



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.

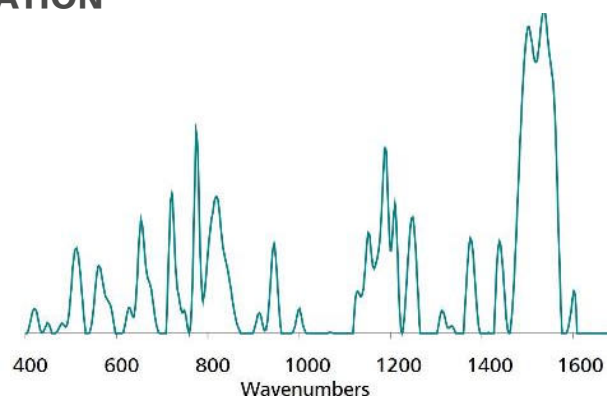
## INTRODUCTION

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  $\mu\text{g/mL}$ , 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  $\mu\text{g/g}$ . 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  $\mu\text{L}$  of the top EA layer was added to a vial containing 400  $\mu\text{L}$  of Au NPs and 50  $\mu\text{L}$  of 0.5 mol/L NaCl. The vial was shaken to mix and immediately placed in the vial attachment on Misa for measurement.



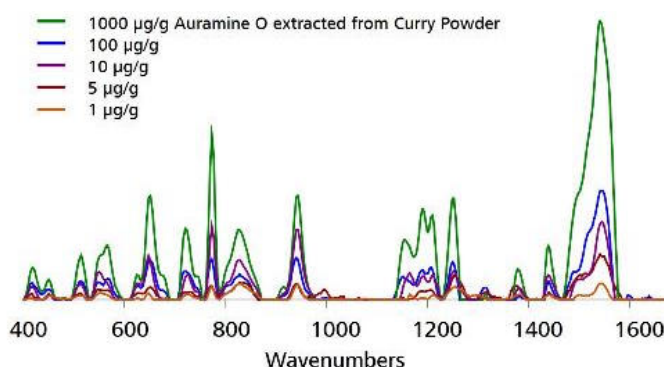
**Table 1.** Experimental parameters

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  $\mu\text{g/g}$  (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
<b>Reagents</b>	
NaOH solution	0.4 g NaOH in 100 mL water
Solid NaCl	
Ethylene acetate	
NaCl solution	3 g NaCl in 100 mL water
<b>Test settings</b>	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.

## CONTACT

メトロームジャパン株式会社  
143-0006 東京都大田区平  
和島6-1-1  
null 東京流通センター アネ  
ックス9階

[metrohm.jp@metrohm.jp](mailto:metrohm.jp@metrohm.jp)

## CONFIGURATION



### MISA Advanced

Metrohm Instant SERS Analyzer (MISA) は、微量レベルでの違法物質、食品添加物、および食品汚染物質の迅速な検出 / 同定のための高性能な携帯可能分析システムです。MISAは、Metrohm 独自の軌道ラスタースキャン技術 (Orbital Raster Scan Technologie, ORS) を備えた高効率の分光器を有しています。これは省スペースで、より長いバッテリー寿命を持ち、現場やラホでの移動式用途にも完璧に適しています。MISA ではフレキシブルなサンプル採取を可能にする、レーザークラス1の様々なアタッチメントをご利用いただけます。アナライザーはBluetoothまたはUSBコネクタを介して操作可能です。

MISA Advanced ハッチケースは、ユーザーに Metrohmのナノ粒子溶液とP-SERSストリップを用いたSERS分析を可能にするコンフリートハッチケースです。

MISA Advanced ハッチケースには、MISAハイアルアタッチメント、P-SERSアタッチメント、ASTM校正標準、USBミニケーブル、USB電源装置、ならびにMISA装置を操作するためのMISA Calソフトウェアが含まれます。装置と付属品を安全に保管するための頑丈な保護ケースも同梱されています。



### ID - Au NP

IDキット - Au NPには、Mira/Misaユーザーが金コロイド溶液でSERS分析を行うのに必要なコンポーネントが含まれています。このキットには、使い捨てのへら、滴下ヒベット、サンプルホルダー、および金コロイド入りのホルダーが含まれています。