



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  $\mu\text{g/g}$  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

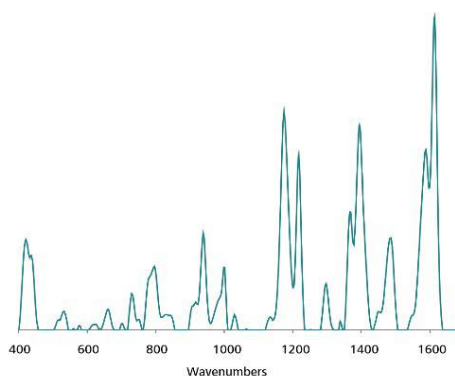
Many food matrices require complicated sample digestion and extraction procedures. This application note describes a very simple water

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  $\text{ng/mL}$ ). 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.

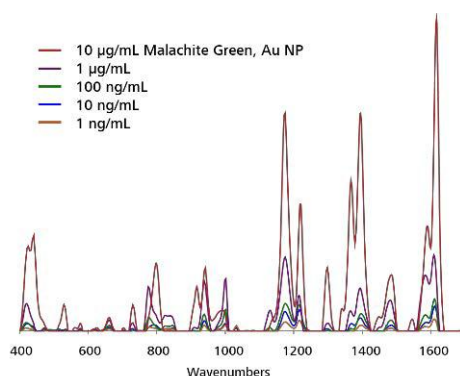


**Table 1.** Experimental Parameters

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
<b>Reagents</b>	
NaCl solution	3 g NaCl in 100 mL water
<b>Test settings</b>	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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## CONFIGURATION



### MISA Advanced

Metrohm Instant SERS Analyzer (MISA) は、微量レベルでの違法物質、食品添加物、および食品汚染物質の迅速な検出 / 同定のための高性能な携帯可能分析システムです。MISAは、Metrohm 独自の軌道ラスタースキャン技術 (Orbital Raster Scan Technologie, ORS) を備えた高効率の分光器を有しています。これは省スペースで、より長いバッテリー寿命を持ち、現場やラホでの移動式用途にも完璧に適しています。MISA ではフレキシブルなサンプル採取を可能にする、レーザークラス1の様々なアタッチメントをご利用いただけます。アナライザーはBluetoothまたはUSBコネクタを介して操作可能です。

MISA Advanced ハッチケースは、ユーザーに Metrohmのナノ粒子溶液とP-SERSストリップを用いたSERS分析を可能にするコンフリートハッチケースです。

MISA Advanced ハッチケースには、MISAハイアルアタッチメント、P-SERSアタッチメント、ASTM校正標準、USBミニケーブル、USB電源装置、ならびにMISA装置を操作するためのMISA Calソフトウェアが含まれます。装置と付属品を安全に保管するための頑丈な保護ケースも同梱されています。



### ID - Au NP

IDキット - Au NPには、Mira/Misaユーザーが金コロイド溶液でSERS分析を行うのに必要なコンポーネントが含まれています。このキットには、使い捨てのへら、滴下ヒベット、サンプルホルダー、および金コロイド入りのホルダーが含まれています。