



Application Note AN-RS-024

Trace Detection of Pyrimethanil in Wine

Protecting consumer safety with Misa

Pyrimethanil is a broad-spectrum fungicide. As grapevines are susceptible to fungal pathogens, large-scale viticulture operations apply pyrimethanil as part of a mixed treatment. Although chemical analysis of wines post-fermentation finds low to undetectable amounts of residue, pyrimethanil is a suspected human carcinogen and chronic exposure can result in multi-organ toxicity in some animal species. The US FDA and EU have established a maximum permissible level of 5 $\mu\text{g/mL}$ pyrimethanil in finished wine products to balance potential

health risks with a sustainable wine industry.

Standard methods for detecting pyrimethanil in bottled wines include laboratory-based GC, LC, and immunoassays. Misa (Metrohm Instant SERS Analyzer) integrates detection, data processing, and results sharing into a user-friendly system for high-throughput, onsite testing. In this application, trace detection of pyrimethanil in wine with Misa requires few laboratory supplies and minimal sample processing, yet returns rapid results.

INTRODUCTION

This application note describes a procedure for trace detection of pyrimethanil in white wine. A very simple sample extraction process results in

very sensitive SERS detection of pyrimethanil with Misa and gold nanoparticles (Au NPs).

REFERENCE SPECTRUM AND LIBRARY CREATION

To establish a reference spectrum, pure pyrimethanil standard at a concentration of 10 $\mu\text{g/mL}$ in ethanol was analyzed with Au NPs.

The unique SERS spectrum shown in **Figure 1** can be used to create a library entry for pyrimethanil.



Figure 1. Unique standard reference Au NP SERS pyrimethanil spectrum.

EXPERIMENT

White wine was spiked with a stock solution of pyrimethanil in ethanol to provide a concentration range of test samples: 10, 5, and 1 g/mL, 500 and 100 ng/mL. Chloroform (0.5 mL) was added to 1 mL of each sample concentration in a glass vial. This mixture was vigorously shaken and allowed to rest for at least 5 minutes to allow phase separation. Note that longer rest times improve results. Taking care to not disturb the lower chloroform layer, 200 μL of the top layer was transferred to a second vial and dried on a hot plate. The dried residue was resuspended in 450 μL of Au NP solution and 50 μL of 0.5 mol/L NaCl and shaken well to mix. This vial was inserted into the Misa vial attachment for measurement.



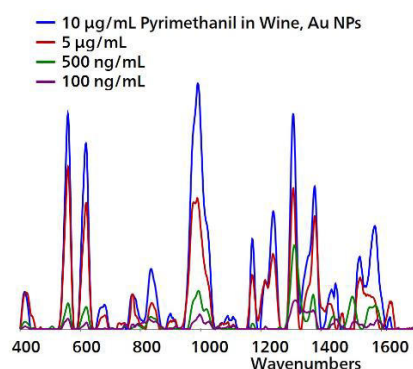
Table 1. Experimental parameters

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

RESULTS

Overlaid baseline-corrected Au NP SERS spectra acquired for the concentration range of test extracts demonstrates detection down to 100 ng/mL (**Figure**

2), a level significantly lower than the maximum permissible levels for pyrimethanil residue in wine.

**Figure 2.** Overlaid, baseline corrected, and background subtracted Au NP SERS spectra of pyrimethanil extracted from wine.

FIELD TEST PROTOCOL

Detection of pyrimethanil in the field

Fill a vial halfway with white wine. Using a pipette, add 10 drops of chloroform to this vial. Cap and shake very well to mix, and let rest for at least 5 minutes to allow layers to separate. Carefully remove a portion of the top layer with a clean

pipette, and place 4 drops of this solution into a *clean vial*. Evaporate the liquid on a hot plate. Fill this vial halfway full with Au NPs and add 1 drop of NaCl, then cap and shake. 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		
Chloroform		
NaCl solution	3 g NaCl in 100 mL water	
Test settings		Use ID Kit OP on MISA

CONCLUSION

Misa provides a highly-sensitive, cost-effective solution for detecting pyrimethanil in wine. With Misa's portability, levels of pesticide residue can be

rapidly and reliably assessed in wineries during the production process, as well as in commercial storage, shipping, and receiving facilities.

CONTACT

143-0006 6-1-1
null 9

metrohm.jp@metrohm.jp

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、現場やラボ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 パッケージは、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プリートパッケージです。

MISA Advanced パッケージには、MISAバ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、滴下ピペット、サンプルボトル、および金コロイド入りのボトルが含まれています。