Targeted Fentanyl Screening Utilizing Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS) Applied to Authentic Seized Drug Casework Samples

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Abstract

Effective and rapid screening methods for seized drug analysis are crucial to ensure the safety of first responders and laboratory personnel, while reducing overall analysis time and improving reliability. The drug landscape has been overwhelmed by fentanyl and fentanyl analogs that are extremely potent and generally present in low concentrations with other drugs and diluents. We have previously reported the use of electrochemical surface-enhanced Raman spectroscopy (EC-SERS) as a novel screening method for detecting fentanyl and fentanyl analogs in the presence of commonly encountered analytes. Herein, we present the application of this targeted method to authentic seized drug casework samples to assess the performance and fit-for-purpose of the developed method to accurately identify fentanyl and fentanyl-like substances. Authentic sample sets contained a wide range of analytes, and a varying number of compounds present in each sample, representing both true positive and true negative samples. EC-SERS results were compared to the ground-truth as established by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), as well as the results of chemical color tests. Application to authentic samples allowed for identification of fentanyl and fentanyl analogs. The targeted approach was shown to provide preferential enhancement of the fentanyl signal. The overall accuracy for the targeted screening method for the presence of a fentanyl/fentanyl-like substance was 87.5 % and the fentanyl samples averaged between 6 wt. % to 9 wt. % fentanyl or fentanyl analog. EC-SERS provided an alternative fentanyl screening approach demonstrating results within minutes and the absence of false positives.

Keywords: Electrochemical surface-enhanced Raman spectroscopy (EC-SERS), targeted fentanyl screening, *in situ* SERS, forensic drug chemistry, authentic seized drug samples, drug screening

1. Introduction

Screening methods are common practice in the field of forensic drug analysis and have been used routinely for many years. Indeed, chemical color tests are a common method employed for this process [1–3]. However, these simple tests may be prone to false positive and negative results and may lack specificity, especially in today's changing drug landscape [1,4–7]. Also, multiple different reagents and tests may be required, increasing the handling of potentially fatal substances. These drawbacks demonstrate the need for novel and improved screening methods for seized drugs. In 2021, fentanyl reports significantly increased ($p < 0.05$, total of ≈154,000 reports and an increase of ≈37,000 reports), accounting for 60 % of narcotic analgesics, while methamphetamine, cannabis/THC, cocaine, fentanyl, and heroin remained the top five drugs reported by laboratories

within the United States, according to the National Forensic Laboratory Information System (NFLIS) [8]. The prevalence of these drugs within the United States, as well as novel psychoactive substances (NPS), requires screening methods that provide sensitive, selective, and rapid results to prevent backlogs and provide reliable down-the-line decision making. Safety and analysis protocol may be affected by the presence of fentanyl-related compounds both at the scene and the laboratory, making the screening of these substances an important step. Both low weight percent contribution of potent opioids like fentanyl analogs and the presence of diluent compounds/mixture samples are a challenge to current methods.

More recently, portable Raman instruments have been utilized for this purpose, although several disadvantages have been demonstrated with seized drug samples, including failed identifications with some mixture samples and where the concentration of the analyte of interest is low [9]. However, Raman spectroscopy is considered a Category A technique by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), demonstrating a high degree of discrimination through structural characterization [3]. As such, Raman has been a promising technique in forensic science for many years and has been utilized within the literature [9–13], but improvements are needed in order to advance applications for successful, rapid screening.

The discovery of the surface-enhanced Raman scattering (SERS) phenomenon in 1974 by Fleischmann et al. [14] provided needed improvements in Raman scattering, leading to the propagation of many new Raman applications and technologies. The SERS effect is most commonly achieved using metallic surfaces and nanoparticles, where the overall enhancement in signal can be attributed to both an electromagnetic effect and a chemical effect [15–18]. Since its discovery, SERS has been utilized in a number of fields with great success as evidenced by tens of thousands of publications [19], and the analysis of drugs of abuse is no different. Many applications related to drug detection have also been explored including barbiturates, amphetamine-like substances, cathinones, cocaine, and a range of opioid compounds including fentanyl, morphine, and heroin [14–29].

Interestingly, the first discovery by Fleischmann et al. of the SERS phenomenon was achieved using electrochemical roughening to obtain a SERS substrate. Electrochemistry provides a direct way of controlling and generating an effective SERS substrate, providing enhanced signals and improved detection limits over traditional Raman. The electrochemical-SERS (EC-SERS) process is described and explained in-depth in several manuscripts in the literature [36–40]. Briefly, the application of potential to a metallic electrode results in the oxidation of the metal surface, producing metal ions in solution which can then be reduced to form nanostructures on the electrode surface capable of producing the SERS phenomenon. In these applications, electrochemistry is used to generate or influence the SERS substrate either in situ or ex situ; however, some applications have also used the electrochemical response of the analyte for detection along with SERS [41], although this is not as common. A major benefit to EC-SERS approaches is the speed and simplicity of generating a SERS substrate, eliminating the need for costly, timely, or intensive methods for nanoparticle synthesis or substrate manufacturing. However, one drawback to this type of substrate is that it may be less reproducible and more random than other manufactured substrates.

While EC-SERS continues to be an effective and simple approach to SERS substrate generation, exploration into the forensics realm has remained limited. In fact, EC-SERS applications represent a significantly smaller portion of the literature than SERS, with only a few hundred publications [42] compared to the over 40,000 for SERS applications [19]. EC-SERS presents many advantages and desirable characteristics for forensic drug screening including being rapid, inexpensive, and simple. Therefore, it is of great interest to the forensic community to investigative the effectiveness and utility of EC-SERS for seized drug screening. To date, very few EC-SERS applications have been demonstrated for drugs of abuse: Bindesri et al. for the detection of THC (delta-9-tetrahydrocannabinol) [43], Gonzalez-Hernandez et al. for the detection of mephedrone and 4-methylethcathinone [41], and Ott et al. for fentanyl and its analogs [44].

Our previous work provided the foundation for an EC-SERS method aimed at detecting fentanyl and fentanyl analogs in seized drug samples [44]. This work provides the application of that method toward authentic seized drug specimens from the Maryland State Police Forensic Sciences Division. Herein we describe how EC-SERS can be used in a targeted fashion for time-resolved spectroelectrochemical measurements of drugs of abuse using silver screen-printed electrodes. This work provides a technological step toward novel drug screening approaches using techniques with high discrimination power such as EC-SERS to assist forensic laboratories with case triage, preventing backlogs, and addressing the changing drug landscape.

2. Materials and Methods

Authentic seized drug samples (n=24) were provided by the Maryland State Police Forensic Sciences Division (MSP) for analysis by EC-SERS. These samples were prepared and analyzed onsite at the MSP using the portable SPELEC combination potentiostat and 785-nm Raman spectrometer from Metrohm DropSens, USA running DropView data analysis software (version 3.2.2.18LZ04). Silver screen-printed electrodes ($SP_{Ag}Es$, DRP-C013) were used as cost-effective and disposable analysis platforms and contained silver working (geometric area of 0.02 cm^2) and reference electrodes and a carbon counter electrode (Metrohm DropSens, USA, Riverview, FL) in a single platform.

Sample preparation of authentic specimens consisted of dissolving a small amount of sample (several milligrams or the tip of a small spatula) in the supporting electrolyte of 0.1 mol/L perchloric acid (Fisher Scientific, Fair Lawn, NJ) supplemented with 0.01 mol/L potassium chloride (Sigma-Aldrich, St. Louis, MO) and then vortexed. Ultrapure water (18.2 MΩ) was obtained from a Millipore Direct-Q UV water purification system (Billerica, MA) for preparation of the supporting electrolyte. Samples were then analyzed by placing a 50-µL drop on the electrode, ensuring full coverage of the working, reference, and counter electrodes.

The targeted fentanyl screening method was achieved using multi-pulse amperometric detection (MAD) method for a tailored in situ generation of the SERS substrate with simultaneous SERS analysis of the sample as described in Ott et al. [44]. Briefly, the MAD method was carried out in five steps with a sampling interval of 0.1 s: +0.45 V for 3 s, -0.05 V for 30 s, OCP (open circuit potential) for 240 s, +0.45 V for 3 s, and -0.05 V for 50 s to generate the substrate (**Figure 1**). These potentials were optimized to achieve the largest enhancement of the Raman signal for fentanyl. **Supplementary Figures S1-S2** demonstrate the MAD process and examples of

amperoRamangrams and MAD amperograms. Raman spectra were collected at a laser power of 379.1 mW and an integration time of 5 s. Higher concentration samples can be observed within the first 30 s of the experiment, while low concentration samples require approximately 300 s for maximum enhancement. Sample preparation time was minimal, less than 2 min.

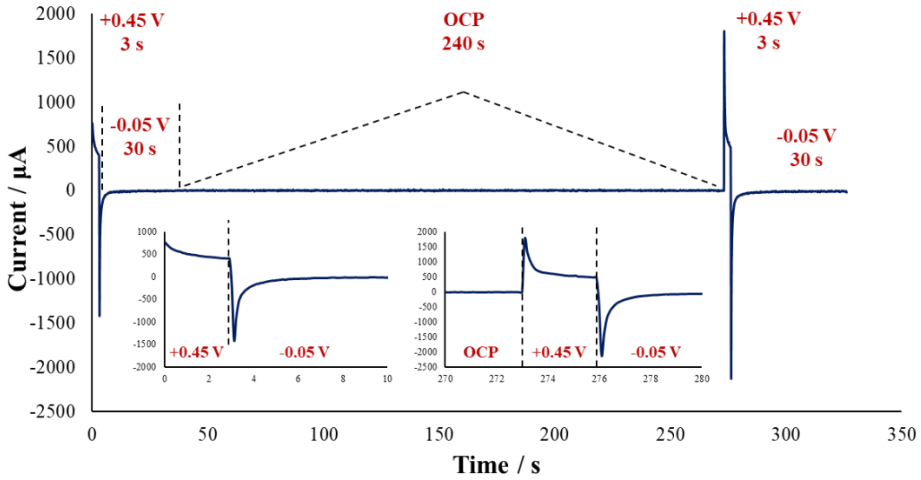

Figure 1. *Example of the multipulse amperometric detection technique for the generation of the SERS substrate via electrochemistry, demonstrating the enhancement regions due to the electrogeneration of nanoparticles.*

Based on amperoRamangram analysis, it was seen that the largest enhancement of signal occurred between 290 seconds and 300 seconds. Therefore, the spectrum with the highest intensity within this range was chosen for analysis. Spectra were assessed and baseline subtracted via the DropView software and exported for further visual comparison of diagnostic Raman bands and peak ratios via spectral overlay. Normalization and spectral subtraction were also employed for comparison purposes. Critical bands for identification of fentanyl included 463 cm^{-1}, 746 cm^{-1}, 830 cm^{-1}, 1004 cm^{-1}, 1029 cm^{-1}, 1182-1201 cm^{-1}, and 1600 cm^{-1}. Similar bands are noted for several fentanyl analogs and can be observed in Ott et al. [44] including a prominent band at 1467 cm^{-1} for furanyl fentanyl. The majority of other tested substances did not demonstrate similar spectra with the exception of cocaine and methamphetamine, although signal intensity and sensitivity were drastically lower for these compounds. Differentiation from cocaine was determined by the presence of the cocaine Raman bands at approximately 777 cm^{-1}, 830 cm^{-1}, 887 cm^{-1}, and 932 cm^{-1} and the absence of the 463 cm^{-1} band, as well as overall intensity and comparison of the overall spectrum. Methamphetamine was differentiated based on a rectangular pattern of Raman bands of low intensity between approximately 1400 cm^{-1} and 1530 cm^{-1}, the lack of the 463 cm^{-1} band, overall spectral comparison, and significantly low signal intensity. Identification in the presence of quinine could be achieved based on the main Raman bands of fentanyl, changes to the peak ratios due to the presence of fentanyl, and overall comparison. The results from this study were compared with ground-truth data obtained using gas chromatography-mass spectrometry (GC-MS) for qualitative identification and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for quantitative analysis. Quantitative data was not provided for all samples as some samples were unable to be quantitated for various reasons via LC-MS/MS. As such, these samples are marked in **Figure 2.** Instrument parameters can be found in the **Supplemental Information Tables S1 and S2**. Future work will focus on objective chemometric and library-based analysis approaches.

3. Results and Discussion

The targeted EC-SERS screening method was previously demonstrated as fit-for-purpose through a method optimization, demonstration of sensitivity and selectivity, and analysis of simulated seized drug samples as outlined in Ott et al. [44]. To assess the applicability of the targeted method within a forensic laboratory setting, authentic seized drug samples were provided by the MSP for analysis. It is important to note that these samples were assessed onsite within the MSP laboratory, demonstrating the portability and simplicity of the EC-SERS method and instrumentation. Set-up of the instrument and performance check took approximately 10 min from the time the instrument was removed from the carrying case before samples were ready to be analyzed. This rapid and portable nature makes this technology amenable to a wide array of testing applications in the field and in the laboratory.

3.1. Overview and Composition of Authentic Seized Drug Samples

The targeted EC-SERS method previously demonstrated limits of detection in the low-to-mid ng/mL range with limited interferences from other common drugs of abuse [44]; however, authentic samples may present several challenges over simulated laboratory specimens including low weight percent contributions, complex mixtures, and different analogs. Therefore, it was of interest to expand this study and apply the EC-SERS method to authentic casework specimens to demonstrate fit-for-purpose as a targeted fentanyl screening method to identify the presence of fentanyl or a fentanyl analog in samples.

The authentic seized drug sample set (n=24) was assessed using the targeted fentanyl EC-SERS screening method employing *in situ* SERS substrate generation via the MAD method and simultaneous SERS sample analysis. This set of authentic samples was assessed via GC-MS, the results of which were used as ground-truth, in addition to LC-MS/MS results for a subset of the samples, which also provided quantitative data (**Supplementary Table S3** contains the quantification data) to better understand the weight percent of different compounds. Based on these confirmatory techniques, this set of samples contained fentanyl and fentanyl analog positive samples, as well as negative samples that did not contain a fentanyl-like compound. It is important to remember that the EC-SERS method is intended to serve as a screening tool and is not intended to replace mass spectrometry methods for confirmatory testing. EC-SERS could be used for casework triage and to assist in informed decision-making regarding confirmatory methods and analyst safety. These samples also represented a variety of scenarios, from simple one-compound samples to complicated mixtures of up to seven different compounds encompassing other drugs of abuse and diluents. **Table 1** provides a breakdown of the number of compounds found and how many samples contained that number of compounds.

Table 1*. Breakdown of the number of compounds identified in the targeted authentic sample set and how many of the samples contained that number of compounds.*

Number of Compounds	Number of Authentic Samples
1	10
2	5
3	0
4	5
5	1
6	2
7	1

Based on the GC-MS and LC-MS/MS data, of the 24 authentic samples, seven were true negatives for fentanyl (Unknowns 15, 16, 17, 21, 22, 23, and 24) and ten contained fentanyl (Unknowns 1-5, 7, 8, 11, 12, and 19). In addition, nine samples contained at least one fentanyl analog (Unknowns 4, 6, 7, 9, 10, 13, 14, 18, and 20). **Figure 2** provides a graphical representation of the analytes identified via GC-MS and LC-MS/MS and an overview of the results discussed within the following sections. Several differences can be seen between the two methods, highlighting difficulties surrounding the sampling of a seized material, where heterogeneity of seized drug materials may complicate analysis due to the prevalence of multiple drug compounds, diluents, and presence of some analytes in low percent contribution. In addition, it should be kept in mind that since quantitative analysis via LC-MS/MS was not performed on all samples, including the true negatives, these samples may have contained other analytes not identified via GC-MS.

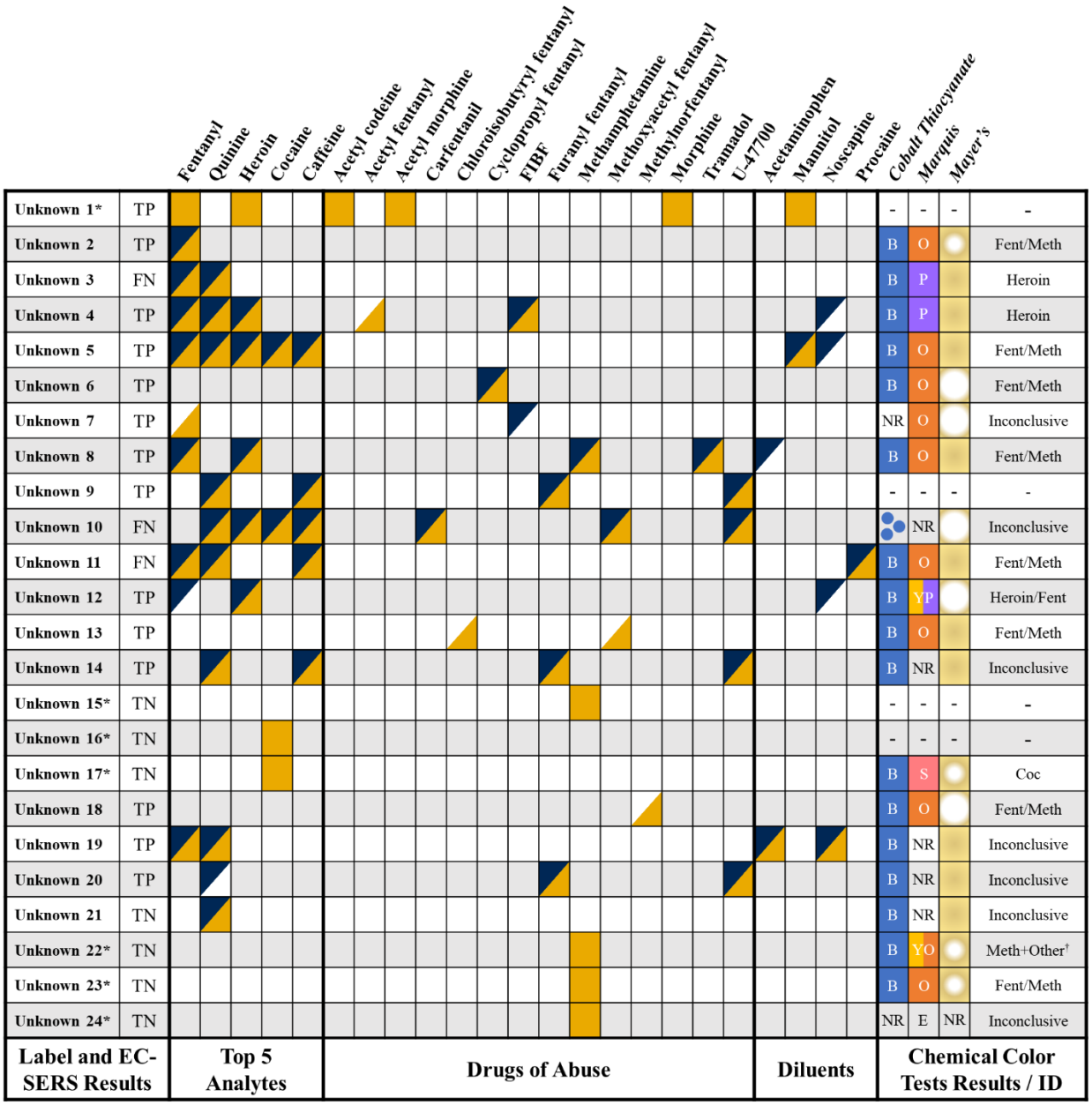

Figure 2. *Graphical comparison between identifications provided by GC-MS (gold/yellow), LC-MS/MS (blue), chemical color tests, and EC-SERS. Solid, gold-colored squares and asterisk indicate samples not analyzed via LC-MS/MS. EC-SERS results for identification of fentanyl are provided in the second column, where TP = true positive, TN = true negative, FP = false positive, and FN = false negative. For chemical color tests, square color corresponds to the color change result of the test. Color test legend: blue circles = blue specs, two colors = mix of the colors, 'NR' = no reaction, 'E' = effervescence, for Mayer's: all yellow = yellow precipitate, large white circle = white precipitate, yellow gradient to medium white circle = yellowish-white precipitate, 'B' = blue, 'O' = orange, 'Y' = yellow, 'S' = salmon, and 'P' = purple Presumptive identification is provided based on the color test with the following abbreviations: Fent = fentanyl, Meth = methamphetamine, Quin = quinine, Coc = cocaine. Dagger (†) indicates that this color combination could represent methamphetamine and a bath salt or fentanyl and diphenhydramine.*

3.2. Targeted Fentanyl EC-SERS Performance

As noted earlier, this MAD EC-SERS method was employed onsite at the MSP laboratory for rapid and simple analysis of the authentic samples. **Figure 3** provides the Raman spectra from the EC-SERS analysis for six of the authentic samples of interest and include single compound and multi-drug samples (spectra for all 24 authentic samples can be found in the **Supplementary Information**). These spectra are compared to that of a known fentanyl standard analyzed using the same method. Unknown 7 was confirmed to be fentanyl by GC-MS and provided excellent correlation to the fentanyl standard by EC-SERS, providing a positive identification. Unknown 1 demonstrated a very different type of sample due to identification of six compounds in the sample by GC-MS. Despite the large number of compounds in this sample, the targeted EC-SERS method demonstrated excellent selectivity, where the applied potentials and adsorption characteristics on silver provided preferential amplification of the fentanyl signal, allowing identification through visual correlation of major Raman bands with the fentanyl standard. Similar results are seen for Unknowns 8 and 13. During the development of the MAD method, quinine was found to be the most significant interfering compound, demonstrating enhancement of the Raman signals. Unknowns 9 and 19 demonstrate this interference from quinine. However, these samples still allowed tentative identification of a fentanyl compound in the sample. It is also interesting to note that Unknown 9 demonstrated a large Raman band around 1467 cm^{-1}, correlating with furanyl fentanyl. In combination with the two major peaks around 1000 cm^{-1}, the change in peak ratio of the peaks at ≈1600 cm^{-1} compared to quinine, and the presence of several other diagnostic fentanyl bands provided a tentative identification of furanyl fentanyl.

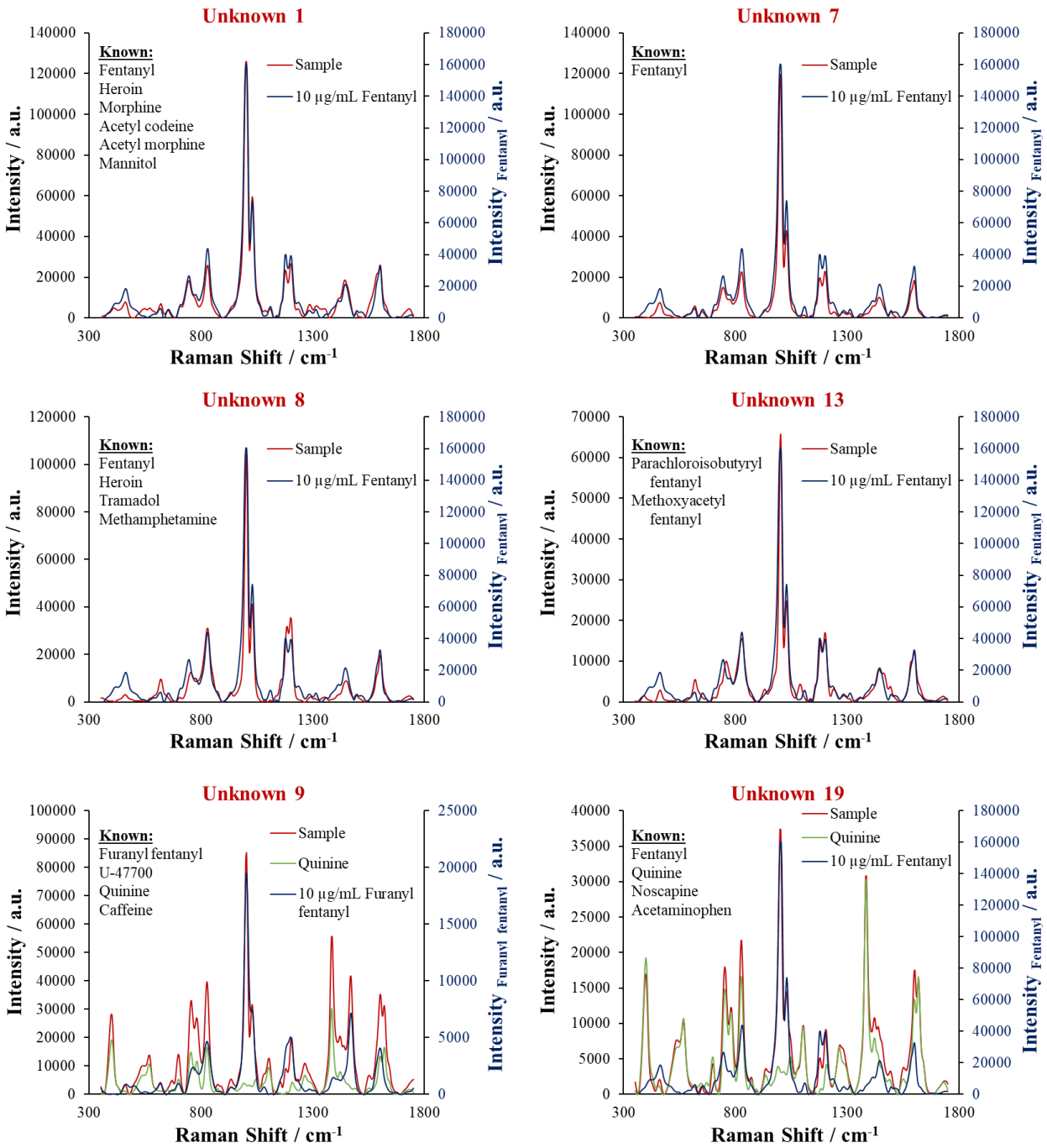

Figure 3. *Targeted EC-SERS spectra for the analysis of authentic seized drug samples demonstrating the correlation and identification of fentanyl and fentanyl-like compounds within the samples providing evidence toward the screening capabilities of EC-SERS for fentanyl compounds. Note that the secondary axis is provided for the intensity of the Raman signal for a fentanyl or furanyl fentanyl standard.*

It is worth noting another sample with interesting results, Unknown 12. Analysis of this sample via GC-MS did not provide sufficient signal for identification of fentanyl but did identify heroin. However, analysis by the targeted EC-SERS method provided a presumptive identification of fentanyl. This was confirmed via the LC-MS/MS analysis of the sample. This provides an excellent

demonstration of the increased sensitivity and reliability of this targeted EC-SERS method for the screening of fentanyl. **Figure 4** shows the EC-SERS spectrum for Unknown 12.

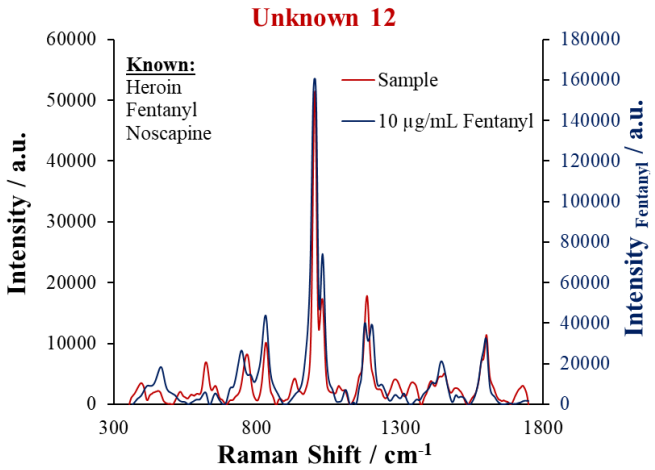

***Figure 4**. Screening identification of fentanyl in an authentic seized drug sample that was approximately 4 % fentanyl by weight according to LC-MS/MS analysis, demonstrating the sensitivity of the EC-SERS approach. Note that the secondary axis displays the intensity for the fentanyl standard.*

While interference from quinine was demonstrated previously, it is important to present examples where this interference could result in a false negative conclusion, likely due to competitive adsorption between quinine and fentanyl. **Figure 5** demonstrates two examples of authentic samples where the quinine signal overwhelmed the signal from fentanyl, although the main Raman band for fentanyl can still be observed. Unknown 3 provides an excellent opportunity to observe this effect since this sample contained only fentanyl and quinine. The LC-MS/MS data was used to determine the ratio of fentanyl to quinine that may prevent successful identification. The ratio of fentanyl to quinine in Unknown 3 was 1:29 and the ratio of furanyl fentanyl to quinine in Unknown 20 was 1:9. It is interesting to note the presence of the furanyl fentanyl bands at ≈1458 cm^{-1}, ≈1004 cm^{-1}, and 1039 cm^{-1}. While these peaks may allow identification of a fentanyl compound, caution should be taken due to the low relative signal of the bands, although a presumptive identification could be made. EC-SERS is a dynamic process involving diffusion, migration, and adsorption of analytes and these processes may be affected by many things including the presence of other molecules or concentration differences. Competition for adsorption area, different adsorption properties, and orientation differences may have an effect on the resulting spectrum. On average, the threshold ratio in samples that still afforded identification of a fentanyl or fentanyl-like compound was around 1:3 for quinine, although exceptions were observed and it should be noted that the number of compounds and their identity could also affect detection, along with considerations of the heterogeneity of the original seized sample, where samples taken for EC-SERS and for LC-MS/MS may have contained different quantities/ratios due to possible heterogeneity in the seized samples. Further, the Drug Enforcement Administration's Fentanyl Profiling Program (DEA FPP) determined that the top five encountered secondary substances in seized powder fentanyl samples from 2021 was mannitol (59 % of samples), inositol (46 % of samples), lactose (30 % of samples), xylazine (14 % of samples), and fluorofentanyl (14 % of samples) [45]. Also, a report conducted by The Center for Forensic Science Research & Education (CFSRE) between the first quarter of 2018 and the first quarter of

2022 identified quinine in 18.3 % of exhibits (n=2,151), where positivity was dependent on the location within the United States (for example Washington DC = 53.8 % of samples, Pennsylvania = 7.5 % of samples, and Texas = 0.7 % of samples) [46]. This suggests that problems associated with quinine interference may be region-dependent and will need to be assessed based on representative casework samples from individual laboratories or regions, as quinine interference may not pose a significant issue in regions where quinine is rarely observed. For this study, the forensic laboratory was responsible for selecting samples from authentic casework with the only requirement being that a portion of samples had to contain fentanyl and/or a fentanyl analog and another portion had to be negative for fentanyl and/or a fentanyl analog. Therefore, the analyzed samples represented what this forensic laboratory might receive on a given day, including the large number of quinine positive samples, which is quite common for the region where the MSP laboratory is located.

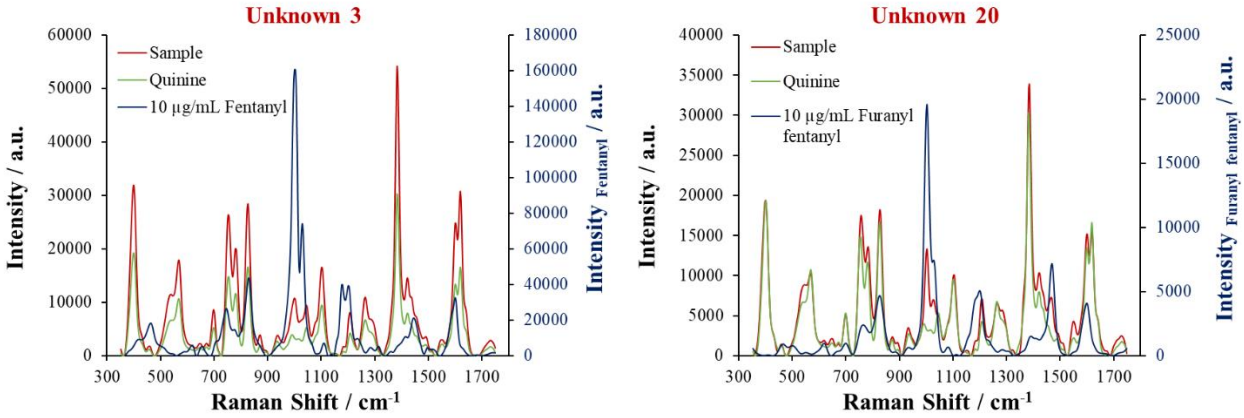

Figure 5*. Targeted EC-SERS spectra for authentic samples containing quinine, demonstrating the interference exhibited by this molecule when present in the seized samples. Ground truth identifications were as follows: Unknown 3 contained fentanyl and quinine and Unknown 20 contained furanyl fentanyl, U-47700, and quinine. Note that the secondary axis is reserved for the intensity of the fentanyl or furanyl fentanyl standard.*

Although this *in situ* EC-SERS method was targeted toward fentanyl, high concentrations of other analytes may still present some Raman signal due to the enhancement from the SERS substrate. However, it is important to note that the signal from these other molecules is significantly smaller than that seen for fentanyl. **Supplementary Figures S3 and S4** demonstrate example spectra from the true negative authentic samples. It is worth noting that cocaine presented similar Raman bands, and both cocaine and fentanyl shared the ≈1000 cm^{-1} band in common, raising the potential for a false positive result. Methamphetamine also demonstrated similar peaks. However, as seen in **Supplementary Figures S3 – S6**, there are differences in the Raman spectra that can be observed, as well as a significant reduction in signal intensity for cocaine and methamphetamine samples. The future incorporation of chemometric approaches for spectral comparison is expected to minimize false positive results and improve overall accuracy.

Using the ground-truth data from the GC-MS analysis, the performance of the *in situ* targeted EC-SERS method was assessed based on correct identification of fentanyl or a fentanyl-like substance in the authentic data set. Out of the 24 authentic seized samples, there were no false positives for fentanyl. However, there were several false negatives, which were all a result of interference from

quinine in the sample. As quinine was deemed the major interfering compound, and its combination with fentanyl in street samples is common within the Maryland/Washington D.C. region, an analyst may decide to treat EC-SERS quinine positive samples as potential fentanyl-containing specimens, essentially limiting false negative results, especially when the main Raman band at ≈1004 cm^{-1} is observed. It is also important to note that the LC-MS/MS data provided an opportunity to assess the relative percent contribution of fentanyl or fentanyl analogs to the overall sampled mass from the seized specimens. As demonstrated in our previous work, identification of fentanyl was possible at low percent contributions, a significant advantage to this type of screening method. When considering fentanyl, the average weight percent of fentanyl in this data set was approximately 6 wt. %. When taking into account all fentanyl and fentanyl analogs individually, the average weight percent of these was also approximately 6 wt. %; however, when considering the total contribution of fentanyl-like compounds in a sample, the average weight percent increased to approximately 9 wt. % due to some samples containing multiple fentanyl-like compounds. **Table 2** provides the performance measures of the targeted fentanyl EC-SERS method for screening authentic seized samples, demonstrating the effectiveness of this screening approach.

***Table 2**. Performance rates calculated based on the authentic seized samples as assessed via EC-SERS. A total of 14 of the samples were identified as containing fentanyl or a fentanyl analog (TP), 7 samples did not contain a fentanyl-like substances (TN), 3 samples contained a fentanyl-like substances but were not identified by the EC-SERS screening (FN), and there were no false positives (FP).*

$FPR = \frac{100 * FP}{TN + FP}$	False Positive Rate	0 %
$FNR = \frac{100 * FN}{TP + FN}$	False Negative Rate	17.6 %
$TNR = \frac{100 * TN}{TN + FP}$	True Negative Rate (Specificity)	100 %
$TPR = \frac{100 * TP}{TP + FN}$	True Positive Rate (Sensitivity)	82.4 %
$Accuracy = \frac{100 * (TP + TN)}{TP + TN + FP + FN}$	Accuracy	87.5%

3.3.Comparison to Chemical Color Tests

Current screening protocols commonly utilize chemical color tests, but these tests may struggle with the presence of multiple compounds in the same sample, may be subjective, and may be problematic considering the prevalence of novel psychoactive substances in the drug landscape, including fentanyl analogs [1,4–7]. Subjectivity may be introduced in these tests, where results could depend on the experience of the chemist in interpreting colors, especially those colors where the shade may denote differences. One example is the Marquis test, where an orange color may indicate methamphetamine or fentanyl, while a salmon color may indicate cocaine, and a range of yellows to oranges to reds may indicate a number of drugs [47–50]. Generally, a color testing scheme is utilized with multiple color tests to overcome these challenges and provide discrimination between drugs and drug classes, but the number of tests must be considered in terms of time, sample size limitations, and solvent/waste issues. As such, color will generally only provide a class of drug or tentative identification of a single drug or group of drugs.

As a comparison, the results of chemical color tests performed on a twenty of the samples at the MSP forensic laboratory are also shown in **Figure 2**, along with presumptive identifications and remarks. Due to the subjective and presumptive nature of the color tests, it is difficult to provide an accurate assessment of the results as there were multiple instances where several analytes could have been the correct conclusion. However, there was one instance (5 % (1/20) of samples, Unknown 3) of a false positive for heroin when no heroin was detected via GC-MS or LC-MS/MS. There were multiple instances (35 % (7/20) of samples, Unknowns 7, 10, 14, 19 to 21, and 24) where the results of the testing scheme were not as expected for the target compounds, and these were categorized as inconclusive without providing a suggested identification for the purposes of this paper. Finally, the remaining 60 % (12/20) of the samples were correctly identified presumptively for at least one controlled compound or samples with no controlled substance, with the caveat that more than one compound may produce the observed result, lowering the analytical significance. For identification of fentanyl or a fentanyl analog specifically, the color test results had an accuracy of 60 %, with a true positive rate of 53.3 % and a true negative rate of 80 %.

The presented targeted EC-SERS method provides an opportunity to selectively monitor and screen samples for fentanyl-like compounds with the possibility of analog differentiation and identification. The benefit of this approach is increased analytical significance added from the addition of a vibrational spectroscopic technique providing structural information. Therefore, the EC-SERS method provides an improvement over the current chemical color test scheme, allowing improved differentiation between fentanyl/fentanyl analogs and other drugs of abuse. Another point of comparison is chemical consumption and waste. Preparation of the color test reagents used here required cobalt thiocyanate, concentrated sulfuric acid and formaldehyde (Marquis), and mercuric chloride and potassium iodide (Mayer's) [47,51]. Aside from the preparation and chemical waste generated from these tests, these compounds also include many hazards including targeting lung, thyroid, eyes, and kidneys, toxicity, corrosivity, carcinogenicity, heritable genetic damage, reproductive toxicity, and long-lasting aquatic environmental impact [47,52]. This EC-SERS method uses only dilute perchloric acid and water as the solvent for analysis, reducing waste and improving on safety hazards, although perchloric acid still has its own hazards to be aware of including corrosivity and targeting the thyroid on repeated exposure [53], these hazards are reduced in diluted form. However, EC-SERS will have the addition of single-use SPE waste. Additionally, EC-SERS requires interaction with a powdered sample one time versus multiple times for color testing. Finally, the time required to complete presumptive testing should be considered. This color testing scheme was previously used in Sisco et al. and took 18.6 min for a set of five samples [54]. This targeted EC-SERS method is versatile in the fact that there are two enhancement regions for the SERS effect. The first occurs within the first 30 s and the second is near the end of the experiment around 300 s. In this way, samples with higher concentrations of target analyte can be easily observed and enhanced in the first 30 s, while samples with low percent contributions will demonstrate increased sensitivity during the second enhancement. Therefore, a set of five samples could take between 10 min and 30 min to be screened, including sample preparation. As such, the time requirement is similar between the color testing scheme and the EC-SERS method. For the purposes of this paper, all samples were allowed to undergo the full procedure, even if fentanyl or a fentanyl-like compound was identified during the first 30 s.

4. Conclusions

This electrochemical surface-enhanced Raman spectroscopy (EC-SERS) screening method provides several advantages over traditional color testing including improved selectivity, reduced chemical waste and hazards, the ability for targeted approaches, and improved analyst safety from reduced exposure to seized samples compared to a color testing scheme. Identification of potentially hazardous substances like fentanyl and fentanyl analogs is critical since analysis tests, operating procedures, and safety practices may be altered by the presence of these dangerous compounds. Unlike other SERS applications that require many synthesis steps and materials, EC-SERS utilizes a simple and effective electrochemical generation of the SERS substrate (disposable screen-printed electrodes), improving reproducibility for a fast, simple, and inexpensive method. The EC-SERS approach presented here is a targeted screening for fentanyl-like substances that is rapid and effective, providing a powerful technique with high discrimination ability for fentanyl. Using a screen-printed electrode platform allows this methodology to be portable for laboratory or field operation, increasing the usefulness and ability of the EC-SERS method. In addition, a simple sampling approach using just the tip of a spatula was implemented, allowing for a small amount of the seized sample to be tested easily.

This screening approach, previously developed by our group [44], was applied to authentic samples from the Maryland State Police. Excellent detection capabilities were demonstrated with positive fentanyl identifications on samples that averaged 6 wt. % to 9 wt. % fentanyl or fentanyl-like substances, which compares with seizures made throughout the United States. According to the most recent information from the DEA, the average purity of fentanyl in powder samples was 14.4 % and ranged between 0.1 % and 75.6 % for samples examined by the DEA FPP in 2021 (n=666 samples) [45]. This targeted method was selective, demonstrating preferential enhancement of the fentanyl signal compared to other drug analytes, allowing for identification of fentanyl in the presence of cocaine, methamphetamine, and other analytes, as well as identification of these other compounds in some instances. Quinine was identified as the major interfering compound, solely leading to the false negative results of approximately 18 % (3 samples) in the authentic samples. The overall accuracy for screening for fentanyl-like substances in the authentic data set was approximately 88 %, an increase from the accuracy of the color test results of 65 %. Future work will focus on chemometric approaches for objective spectral comparisons and score-based identifications, as well as the development of untargeted methods. This EC-SERS method represents one of the first demonstrations of EC-SERS applications toward forensic drug analysis and represents a step forward in developing novel screening methods for drugs of abuse that can improve the reliability of analysis, safety of first responders, and selectivity in a changing drug landscape, while helping to streamline further confirmatory testing.

Future work will focus on chemometric approaches for objective spectral comparisons and score-based identifications. In addition, the development of untargeted screening methods utilizing EC-SERS approaches will be studied, as well as exploring EC-SERS mechanisms.

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Disclaimers

Certain commercial products are identified in order to adequately specify the procedure; this does not imply endorsement or recommendation by the National Institute of Standards and Technology (NIST), nor does it imply that such products are necessarily the best available for the purpose.

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