White Paper

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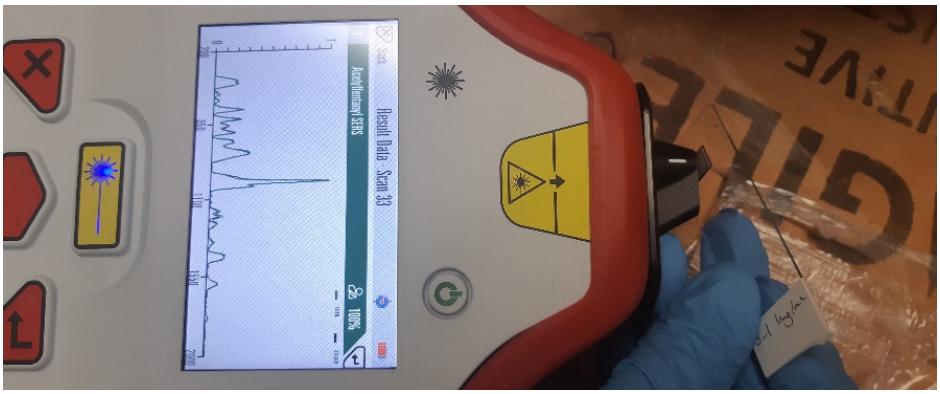
Detection of Narcotics Using Surface-Enhanced Raman Spectroscopy with Agilent Resolve

Creating a custom SERS library of hazardous narcotics using Agilent Command software



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Abstract

The Agilent Resolve handheld Raman spectrometer enables the fast, easy detection of street drugs out of container, through a variety of difficult barrier materials, and on substrates. While Raman spectroscopy is traditionally used for bulk analysis of samples, it can also be used for detection of trace compounds when coupled with surface-enhanced Raman spectroscopy (SERS) substrates.

In this study, the Resolve was used with Nikalyte gold SERS substrates for the trace detection of fentanyl analogs and other narcotics. The steps taken to create a user library of narcotics using Agilent Command Fleet Management software are outlined. To check its validity, the narcotics library was installed on the Resolve and used to analyze a range of street samples.

This white paper demonstrates the ease-of-use of Command to build a narcotics detection library and the compatibility of the portable Resolve analyzer with SERS for trace detection of fentanyl analogs.

Introduction

Fentanyls are highly potent synthetic opioids (10 to 1,000 times more potent than heroin), which have contributed to many overdose deaths around the globe in recent years. Since fentanyl analogs are typically present at trace-level concentrations in street samples, they can be difficult to detect using spectroscopic techniques.

Raman spectroscopy is a well-established analytical technique that provides molecular specific data, enabling the identification of bulk concentration narcotics. To enable trace detection of drug compounds, such as fentanyl analogs, Raman spectroscopy can be coupled with a roughened metallic surface in a technique known as SERS. By adsorbing the drug of interest onto the metallic surface, the Raman signal of the drug can be enhanced, increasing the sensitivity of the Raman spectrometer to enable trace-level detection.

This white paper explores the compatibility of SERS substrates bought from Nikalyte with the Agilent Resolve Raman handheld analyzer to detect a range of narcotics, including fentanyl analogs. A small selection of fentanyl variants and other drug-related materials were analyzed using the SERS technique. From the data set, the Agilent Command Fleet Management software was used to build a custom Resolve library of SERS narcotic entries. Once installed on the Resolve analyzer, the custom-built library was used to identify fentanyl analogs in a range of street samples.

Experimental

SERS substrate slides: Gold and silver SERS substrate slides were used for all SERS trace experiments (Nikalyte Ltd, Bicester, UK, https://www.nikalyte.com/sers-substrates). Nikalyte Ltd worked with Agilent to develop the substrates for use on the Resolve analyzer (Figure 1).

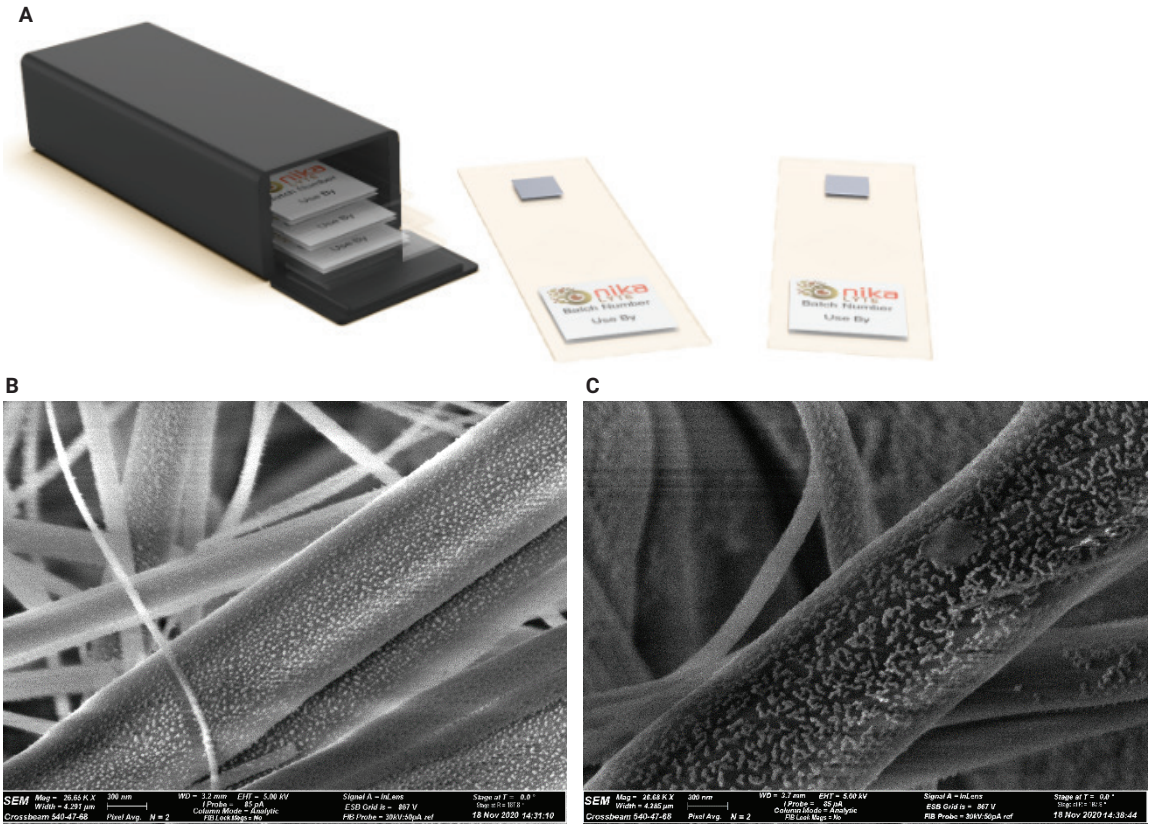

Figure 1. (A) SERS gold substrates used to enhance the sensitivity of the Agilent Resolve handheld Raman analyzer. Images of the SERS substrates before (B) and after (C) the addition of caffeine captured using scanning electron microscopy (SEM). (B) The gold nanoparticles appear as uniform spheres deposited onto the cellulose strands. (C) After the addition of caffeine, the gold nanoparticles form non-uniform aggregates on the cellulose strands. Photos courtesy of Nikalyte Ltd, https://www.nikalyte.com/sers-substrates.

Narcotics: Eleven narcotic certified standards were used to build the custom Command library. The standards included: lorazepam, nimetazepam, heroin HCl, phencyclidine HCl (PCP), 3,4-methylenedioxyamphetamine HCl, DL-methylamphetamine, DL-amphetamine, fentanyl HCl, alfentanil HCl, remifentanil HCl, and acetylfentanyl.

Cutting agents: Lab standard quinine bisulphate and alpha lactose monohydrate.

Collecting standard spectra of narcotics and cutting agents: Standard solutions of the 11 certified standard narcotics were prepared at 1 mg/mL in de-ionized water. As shown in Figure 2A, 14 µL of each solution was slowly added to the bottom corner of the nanoparticle pad on a Nikalyte slide, taking care not to oversaturate the pad. The loaded nanoparticle pad was positioned alongside the white line on the Resolve nose cone (Figure 2B). The Resolve, which was set to glass vial mode and the highest laser power setting of 475 mW, collected data from three continuous measurements. The process was repeated on three separate Nikalyte glass slides for each narcotic.

Standard solutions of the cutting agents, alpha lactose monohydrate and quinine bisulphate, were prepared at 10 mg/mL in de-ionized water. To obtain scans of the pure cutting agents, 14 µL was slowly added to the bottom corner of the nanoparticle pad on a Nikalyte slide. Three spectra were collected using the glass vial setting and full laser power on the Resolve. This process was repeated on two separate Nikalyte slides.

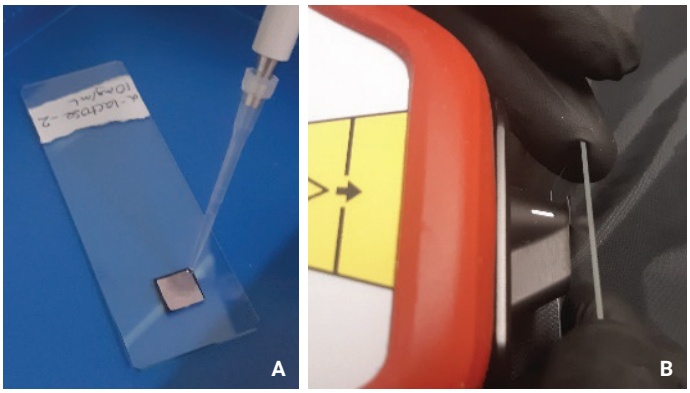

Figure 2. (A) Addition of 14 µL of narcotic standard added to the corner of the SERS substrate. (B) Alignment of the SERS substrate with the line on the Agilent Resolve handheld Raman analyzer nose cone.

Making a command library

A custom library was created using the "Library Build Functions" in the PC-based Command Fleet Management software, as outlined in Figure 3. Scans corresponding to each measured item were processed to make a robust library entry using the "Add Library Item" option. To ensure the entry corresponded to the chemical only, and not the glass slide, the "Glass Removal" tool was used to remove the background of most standards. Any scans that showed high glass background features were discarded. The name of the chemical, hazard class, and any further information was logged in the software before the chemical entry was complete. Once this process had been repeated for each narcotic standard and cutting agent, the library items were compiled into a library labeled as "SERS Narcotics" using the Build Library software tool.

The SERS Narcotics library was then exported via a USB and loaded onto the Resolve analyzer. The Command library was set as "matchable" on the Resolve handset, making it available for spectral matching of Resolve measurements of "unknown" drug compounds.

The new library was tested by measuring the standard solutions of narcotics using the Resolve and checking the match to the SERS-labeled entry within the library.

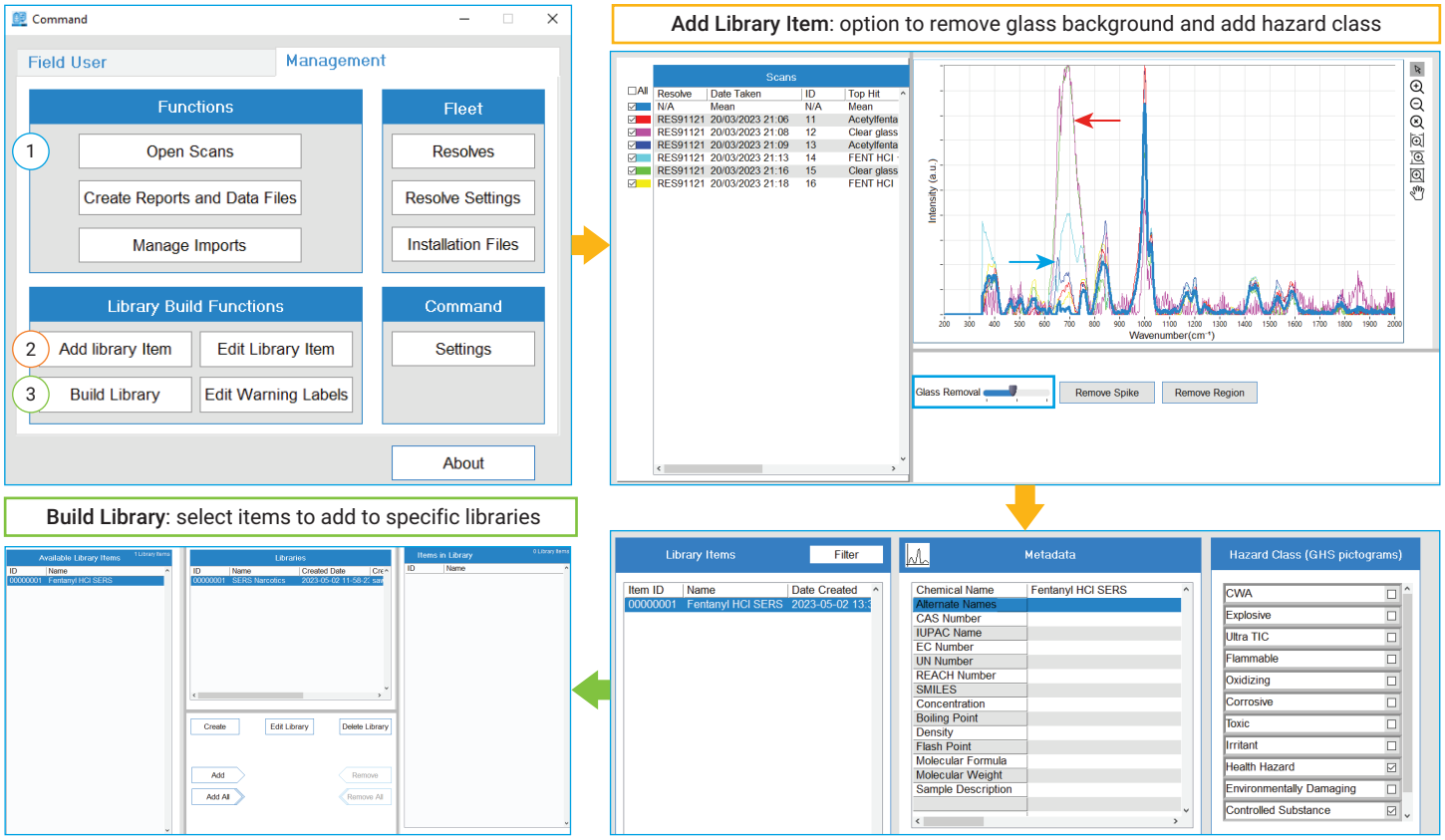

Figure 3. Workflow to build a custom library using the Agilent Command Fleet Management software. (1) The scans from the Agilent Resolve handheld Raman analyzer are imported using a database or Reachback file. (2) To build an entry for the library, the different repeat scans for a chemical are opened by clicking the "Add Library Item" option. This function shows the mean spectrum compared to the repeats, facilitates "glass background" removal, and allows for the addition of chemical information (metadata and hazard class). (3) Once the entries are complete, they can be added to libraries in Command with the Build Library function.

Detecting narcotics with cutting agents

To determine if the narcotic could be detected above the cutting agents, a solution was made at a 1:10 ratio of narcotic (0.5 mg/mL) to cutting agent (5 mg/mL). Solutions were prepared using acetylfentanyl, fentanyl HCl, MDA, and heroin HCl with each of the two cutting agents, quinine bisulfate and alpha lactose monohydrate. Each solution was spotted onto SERS substrates as described in Figure 2, and three scans were collected for each slide using the Resolve in vial mode. The top five spectral matches of the sample scans and library spectra were automatically recorded and reported by the Resolve software.

Detecting narcotics in street samples

A range of street narcotic samples were analyzed in this study. A single scan was collected of the street sample in native powder or tablet form using the Resolve in vial mode. The highest laser power setting of 475 mW was used.

For SERS data collection, approximately 3 to 5 µg of the drug sample was dissolved in 3 mL of distilled water and 15 µL was spotted onto the SERS substrate using the same method described in Figure 2. The scan data was processed using the Command custom library for narcotics and the top five matches were recorded. Only the SERS Narcotics library was used for matching.

Results and discussion

Using SERS substrates to detect narcotics

Street samples that are consumed for recreational and nonmedical reasons can contain a variety of narcotics and cutting agents. These components can include different variants of fentanyls or other narcotics such as heroin or MDMA (also known as ecstasy). Some of these materials can pose a challenge in the detection of fentanyl analogs, as they may have spectral features in common with fentanyls. Amphetamine, methamphetamine, and phencyclidine (PCP), which all contain a doublet at 1,004 and 1,029 cm^{-1} due to the ring breathing mode and the C=C CH stretch, respectively, are examples of possible interferents. To thoroughly evaluate the Resolve analyzer, a selection of narcotics likely to be found alongside fentanyl analogs and which may share spectral features with fentanyls were selected for this study.

For the initial investigation, the narcotics were adsorbed onto the Nikalyte SERS substrates by dissolving the narcotics in water and spotting the samples onto the corner of the SERS substrates (Figure 2). The Resolve was used in vial mode to reduce the background contribution from the Nikalyte slides. Three measurements were collected per substrate and three substrates were prepared for each sample (Figure 4).

Figure 4. Averaged normalized spectra of the 11 narcotic certified standards used to build the SERS Narcotics library.

For fentanyl HCl and its respective derivatives (alfentanil HCl, remifentanil HCl, and acetylfentanyl), the spectra were similar regarding peak position and intensity. The most intense peak was a doublet at 1,004 and 1,029 cm^{-1}, corresponding to a ring breathing mode and a C=C CH stretch, respectively.[1] See Figure 5 for structures.

The largest visible spectral difference arises from a broad peak in remifentanil, between 804 and 889 cm^{-1} from the C-C(O)-O ester bond. Similarly, the doublet at 1,004 and 1,029 cm^{-1} is the most distinctive in both DL-amphetamine and DL-methylamphetamine. The main spectral changes between amphetamine and methylamphetamine from the fentanyl derivatives arise at ~1,200 cm^{-1}, further attributed to C-ring breathing mode. The doublet at 1,004 and 1,029 cm^{-1} is detected in PCP and a weak peak at ~1,000 cm^{-1} is present in nimetazepam and lorazepam. These peaks differ from fentanyls and amphetamines, however, they are not the main spectral features.

Heroin is a highly fluorescent compound, which can make its identification using Raman spectroscopy challenging. However, when heroin was measured using the SERS substrates, the main peak was present at 624 cm^{-1} due to ring breathing mode and weak fluorescent background was detected. The background was minimized due to the gold nanoparticle surface that is known to quench fluorescence—further supporting the use of SERS substrates in drug detection.

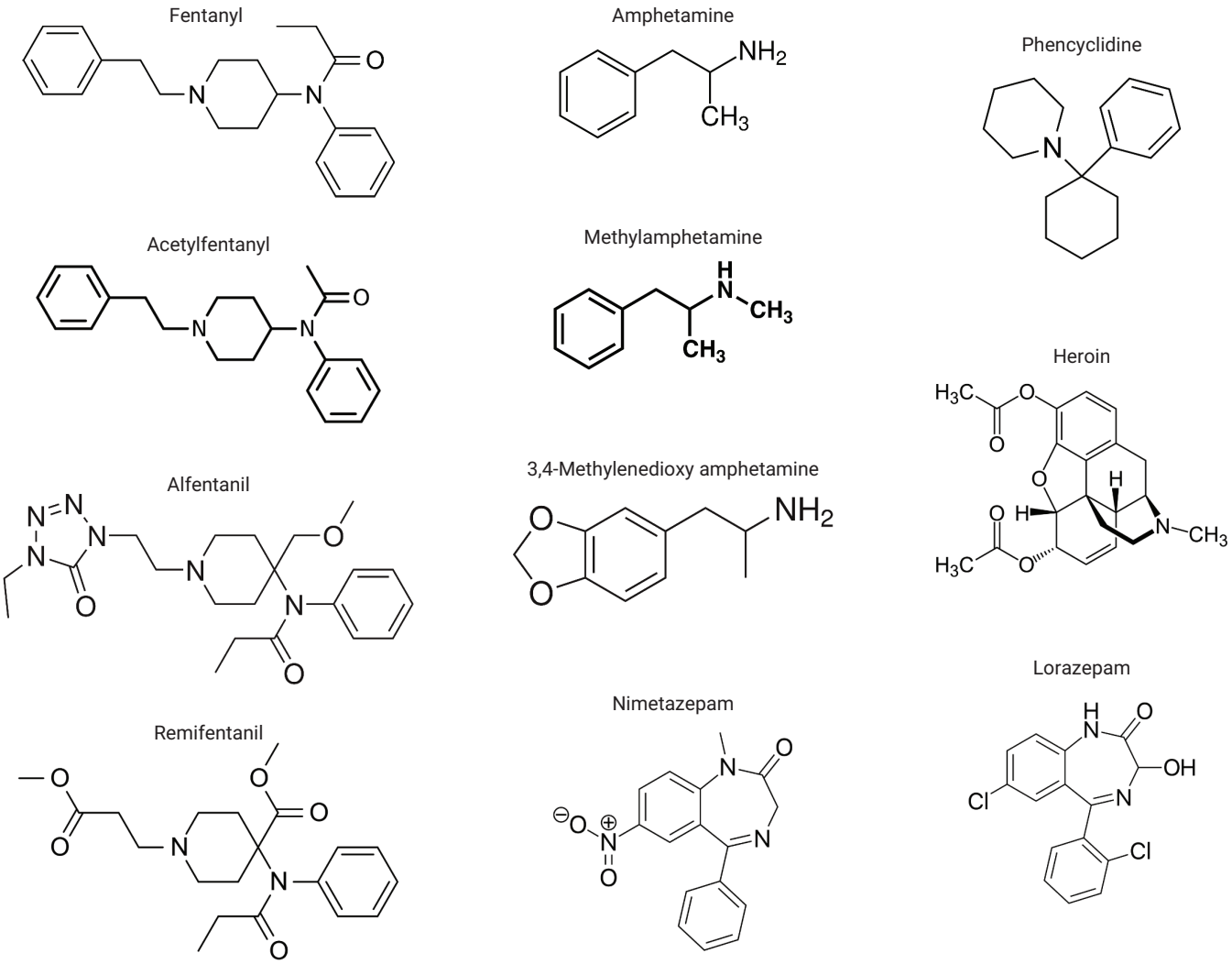

Figure 5. Structures of the 11 compounds, including four fentanyl analogs (left), measured by the Agilent Resolve analyzer to create the SERS Narcotics library for Resolve using Agilent Command software.

Using SERS substrates to detect narcotics mixed with cutting agents

Following the initial investigation of pure narcotics, fentanyl and acetylfentanyl were added to common cutting agents, quinine and lactose, at a ratio of 1:10. The samples were spotted onto gold SERS substrates and analyzed using the Resolve. Average normalized spectra from three scans of each sample are shown in Figure 6.

As shown in Figure 7, the custom-built SERS library was able to identify the fentanyl and lactose in the mixture. However, no fentanyl identification was made in the quinine mixed-samples.

As can be seen in the Raman spectra for the fentanyl and quinine mixture (Figure 6), there is no fentanyl doublet at 1,004 and 1,029 cm^{-1}, but the quinine peak at 1,366 cm^{-1} is present. These spectral features indicate that only quinine interacted with the gold SERS substrate. This behavior was replicated with both heroin and MDA, where the narcotics were detectable in the presence of lactose but not in the presence of quinine. Therefore, it is recommended that any sample where quinine is detected is questioned and is further analyzed using another analytical technique.

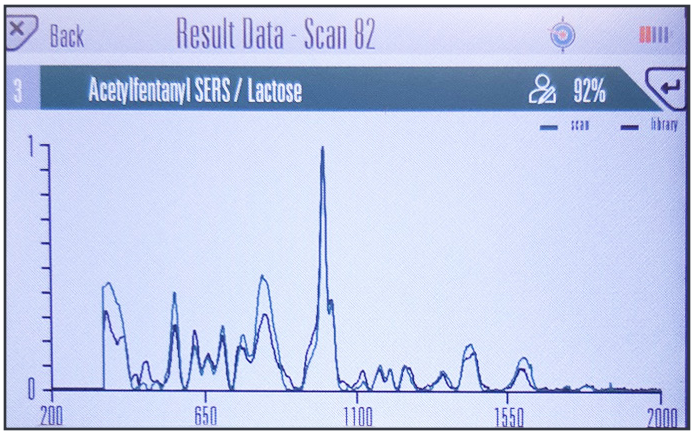

Figure 7. Screenshot of Agilent Resolve analyzer showing SERS library match result (92%) for acetylfentanyl mixed with lactose.

Figure 6. Averaged normalized spectra of fentanyl and acetylfentanyl mixed 1:10 with common cutting agents quinine and lactose.

Investigation of silver SERS substrates

In addition to the standard SERS gold substrates, silver substrates synthesized by Nikalyte were also evaluated in this study. Gold substrates were expected to yield a stronger Raman signal than silver substrates, as the SERS signal from gold is more greatly enhanced by the Resolve analyzer's 830 nm laser. Silver substrates typically enhance Raman signals more at laser wavelengths, such as 785 and 532 nm.

Gold substrates outperformed silver for all the narcotics tested in this study except for acetylfentanyl and MDA. Some acetylfentanyl Raman peaks produced a stronger signal with silver substrates and the MDA signal was equal to that observed with the gold substrates. Different Raman peaks were enhanced in the acetylfentanyl between the gold and silver substrate. Both silver and gold nanoparticle SERS substrates enhanced the Raman signals of the narcotics to varying degrees, likely due to differing surface chemistry between the SERS active materials.

Identification of fentanyls in street samples using the Command-built library

The range of street samples used to test the Resolve analyzer and SERS Narcotics library for the identification of fentanyl-based drugs is summarized in Table 1. The composition of the samples was verified using paper spray mass spectrometry.

Before SERS analysis, the street samples were analyzed using Raman spectroscopy, which is typically used for the identification of bulk (high concentration) compounds. Only one sample, which contained 40% fentanyl, was correctly identified using Raman spectroscopy. The 40% fentanyl sample was the only sample in the set with a fentanyl concentration above 15%, which is the expected cutoff concentration for bulk Raman analysis.

Figure 8 shows the comparison of the Raman and SERS spectra for the 40% fentanyl, 60% caffeine street sample. In the Raman spectrum (Figure 8, green trace), the typical fentanyl doublet at ~1,000 cm^{-1} is weak but recognizable. However, the predominant spectral feature can be seen at 566 cm^{-1}, which arises from the C=O-N deformation vibration in caffeine.[2] In the SERS spectra (Figure 8, blue trace), the fentanyl doublet is the predominant feature. The amplification of the fentanyl doublet is likely due to the fentanyl preferentially adsorbing onto the gold surface over caffeine, resulting in a stronger enhancement.

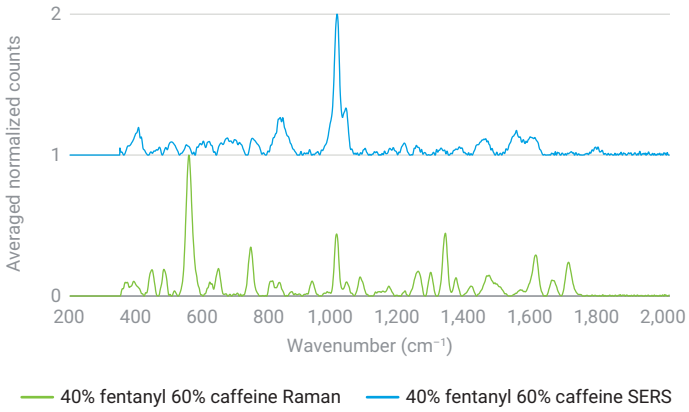

Figure 8. Comparison of Raman (green) and SERS (blue) spectra for the detection of fentanyl in the 40% fentanyl, 60% caffeine street sample.

For trace analysis using the Resolve analyzer, a small amount (~3 µg) of each of the street samples was dissolved in water and spotted onto a gold SERS substrate. The acquired measurements for each sample were compared with the SERS Narcotics spectral library.

As summarized in Figure 9, the Resolve correctly identified the presence or absence of fentanyl in the 33 street samples 70% of the time; 35% of samples were correctly identified as having fentanyl present in the sample and 35% were correctly identified as having no fentanyl present.

When a sample matched to fentanyl, "fentanyl HCl" was most often the top match result with the SERS library entry, with a match score over 80%. Missed detection of fentanyls occurred in eight samples and two fentanyl-free samples were falsely identified as containing fentanyl.

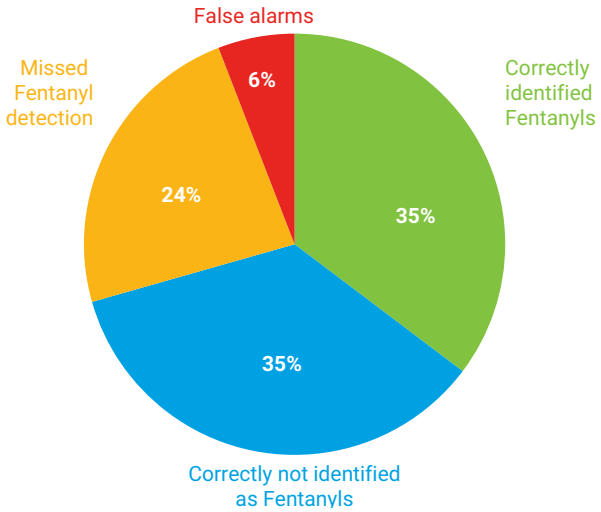

Figure 9. Overall detection of fentanyls in a range of street samples using the Agilent Resolve analyzer and Agilent Command-built SERS Narcotics library. The narcotics were spotted onto gold SERS Nikalyte substrates.

Table 1. Street drug substances analyzed using the Agilent Resolve analyzer. The box colors correspond with Figure 9.

Color Key			
Correctly identified as fentanyl	Missed fentanyl detection	Correctly identified as not fentanyl	Falsely identified as fentanyl

Below 1% Fentanyl	Above 1% Fentanyl	No Fentanyl
0.26% fentanyl, 99% caffeine	1.6% fentanyl, 64% etizolam, 34% caffeine, 0.4% erythritol	Gamma hydroxybutyrate (GHB)
0.5% carfentanil, 99% xylitol, 0.14% 4-anilino-N-phenethylpiperidine (ANPP)	1.52% fentanyl, 0.29% carfentanil, 0.47% ANPP, 0.1% erythritol, 98% caffeine	MDMA
0.5% fentanyl, 99% caffeine	1.7% fentanyl, 0.13% carfentanil, 58% caffeine, 40% erythritol	2-(4-bromo-2,5-dimethoxyphenyl) ethanamine (2C-B)
0.55% fentanyl, 99.5% caffeine	2% fentanyl, 98% caffeine	68% cocaine, 30% creatine, 2% levamisole
0.44% fentanyl, 0.23% carfentanil, 32% heroin, 4% etizolam, 60% caffeine	4% fentanyl, 96% caffeine	Methylamphetamine
0.12% fentanyl, 0.43% carfentanil, 0.23% ANPP, 50% caffeine, 49% xylitol	6.82% fentanyl, 0.11% etizolam, 0.15% flualprozolam, 90% caffeine	Ketamine
0.61% fentanyl, 50% caffeine, 49% erythritol	7.54% fentanyl, 0.11% etizolam, 2% mannitol, 90% caffeine	Diazepam, lactose
0.25% fentanyl, 0.4% carfentanil, 0.5% heroin, 2% etizolam, 96% caffeine	7.85% fentanyl, 5.7% cocaine, 44% caffeine, 44% erythritol	Hydromorphone, lactose
	11.8% fentanyl, 19.2% etizolam, 60% caffeine, 20% sugar	50% heroin, 46% caffeine, 3.3% etizolam
	12.6% fentanyl, 3% fluorofentanyl, 2.9% etizolam, 80% caffeine	50% MDA, 49% MDMA, 0.25% 3,4-methylenedioxy -N-ethylamphetamine (MDEA)
	13.7% fentanyl, 3.98% xylazine, 1.64% etizolam, 0.31% alprazolam, 80% caffeine	Oxycodone pill
	40% fentanyl, 60% caffeine	39% cocaine, 60% phenacetin, 0.12% levamisole
		10% flualprazolam, 90% lactose

Missed fentanyl detection results

Three of the missed fentanyl detections occurred in the presence of a high concentration of etizolam (Table 1). It is likely that the high concentration of etizolam can affect detection of fentanyl due to a large peak at 1,490 cm^{-1} in the SERS spectrum.[3]

To demonstrate the effect of additional materials, such as etizolam, in a fentanyl sample, a couple of examples were reviewed using the Agilent Reachback offline tool. This tool is designed for Raman spectral analysis and the comparison of non-SERS spectra with non-SERS Resolve library data. Therefore, the comparison of SERS spectra with non-SERS Resolve data is intended for indicative purposes only.

Comparing spectra for the sample comprising 11.8% fentanyl, 19.2% etizolam, 60% caffeine, and 20% sugar, the SERS spectra had a match score of 68.07% to non-SERS fentanyl HCl. The match score increased to 81.1% with the addition of etizolam, and further increased to 85.32% by adding caffeine (Figure 10). The improvement in match scores demonstrates the improvement in performance expected with inclusion of more items to the spectral library. Detection accuracy could be further improved by adding etizolam or known mixtures containing etizolam into the SERS Narcotics library.

It is also important to ensure that matching of SERS sample spectra is done against a SERS spectral library, as shifts can be seen between the Raman spectra and the SERS spectra. Figure 10 shows a shift in the peak at 1,490 cm^{-1} for etizolam and shifts in the fentanyl peaks when adsorbed onto the gold substrates.[1]

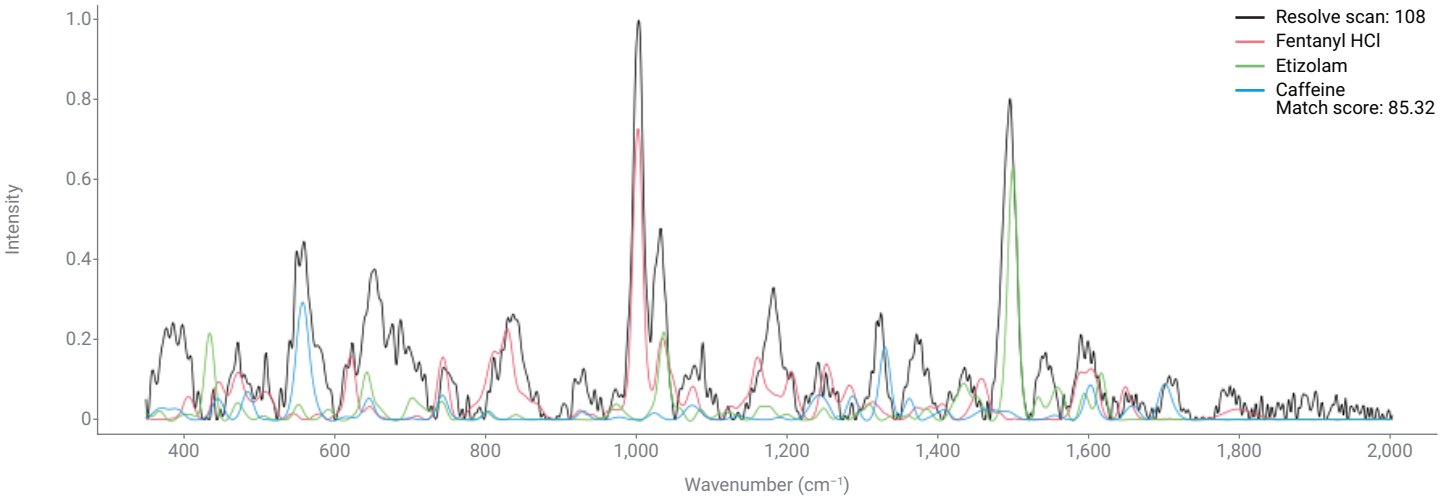

Figure 10. SERS measurement of street sample containing 11.8% fentanyl, 19.2% etizolam, 60% caffeine, and 20% sugar (black) plotted against Raman library entries for fentanyl HCl (red), etizolam (green), and caffeine (blue). The combination of the three Raman library entries has an 85.32% match score with the street sample.

The other missed detection samples predominantly contained caffeine with fentanyl, with one other highly mixed sample that contained both caffeine and ANPP (Table 1). Using the Reachback offline tool to compare the non-SERS spectra of fentanyl HCl, 4-ANPP, and caffeine, there was a 71% match with the SERS spectra of the street sample. However, there was a large unexplained peak at 1,536 cm^{-1}. This peak was also present in the remaining street samples that contained fentanyl with caffeine and resulted in missed fentanyl detection (Figure 11). This peak was found to originate from the SERS substrates themselves. Therefore, a SERS measurement should be collected from any solvent, including water, which was used to dissolve the test samples, and the spectrum should be added to the user library.

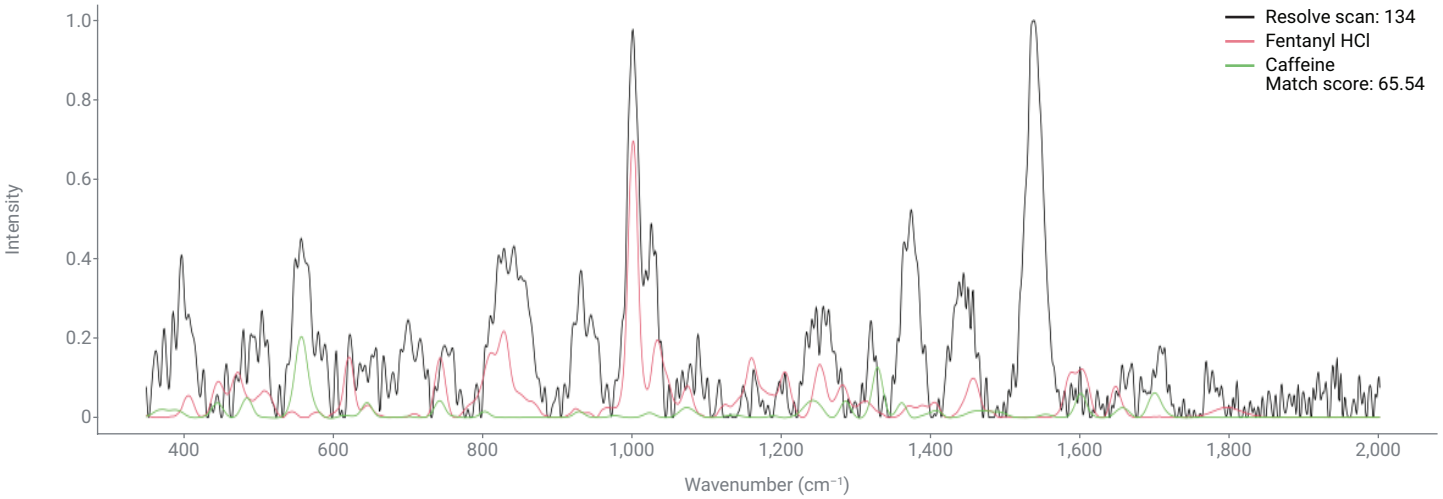

Figure 11. SERS street sample containing 0.26% fentanyl and 99% caffeine (black) plotted against Raman library entries for fentanyl HCl (red) and caffeine (green).

False alarms

The two false alarms in the street samples were methamphetamine and a cocaine-based mixture (Table 1, red). These results were not unexpected as both methamphetamine and cocaine share a doublet peak at 1,004 and 1,029 cm^{-1} (Figure 12). Adding methamphetamine into the SERS Narcotics library did result in two correct matches for street samples containing MDA and MDMA. However, methamphetamine is a common false alarm with fentanyl due to a high similarity in their structures. This similarity is exemplified by the 85.28% match score between the SERS spectra for methamphetamine and the Raman library entry for fentanyl HCl (Figure 12A).

For cocaine, there were some larger peaks in the cocaine sample that are absent in the fentanyl HCl spectra, such as the peaks at 1,380, 1,444, 1,532, and 1,790 cm^{-1} (Figure 12B). Adding cocaine into the SERS Narcotics library would help reduce this false alarm.

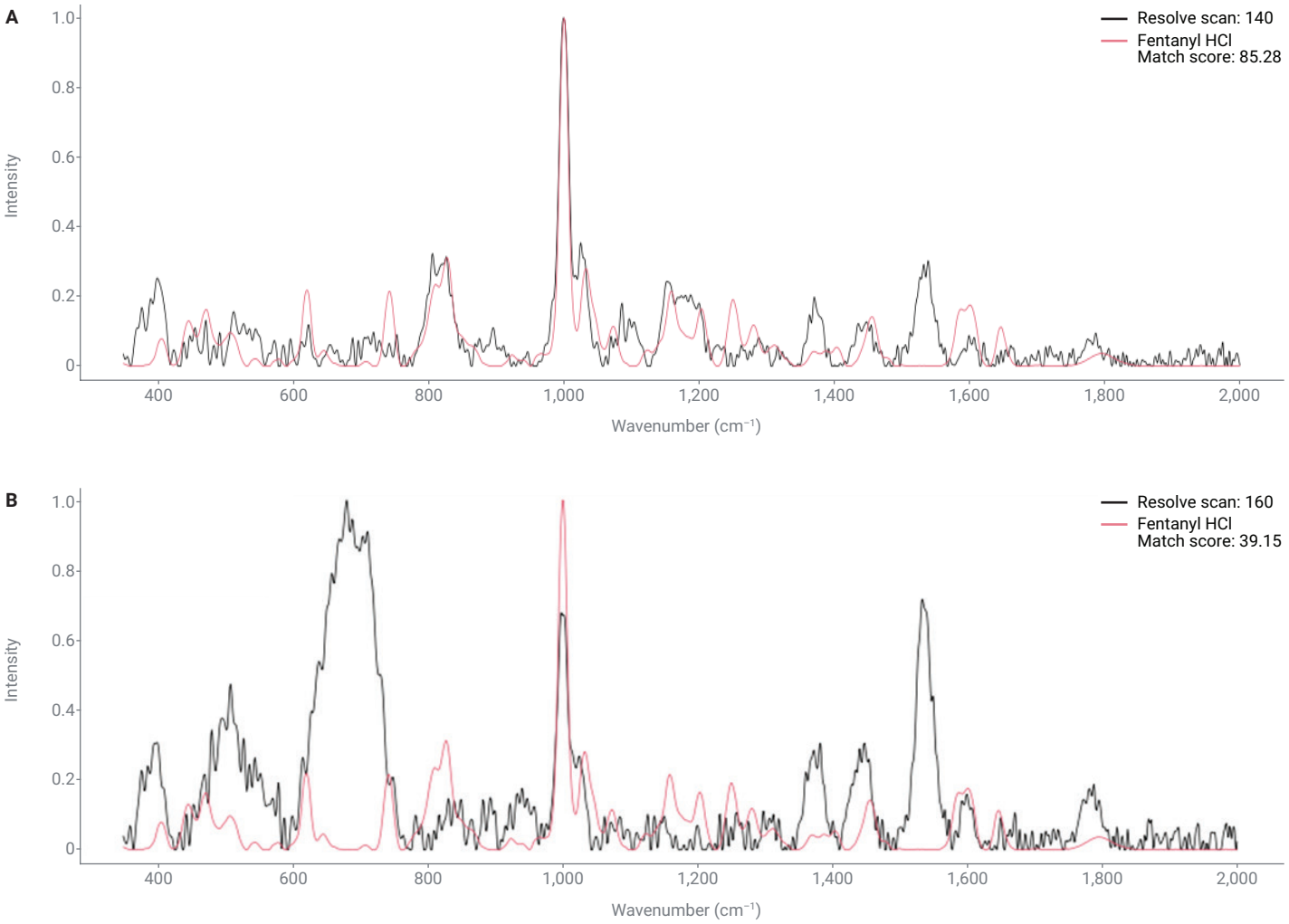

Figure 12. Fentanyl false alarms from street samples containing methamphetamine (A) and cocaine (B). SERS scans of street samples (black) plotted against Raman library entry for fentanyl HCl (red scan, A, and red scan, B).

Conclusion

The Agilent Resolve handheld Raman spectrometer is typically used for the direct analysis of bulk materials, including samples contained in opaque packaging in spatially offset Raman spectroscopy (SORS) through-barrier mode. The versatility and adaptability of the instrument also allow it to be employed for detection of trace compounds using surface-enhanced Raman spectroscopy (SERS) substrates. In this paper, the capabilities of the Resolve analyzer were evaluated for the identification of fentanyl compounds in 33 street drugs. Key points include:

- Agilent Command Fleet Management software enabled a custom library for Resolve to be built for fentanyl analogs and other narcotics using gold SERS substrates bought from Nikalyte.
- The SERS Narcotics library confirmed the presence or absence of fentanyls in 70% of a range of street samples.
- Caution is recommended when dealing with samples that contain the cutting agent quinine, as the narcotic did not interact with the gold SERS substrate in its presence. A complimentary technique is recommended for further analysis of samples found to contain quinine.
- To improve the accuracy and robustness of the narcotics library, SERS spectra for etizolam, caffeine, ANPP, and cocaine reference samples should be included in any SERS custom-built libraries for fentanyl detection. A complimentary technique is recommended for further analysis of samples found to contain these compounds.
- Further method development is required to account for the contribution of the solvent on the SERS spectra.

With further improvements to the SERS Narcotics library, the Resolve analyzer represents a valuable tool for the detection of hazardous narcotics—including low concentrations of highly potent fentanyl analogs.

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