

Application News

No. C166

Liquid Chromatograph Mass Spectrometry

Analysis of Residual Pesticides in Vegetable Extracts Using LC/MS/MS <LCMS-8050>

In order to prevent residual pesticides and chemicals in food items from adversely affecting human health, the maximum residue limit of all pesticides, feed additives, and veterinary pharmaceuticals is set for each food item within the range of amounts that were determined to be safe for people to consume (the Positive List System). The Food Sanitation Act prohibits selling and importing food items with amounts of pesticides or other chemicals that exceed the maximum residue limit. Furthermore, maximum usage limits of pesticides and other chemicals are prescribed in the Agricultural Chemicals Regulation Law to prevent them from remaining in food items at amounts that exceed the limits.

This article introduces an analysis of pesticides contained in green onion extracts using liquid chromatography tandem mass spectrometry (LC/MS/MS). The analyzed pesticides include endosulfan (benzoepin) which is a substance that was added on April 1, 2012 to the Ministerial Ordinance on Prohibition of Agricultural Chemical Sales.

While endosulfan has conventionally been analyzed by gas chromatography mass spectrometry (GC/MS), detection by liquid chromatography mass spectrometry (LC/MS) with high sensitivity has become possible by optimizing the temperature and other conditions of the electrospray ionization interface for LC/MS. Good recovery factor resulted even for the green onion extracts obtained by the QuEChERS method which is known to have a relatively strong matrix influence. The example analyses introduced here cover endosulfan, thiamethoxam, dinotefuran, and an iprodione metabolite in green onion extract as well as imazalil, fludioxonil, pyrimethanil, and azoxystrobin in orange extract.

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Analytical Conditions

The analytical conditions of high performance liquid chromatography (HPLC) and Mass Spectrometry (MS) are shown in Tables 1 and 2.

Table 1 Analytical Conditions for HPLC

Column	: Shim-pack™ XR-ODS II (75 mm L×2.1 mm I.D., 2.2 μm)	
Mobile phase	: A) 0.5 mmol/L ammonium acetate-water B) 0.5 mmol/L ammonium acetate-methanol	
Time programs	: B conc. 10% (0 min) → 95% (4.50-8.00 min) → 10% (8.51-12.00 min)	
Column temp.	: 40 °C	
Flow rate	: 0.2 mL/min	
Injection Volume	: Green Onion 1 μL, Orange 2 μL	

Table 2 Analytical Conditions for MS

	Green Onion	Orange
Probe Voltage	0.5 kV (ESI-Positive) / -0.5 kV (ESI-Negative)	0.5 kV (ESI-Positive) / -0.5 kV (ESI-Negative)
DL Temp.	150 °C	150 °C
Block Heater Temp.	300 °C	500 °C
Interface Temp.	100 °C	400 °C
Nebulizing gas flow	3.0 L/min	3.0 L/min
Drying gas flow	13.0 L/min	10.0 L/min
Heating gas flow	7.0 L/min	10.0 L/min

MRM Transition Conditions

Data of the multiple reaction monitoring (MRM) transition and the calibration curve of each pesticide are shown in Table 3. An acceptable coefficient of determination was obtained for each pesticide in the calibration curve concentration range with each value being higher than 0.99.

Table 3 MRM Transition Conditions

Pesticide	Polarity (+/-)	Precursor (m/z)	Product (m/z)	Calibration Curve Conc. Range (ng/mL)	Coefficient of Determination (r ²)
Dinotefuran	+	203.10	129.2	0.05-100	0.9981
Thiamethoxam	+	292.10	211.0	0.05-100	0.9978
Dinotefuran	-	201.00	61.0	0.5-100	0.9979
Iprodione metabolite	-	328.05	141.0	0.02-100	0.9992
Endosulfan (α+β)	-	404.70	268.9	0.2-100	0.9996
Azoxystrobin	+	404.10	372.1	0.01-50	0.9999
Pyrimethanil	+	200.20	107.1	0.02-50	0.9981
Imazalil	+	296.90	159.0	0.05-50	0.9949
Fludioxonil	-	246.90	180.1	0.05-50	0.9995

Linearity of Calibration Curves

The calibration curves of common pesticides, an iprodione metabolite and endosulfan (α+β), are shown in Figs. 1 and 2.

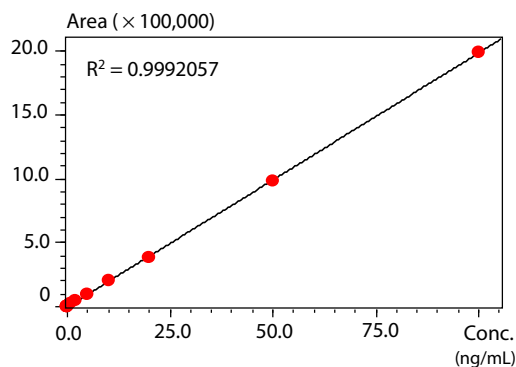


Fig. 1 Calibration Curve (Iprodione Metabolite)

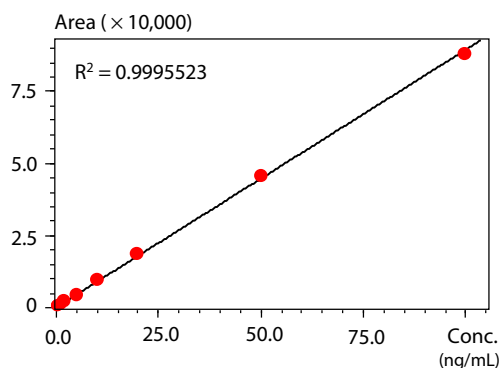


Fig. 2 Calibration Curve (Endosulfan (α+β))

■ Chromatograms of Samples

The chromatograms of samples are shown in Figs. 3 and 4. Through selective analysis in which impurity peaks are not

detected, each pesticide was well separated and chromatograms with good peak shapes were obtained.

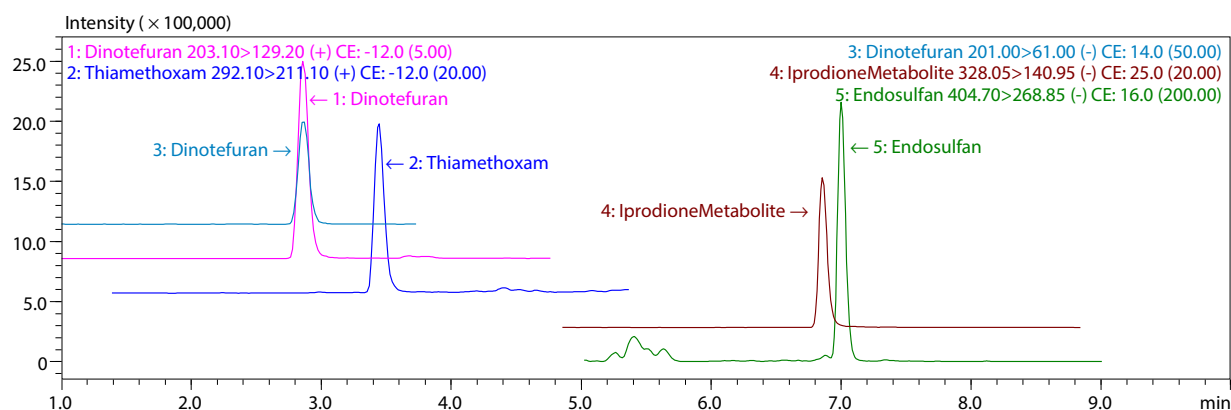


Fig. 3 Chromatograms of the Sample (Green Onion)

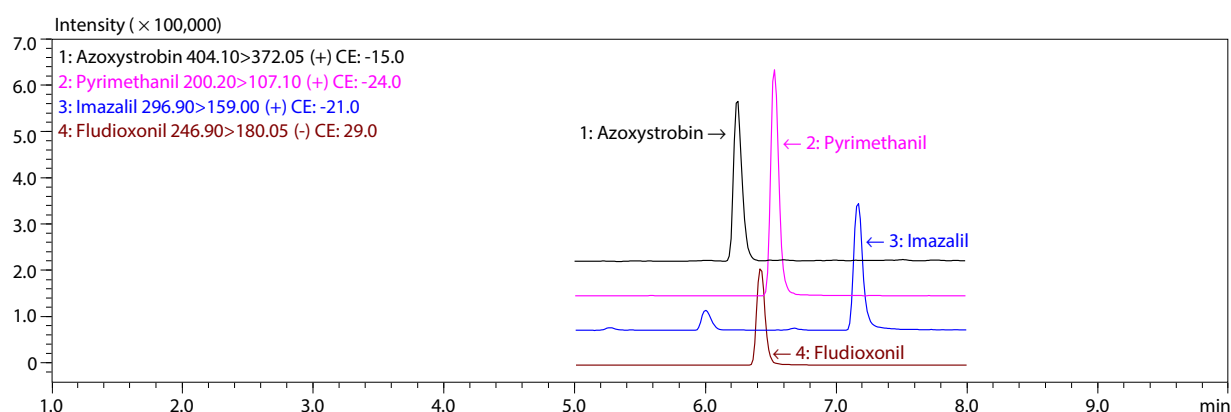


Fig. 4 Chromatograms of the Sample (Orange)

■ Recovery Factor

Recovery factor of all pesticides in the green onion and orange extracts are shown in Table 4.

The recovery factor was obtained using the following formula:

$$\text{Recovery factor (\%)} = \frac{(\text{Measured concentration} - \text{Concentration of blank sample})}{\text{Actual spike concentration}} \times 100$$

Good recovery factors were obtained for the green onion and orange extracts in the range of 88 to 104 % and 74 to 98 %, respectively.

The peak area repeatabilities (%RSD, n = 3) of the samples are shown in Table 4. The %RSD for each pesticide is favorable with all values being lower than 8 %.

Table 4 Recovery Factor and Peak Area Repeatability

Pesticide	Spike Concentration (ng/mL)	Recovery Factor (%)	%RSD	Pesticide	Spike Concentration (ng/mL)	Recovery Factor (%)	%RSD
Dinotefuran	100	101	0.70	Azoxystrobin	2	93	2.66
Thiamethoxam	10	104	5.50	Pyrimethanil	40	74	5.08
Iprodione metabolite	25	88	2.80	Imazalil	10	98	0.66
Endosulfan (α + β)	50	104	5.36	Fludioxonil	20	83	7.78