



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

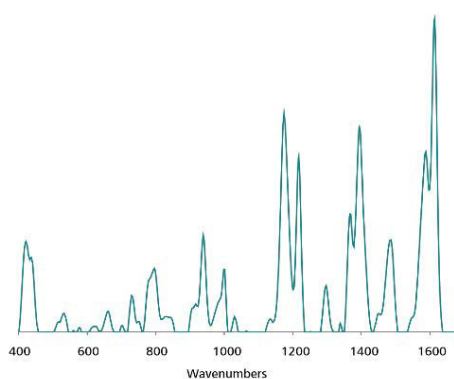
Many food matrices require complicated sample digestion and extraction procedures. This application note describes a very simple water test that can be

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.

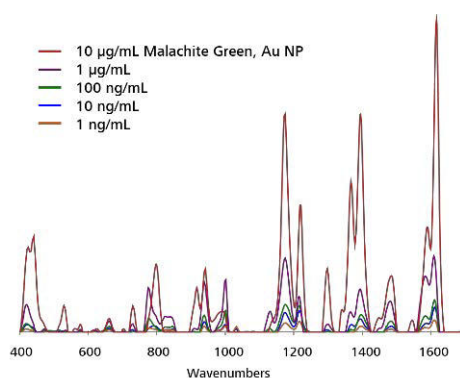


**Table 1.** Experimental Parameters

| 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.

## CONTACT

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