SHIMADZU

Application News

No.C86

Liquid Chromatography Mass Spectrometry

Analysis of Malachite Green Using a Triple Quadrupole LC/MS/MS [LCMS-8030]

Malachite green, besides being used as a dye in the textile and paper industries in Japan, is also used as a synthetic antibacterial drug to treat diseases such as water mold disease in aquarium fish. Due to concern related to its carcinogenicity and genotoxicity, not to mention the persistence of its metabolite, leucomalachite green, application of malachite green with aquaculture animals is prohibited under the Pharmaceutical Affairs Act. The United States in 1981, and the European Union and China in 2002 prohibited its use with all food-related items. However, due to its low price, effectiveness, and easy availability, cases of its detection in eel, salmon and other farmed fish continue to appear, resulting in strengthened worldwide monitoring.
Here, we show the quantitative analysis of malachite green and leucomalachite green using the LCMS-8030. In addition, we report the results of spiked-recovery measurements conducted using a salmon extract solution as an actual sample.

MRM Optimization and Quantitative Analysis

Optimization was conducted to determine the product ions (quantitation and reference ions) and collision energies for malachite green, malachite green-d5, leucomalachite green and leucomalachite green-d6.

Fig. 1 and Fig. 2 show the respective product ion mass spectra and the calibration curves generated using the internal standard method. Excellent linearity was obtained over the range of 0.5-50 ng/mL.

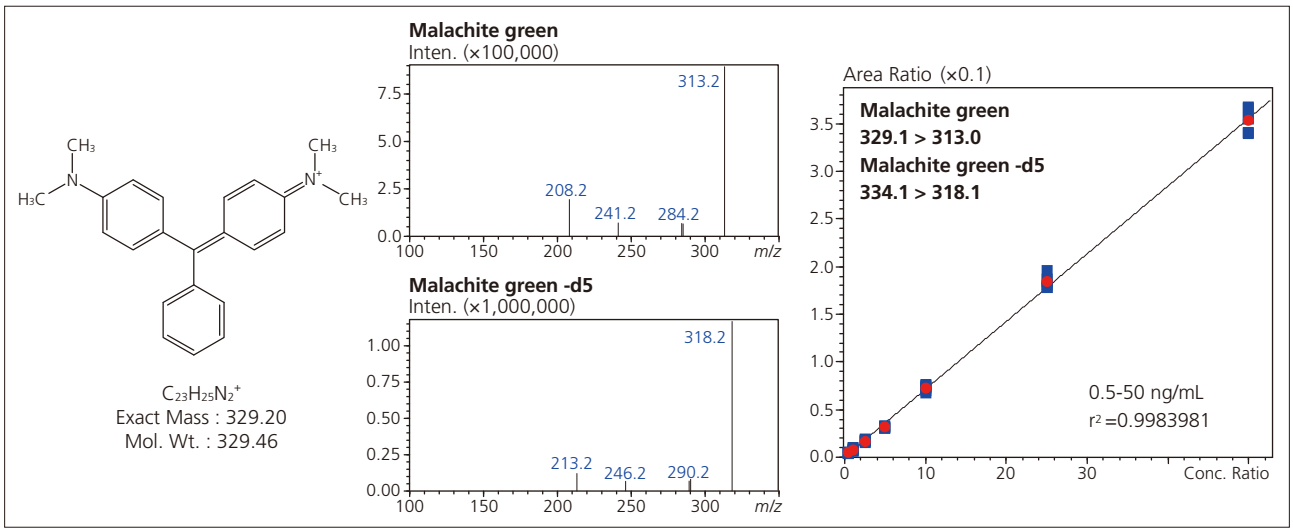

Fig. 1 Product Ion Mass Spectra and Calibration Curve for Malachite Green

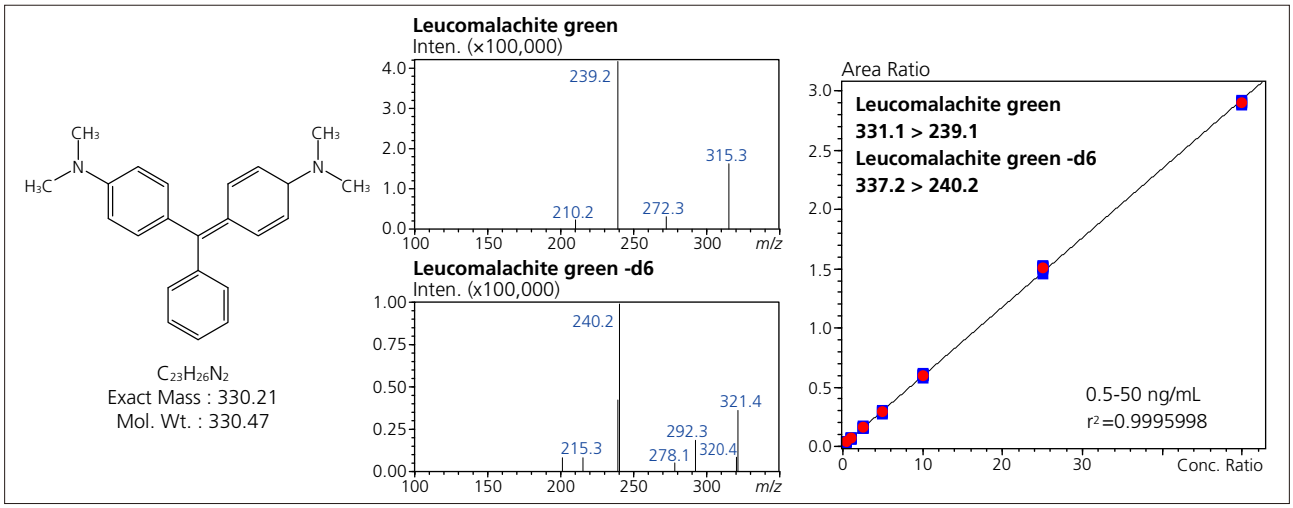

Fig. 2 Product Ion Mass Spectra and Calibration Curve for Leucomalachite Green

Salmon extracts were prepared according to the "Malachite Green Analytical Method" specified by Japan's Ministry of Health, Labour, and Welfare. Each extract was spiked with standard samples of malachite green and leucomalachite green at concentrations corresponding to 10 ng/mL and was then analyzed by LC/MS/MS. Fig. 3 shows the MRM chromatograms of the standard sample spiked salmon extract. Table 1 shows the peak area ratios of the standard sample and the salmon extract solution spiked with the standard (n = 6), in addition to the respective rates of recovery. Excellent recovery was obtained with little variation, permitting quantitation at 10 ng/mL without any adverse effects from the matrix.

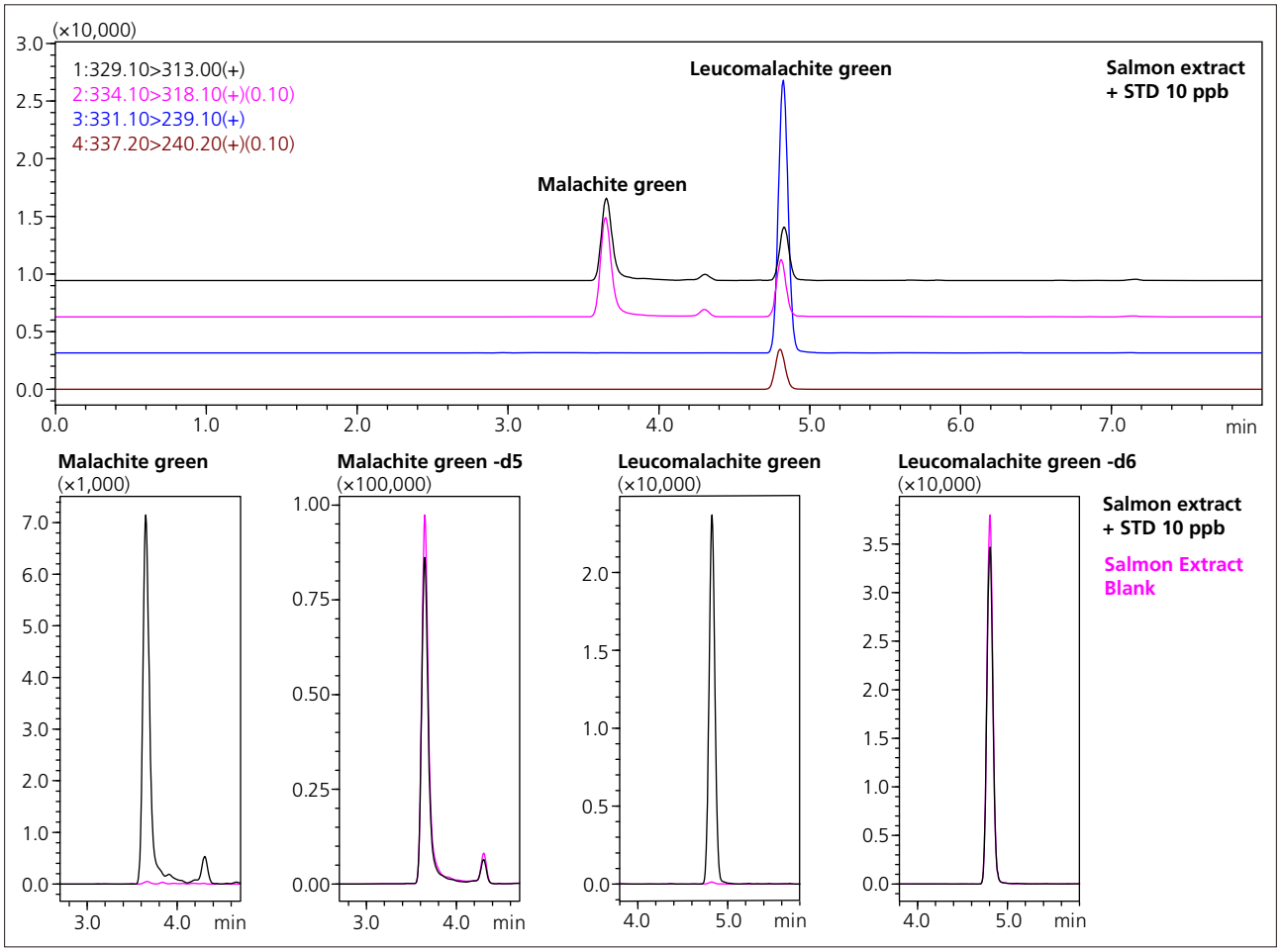

Fig. 3 MRM Chromatograms of Salmon Extract Blank and Salmon Extract Spiked with 10 ppb STD

Table 1 Recovery Ratio of Salmon Extract Spiked with 10 ppb STD

Malachite green

	Area ratio								Recovery (%)
	1	2	3	4	5	6	Average	%RSD	
STD 10 ppb	0.0750	0.0756	0.0732	0.0673	0.0699	0.0728	0.0723	4.39	
Sarmon + STD 10 ppb	0.0845	0.0851	0.0823	0.0847	0.0824	0.0837	0.0838	1.41	115.9

Leucomalachite green

	Area ratio								Recovery (%)
	1	2	3	4	5	6	Average	%RSD	
STD 10 ppb	0.6149	0.5795	0.5929	0.6105	0.6064	0.5985	0.6004	2.1611	
Sarmon + STD 10 ppb	0.6794	0.6732	0.6693	0.6931	0.6701	0.6689	0.6757	1.3938	112.5

Table 2 Analytical Conditions

Mobile Phase A	: 10 mmol/L Ammonium acetate - water	Probe Voltage	: +4.5 kV (ESI-positive mode)
Mobile Phase B	: Acetonitrile	Nebulizing Gas Flow	: 3.0 L/min
Gradient Program	: 10 %B (0 min) – 100 %B (2-5 min) – 10 %B (5.01-8 min)	Drying Gas Flow	: 10 L/min
Column	: Shim-pack XR-ODS Ⅱ (75 mmL. × 2.0 mmI.D., 2.2 µm)	DL Temperature	: 250 °C
Flow Rate	: 0.2 mL/min	BH Temperature	: 400 °C
Injection Volume	: 2 µL	DL Voltage / Q-array Voltage	: Using default values
Column Temperature	: 40 °C		

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