



Application Note AN-RS-024

葡萄酒中嘧霉胺的痕量检测

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 µg/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 $\mu\text{g/mL}$, 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.



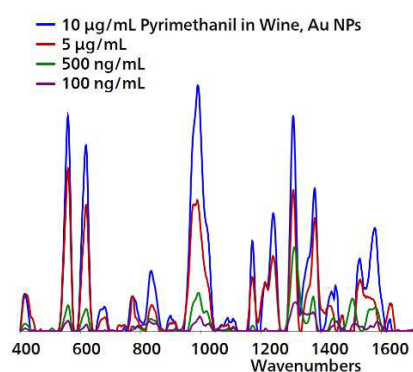
Table 1. Experimental parameters

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.

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CONFIGURATION



MISA Advanced

Metrohm Instant SERS Analyzer (MISA) 是一款高性能、便携式分析系统,可快速检测/鉴定非法物质、食品添加剂和微量食品污染物。MISA 的特点是配备了 Metrohm 的轨道光栅扫描 (ORS) 技术设备的光谱仪。其空间需求小和并且电池寿命有所延长,适用于现场测试或移动实验室应用。MISA 提供各种 1 级激光附件,可灵活选择取样。该分析仪可通过 Bluetooth 或 USB 连接运行。

MISA Advanced 套件是一个完整套件,其作用是让用户能用 Metrohms 纳米颗粒溶液和 P-SERS 试剂条进行分析。

MISA Advanced 套件包含了一个 MISA 小管附件、一个 P-SERS-附件、一个 ASTM 校正标准件、一个 USB 迷你电缆、一个 USB 供电单元和用于运行 MISA 仪器的 MISA Cal 软件。还随供了一个用来安全保管仪器和附件的坚固保护箱。



ID – Au NP

ID 套件 - Au NP 包含了 Mira/Misa 用户使用胶体金溶液进行 SERS 分析所需的组件。该套件包含了一个一次性抹刀、一个移液管、样品小瓶和一个含金胶体的瓶子。