A Rapid iMethod™ Test for the Quantification of Malachite Green and Related Triphenylmethane Dyes

iMethod™ Test for Malachite Green Version 1.0 for Cliquid® Software

Malachite green (MG) and its hydrolyzed metabolite, leucomalachite green (LMG) are organic compounds that possess powerful anti-fungal properties and hence, are commonly used in fish farms as prevention against fungus and infection. In addition, its anti-parasitic properties have made its use a common treatment for skin flukes, gill flukes, white spot and other parasites found in aquaculture. While MG and LMG are not listed as veterinary medicines under Annex I, II or III of the EU council regulation 2377/90, there has been increasing concerns about its usage due to harmful effects on humans, leading to a ban on the use of MG in most countries.

The following description outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Analysis of Malachite Green and Related Triphenylmethane Dyes in Food when using an AB SCIEX 3200 Series instrument. This method has also been developed and verified for use with 4000 Series instrumentation. More in depth sample preparation, and instrument parameter information is included as part of the standard operating procedure provided with the malachite green and triphenylmethane dyes iMethod test upon purchase.

Example sample preparation procedures are provided that are based upon a simple sample homogenization, centrifugation, extraction and dilution. A deuterated internal standard D5-LMG at known concentrations are added during sample preparation to monitor sample recovery.

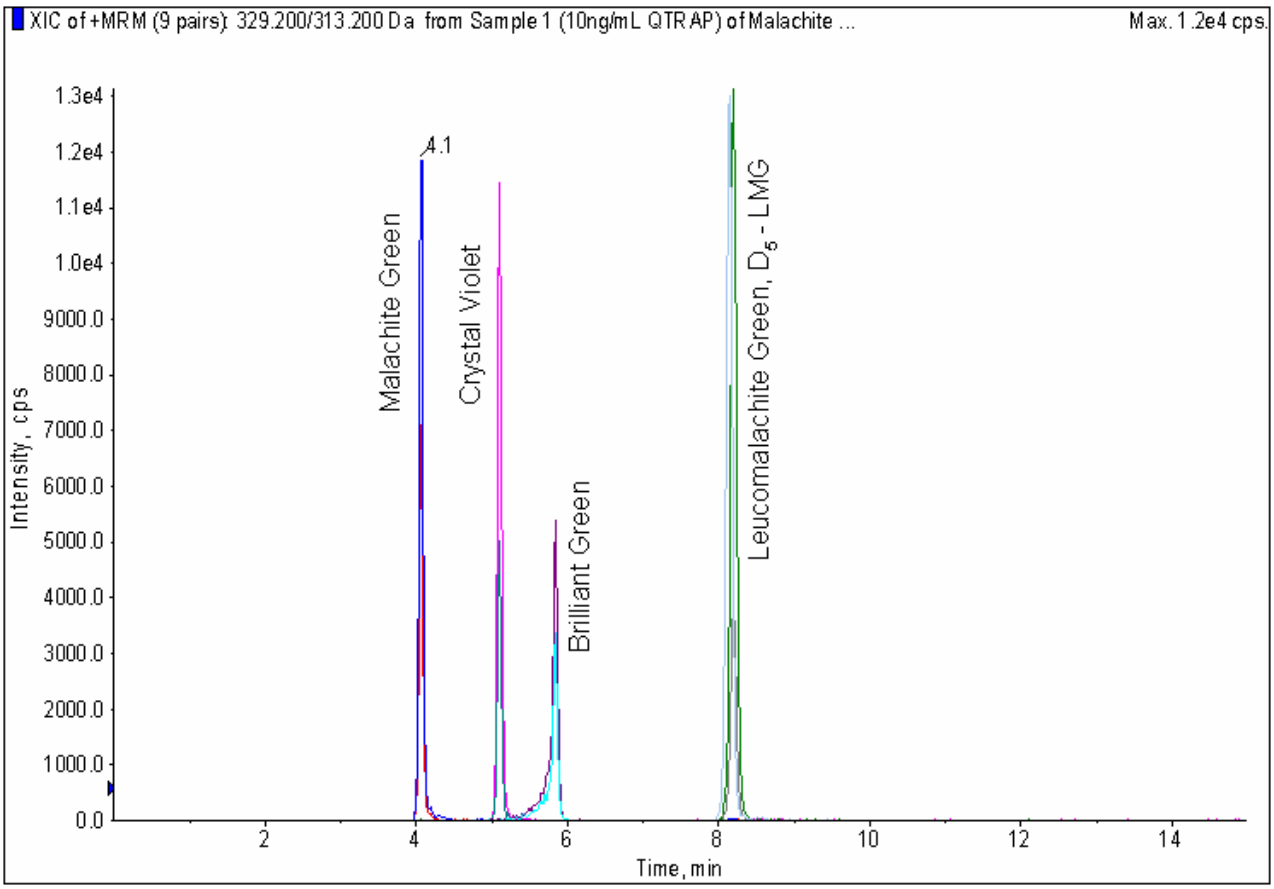

Figure 1: Chromatogram of a malachite green and related triphenylmethane dyes analyzed by LC/MS/MS

Results

An example chromatogram of MG, LMG and related triphenylmethane dyes is shown in Figure 1 with retention times and the MRM transitions used listed in Table 2. The excellent sensitivity of the method is highlighted by the limits of detection shown. Signal-to-Noise varies depending on the ionization and fragmentation efficiency of each analyte.

Table 1: MRM Transitions, Retention Times, and Limit of Detection

Compound	MRM 1	MRM 2	RT (min)	LOD
Malachite Green	329 / 313	329 / 208	4.1	<1ppb
Leucomalachite Green	331 / 239	331 / 223	8.2	<1ppb
Crystal Violet	372 / 356	372 / 340	5.1	<1ppb
Brilliant Green	385 / 341	385 / 297	5.8	<1ppb
D_5-LMG	336 / 239		8.1	

Calibration

The following calibration curves using the calibrator, low and high level controls are provided as examples, showing the range and linearity expected for this assay.

Please note that the results were obtained using a single instrument and single set of standards and samples, and the results here may not be typical for all instruments. Prior to production use, the method should be fully validated with real samples. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

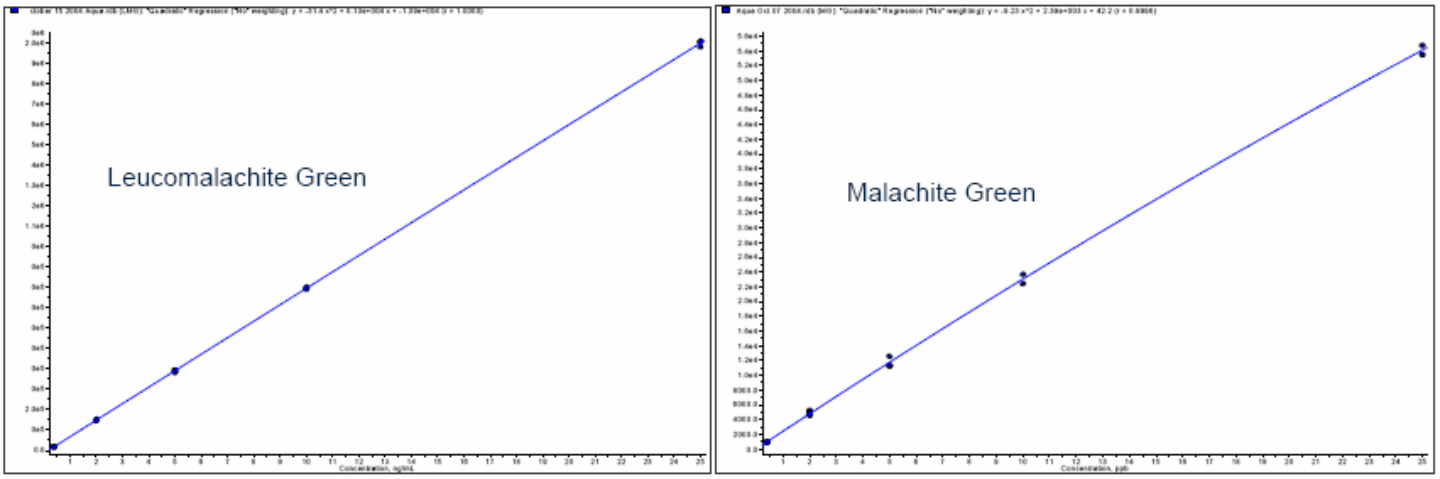

Figure 2: Example calibration curves for malachite green and leucomalachite from .4 to 25 ppb.

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 Series (3200 QTRAP® or API 3200™) or 4000 Series (4000 QTRAP® or API 4000™) LC/MS/MS System
- A Shimadzu Prominence 20A LC System with reservoir tray and bottles, system controller CBM-20A, 100 µL mixer, 2 isocratic pumps LC-20AD, 3-channel degasser autosampler SIL-20AC, column oven CTO-20AC or Agilent 1100/1200 LC system with binary pump G1312A (without static mixer), well plate autosampler, and thermostated column oven
- MG, LMG and triphenylmethane dyes (www.sigmaaldrich.com)
- D5-LMG internal standard (www.customlabelling.com)
- LC/MS-grade water, acetonitrile, formic acid, and ammonium acetate
- A Phenomenex Synergi 4 µm Max-RP 80A 50 x 2 mm column (included with the iMethod™ test)
- 1.5 mL Eppendorf tubes
- A centrifuge able to accommodate Eppendorf tubes and run at 14000 rpm
- Pipettes and standard laboratory glassware

Ordering Information

Product Name	Part Number
iMethod™ Test for Malachite Green Version 1.0 for Cliquid® Software	1034369

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Analysis of Malachite Green, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod test and are recommended for inexperienced users. Please consult your local sales representative for more details.

Important Note

The iMethod™ Test described above has been designed by AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It us up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

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