



Application Note AN-RS-028

# SERS Detection of 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 g/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.

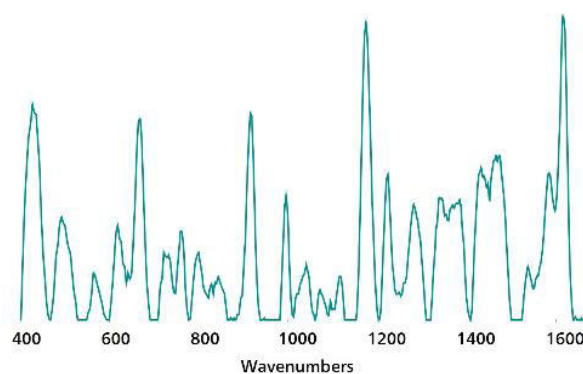
This application note describes a procedure for detection of BB in a flavored drink mix. The assay is based on the acquisition of SERS-specific spectra

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 g/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.



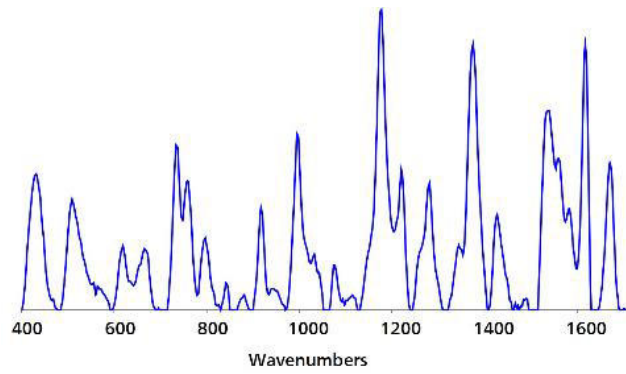
**Figure 1.** Standard Au NP SERS reference spectrum for Brilliant Blue.

## 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 L of this solution was added to a vial containing 450 L of Au NPs, followed by 50 L 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.

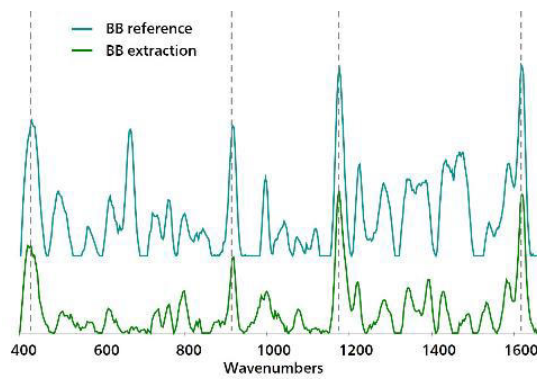




**Figure 2.** Direct Au NP interrogation for BB in a flavored drink mix.

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 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 L of Au NPs and 50 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.

## EXPERIMENT AND RESULTS

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

<b>ID Kit - Au NP</b>	<b>6.07506.440</b>
includes:	Gold nanoparticles (Au NP)
	Scoop
	Disposable pipettes
	2 mL glass vials
<b>Reagents</b>	
Benzethonium Cl	0.2 g in 100 mL water
Chloroform	
NaCl solution	3 g NaCl in 100 mL water
<b>Test settings</b>	Use <b>ID Kit OP</b> 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.

## CONTACT

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## CONFIGURATION



### MISA Advanced

Metrohm Instant SERS Analyzer (MISA) / MISA Metrohm (Orbital Raster Scan Technologie, ORS)

MISA 1BluetoothUSB

MISA Advanced MetrohmP-SERSSERS

MISA Advanced MISAP-SERSASTMUSBUSBMISA

MISA Cal



ID - Au NP

ID - Au NPMira/MisaSERS