



Application Note AN-RS-014

# Trace Detection of Rhodamine B in Cayenne Powder

## Protecting consumer safety with Misa

The addition of dyes to provide uniform coloration and enhance visual appeal in food products is a common practice. Rhodamine B is a dye utilized extensively in biotechnology and industrial applications and is one of several colorants banned for use as food additives in Europe and North America. The most common analytical methods for detection of illicit dyes in food products, GC/MS and HPLC, are laboratory-based instrumental methods that require

specialized training. With Misa (Metrohm Instant SERS Analyzer), detection of trace amounts of Rhodamine B in ground cayenne pepper is quick and easy after a facile extraction procedure with minimal material consumption. Rhodamine B can be detected in cayenne powder at a concentration of 50 µg/g. However, a simple concentration step improves that limit to 10 µg/g.

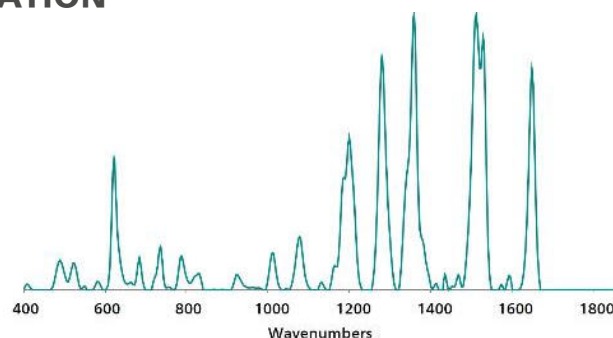
## INTRODUCTION

Ground cayenne pepper bought commercially was doped with Rhodamine B (RhB) and tested

with Misa to simulate a realistic food screening scenario.

## REFERENCE MATERIAL AND LIBRARY CREATION

To establish a reference spectrum, a pure RhB standard (50 µg/g in ultrapure water) was analyzed using gold nanoparticles (Au NPs). The unique SERS spectrum shown in **Figure 1** can be used to create a library entry for RhB.



**Figure 1.** Gold NP SERS standard reference spectrum of Rhodamine B.

## EXPERIMENT

A stock solution of RhB in methanol was prepared. Purchased cayenne powder was treated with serial dilutions of the stock (3 mL stock to 1 g cayenne) to yield samples with 1000, 500, 100, 50, 10, 5, and 1 µg/g of RhB. Samples were thoroughly mixed and air-dried. To prepare extracts, 0.1 g of each spiked sample was added to a vial with 400 µL of methanol, shaken to mix, and left to settle for 10 minutes. To prepare test samples, 50 µL of the methanol extract was pipetted into a vial with 400 µL of Au NP solution and 50 µL of 0.5 mol/L salt solution. The vial was shaken to mix, and then placed into the vial attachment on Misa for testing.



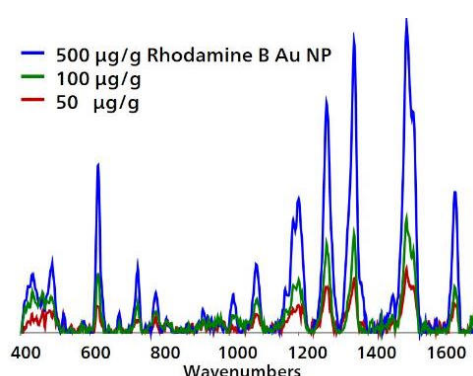
**Table 1.** Experimental Parameters

| 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

In **Figure 2**, overlaid spectra of RhB indicate detection down to 50 µg/g. For each concentration tested, the baseline spectrum

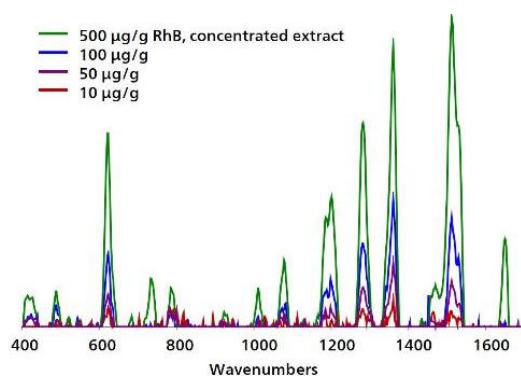
from unadulterated cayenne was subtracted from the average of baseline-corrected, replicate measurements.



**Figure 2.** Gold NP SERS concentration profile of RhB extracted from adulterated cayenne powder. Spectra are baselined, with Au NP and control subtracted.

To improve trace detection and spectral signal-to-noise, a very simple concentration method was applied to each extract. All extracts were fully air-dried, then resuspended in methanol to

yield a 5x increase in concentration. The spectra in **Figure 3** demonstrate detection of RhB down to 10 µg/g.



**Figure 3.** RhB profile after 5x concentration.

## FIELD TEST PROTOCOL

### Detection of Rhodamine B in the field

Using the large end of the scoop, add 3–4 scoops of sample to a 2 mL vial. Add methanol to the vial until ~1/3 full. Cap and shake the vial gently to mix, then let sample rest for 2 minutes. Fill a clean vial halfway full with Au NPs. Using

pipettes, add 1 drop each of sample solution and NaCl solution to the 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       |                            |
| Methanol       |                            |
| NaCl solution  | 3 g NaCl in 100 mL water   |
| Test settings  | Use ID Kit OP on MISA      |

## CONCLUSION

Trace levels of detection, ease of sample preparation, and rapid assay times collectively recommend Misa as a reliable, cost-effective

solution for high-throughput, on-site identification of adulterated food products.

## CONTACT

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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 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。

