RAMAN APPLICATION NOTE RS-021

Trace Detection of Erythrosine B in Sugar

Protecting consumer safety with Misa

Erythrosine B (EB), also known as Red Dye #3, is a synthetic dye approved for use in candy in the US, and in pharmaceuticals and cosmetics in the EU and elsewhere. However, rodent studies suggest that ingestion of EB can promote thyroid tumor formation. EB may also be implicated as a dietary factor contributing to hyperkinesis in children. WHO recommends a daily intake of EB less than 0.1 mg/kg of body weight. While consumption of EB below this threshold is deemed acceptable, it is important to monitor the use of EB to ensure that dietary guidelines are both appropriate and adequately enforced.

With Misa (Metrohm Instant SERS Analyzer), the sensitive and selective detection of EB is demonstrated in a simple assay format easily adapted for on-site surveillance testing. The ability to obtain fast results with a portable test platform recommends Misa as a competitive and cost-effective alternative to laboratory technologies (e.g., CE, HPLC) currently employed for detecting EB in foodstuffs.

INTRODUCTION

Misa is a portable screening tool for detecting food additives, including chemical colorants. In this application note, a facile extraction and analysis procedure is used to detect EB in spiked sugar and colored decorating sugar.

REFERENCE SPECTRUM AND LIBRARY CREATION

To establish a reference spectrum for EB, a pure standard in water (5 µg/mL) was analyzed using gold nanoparticles (Au NPs). The unique SERS spectrum shown in **Fig. 1** can be used to create a library entry for EB.

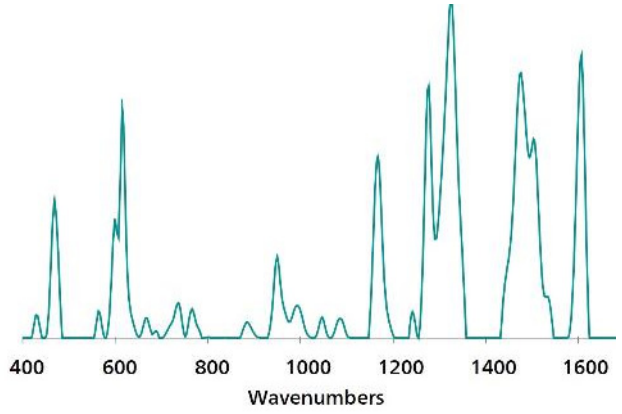

Figure 1. Standard SERS reference spectrum of erythrosine B.

EXPERIMENT

To simulate testing for EB in a food product, solid EB was mixed thoroughly with pure sugar to prepare a concentration range of test samples: 100, 50, 25, and 10 µg/g. To extract EB, 100 mg of each dry sample was dissolved in 1 mL of ethanol and allowed to sit for 5 minutes. A test sample was prepared by pipetting 100 µL of the ethanolic extract into a glass vial containing 800 µL of Au NPs and 100 µL of 0.5 mol/L NaCl. The sample was shaken and placed into the Misa vial attachment for analysis.

To test for EB in Betty Crocker Pink™, a commercially available colored decorating sugar, samples were prepared as previously described.

Table 1. Experimental Parameters

Instrument		Acquisition	
Firmware	0.9.33	Laser Power	5
Software	Misa Cal V1.0.15	Int. Time	1 s
Misa Vial Attachment	6.07505.040	Averages	10
ID Kit - Au NP	6.07506.440	Raster	ON

RESULTS

Overlaid SERS spectra for ethanolic extracts of sugar spiked with EB demonstrate detection down to 10 µg/g.

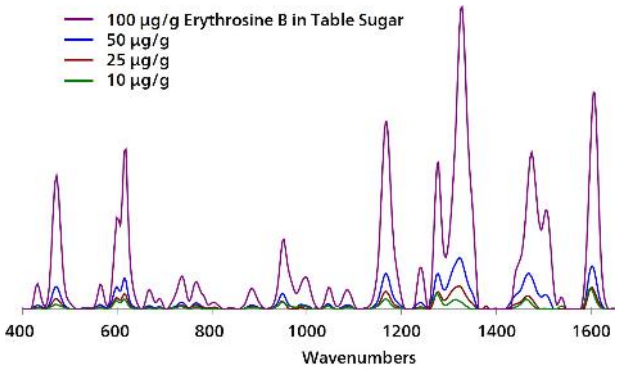

Figure 2. Overlaid, baselined, background-subtracted SERS spectra of EB in sugar with Misa and Au NPs.

Based on peak comparison to reference spectra recorded for the spiked sugar samples, the presence of EB in pink sugar is confirmed. Note: Minor peak shifts, as evident in **Fig. 3,** do not compromise library identification capabilities.

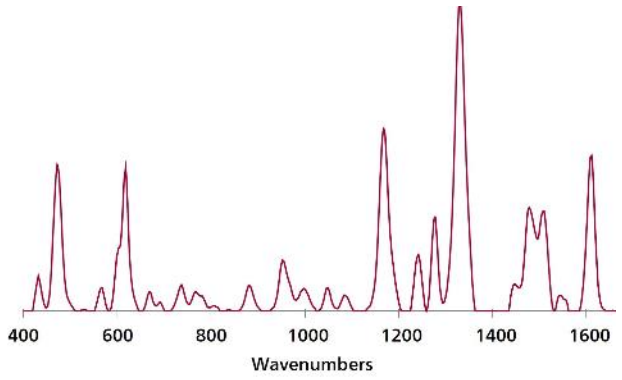

Figure 3. Overlaid, baselined, background-subtracted SERS spectra of EB in sugar with Misa and Au NPs.

FIELD TEST PROTOCOL

Detection of erythrosine B in the field.

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	
Ethanol	
NaCl Solution	3 g NaCl in 100 mL water
Test Settings	Use **ID Kit OP** on Misa

Using the large end of the scoop, add 3–4 scoops of sample to a 2 mL vial. Add ethanol to the vial until halfway full. Cap and shake the vial gently to mix, then let sample rest for 5 minutes. Fill a *clean vial* halfway full with Au NPs. Using pipettes, add 2 drops each of sample solution and NaCl solution to Au NPs, then cap and shake the vial gently to mix. Insert into vial attachment on Misa for measurement.

CONCLUSION

Misa provides a robust solution for detecting EB in processed foods. This analysis requires minimal user training and consumables, thereby providing a facile analytical methodology for on-site chemical surveillance in both high- and low-resource testing environments.

Analytes:	**Colorants – dyes, pigments, inks**
Matrix:	**Food – additives**
Method:	**Spectroscopy (NIRS/Raman)**
Industry:	**Food & beverage**