



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 21st 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 $\mu\text{g/g}$ 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.

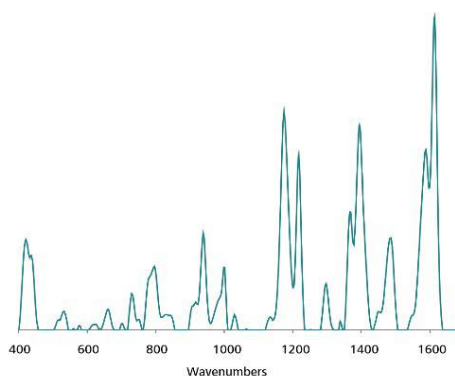


Figure 1. Standard 1 $\mu\text{g/mL}$ Au NP SERS reference spectrum of malachite green.

EXPERIMENT

Simulated testing of MG in aquaculture media was conducted by spiking stream water samples with a range of analyte concentrations (10 and 1 $\mu\text{g/mL}$, 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.

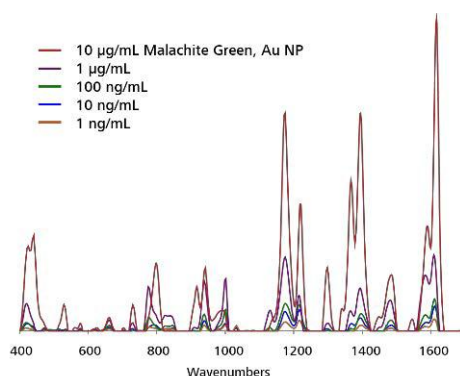


Figure 2. Au NP SERS concentration profile of MG extracted from stream water. Spectra are baselined, with background subtracted.

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) 是一款高性能、便携式分析系,可快速/定非法物、食品添加和微量食品染料。MISA 的特点是配了 Metrohm 独特的道光栅描 (ORS) 技的高效光。其空需求最小和并且池寿命有所延,是或移室用的理想。MISA 提供各 1 激光附件,可活取。分析可通 BlueTooth 或 USB 接行。MISA Advanced 套件是一个完整套件,其作用是用能用 Metrohms 米粒溶液和 P-SERS 条行 SERS 分析。MISA Advanced 套件包含了一个 MISA 小管附件、一个 P-SERS-附件、一个 ASTM 校正准件、一个 USB 迷、一个 USB 供元和用于行 MISA 器的 MISA Cal 件。随供了一个用来安全保管器和附件的固保箱。



ID – Au NP

ID 套件 - Au NP 包含了 Mira/Misa 用使用体金溶液行 SERS 分析所需的件。套件包含了一个一次性抹刀、一个移液管、品小瓶和一个含金体的瓶子。