Application Note AN-RS-051

拉曼光谱技术快速检测膳食补充剂中的非法添加物

Interrogation of «honey for men» with Raman spectroscopy

Often advertised as alternative medicines, dietary supplements are actually classified as food in both the European Union and the United States. As such, these products don't get the same testing, quality control, labeling, or regulation as pharmaceuticals. This provides motivation for some manufacturers to inflate the effects of a supplement through illegal adulteration with undeclared substances like pharmaceuticals, analogues, or unauthorized additives. This practice provides rapid results and increases sales, but it can also lead to health complications from side effects and acute intoxication. For instance, sexual and sport performance supplements commonly contain undeclared caffeine, steroids, hormones, and pharmaceuticals for treating impotence. From increased aggression to heart attack and overdose, the resulting side-effects can be dire. One of these products, «hot honey» or «honey for men», is an easily sourced dietary supplement that promises its users power and vitality. Metrohm's SERS solutions offer a fast, sensitive, and portable way to analyze such supplements on-site, without interference from the sample matrix.

INTRODUCTION

Analysis of dietary supplements is traditionally time-consuming, costly, and dependent on laboratory-based techniques like HPLC and GC-MS. Rapid on-site methods are needed for surveying the supplement landscape. Surface-enhanced Raman scattering (SERS) meets these requirements; it is easy to use, highly sensitive, fast, inexpensive, and portable. SERS utilizes nanoparticle substrates to amplify the Raman signal and produce a fingerprint spectrum of very low-level analytes. Metrohm's P-SERS substrates – convenient, single-use tests featuring nanoparticles printed onto paper strips – are ideal for detecting diverse trace materials in a quick and cost-effective way.

PROOF OF CONCEPT

A publication from Wageningen University & Research detailed an extensive study on the development of a SERS-based method for rapid detection of illegal adulterants in dietary supplements [1]. Prof. van Ruth's group carried out SERS studies with the goal of providing a qualitative screening method for detection of illicit adulterants in dietary supplements.

Qualitative screening and detection

Twenty-three pharmaceutically active adulterants were examined for SERS activity. Eleven of these analytes gave very good signals when tested with silver P-SERS substrates and the Metrohm Instant SERS Analyzer (MISA). These were included in a custom reference library of adulterants used for the screening of experimental samples. The active analytes and their current status in dietary supplement formulations as established by the European Commission (EC) are listed in Table 1.

Table 1. SERS-active adulterants, their type, and current EC status in dietary supplement formulations as established by the European Commission, and a summary of results including Hit Quality Index (HQI) indicating the level of correlation between sample and library spectra and limit of identification (LOI) for each adulterant.

Analyte	Supplement Type	EC Status	HQI	LOI (% w/w)
Acetildenafil	Erectile dysfunction	Forbidden	0.79–0.99	0.1
Fluoxetine HCl	Weight loss	Forbidden	0.71–0.83	5.0
Homo sildenafil	Erectile dysfunction	Forbidden	0.79–0.99	0.1
Melatonin	Sleep regulation	Allowed	0.79–0.93	5.0
Phenethylamine	Energy	Forbidden	0.81–0.94	0.5
Piperin	Weight loss	Allowed	0.70–0.92	2.5
Sildenafil citrate	Erectile dysfunction	Forbidden	0.79–0.99	0.1
Synephrine	Weight loss	Allowed	0.70–0.81	5.0
Thiosildenafil	Erectile dysfunction	Forbidden	0.60–0.99	0.1
Vardenafil HCl	Erectile dysfunction	Forbidden	0.81–0.95	0.1
Vinpocetine	Memory support	Allowed	0.61–0.98	0.5

Interference from excipients

SERS's unique insensitivity towards many excipients is a crucial advantage of this technique. A mixture of common excipients was carefully formulated to represent the filling material used in market supplements. This filler consisted of microcrystalline cellulose (95.0% w/w), magnesium stearate (3.0% w/w), silicon dioxide (1.0% w/w), hydroxypropyl methylcellulose (0.5% w/w), and titanium oxide (0.5% w/w)—each of which was evaluated for SERS activity. These were confirmed to exhibit low/no SERS activity and were not expected to interfere with detection of the target adulterants.

Limit of identification (LOI)

To estimate the detectability range of each adulterant, different concentrations were mixed with the excipient mixture (0.1–50.0% w/w). Each artificially adulterated sample was processed as follows: 10 mg of each sample was weighed into an Eppendorf tube and diluted with 100 µL of ethanol. Each sample was vortexed and centrifugated, then 10 µL of the supernatant was dropped onto the silver P-SERS strip, dried for 15 minutes, and analyzed.

DIETARY SUPPLEMENT ADULTERATION

Eighteen different commercial supplements, including nine weight-loss and lifestyle and nine sexual and sport enhancement supplements, were sourced, tested for SERS activity, and adulterated within the 0.1% to 5.0% w/w range. Ethanol, used to extract the target without dissolving the matrix, was simply shaken in a small vial with the sample, then the supernatant was applied directly to a P-SERS strip. After drying, instrumental analysis commenced. MISA Cal software produces a result within seconds, accompanied by a Hit Quality Index (HQI) indicating the level of correlation between sample and library spectra. The LOI for each adulterant indicates the lowest concentration at which the HQI > 0.70 (Table 1).

RESULTS

SERS was highly effective in detecting all of the target sexual and sport enhancement adulterants (i.e., acetildenafil, homo sildenafil, sildenafil citrate, thiosildenafil, and vardenafil HCl), with high match scores (0.79–0.99), even at the lowest concentration level tested (LOI of 0.1% w/w). This indicates successful detection of some of the best-known pharmaceutical products on the market (Viagra® for sildenafil and Levitra® for vardenafil) and their unapproved analogues well below active therapeutic doses. SERS was also capable of detecting the weight-loss and lifestyle analytes vinpocetine and phenethylamine with a LOI of 0.5% w/w, piperin with a LOI of 2.5% w/w, and melatonin, fluoxetine HCl, and synephrine with a LOI of 5.0% w/w.

REAL-WORLD APPLICATION

«Hot honey» or «honey for men» is an easily sourced dietary supplement that promises power and vitality. However, the FDA warns that such honey products may contain undisclosed drugs for erectile dysfunction, including sildenafil and tadalafil, in active amounts [2].
Metrohm Nederland used MISA to test seized honey samples and illustrate the utility of Metrohm's SERS solutions in the fight against illegal adulteration. An essential part of this application is the testing of multiple SERS substrates to optimize the target signal. While Prof. Saskia's group focused largely on silver P-SERS strips for their convenience, the Metrohm group also tested gold P-SERS strips in addition to gold and silver colloid solutions.

Sample preparation

Four samples of honey were provided, each with known concentrations of sildenafil and tadalafil confirmed by GC-MS (Table 2).

To prepare the samples for SERS analysis, a portion of each sample was simply dissolved into 1 mmol/L sodium hydroxide, then shaken with sodium chloride and isopropyl alcohol (IPA). After separation, the organic fraction was applied directly to P-SERS and colloid SERS substrates (Figure 1).

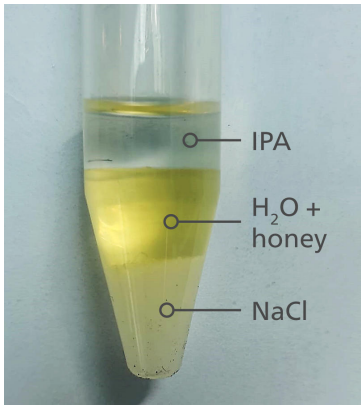

Figure 1. A simple solvent extraction procedure proved effective at moving target substances into an organic layer (IPA) in order to isolate them from the honey matrix.

Table 2. Seized samples of honey which were laboratory tested and shown to have undeclared amounts of the known erectile dysfunction drugs sildenafil and tadalafil.

Sample	Analyte	Concentration
1	Sildenafil	20–50 mg/15 g
2	Sildenafil	20–50 mg/15 g
3	Tadalafil	50 mg/10 g
4	Tadalafil	20–50 mg/15 g

RESULTS

Sildenafil

Honey samples 1 and 2 were easily analyzed with both silver (Ag) P-SERS strips and gold (Au) nanoparticle colloid solution (NP); this is characteristic of substances that are both strongly Raman-active and those that bind well with SERS substrates. The spectra of samples 1 and 2 are shown in **Figure 2**, compared with reference spectra of sildenafil from Metrohm.

Notice that honey itself does not contribute significantly to the spectrum, even after minimal sample preparation. The similarities are obvious, and MISA Cal software correctly matches the substance to three different reference spectra of sildenafil with very high HQI scores (**Figure 3**). The evidence here for sildenafil contamination is particularly strong, because not only does the honey sample match within a custom SERS library, but the spectrum also matches to sildenafil from Metrohm's larger Illicit Library.

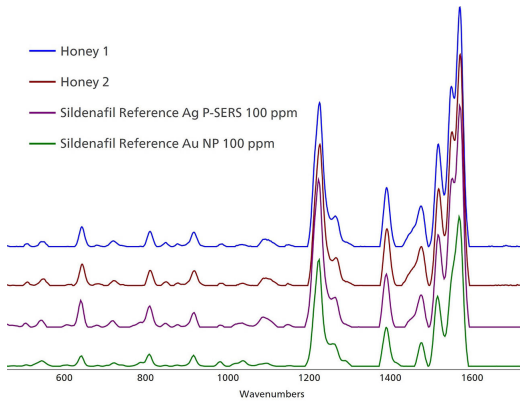

Figure 2. A simple solvent extraction procedure proved effective at moving target substances into an organic layer (IPA) in order to isolate them from the honey matrix.

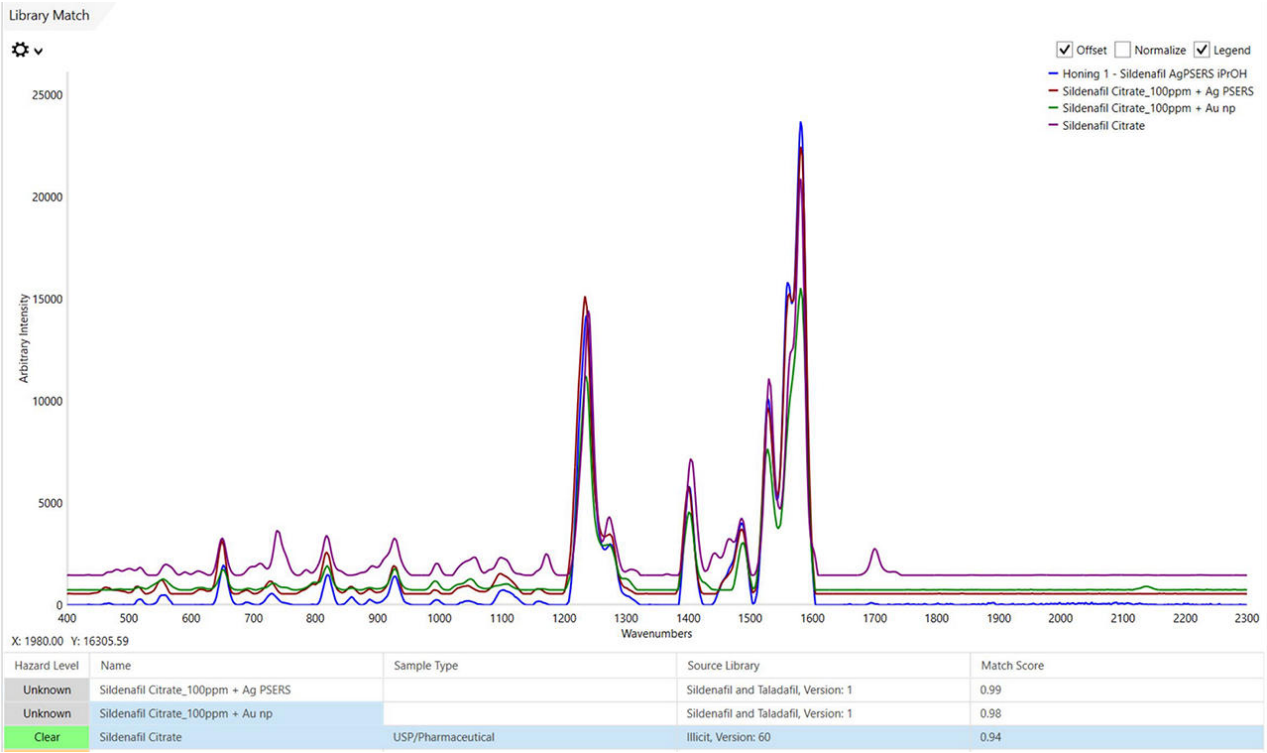

Figure 3. Library matches between honey sample 1 (blue) and Ag P-SERS (red) and Au NP SERS (green) spectra, in addition to a sildenafil spectrum from Metrohm's Illicit Library (purple). Note the very high HQI values achieved for all sildenafil matches (HQI values closest to 1 indicate very strong correlation of sample spectrum with library spectra).

Tadalafil

Both silver and gold P-SERS substrates provided weak SERS enhancement for tadalafil, and so a gold nanoparticle colloid solution was tested and found to provide a good SERS signal (**Figure 4**).

When the gold NP solution was used to analyze honey samples 3 and 4, both compared favorably with a tadalafil reference spectrum and both samples were correctly identified as containing tadalafil. Sample 3 is shown below in **Figure 5** with the good confidence provided by a high HQI score through library matching routines in MISA Cal software.

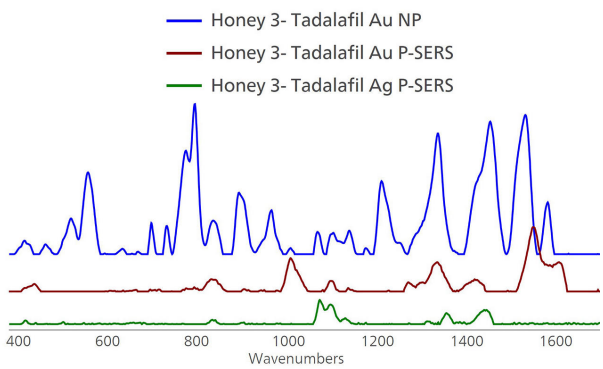

Figure 4. Out of three SERS substrates tested, including Au NP, Au P-SERS, and Ag P-SERS, only the Au NP solution (in blue) provided a good SERS spectrum of tadalafil.

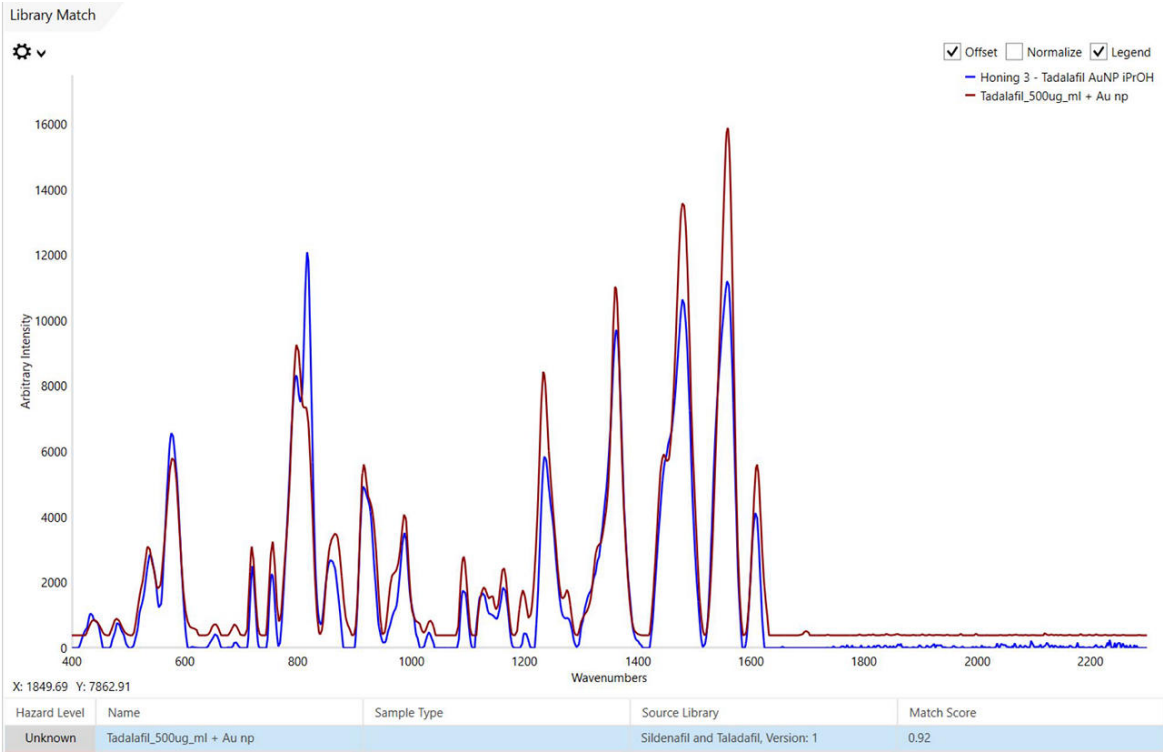

Figure 5. The spectrum of honey sample 3 after a simple extraction and analysis with gold NP colloid solution and MISA. This honey sample spectrum (blue) is overlaid with the reference tadalafil SERS spectrum (red) with which it was matched.

The signal of sample 4 is clearly weaker than that from sample 3 (Figure 6). This is consistent with the lower known concentration of tadalafil in the sample.

In all four honey samples, the presence of sildenafil and/or tadalafil is confirmed with SERS analysis. Sample preparation time averages 10 minutes per sample, most of which is needed to dissolve the honey in the NaOH solution. MISA only requires a maximum of 10 seconds for sample measurement.

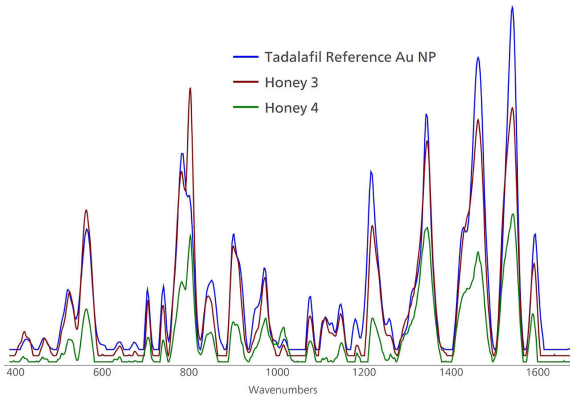

Figure 6. The presence of undeclared tadalafil in honey is confirmed through favorable comparison of honey samples with a tadalafil gold NP SERS reference spectrum.

CONCLUSION

SERS is growing in popularity as an analytical tool because of its high sensitivity, low cost, and ability to provide a rapid, portable method for in-situ trace detection. Detection of illegal adulterants no longer requires high-resource laboratory techniques like gas or liquid chromatography and mass spectrometry. Valuable features of the Metrohm's SERS solutions include:

- high selectivity for target adulterants
- lack of matrix interference
- simplicity of both sample pretreatment and analysis
- low-cost analysis that avoids the expense of laboratory equipment, consumables, and highly trained personnel

As demonstrated by external and internal research, SERS provides target identification in the presence of expected excipients with very little sample processing. Ultimately, this can help protect unsuspecting consumers from harmful adulterants.

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AUTHORS

Dr. Melissa J. Gelwicks, Metrohm Content Specialist
Prof. Ruth van Saskia, Professor of Food Supply Chain Integrity at University College Dublin
Dennis Zijderveld, Metrohm Product Manager Spectroscopy
Martijn van der Plas, Metrohm Application Support Engineer Spectroscopy
Dr. Wei W. Yu, Metrohm SERS Product Manager

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CONTACT

Metrohm Taiwan Ltd.
開封街二段 46 號 5 樓
108 台北市
info@metrohm.com.tw

CONFIGURATION

MISA Advanced

MISA Advanced 套装是一个完整的套装,允许用户使用 Metrohm's 的纳米颗粒溶液和 P-SERS 试纸条进行 SERS 分析。

MISA Advanced 套装包含一个 MISA 样品瓶附件、一个 P-SERS 附件、一个 ASTM 校正标准件、一根 USB Mini 电缆、一个 USB 电源,以及用于操作 MISA 仪器的 MISA Cal 软件。还提供了一个坚固的防护箱,用于安全存放仪器及附件。