

Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

a) ODS column

b) Biphenyl column

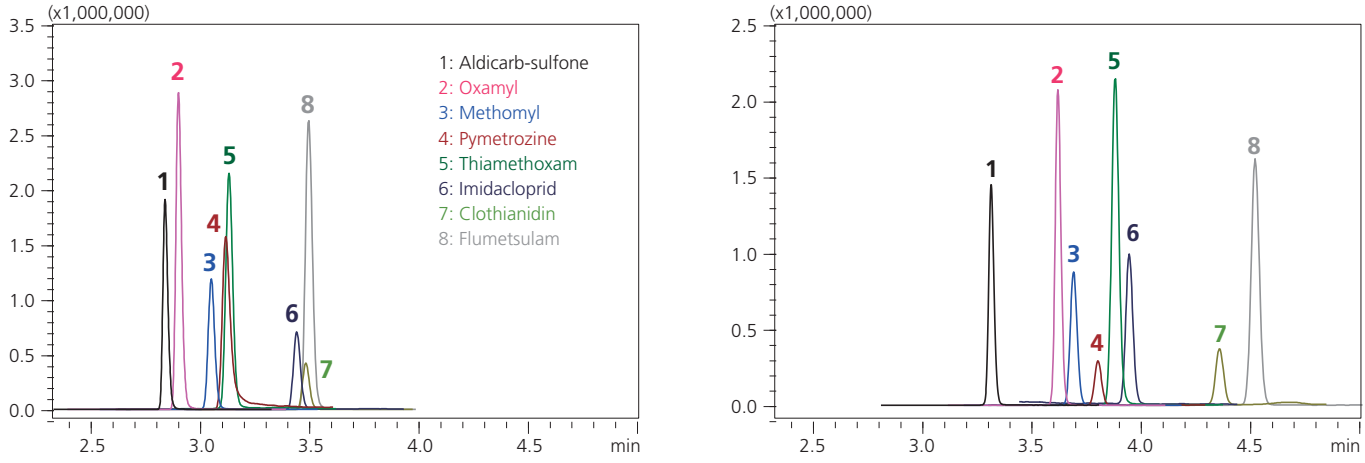

Figure 4 MRM chromatograms for early eluting 8 pesticides.

Table 2 Retention time (RT) of 8 compounds.

#	Compound	Tranisition	Retention Time (RT) / min		ΔRT* / min
			ODS column	Biphenyl column	
1	Aldicarb-sulfone	240.20>86.10	2.844	3.318	0.474
2	Oxamyl	237.10>72.10	2.905	3.624	0.719
3	Methomyl	163.10>88.10	3.055	3.696	0.641
4	Pymetrozine	218.10>105.10	3.121	3.886	0.765
5	Thiamethoxam	292.00>211.10	3.136	3.951	0.815
6	Imidacloprid	256.10>175.10	3.446	4.364	0.918
7	Clothianidin	250.10>132.00	3.488	3.808	0.32
8	Flumetsulam	326.10>129.10	3.501	4.527	1.026

* ΔRT = RT (Biphenyl column) – RT (ODS column)

The separation was also enhanced with the biphenyl column, even when the retention was not improved, such as the cis-trans isomers of (*E*)-Ferimzone and (*Z*)-Ferimzone.

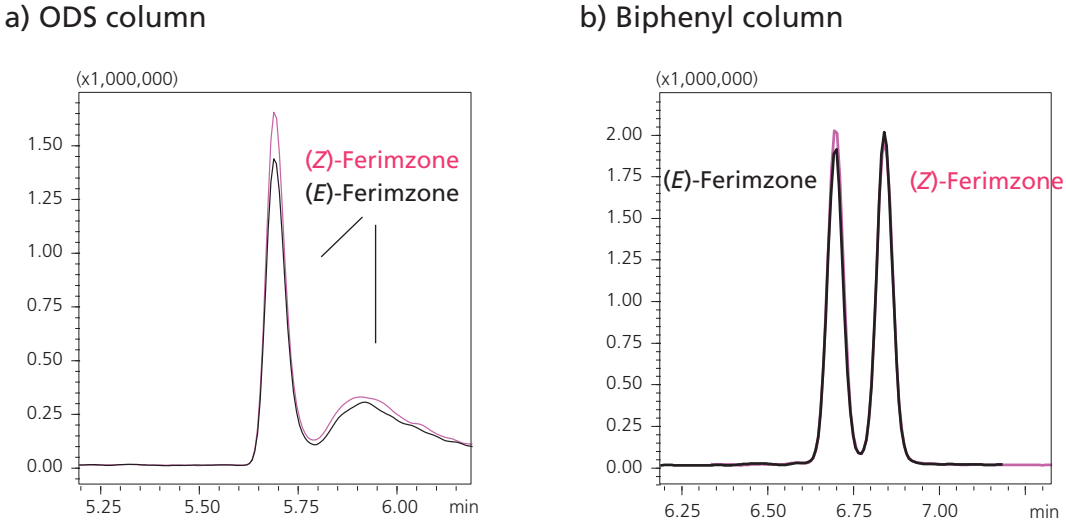

Figure 5. MRM chromatograms for (*E*)-Ferimzone and (*Z*)-Ferimzone.

Using the biphenyl column, the peak shapes improved for Dimethirimol and Iprovalicarb, which have aromatic groups, and hence sensitivity was higher than when using the ODS column. This tendency was also observed for other aromatic compounds.

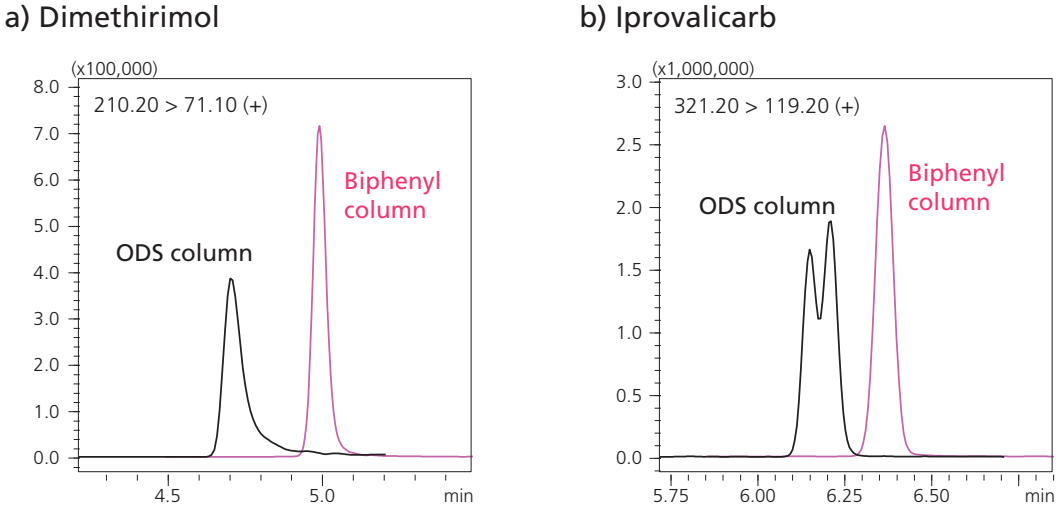

Figure 6. MRM chromatograms for Dimethirimol (a) and Iprovalicarb (b), black: ODS column and pink: biphenyl column

Multi-residue pesticides analysis by LC-MS/MS using the ODS column and the biphenyl column

Conclusions

- A biphenyl column as well as an ODS column is useful for the multi-residue pesticides analysis by LC-MS/MS.
- By developing unique technologies for fast data acquisition, the LCMS-8060 system creates new opportunities for high density analysis and expanded pesticides screening programs.

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

First Edition: June, 2016

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/