



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 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

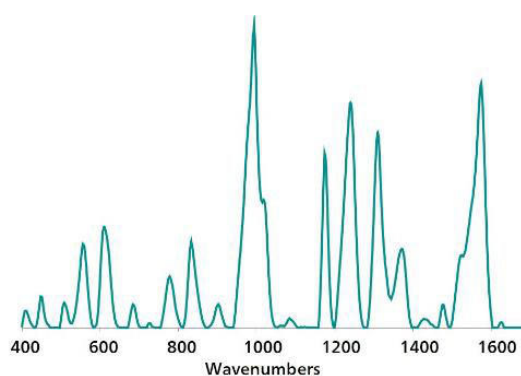
This application note describes a procedure for trace detection of pyrimethanil in white wine. A very simple sample extraction process results in very sensitive

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 µg/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 µg/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.



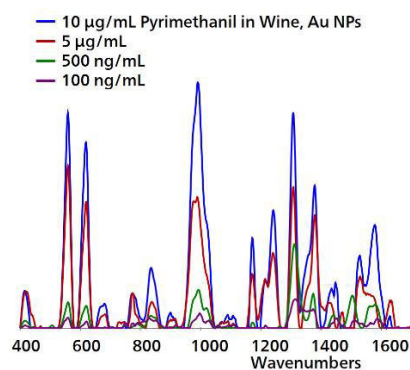
**Table 1.** Experimental parameters

| 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

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