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 stamens and stigmas 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 µL each of extract and 0.5 mol/L NaCl to 800 µL 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.

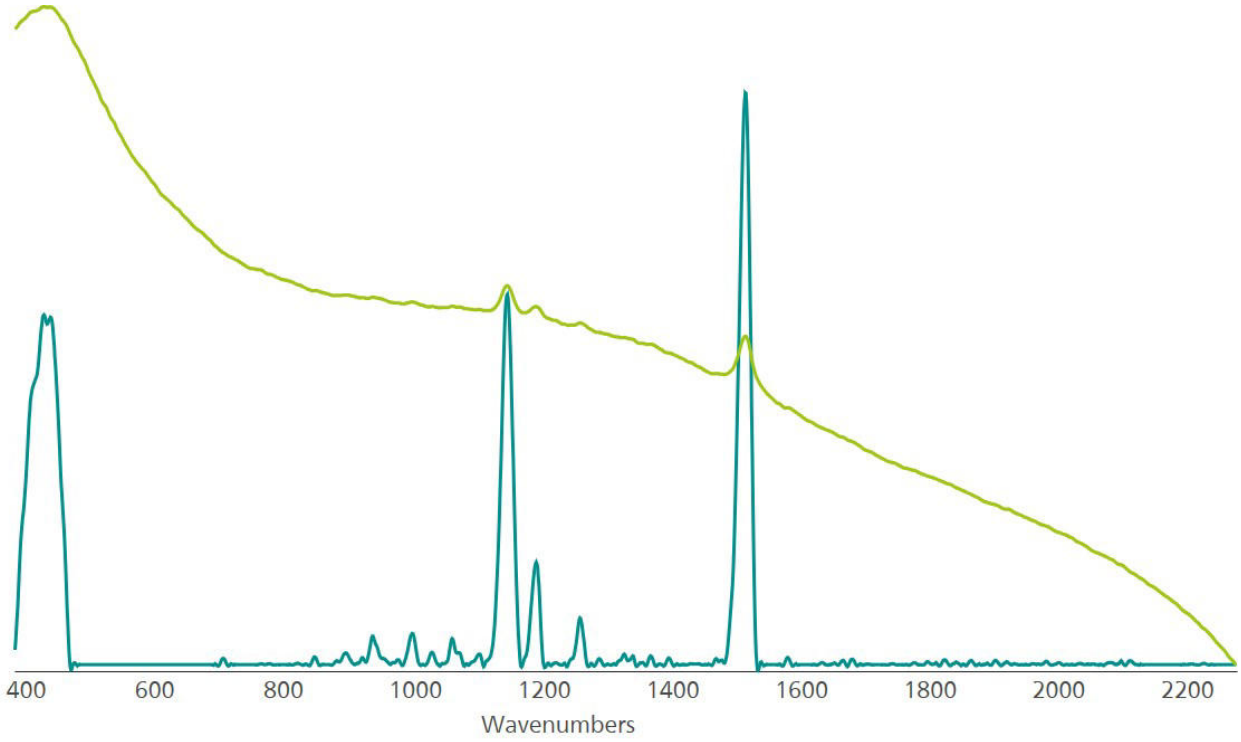

Figure 1. Saffron's distinctive Raman spectrum: unaltered (top) and baselined (bottom).

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.

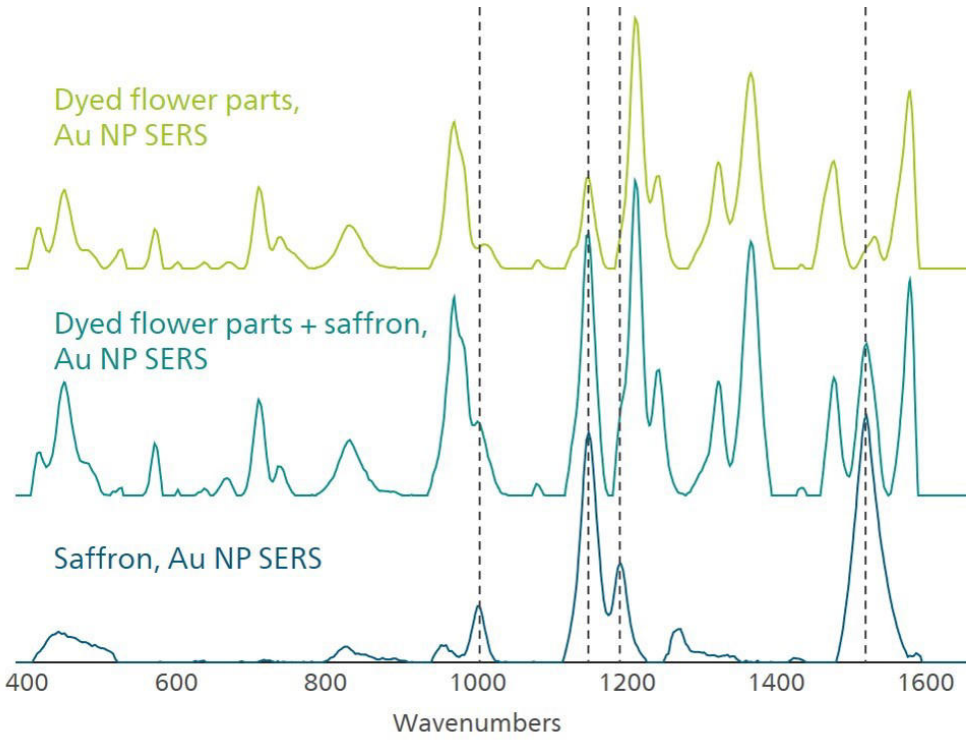

Figure 2. Visual confirmation in the SERS spectra of Sudan 1 (top), Saffron (bottom), and an experimental mixture of both.

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.

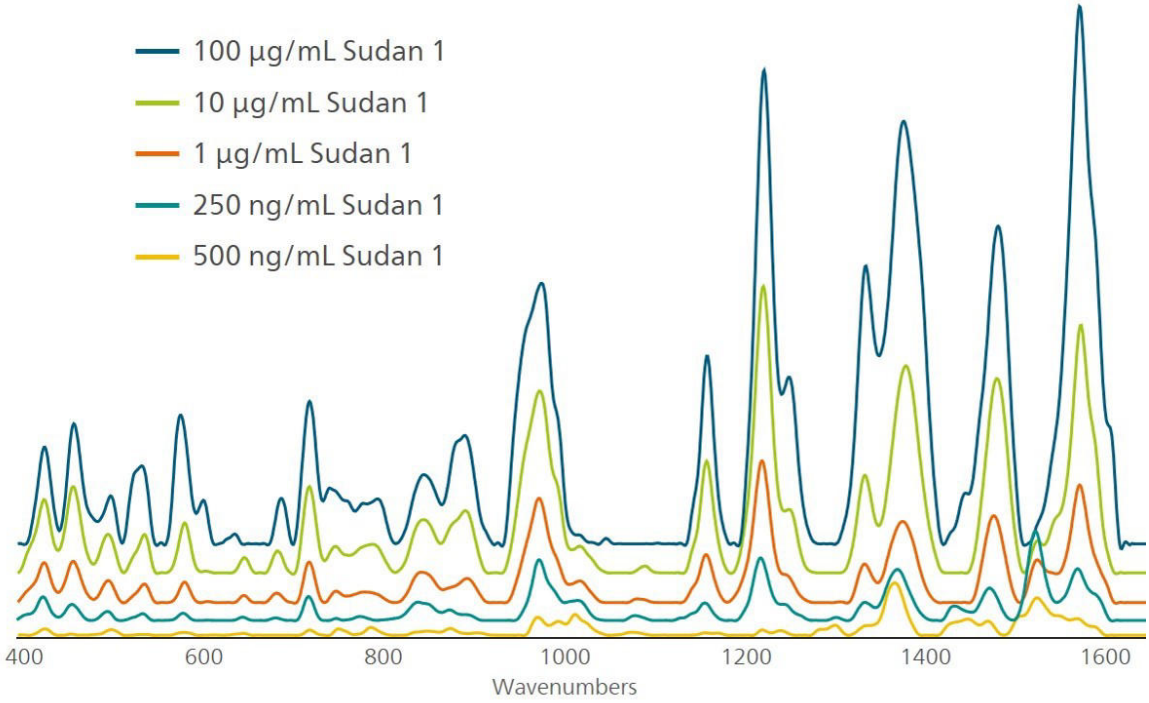

Figure 3. Concentration profile for Sudan 1, demonstrating MISA's detection capabilities down to 500 ng/mL.

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) is a high performance, portable analyzer system used for rapid, trace level detection / identification of illicit materials, food additives and food contaminants. MISA features a high-efficiency spectrograph equipped with Metrohm's unique Orbital-Raster-Scan (ORS) technology. It has a minimal footprint and extended battery life, perfect for on-site testing or mobile laboratory applications. MISA offers various Laser Class 1 attachments for flexible sampling options. Analyzer operation is available through BlueTooth or USB connectivity.

The MISA Basic package allows users to customize MISA to suit their needs. It is a starter package that contains the basic components for performing SERS analyses using Metrohm's nanoparticle solutions.

The MISA Basic package includes the MISA instrument, a MISA Vial Attachment, a USB Mini Cable, a USB Power Supply and MISA Cal software for operating the instrument.

MISA Advanced

Metrohm Instant SERS Analyzer (MISA) is a high performance, portable analyzer system used for rapid, trace level detection / identification of illicit materials, food additives and food contaminants. MISA features a high-efficiency spectrograph equipped with Metrohm's unique Orbital-Raster-Scan (ORS) technology. It has a minimal footprint and extended battery life, perfect for on-site testing or mobile laboratory applications. MISA offers various Laser Class 1 attachments for flexible sampling options. Analyzer operation is available through BlueTooth or USB connectivity.

The MISA Advanced package is a complete package that allows the user to perform SERS analyses using Metrohm's nanoparticle solutions and P-SERS strips.

The MISA Advanced package includes a MISA Vial Attachment, a P-SERS Attachment, a ASTM Calibration Standard, a USB Mini Cable, a USB Power Supply and MISA Cal software for operating the MISA instrument. A ruggedized protective case is also provided to securely store the instrument and accessories.

MISA Vial Attachment

Suitable for 15 x 26 mm glass vials. MISA vial attachment can be used for measurements with Metrohm's gold or silver SERS nanoparticles. Laser Class 1 operation.