



Application Note AN-RS-036

Trace Detection of Toxic Dye in Saffron

Protecting consumer safety with MISA

Saffron, a precious spice comprised of dried stigmas from the purple *Crocus sativa* flower, is prized for its many health benefits and for its distinctive color, aroma, and delicate flavor. It is the most expensive spice in the world by weight, due to the labor- and time-intensive process of collecting individual filaments by hand. Saffron quality in the international trade market is certified under ISO 362-1. However, illicit producers sell low-grade or counterfeit saffron to unsuspecting consumers and reap substantial profits. The challenge in authenticating saffron is due to the variety of strategies used to mimic a pure product,

including dyes and the inclusion of flower parts from different species. Successful methods for authentication of saffron typically require complex analytical methodologies in combination with chemometric methods, which does not address the rising demand for cost-effective, on-site analysis and interdiction of counterfeit saffron in the field. SERS (Surface-Enhanced Raman Scattering) detection of a toxic dye (Sudan 1) used to adulterate saffron demonstrates the power of MISA (Metrohm Instant SERS Analyzer) for simple, portable food authentication in this Application Note.

AUTHENTICATION OF SAFFRON

The most common form of saffron adulteration is the marketing of dyed and dried stigmas and stamens from other flower species to imitate saffron's distinctive visual and sensory properties. In this application, crocin, a carotenoid ester that is primarily responsible for saffron's distinct coloration, is extracted and compared with Sudan 1, an orange-red azo dye and a known carcinogen. Sudan 1 is banned

for use in foods worldwide, yet is frequently used for the illicit coloring of costly spices. In a manner consistent with saffron counterfeiting, non-saffron flower parts are dyed and mixed with authentic saffron for this application. This mixture is extracted and compared to both saffron and Sudan 1 standards to demonstrate the ability of SERS to differentiate these strongly colored compounds.

MATERIALS AND METHODS

High-quality Negin saffron from Iran was purchased from a commercial supplier. Plant material used for simulating saffron adulteration with biological material consisted of dried stigmas and stamens harvested from flowers purchased at a local grocer. Sudan 1 was sourced from a chemical supplier. Raman spectra were collected directly from purchased saffron, placed in a glass vial, and inserted into the vial holder on MISA. A SERS reference spectrum for saffron required extraction of 100 mg of pure saffron

with 1 mL of methanol, then addition of 100 μ L each of extract and 0.5 mol/L NaCl to 800 μ L of Au nanoparticles in a glass vial. The SERS sample was mixed, inserted into the vial holder, and analyzed with the SERS OP on MISA. SERS analysis of saffron adulterated with Sudan 1 consisted of a 1:1 (w/w) mixture of pure saffron and flower parts which had been soaked in a 1 mg/mL solution of Sudan 1 in methanol and dried. Extracts for analysis were prepared as for pure saffron above.

RESULTS AND DISCUSSION

Saffron can be detected directly with Raman analysis, although authentication would likely require a combination of Raman and chemometrics. This is primarily because highly colored materials, such as the

crocin compounds that lend their color to authentic saffron and the dyes used in counterfeit products, can exhibit fluorescence that overwhelms the Raman signal.

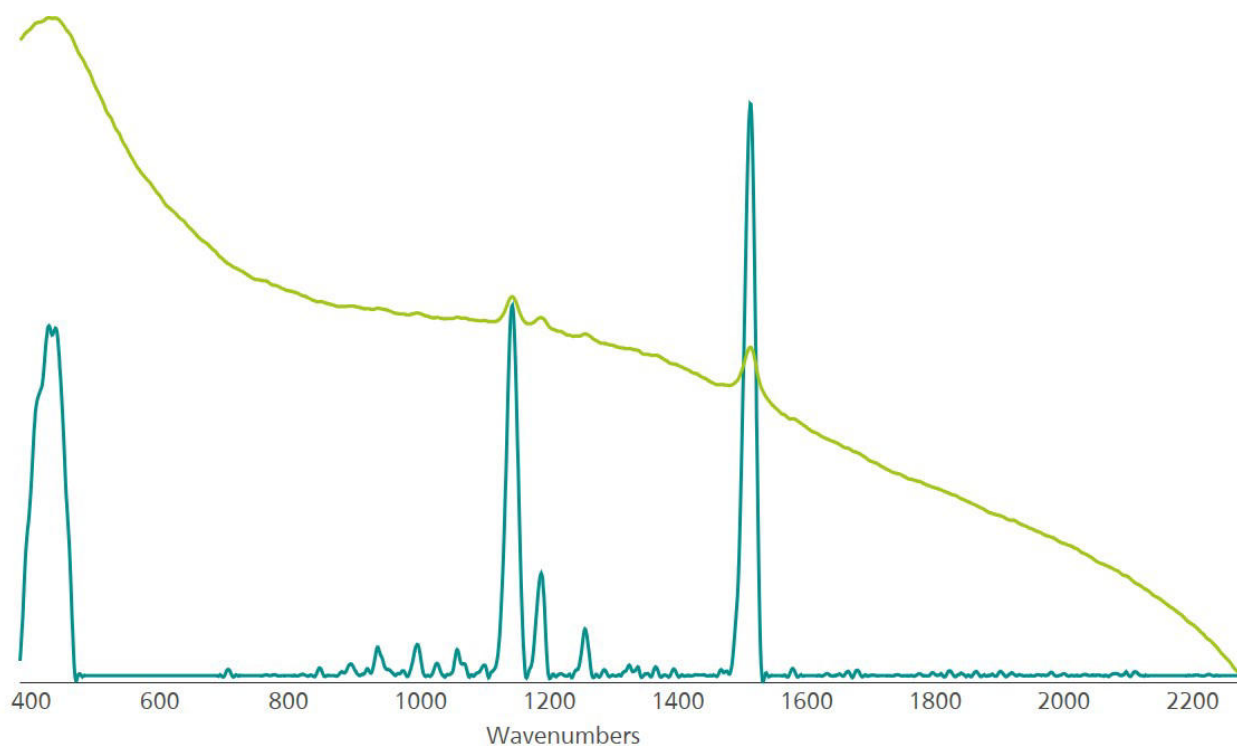


Figure 1. Saffron's distinctive Raman spectrum: unaltered (top) and baselined (bottom).

This is demonstrated in **Figure 1**, where the unaltered Raman spectrum of saffron (top) displays the broad, distinctive signal of fluorescence. The baselined spectrum (bottom) contains signature peaks from crocin, but of low intensity. This is a classic example of an application where SERS can more sensitively

evaluate a specific analyte, because fluorescence has less of an influence on the SERS spectrum.

The SERS spectrum for pure saffron provides a useful standard for evaluating the authenticity of saffron, as shown in the bottom spectrum in **Figure 2**.

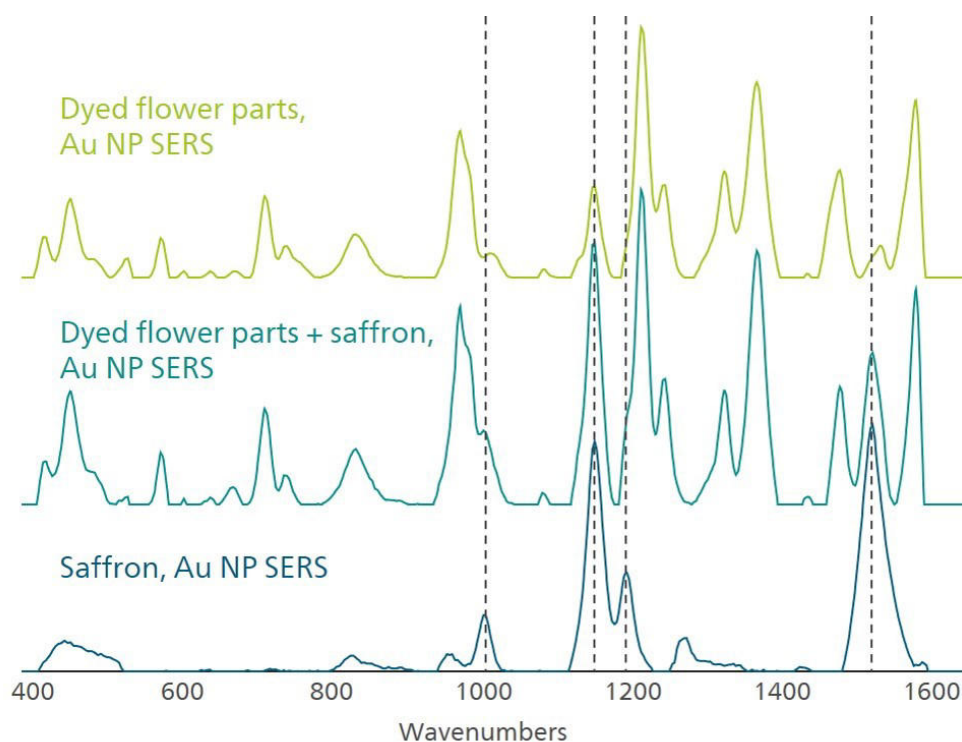


Figure 2. Visual confirmation in the SERS spectra of Sudan 1 (top), Saffron (bottom), and an experimental mixture of both.

The acquired peak profile agrees with reported spectra of crocin extracted from saffron. Sudan 1 at a concentration of 0.01 mg/mL also has a distinct and complex SERS spectrum, as seen in the top spectrum in **Figure 2**. When these distinct spectra are overlaid with the SERS spectrum of a mixture of pure and counterfeit saffron, both pure saffron and Sudan 1 can be distinguished. Finally, detection limits are

important for any trace detection application with SERS. Serial dilutions of 1 mg/mL Sudan 1 dye stock solution in methanol were used to demonstrate detection of Sudan 1 at concentrations as low as 500 ng/mL (**Figure 3**). With this level of sensitivity, the use of virtually any amount of this dye for saffron authentication can be detected with MISA.

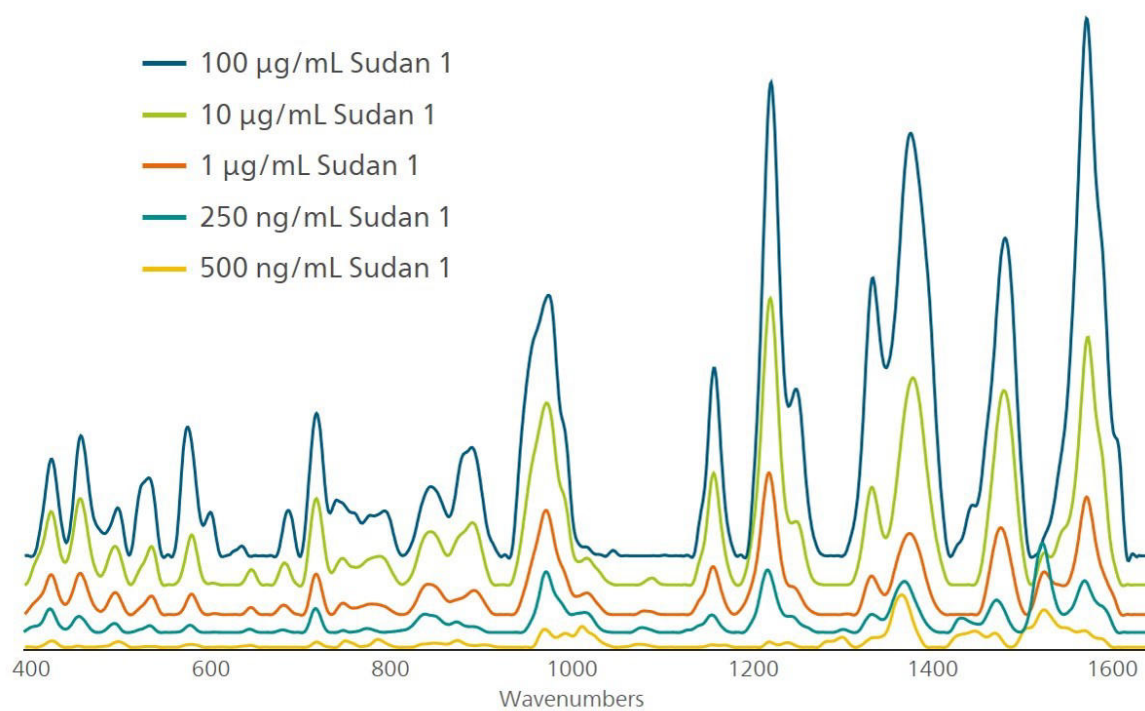


Figure 3. Concentration profile for Sudan 1, demonstrating MISA's detection capabilities down to 500 ng/mL.



CONCLUSION

Herein, saffron is authenticated and Sudan 1 is detected by SERS in a demonstration of the growing potential of portable Raman spectroscopy for the frontline defense of food safety and integrity. Given the strong SERS response of chemical structures

common to dyes and artificial food colorants, this application is likely to extend to other coloring agents used to enhance spices and mask inferior product. MISA from Metrohm Raman holds great promise as a versatile tool for protecting food safety.

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CONFIGURATION



MISA Basic

Metrohm Instant SERS Analyzer (MISA) ist ein hochleistungsfähiges, tragbares Analysensystem zur schnellen Detektion / Identifizierung von illegalen Substanzen, Lebensmittelzusatzstoffen und Lebensmittelverunreinigungen im Spurenbereich. MISA verfügt über einen hocheffizienten Spektrographen, der mit der einzigartigen Orbital-Raster-Scan (ORS)-Technologie von Metrohm ausgestattet ist. Er hat einen minimalen Platzbedarf und eine verlängerte Batterielebensdauer, perfekt für Tests vor Ort oder mobile Laboranwendungen. MISA bietet verschiedene Laser-Klasse-1-Aufsätze für flexible Probenahmeoptionen. Der Betrieb des Analysators ist über Bluetooth oder USB-Konnektivität möglich.

Das MISA Basic-Paket ermöglicht es den Benutzern, MISA an ihre Bedürfnisse anzupassen. Es ist ein Starterpaket, das die Basiskomponenten für die Durchführung von SERS-Analysen mit den Nanopartikellösungen von Metrohm enthält.

Das MISA Basic-Paket enthält das MISA-Gerät, ein MISA Vial Attachment, ein USB-Minikabel, ein USB-Netzteil und die Software MISA Cal zum Betrieb des Gerätes.



MISA Advanced

Metrohm Instant SERS Analyzer (MISA) ist ein hochleistungsfähiges, tragbares Analysensystem zur schnellen Detektion / Identifizierung von illegalen Substanzen, Lebensmittelzusatzstoffen und Lebensmittelverunreinigungen im Spurenbereich. MISA verfügt über einen hocheffizienten Spektrographen, der mit der einzigartigen Orbital-Raster-Scan (ORS)-Technologie von Metrohm ausgestattet ist. Er hat einen minimalen Platzbedarf und eine verlängerte Batterielebensdauer, perfekt für Tests vor Ort oder mobile Laboranwendungen. MISA bietet verschiedene Laser-Klasse-1-Aufsätze für flexible Probenahmeoptionen. Der Betrieb des Analysators ist über Bluetooth oder USB-Konnektivität möglich.

Das MISA Advanced-Paket ist ein Komplettpaket, das es dem Anwender ermöglicht, SERS-Analysen mit Metrohms Nanopartikelösungen und P-SERS-Streifen durchzuführen.

Das MISA Advanced-Paket enthält einen MISA Vial-Aufsatz, einen P-SERS-Aufsatz, einen ASTM-Kalibrierstandard, ein USB-Minikabel, ein USB-Netzteil und die MISA Cal-Software zum Betrieb des MISA-Geräts. Ein robuster Schutzkoffer zur sicheren Aufbewahrung des Instruments und des Zubehörs wird ebenfalls mitgeliefert.



Misa Probengläschenaufsatz

Geeignet für 15 x 26 mm Glasfläschchen. Der Misa-Fläschchenaufsatz kann für Messungen mit den Gold- oder Silber-SERS-Nanopartikeln von Metrohm verwendet werden. Betrieb in Laserklasse 1.