



Application Note AN-RS-028

SERS检测亮蓝(Brilliant Blue)

Overcoming fluorescence issues with Misa

Brilliant Blue (BB) FCF, more commonly known as FD&C Blue #1, is the most commonly used blue dye worldwide for food and beverages. It is generally accepted as safe and non-toxic. Aside from foods labelled as organic or as free from artificial dyes, there is little objection to the use of BB at levels at or exceeding 100 µg/g in foods. **This application for Misa (Metrohm Instant SERS Analyzer) is unique.** The benefit is twofold — successful detection of a fluorescent dye, and a unique sample cleanup technique that permits detection of a target that does not exhibit a strong SERS signal and is present in a complex

matrix. It is well known that Raman identification can be overwhelmed by fluorescence, and sometimes SERS can be used as an alternative method of detection. In addition to being a strongly fluorescent dye, BB has a weak SERS signal; detection of such targets often requires extensive sample extraction before the SERS signal is detectable. While Misa successfully detects BB in direct sampling, this application describes a simple extraction method that improves detectability of BB with Misa.

INTRODUCTION

This application note describes a procedure for detection of BB in a flavored drink mix. The assay is based on the acquisition of SERS-specific

spectra for BB in aqueous and chloroform extracts using Misa and gold nanoparticles (Au NPs).

REFERENCE SPECTRUM AND LIBRARY CREATION

To establish a reference spectrum, pure BB standard at a concentration of 500 µg/mL in water was analyzed using Au NPs. The unique

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

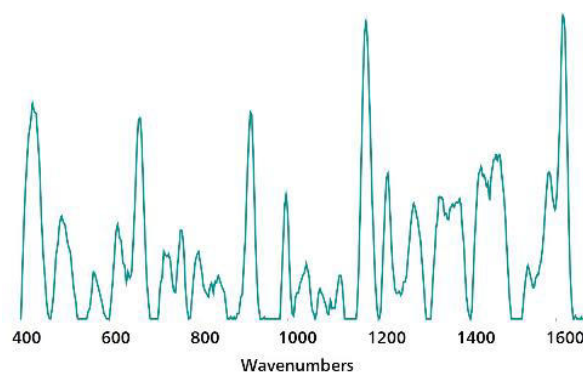


Figure 1. Standard Au NP SERS reference spectrum for Brilliant Blue.

EXPERIMENT AND RESULTS

In a direct test for the presence of BB in a flavored drink mix, 100 mg of «blue raspberry» drink mix was dissolved in 1 mL of water. 50 µL of this solution was added to a vial containing 450 µL of Au NPs, followed by 50 µL of 0.5 mol/L NaCl. The vial was briefly shaken and inserted into the vial attachment on Misa for measurement.

The resulting spectrum, seen in **Figure 2**, shows some peak agreement with the reference spectrum. However, **Figure 2** differs in intensity and shape from the reference spectrum of BB, due to the complex sample matrix. Signals from other components in the mix can compromise library matching and target identification; thus, a simple extraction process was employed to improve the SERS signal for BB.



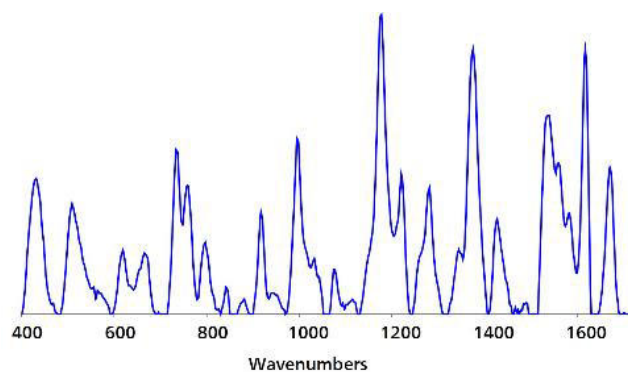


Figure 2. Direct Au NP interrogation for BB in a flavored drink mix.

In a glass vial, 40 mg of sample was dissolved in 1 mL of benzethonium chloride solution (2 mg/mL in water). Benzethonium Cl is a cationic surfactant used to capture the anionic dye. Chloroform (0.5 mL) was added to this vial, the mixture was vortexed for 30 seconds, and then rested for 5 minutes to permit phase separation. 200 μ L of the lower chloroform layer was

carefully transferred by pipette to a fresh vial, which was placed on a hot plate for evaporative drying. Afterward, 450 μ L of Au NPs and 50 μ L of 0.5 mol/L NaCl were added to the dried residue. This vial was capped, shaken to mix, and immediately placed into the vial attachment on Misa for measurement.

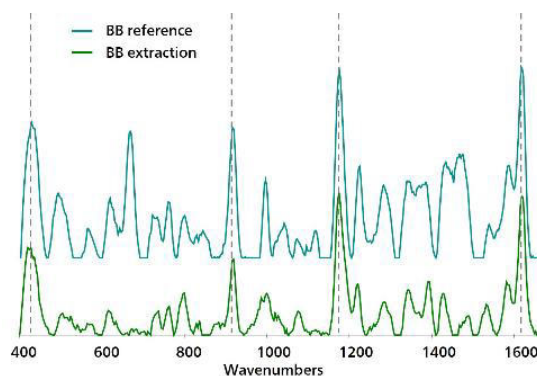


Figure 3. A comparison of the Au NP reference spectrum for BB with the BB spectrum obtained after chloroform extraction.

The stacked spectra in **Figure 3** confirm that this simple sample cleanup yields a BB spectrum with

a profile much closer to the reference spectrum.

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

FIELD TEST PROTOCOL

Detection of Brilliant Blue in the field

Using the large end of the scoop, add 3–4 scoops of sample to a 2 mL vial. Using clean pipettes for each reagent, add benzethonium Cl solution to the vial until halfway full, followed by 10 drops of chloroform. Cap and shake the vial vigorously to mix, then let sample rest for 5 minutes. Using a pipette, carefully remove a

portion of the *lower layer* and add 8 drops of this extract to a *clean vial*, then evaporate the solvent via heating on a hot plate. Fill this vial halfway with Au NPs, add 4 drops of NaCl solution, and then cap and shake the vial gently to mix. Insert into the 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	
Benzethonium Cl	0.2 g in 100 mL water
Chloroform	
NaCl solution	3 g NaCl in 100 mL water
Test settings	Use ID Kit OP on MISA

CONCLUSION

Misa successfully confirms the presence of a fluorescent dye in a complex food matrix. The identification of **Brilliant Blue** in a flavored drink mix is unique in that it overcomes fluorescence while avoiding extensive sample cleanup,

advanced spectral processing, and the complexity and expense of laboratory instrumentation. Contact Metrohm Raman for advice in adapting your custom application for Misa.

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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。