



Application Note AN-RS-018

# Trace Detection of Malachite Green in Stream Water

## Protecting consumer safety with Misa

Malachite green (MG) is a dye used widely as a textile colorant and biological stain. It was also a remarkably effective fungicide used in aquaculture operations until the turn of the 21<sup>st</sup> century. MG is acutely toxic to fish and mammals, and it and its metabolites persist in the flesh, making it a threat to the human food chain. The EU has concluded that contaminated foods containing levels higher than 2 µg/g MG constitute a credible health risk, and several countries

have banned malachite green as an aquaculture additive. Despite tight regulation, seafood products contaminated with MG continue to find their way to consumers from illegal operations that evade strict oversight procedures and health safety standards. Using Misa (Metrohm Instant SERS Analyzer) to ensure food safety, the rapid and highly sensitive detection of malachite green is achieved in a facile assay format.

## INTRODUCTION

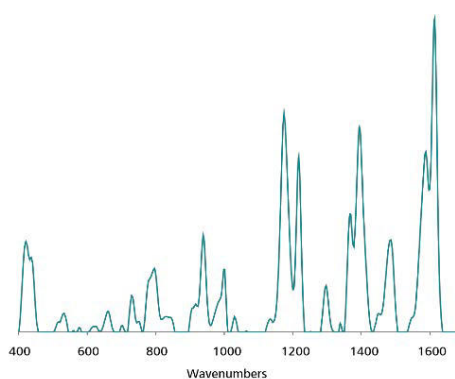
Many food matrices require complicated sample digestion and extraction procedures. This application note describes a very simple water test that can be

performed directly at the aquaculture site, to determine if malachite green is being illegally used as a fungicidal treatment.

## REFERENCE MATERIAL AND LIBRARY CREATION

To establish a reference spectrum for MG, a pure standard in water is first analyzed using gold

nanoparticles (Au NPs). This unique SERS spectrum (Figure 1) can be used to create a library entry for MG.



**Figure 1.** Standard 1 µg/mL Au NP SERS reference spectrum of malachite green.

## EXPERIMENT

Simulated testing of MG in aquaculture media was conducted by spiking stream water samples with a range of analyte concentrations (10 and 1 µg/mL, 100, 10, and 1 ng/mL). Test samples were prepared by pipetting 0.1 mL of each analyte concentration (at pH 7.0) into vials containing 0.8 mL of Au NPs and 0.1 mL of 500 mmol/L NaCl. After thorough mixing, samples were inserted into the Misa vial attachment for the acquisition of SERS spectra.

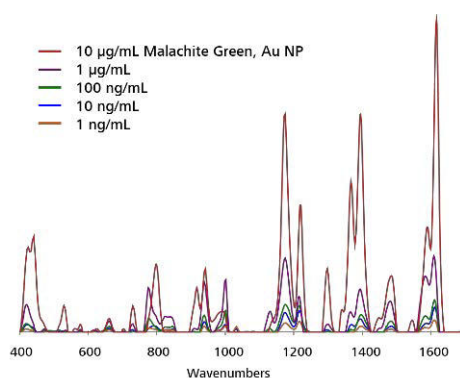


**Table 1.** Experimental Parameters

Instrument		Acquisition	
Firmware	0.9.33	Laser Power	5
Software	Misa Cal V1.0.15	Int. Time	1 s
Misa Vial Attachment	6.07505.040	Averages	10
ID Kit - Au NP	6.07506.440	Raster	ON

## RESULTS

Overlaid spectra in **Figure 2** at each MG concentration show clear detection down to 1 ng/mL.



**Figure 2.** Au NP SERS concentration profile of MG extracted from stream water. Spectra are baselined, with background subtracted.

## FIELD TEST PROTOCOL

### Detection of malachite green in the field

Fill a clean vial halfway full with Au NPs. Using pipettes, add 2 drops each of aquaculture medium and NaCl solution to Au NPs, then cap and shake the

vial gently to mix. Insert into vial attachment on Misa for measurement.

**Table 2.** Requirements for Field Test Protocol

ID Kit - Au NP	6.07506.440
includes:	Gold nanoparticles (Au NP)
	Scoop
	Disposable pipettes
	2 mL glass vials
Reagents	
NaCl solution	3 g NaCl in 100 mL water
Test settings	Use ID Kit OP on MISA

## CONCLUSION

The highly sensitive detection of MG in a potentially difficult test matrix is reduced to a very simple assay with Misa and Au NPs. This analysis requires minimal user training and can be performed on-site for rapid, high-throughput test applications. The lower limit of

MG reported here is accurate even below the threshold for permissible levels in contaminated foods, and compares favorably with the detection limit recently reported for MG in commercial aquaculture media.

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## CONFIGURATION



### MISA Advanced

Metrohm Instant SERS Analyzer (MISA) is a high performance, portable analyzer system used for rapid, trace level detection / identification of illicit materials, food additives and food contaminants. MISA features a high-efficiency spectrograph equipped with Metrohm's unique Orbital-Raster-Scan (ORS) technology. It has a minimal footprint and extended battery life, perfect for on-site testing or mobile laboratory applications. MISA offers various Laser Class 1 attachments for flexible sampling options. Analyzer operation is available through BlueTooth or USB connectivity.

The MISA Advanced package is a complete package that allows the user to perform SERS analyses using Metrohm's nanoparticle solutions and P-SERS strips.

The MISA Advanced package includes a MISA Vial Attachment, a P-SERS Attachment, a ASTM Calibration Standard, a USB Mini Cable, a USB Power Supply and MISA Cal software for operating the MISA instrument. A ruggedized protective case is also provided to securely store the instrument and accessories.



### ID Kit – Au NP

The ID Kit - Au NP contains the components a Mira / Misa user requires to perform a SERS analysis using gold colloidal solution. The kit contains a disposable spatula, dropper, sample vials and a bottle of gold colloid.