

## Analysis of Malachite Green and Leucomalachite Green by LC/MS

Malachite Green is used as a synthetic antimicrobial for treating water mold disease in aquarium fish, but has been identified as carcinogenic, and use in farm fish, etc., for human consumption is banned in Japan. In the Food Sanitation Law in Japan, the maximum residue limit has been specified as “not detected.” In other countries this agent is widely used in farmed salmon and eel, and reports have described detection in farmed eel imported into Japan from China.

The LC/MS procedures used to analyze Malachite Green and its metabolite, Leucomalachite Green, are described as analytical methods in regulations announced by the Ministry of Health, Labour and Welfare. Below is an example of simultaneous analysis of Malachite Green and Leucomalachite Green based on these regulations. As each of these is a basic substance containing a dimethylamino group, the decision was made to use a solvent with an acid composition. The calibration curves created each displayed good linearity in a concentration range of 0.05-50ppb.

Figure 1. Structural formula of Malachite Green and Leucomalachite Green

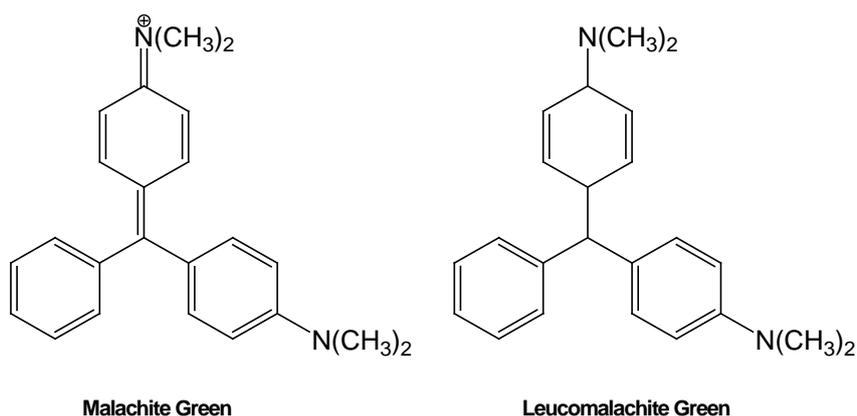
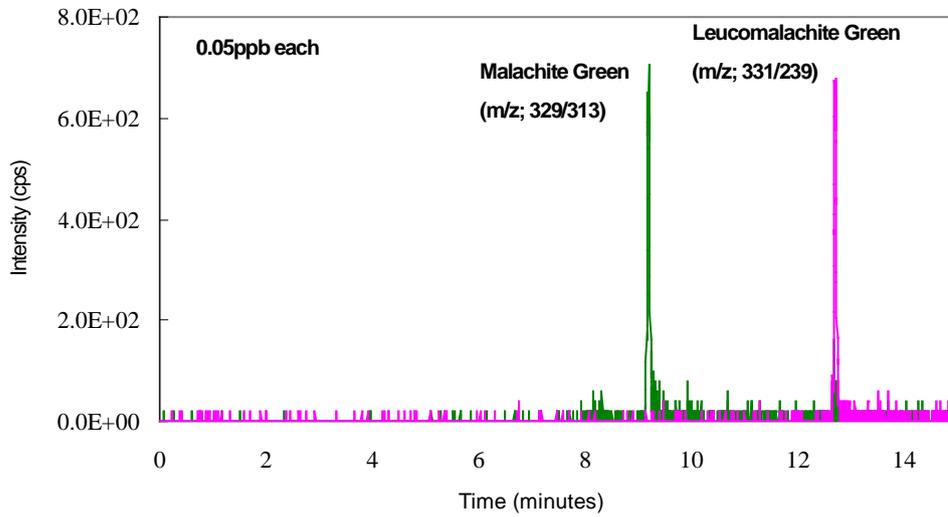
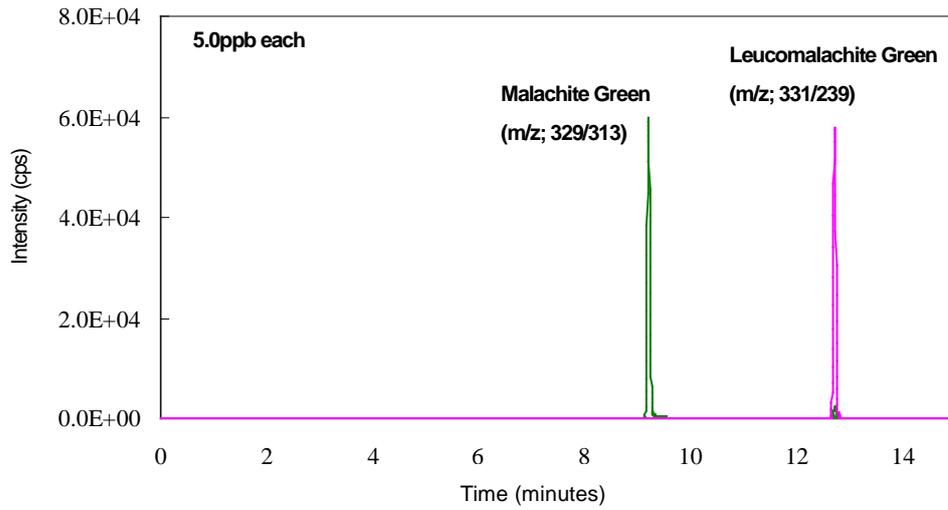


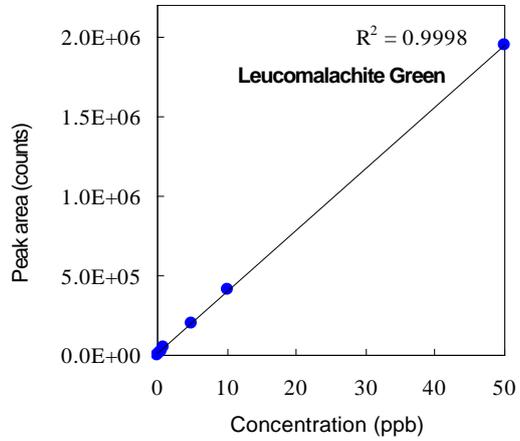
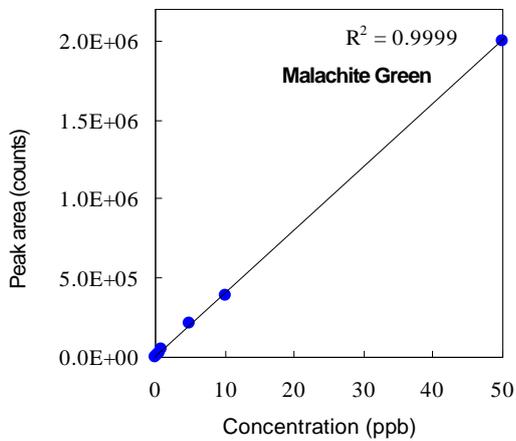
Table: Analysis conditions

Column:	TSKgel ODS-100V, 3 $\mu$ m, 2.0mm ID x 15cm
Mobile phase:	A:10mmol/L ammonium formate , pH 3.75 B: acetonitrile
Gradient:	0min (10%B) → 10 min (100 %B) → 15 min (100 %B)
Flow rate:	0.2mL/min
Temperature:	40°C
Injection vol.:	5 $\mu$ L
Instrument:	QTRAP® (Applied Biosystems)
Ion Source:	ESI
Polarity:	Positive

**Figure 2. Chromatograms of Malachite Green and Leucomalachite Green**



**Figure 3. Malachite Green and Leucomalachite Green calibration curves**





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