Application Note AN-RS-020

Trace Detection of Auramine O in Curry Powder

Protecting consumer safety with Misa

Auramine O (AO) is an industrial dye used for a broad range of manufactured products and as a fluorescent stain for detecting acid-fast bacteria in clinical specimens. Due to its intense yellow coloration, AO is also prized as an additive for enhancing the visual appeal of illicitly processed food products. Curry powder is a likely target for such adulteration, as it is a bright yellow mixture of several spices. Health hazards associated with ingestion, and even improper handling of AO, include a high risk of several cancers, neural and liver toxicity, and even death. Despite bans on AO as a food additive, surveillance testing indicates its persistent use as an adulterant in foods and spices. Misa (Metrohm Instant SERS Analyzer) achieves the rapid and sensitive detection of AO in curry powder in a simple assay format.

INTRODUCTION

Misa is a versatile tool for the rapid and accurate detection of banned food colorants. This application note details a facile extraction procedure for detecting AO in adulterated curry powder.

REFERENCE MATERIAL AND LIBRARY CREATION

To establish a reference spectrum for AO, a pure standard in alkaline water (100 µg/mL, pH 13) was analyzed using gold nanoparticles (Au NPs). The unique SERS spectrum shown in Figure 1 can be used to create a library entry for AO.

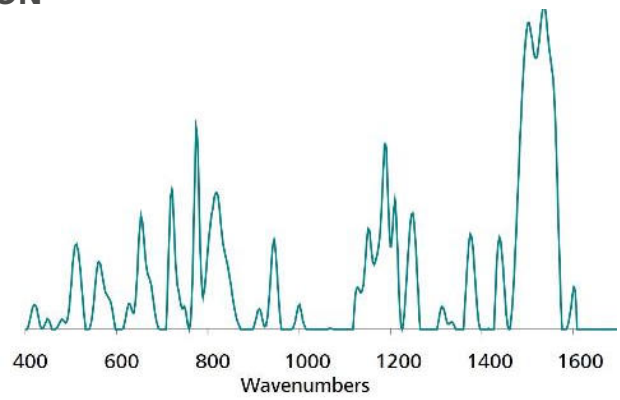

Figure 1. Standard SERS reference spectrum of Auramine O.

EXPERIMENT

In a simulated test for AO in curry powder, solid AO was mixed with purchased curry powder to yield a concentration range of spiked test samples: 1000, 100, 10, 5, and 1 µg/g. Liquid extraction of AO was performed by adding 1 mL of 0.1 mol/L NaOH to 100 mg of sample in a glass vial. This slurry was mixed and allowed to rest for 2 minutes. Ethyl acetate (EA, 1 mL) and NaCl (100 mg) were added to the vial, which was then inverted gently a few times (*do not shake vigorously*) to promote extraction of AO into the EA layer. After 10 minutes, 50 µL of the top EA layer was added to a vial containing 400 µL of Au NPs and 50 µL of 0.5 mol/L NaCl. The vial was shaken to mix and immediately placed in the vial attachment on Misa for measurement.

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

RESULTS

Overlaid, baseline-corrected SERS spectra of basic EA extracts of curry powder spiked with varying concentrations of AO demonstrate reliable detection down to 1 µg/g (Figure 2). Note: Peaks in AO SERS spectra show solvent and pH-related shifts.

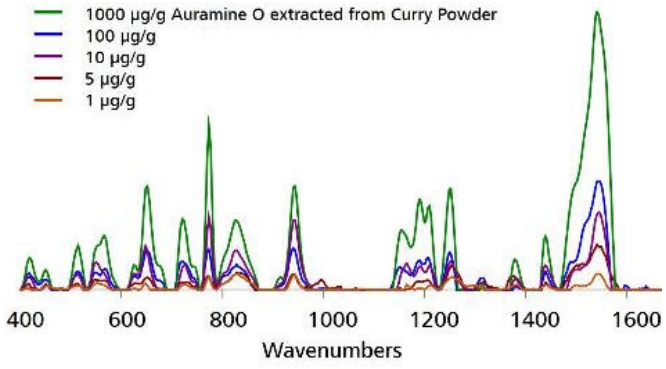

Figure 2. Detection range of AO with Misa and Au NPs.

FIELD TEST PROTOCOL

Detection of Auramine O in the field

Using the large end of the scoop, add 3–4 scoops of sample to a 2 mL vial. Add NaOH solution to the vial until halfway full. Add 3–4 scoops solid NaCl, then fill vial to the top with ethyl acetate. Cap and invert the vial a few times to mix, but *do not shake the vial vigorously*. Let the sample rest for 5 minutes, as distinct layers will form. Fill a *clean vial* halfway full with Au NPs. Using pipettes, add 2 drops each of the *top layer* of the sample solution and NaCl solution to Au NPs, cap and shake the vial gently to mix. Insert into 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	
NaOH solution	0.4 g NaOH in 100 mL water
Solid NaCl	
Ethylene acetate	
NaCl solution	3 g NaCl in 100 mL water
Test settings	Use ID Kit OP on MISA

CONCLUSION

The facile and sensitive detection of AO in adulterated curry powder is demonstrated using Misa. This analysis requires minimal user training and minimal consumables, making it an ideal analytical platform for on-site QC testing in food manufacturing, shipping, and receiving facilities. Misa's portability and ease-of-use in trace detection of illicit colorants outperforms complex extraction and analysis procedures in a laboratory setting.

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CONFIGURATION

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.

ID Kit – Au NP

The ID Kit - Au NP contains the components a Mira / Misa user requires to perform a SERS analysis using gold colloidal solution. The kit contains a disposable spatula, dropper, sample vials and a bottle of gold colloid.