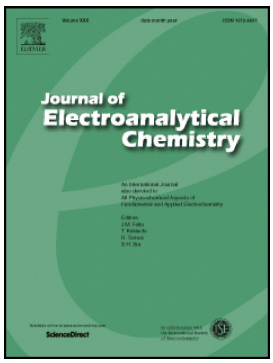


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# Electrochemical detection of fentanyl with screen-printed carbon electrodes using square-wave adsorptive stripping voltammetry for forensic applications

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## Abstract

Square-wave adsorptive stripping voltammetry (SWAdSV) is proposed as a fast, simple, and sensitive approach toward the detection, identification, and semi-quantitation of fentanyl in seized drug samples using screen-printed carbon electrodes. Electrochemical oxidation of fentanyl resulted in the formation of two anodic peaks, one at 0.75 V (peak I) and the second at 0.88 V (peak II) versus a Ag/AgCl pseudo reference electrode. Voltammetric measurements were conducted under optimized experimental conditions using fentanyl standards ranging from 0.076 μg/mL to 6.9 μg/mL, which resulted in a limit of detection of 0.037 μg/mL. Reproducibility was assessed as the average percent relative standard deviation of the slopes of calibration curves and was 2.6% for oxidation peak I and 7.4% for oxidation peak II in cell. Detection capabilities were assessed in a 5-mL cell and in a 100 microliter drop. Interference studies were conducted with cocaine, methamphetamine, quinine, caffeine, and acetaminophen. The mechanism for the electrochemical oxidation of fentanyl to norfentanyl is also described. Accuracy and suitability of the method for seized drug analysis was assessed using 11 simulated samples. Suitability of the method for future analysis of fentanyl in oral fluid was assessed.

## Keywords

Fentanyl, Screen-printed electrodes, Fentanyl Voltammetric Determination, Forensic Science, Oral Fluid, Seized Drugs

## 1. Introduction

Opioid abuse is a severe threat to United States society that presents many challenges to overcome both today and in years to come. In response to the opioid epidemic, the Department of Health and Human Services declared a public health emergency in 2017, which has continued to be renewed quarterly [1,2]. Over 130 people die daily from opioid related drug overdose, where over 28,000 of the 47,000 deaths were attributed to synthetic opioids [1,3]. Opioids represent a class of compounds utilized for their anesthetic and pain-relieving properties and frequently abused due to their euphoric effects [4–6]. Interaction with the $\mu$-opioid receptors within the brain results in decreased response to pain and high physical and psychological dependence. Activation of the $\mu$-opioid receptor has been implicated as the primary receptor sub-type responsible for the effects of fentanyl, demonstrating an affinity for the fentanyl agonist of 100x and 400x greater than for the $\kappa$ or $\delta$-opioid receptors, respectively [7,8].

The newest face of this problem is novel psychoactive substances (NPS) designed to elicit similar or heightened responses compared to their controlled substance counterparts. Fentanyl, a synthetic opioid approximately 100 times more potent than morphine, and fentanyl analogs represent a dangerous class of NPS [9]. Although not new, the last decade has seen increasing knowledge about fentanyl due to its prevalence in the media as a result of the opioid epidemic. In fact, fentanyl has been medically used for its anesthetic and pain relieving properties since 1963, and more commonly for patients with advanced stage cancer[10–12]. Since its synthesis by Paul Janssen, modifications and changes to the core structure have become common and extensive [10].

Fentanyl and its analogs pose many challenges for the forensic community and justice system due to modifications of the fentanyl core structure, potency, presence in mixtures and low concentrations, and difficulty in detecting and identifying newly discovered NPS. Typical analysis for these compounds requires chromatographic separation followed by mass spectrometry. While extremely sensitive and selective, these methods are costly, time consuming, lack portability, and require experienced lab personnel. Further, color field tests generally lack specificity and are subjective. Therefore, there is a need for analytical screening techniques that can offer rapid and accurate results, require minimal sample preparation, and have the ability to be made portable for field use.

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) denotes analytical techniques into three categories (A, B, and C) based on their discriminatory power, with A being considered confirmatory while category C techniques are considered as screening methods. Electrochemistry provides a versatile analytical platform for the detection of drugs with high sensitivity, accuracy, precision, and selectivity [13]. For these reasons, although not currently listed within the SWGDRUG techniques, electrochemistry should be considered a category B technique with intermediate

discriminatory power. Electrochemical techniques have been described previously for the detection of drugs ranging from cannabis to cocaine to synthetic cathinones [14–21]. Nonetheless, few works have described electrochemical methods for the analysis of fentanyl. Table 1 provides a summary of these detection techniques including the work performed in this manuscript; however, these other methods have generally required extensive modifications to the electrode or used methods that are considered outdated [22–26]. For example, in 1994, Guo et al. utilized adsorptive stripping voltammetry (AdSV) at a mercury electrode for the detection of fentanyl. As the toxicity of mercury is well known, the use of mercury electrodes has lost favor. In comparison, screen-printed electrodes (SPEs), have become widely used for electrochemical testing as a result of their small size, low cost to manufacture, robustness, and disposability [27]. The small size of these testing platforms provides excellent use for field applications and allows for the analysis of small volumes of sample. Most recently in 2019, Goodchild et al. and Barfidokht et al. developed a sensor approach for fentanyl using screen-printed carbon electrodes (SPCEs) modified with an ionic liquid [25,26]. Modifications increase the number of steps and time required for the fabrication of these sensors and, invariably, the cost associated with the sensor.

*Table 1. Literature review of fentanyl detection using electrochemical methods*

| Electrode | Technique | LOD (μg/mL) | Linear Range (μg/mL) | $R^2$ | RSD (%) | Reference |
|---|---|---|---|---|---|---|
| GCM-OPFP | CV/ Electrochemi-luminescence | $3.0 \times 10^{-3}$ | 0.003 to 33.6 | 0.999 | 1.9 at 1.7 μg/mL | 14 |
| Graphite SPE | HPLC-AD | 0.77 | 10 to 120 | 0.999 | 0.53 | 15 |
| Hg | ASV | $1.7 \times 10^{-2}$ | 0.034 to 0.336 | 0.999 | 3.6 | 16 |
| SPCE-RTIL | CSWV | 1.7 | 1.7 to 32 | 0.997 | -- | 17 |
| fSPCE-MWCNT/ RTIL | SWV | 3.4 | 3.4 to 33.6 | 0.992 | 3.2 | 18 |
| SPCE-Cell | SWAdSV | $3.7 \times 10^{-2}$ | 0.076 to 0.64<br>1.3 to 6.9 | 0.995<br>0.991 | 2.6 | This work |
| SPCE-Drop | SWAdSV | $2.3 \times 10^{-1}$ | 0.302 to 6.88 | 0.998 | 4.0 | This work |

GCM-OPFP = Glassy Carbon Microspheres with room temperature ionic liquid N-octylpyridium tetrafluoroborate
HPLC-AD = High-Performance Liquid Chromatography-Amperometric Detection
RTIL = Room Temperature Ionic Liquid
fSPCE = Flexible Screen-Printed Carbon Electrode

As fentanyl is commonly encountered in forensic cases, its analysis is of critical importance to aid investigations and reduce case backlogs. A simple sensor for the detection of fentanyl in seized drug cases is presented herein using a square-wave adsorptive stripping voltammetry (SWAdSV) method. Analysis was conducted using SPCEs following parameter optimization. Interference studies, limit of detection, and accuracy was assessed for the method. Following analysis, the electro-oxidative mechanism of fentanyl was explored and tested using electrochemical analysis and liquid chromatography-tandem mass spectrometry (LC/MS/MS).

## 2. Materials and methods

### *2.1. Reagents*

Fentanyl citrate was purchased from Cayman Chemical Company (Ann Arbor, MI). Acetaminophen was purchased from Sigma-Aldrich (St. Louis, MO). Methamphetamine, cocaine hydrochloride, quinine, acetaminophen, and caffeine were used for interference studies. Methanol (Optima®), Acetonitrile (Optima®), and concentrated Hydrochloric acid (Trace grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Tris(hydroxymethyl)amino-methane, Monobasic sodium phosphate, dibasic sodium phosphate, sodium hydroxide, and potassium chloride were purchased from Sigma-Aldrich (St. Louis, MO). A Millipore Direct-Q® UV water purification system (Billerica, MA) was used to obtain purified water (18.2 MΩ).

Fabrication of the screen-printed carbon electrodes required the following inks: Electrodag 6037 SS (silver/silver chloride ink), Electrodag PF-407 A (carbon ink), Electrodag 452 SS (dielectric ink), and Electrodag 418 (silver ink) were obtained from Achenson Colloiden (Scheemda, Netherlands). Polyester films (PET), of 0.5 mm thickness, were used as substrates for printing (HIFI Industrial Film, Dardily, France).

### *2.2. Instrumentation*

Home-made screen-printed carbon electrodes were fabricated using a DEK 248 screen-printing system (DEK, Weymouth, UK) with polyester screens with stencils as described previously [28]. SPCEs contained conductive silver tracks, Ag/AgCl pseudo-reference electrode, and carbon working (0.126 $cm^2$ geometric area) and counter electrodes.

Electrochemical measurements were acquired using the PalmSens3 and PalmSens4 potentiostats with PSTrace software (Randhoeve, Netherlands). The pH of buffer solutions was determined using a FiveEasy Plus pH meter by Metller-Toledo (Columbus, OH).

*2.3. Parameter Optimization*

Parameter optimization was conducted for pH, supporting electrolyte, deposition time, and anodic pre-treatment, as well as technique parameters. Using a fentanyl concentration of 152 μg/mL, buffer pH was tested between pH 5.5 and 9.0 as a drop on the electrode surface. Phosphate buffered saline (PBS, 100 mM supplemented with 100 mM KCl) and Tris-HCl (100 mM) were assessed for use. The effect of adsorptive deposition time on current response for 0.336 μg/mL fentanyl was analyzed in cell for times 0, 10, 20, 40, 80, 160, 320, 640, and 1280 seconds. Finally, anodic pre-treatment of the carbon surface before analysis of fentanyl was assessed in order to improve current response for a range of applied potentials between 1.0 and 1.6 V at times between 20 and 80 seconds. Pre-treatment was shown to increase current response for several measurements using the same electrode. Pre-treatment was conducted on each new electrode before its use in any measurement procedures.

*2.4. Square-Wave Adsorptive Stripping Voltammetry*

First, anodic pre-treatment of the carbon working surface was conducted in Tris-HCl pH 8.5 at a potential of 1.5 V for 40 seconds. Analysis of fentanyl was then accomplished using SWAdSV in either a 5 mL cell or as a 100 microliter drop on the electrode surface. An adsorptive deposition time of 320 seconds (stirred for the cell or quiescent for the drop) was performed prior to the application of the SWAdSV procedure. SWAdSV was carried out between -0.5 V and 1.6 V with a potential step of 0.012 V, amplitude of 0.075 V, and a frequency of 100 Hz. Calibration curves were constructed from voltammograms by measuring peak current heights with increasing concentrations of fentanyl.

*2.5. Interference Studies*

Analysis of commonly encountered drugs and adulterants was conducted using the optimized SWAdSV procedure. Interferents were analyzed alone in Tris-HCl buffer and in various mixture ratios with fentanyl in cell. Identification of anodic peak I for fentanyl was used to assess interference.

*2.6. Analysis of Simulated Samples*

The SWAdSV method was utilized for the analysis of simulated samples prepared in lab to replicate analysis of seized drugs. Various concentrations of fentanyl were prepared in Tris-HCl buffer and measured by a different analyst for qualitative identification of fentanyl and quantified based on calibration curves performed in cell. Expectorated oral fluid from a healthy volunteer was used to prepare spiked oral fluid samples to demonstrate the potential of the method to detect fentanyl in oral fluid samples. To this end, the oral fluid was crashed using cold acetonitrile and Tris-HCl and then centrifuged. An aliquot from the bottom of the sample above the protein pellet was spiked with fentanyl and diluted in

buffer for analysis in cell (4,000 microliters) and in drop (100 microliters) and was measured using the SWAdSV method. The standard addition method was utilized for quantitation of fentanyl within the cell and in drop for simulated oral fluid samples.

### *2.7. Liquid Chromatography-Tandem Mass Spectrometry*

An Agilent 1290 Infinity II Liquid Chromatography system with an Agilent 6470 Triple Quadrupole Mass Spectrometer (Santa Clara, CA) was used in the analysis of fentanyl and its electro-oxidative product with Agilent MassHunter software. A dynamic multiple reaction monitoring method (dMRM) was developed (see Supplementary Material Table S1). An Agilent RR-HD Zorbax Eclipse Plus C18 column (3.0 x 100 mm, 1.8 micron) with an Agilent SB-C18 pre-column (3.0 x 5 mm, 1.8 micron) was used for chromatographic separation. A gradient elution at 0.5 mL/min with a 0.1% formic acid and 5 mM ammonium formate in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B) was used to achieve separation (see Supplementary Material Table S2).

## 3. Results and discussion

### *3.1. Parameter Optimization*

The electrochemical behavior of fentanyl was studied using cyclic voltammetry and resulted in two small anodic peaks, at approximately 0.95 V and 1.05 V (see Supplementary Material Fig. S1). In order to resolve these peaks, supporting electrolyte composition and pH were optimized. Optimization of the pH in PBS demonstrated maximum current response at a pH of 8.5 (Fig. 1). Current response peaked at this pH value and immediately began to decrease at a more basic pH value of 9.0. The pH measurements were tested in triplicate and demonstrated good reproducibility throughout the range. Similar results were obtained in Tris-HCl buffer, which demonstrated loss of the second peak and peak resolution at low and high pH values. The difference between pH 8.5 and 8 is not very important in Tris-HCl; however, a slight improvement in peak current was evident at pH 8.5. Following pH determination, buffer selection was undertaken. Comparisons between PBS and Tris-HCl, both selected for their use in biological analyses, revealed similar current response for both buffers at pH 8.5. However, increased peak current response in lower concentrations of fentanyl was observed in Tris-HCl and was therefore selected for use in subsequent experiments.

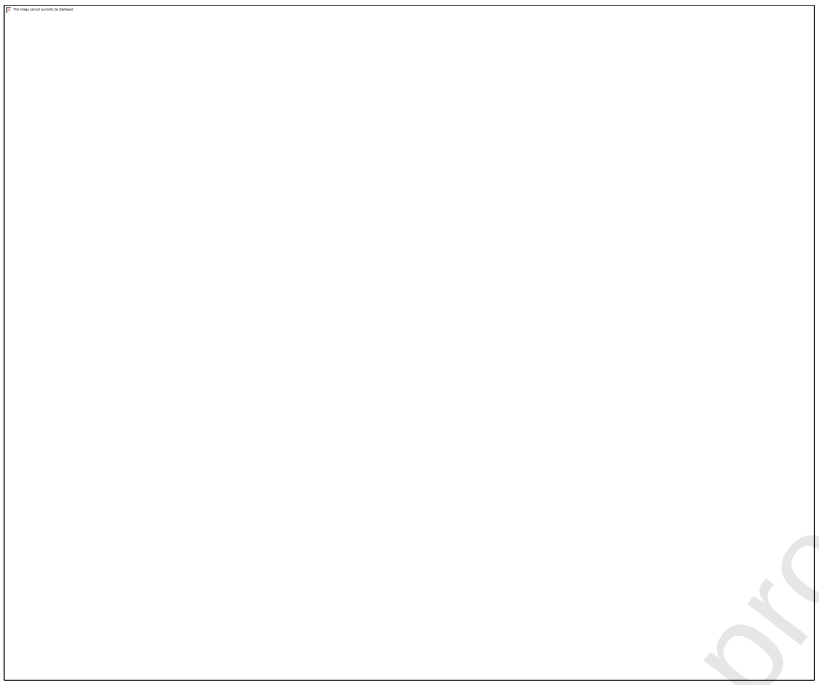

*Fig. 1. Cyclic voltammograms of fentanyl at 151 µg/mL obtained in a 100 microliter drop on SPCE at varying pH values in PBS at a scan rate of 0.1 V/s.*

Anodic pre-treatment of the working electrode surface in Tris-HCl was explored due to observing increased current response for fentanyl. It has been shown in the literature that anodic pre-treatment of a carbon surface results in a roughing of the carbonaceous material, increasing the surface area and creating more opportunities to promote electron transfer, as well as controlling surface functional groups on the electrode that could promote adsorption [29–31]. Furthermore, anodic pre-treatment can remove surface contaminants and lower the required oxidation potentials [29]. After exploration of various potentials and times for pre-treatment, 1.5 V over 40 seconds resulted in the largest current response for both peak I and peak II at the lowest concentration. Finally, an increase in current response was observed during the testing of longer deposition times. Increasing the amount of time, the solution was allowed to stir (for cell measurements) or sit on the electrode surface (for drop measurements) prior to analysis resulted in larger current response. Although increasing the amount of time on the electrode surface increased current response, the ideal time was chosen to be 320 seconds in order to maintain a fast analysis procedure (Fig. 2). The difference in saturation current between the cell and drop methods could result from the ability to stir the solution while in cell compared with drop, as well as, the smaller sample volume for drop analysis. Moreover, a plateau in current response was observed starting around 320 seconds, where increasingly long adsorptive deposition times were not justified by the slightly increased current response.

This behavior was attributed to an adsorption of fentanyl to the carbon working electrode, serving to pre-concentrate the analyte at the surface.

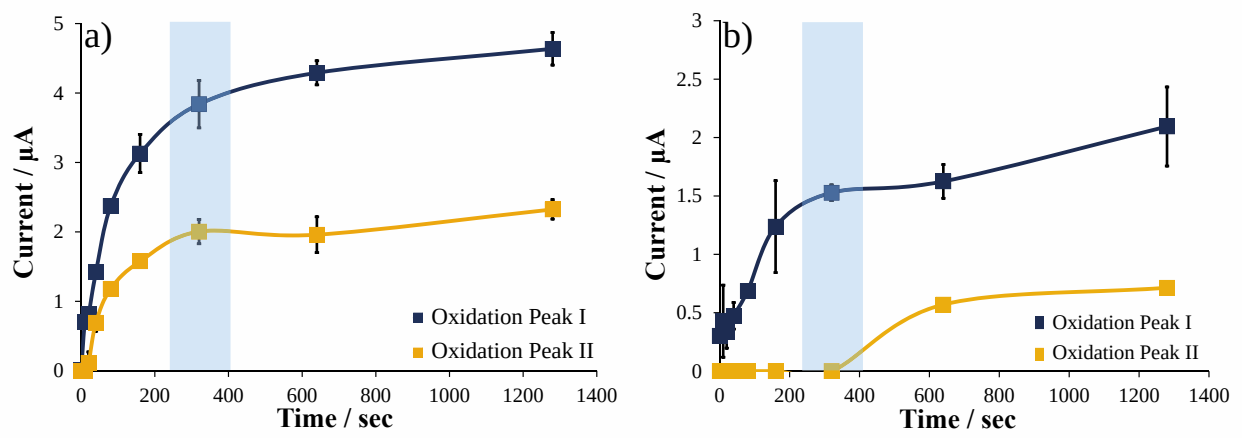


*Fig. 2. Effect of accumulation time on the SWAdSV peak currents for fentanyl=0.336 µg/mL in Tris-HCl pH 8.5 in (a) cell and (b) 100 microliter drop (n=3).*

### *3.2. Fentanyl Detection*

Using the optimum voltammetric conditions and a 100 mM Tris-HCl pH 8.5 buffer as supporting electrolyte, several calibration curves for fentanyl concentrations ranging between 0.075 µg/mL and 6.88 µg/mL were performed. Two oxidation peaks were observed at 0.75 V (peak I) and 0.88 V (peak II), as well as, two areas of linearity across the calibration range. The first linear range was observed below 1 µg/mL between 0.075 µg/mL and 0.637 µg/mL, while the second linear range was between 1.31 µg/mL and 6.88 µg/mL (Fig. 3). However, good linearity was achieved for both regions for oxidation peaks I and II ($R^2 > 0.990$) allowing for analysis of low concentrations of fentanyl. A sensitive method is required for the analysis of fentanyl in seized drug case scenarios as a result of a wide variation in purity of seized samples, which can range between 0.1% and 97.8% pure [32].

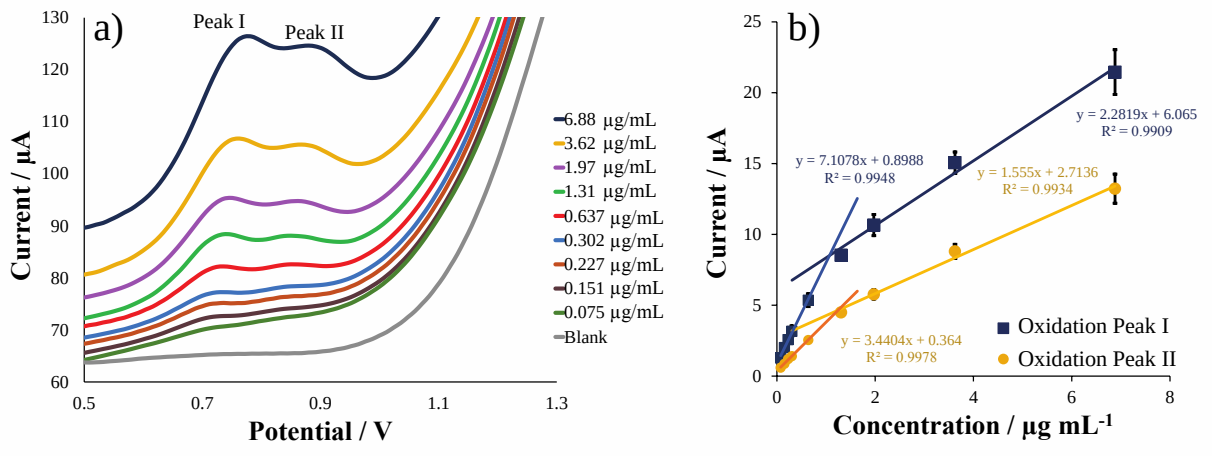


*Fig. 3. SWAdSV of fentanyl (n=3) in Tris-HCl pH 8.5 for (a) Voltammograms in a 5 mL electrochemical cell and (b) average calibration curve and associated deviation for peaks I and II for fentanyl, n=3.*

Analysis as a drop on the electrode surface was conducted between 0.302 µg/mL and 6.88 µg/mL. However, only a single region of linearity was observed for each peak (Fig. 4). This was most likely a result of solution dynamics, adsorption of fentanyl, and saturation of the working electrode surface. In drop analysis, the analyte access to the working electrode is driven by diffusion, resulting in a difference in the adsorption rate of the analyte to the electrode surface, which would be lower than in a cell environment with stirring.

The performance of the proposed method was established in terms of reproducibility and limits of detection for both cell and drop analysis. Sensor reproducibility was determined in terms of the average percent relative standard deviation (RSD) of the slopes of the calibration curves and was 2.6% and 7.4% for peaks I and II, respectively for the cell method (n=6) and 4.0% and 4.6% for the drop method (n=6). The limit of detection (LOD) was assessed statistically as three times the standard deviation in the peak current of the lowest concentration divided by the average slope. The resulting LOD was 0.037 ± 0.017 µg/mL for the cell method (n=6) and 0.233 ± 0.025 µg/mL for the drop method (n=6).

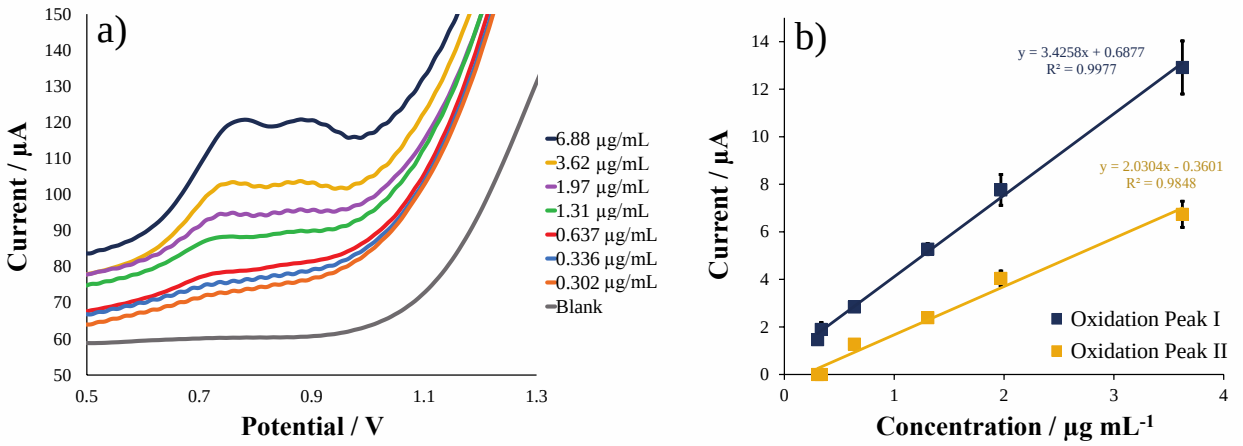


*Fig. 4. SWAdSV of fentanyl (n=3) in Tris-HCl pH 8.5 for (a) Voltammograms in a 100 microliter drop and (b) average calibration curve and associated deviation for peaks I and II for fentanyl, n=3.*

### *3.3. Oxidative Mechanism*

The oxidation of tertiary amines has been previously described in the literature by Masui, Garrido et al., and Frisell et al. to result in the presence of two oxidation peaks. These authors proposed that this process may be due to the oxidation of the tertiary amine followed by the oxidation of the newly formed secondary amine [21,33,34]. Therefore, the peak present at less anodic potentials would result from oxidation of the tertiary amine. However, literature has also detailed the formation of these two oxidation peaks from the oxidation of the tertiary amine due to adsorbed species and diffuse species in solution [35]. In this scenario, oxidation peak I results from oxidation of the adsorbed species and the more anodic peak, peak II, results from the oxidation of the diffuse species. Based on the work presented in this paper, as well as, by Goodchild et al. [25] and Hegde et al. [36], we have proposed the following mechanism for the electro-oxidation of fentanyl.

Electrochemical oxidation of fentanyl occurs through the N—dealkylation of the piperidine nitrogen resulting in the oxidation of fentanyl to norfentanyl. As separate support for this, SWAdSV was performed on different drops of fentanyl for a varying number of scans (0, 10, 20, 40, and 80 scans). Analysis of the resulting product solutions by LC/MS/MS showed the absence of norfentanyl prior to application of the electrochemical procedure and increasing concentration of norfentanyl upon increasing number of square-wave scans (see Supplementary Material Fig. S2). This confirms that the electro-oxidation product was norfentanyl. Norfentanyl was demonstrated by Goodchild et al. to not result in an oxidation peak [25], supporting the hypothesis of both peaks arising from the same oxidation process. Further, through analysis via cyclic voltammetry, the process was shown to be an irreversible electron transfer and demonstrated evidence for both adsorption and diffusion when plotting peak current versus the scan rate and the square root of the scan rate (see Supplementary Material Fig. S3-S4). Adsorption

was also supported through the demonstration of increased peak current with longer stirred adsorptive deposition equilibration time. The proposed oxidation mechanism of fentanyl to norfentanyl can be found in Fig. 5.

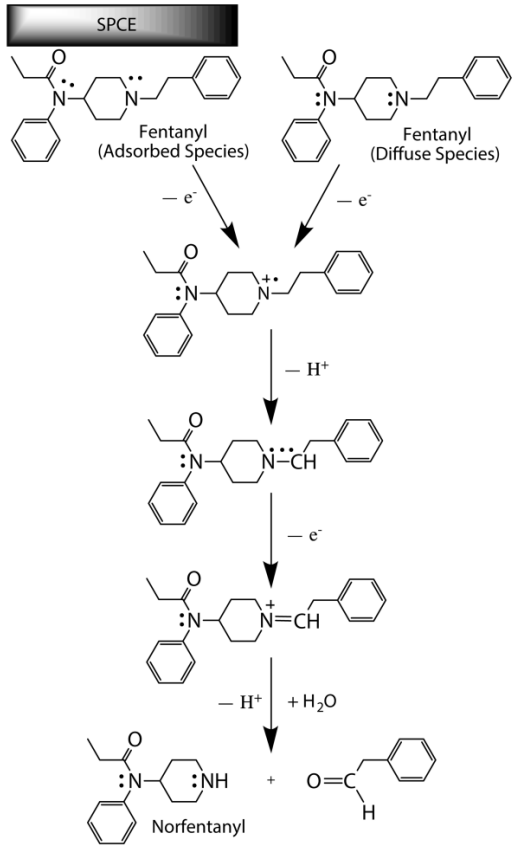


*Fig. 5. Electro-oxidative mechanism for the oxidation of fentanyl to norfentanyl*

### *3.4. Interference Studies*

Fentanyl is commonly encountered as a mixture or used in combination with other seized drugs [37,38]. Interference studies were conducted using methamphetamine, cocaine, quinine, caffeine, and acetaminophen. The interfering compounds were analyzed individually to determine their electroactivity. Caffeine and acetaminophen were found to not interfere with the detection of both peak I and peak II of fentanyl. Caffeine was not electroactive over the potential range tested, however, oxidation of acetaminophen was observed at approximately 0.28 V. Methamphetamine was demonstrated to mask peak II of fentanyl at very dilute concentration ratios (0.3:20 and 0.3:10 fentanyl:methamphetamine), however, in all mixtures, peak I was clearly visible and both peaks were detectable at a ratio of 1:10 of fentanyl:methamphetamine (see Supplementary Material Fig. S5-S8).

Cocaine and quinine both demonstrated oxidation peaks at approximately 0.90 V (see Supplementary Material Fig. S9-S10). As such, peak II of fentanyl could no longer be detected as the presence of a peak

was due to the oxidation of both the interfering compound and fentanyl. However, peak I of fentanyl was visually observable at a ratio of 0.3:10, 1:10, and 1:3 when present with cocaine (Fig. 6). Quinine resulted in the largest interference, where peak I of fentanyl could not be observed at a ratio of 0.3:10. At 1:10 a small shoulder corresponding to peak I was observable. Oxidation peak I for fentanyl was clearly identifiable at a 1:3 ratio of fentanyl:quinine. Table 2 provides a summary of the analysis of the five compounds tested during the interference studies (see Supplementary Material Fig. S11).

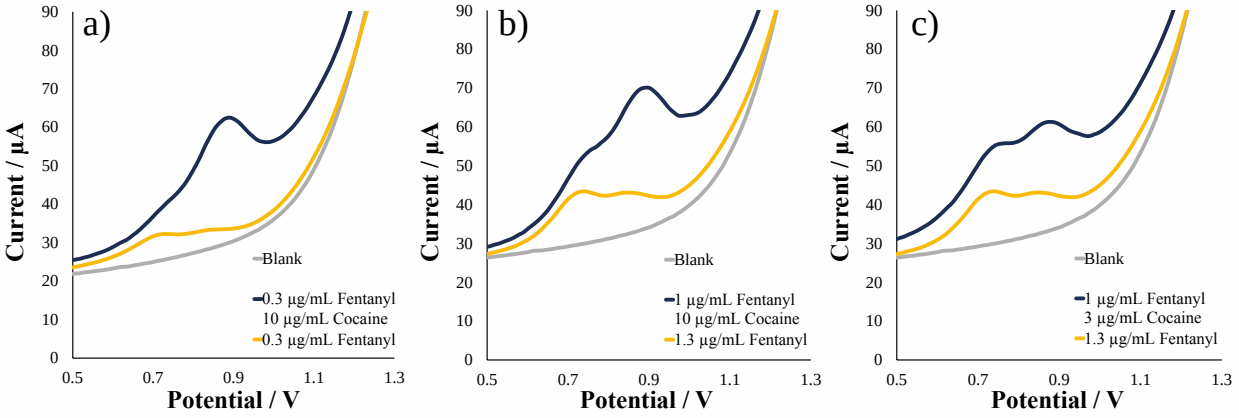


*Fig. 6. SWAdSV for the analysis of fentanyl in the presence of cocaine in a 5 mL cell, 100 mM Tris-HCl pH 8.5 at fentanyl:cocaine ratios of (a) 0.3:10, (b) 1:10, and (c) 1:3.*

Analysis of these potentially interfering compounds demonstrated that lack of interference from acetaminophen and caffeine, as well as, minimal interference problems from methamphetamine. Furthermore, the results of these analyses provide evidence that oxidation peak I serves as a useful identification peak due to the lack of interfering oxidative behavior of other compounds at the corresponding potential.

*Table 2. Summary of interference studies conducted for the compounds tested with fentanyl*

| Compound | Range Tested (μg/mL) | Potential (V) | $R^2$ | Mixture Ratio Tested | Fentanyl Detected? |
|---|---|---|---|---|---|
| Caffeine | 0.193 – 50.1 | NA | NA | NA | NA |
| Acetaminophen | 0.151 – 13.8 | 0.28 | 0.999 | 0.3:10<br>1:10<br>1:3 | Detected at All Ratios |
| Methamphetamine | 0.149 – 33.5 | 0.92 | NA | 0.3:20<br>0.3:10<br>1:10 | Detected at All Ratios |
| Cocaine | 0.303 – 13.4 | 0.90 | 0.978 | 0.3:10<br>1:10<br>1:3 | Peak Shoulder<br>Yes<br>Yes |
| Quinine | 0.099 – 9.00 | 0.90 | 0.966<br>0.999 | 0.3:10<br>1:10<br>1:3 | No<br>Peak Shoulder<br>Yes |

NA = Not applicable due to no electrochemical response with caffeine

### *3.5. Simulated Samples*

Semi-quantitation of the method was explored through the analysis of simulated samples analyzed in the cell to replicate analysis of seized drugs. These simulated samples were prepared to various concentrations in buffer and assessed based on peak current response of the sample compared to calibration curves. A 0% false positive rate was demonstrated for the negative samples, showing none detected with the absence of oxidation peaks across the fentanyl potential window. Ten simulated blank samples were analyzed, which all resulted in no detection of fentanyl oxidation peaks. Improved recovery was observed for samples with concentrations present within the first linear range (between 0.076 and 0.637 μg/mL) and within the second linear range (between 1.31 and 6.88 μg/mL) compared to samples that fell between the two linear ranges. This can be seen in unknown samples 4 and 8 which were 0.6 μg/mL and 1.3 μg/mL, respectively. A possible explanation for this could be due to the cause of these two

linear regions, suggesting an effect resulting from saturation of the working electrode at high concentrations and difference in solution dynamics between adsorbed and diffuse species. This change may occur in the intermediate region, resulting in errors when extrapolating unknown concentrations within this range. Further, a statistical t-test was performed on each concentration tested to demonstrate performance throughout the concentration range. All but one of the concentrations tested were not significantly different from the expected concentration as demonstrated by the p-values for the 95% confidence interval. These p-values demonstrate the ability of the method to perform semi-quantitative analysis in addition to the identification of fentanyl. Table 3 outlines the results for quantitative analysis of the simulated samples.

| Sample Number | Average Current Peak I (μA) | Average Calculated Concentration (μg/mL±SD)[†] | Prepared Concentration (μg/mL) | Recovery (%) | p-value |
|---|---|---|---|---|---|
| Unknown 7 | ND | ND | 0.000 | NA | NA |
| Unknown 1 | 0.89 | 0.091 ± 0.018 | 0.090 | 101 | 0.933 |
| Unknown 2 | 1.19 | 0.132 ± 0.027 | 0.130 | 92 | 0.063 |
| Unknown 6 | 1.30 | 0.147 ± 0.018 | 0.150 | 98 | 0.747 |
| Unknown 3 | 2.48 | 0.305 ± 0.027 | 0.300 | 102 | 0.782 |

*Table 3. Simulated test sample analysis*

| | | | | | |
|---|---|---|---|---|---|
| Unknown 5 | 2.67 | 0.331 ± 0.077 | 0.330 | 96 | 0.723 |
| Unknown 10 | 3.31 | 0.418 ± 0.011 | 0.410 | 102 | 0.339 |
| Unknown 4 | 4.40 | 0.565 ± 0.030 | 0.600 | 94 | 0.185 |
| Unknown 8 | 7.51 | 1.200 ± 0.020 | 1.30 | 92 | 0.014* |
| Unknown 11 | 10.38 | 2.547 ± 0.172 | 2.50 | 102 | 0.681 |
| Unknown 9 | 17.43 | 5.854 ± 0.061 | 6.00 | 98 | 0.053 |

† Calculated concentration based on peak I only, n = 3
ND = none detected, n = 10
* Statistically significant difference based on t-test, $\alpha = 0.05$.

A proof-of-concept application was assessed to determine the potential for future use of this method for the identification of fentanyl in oral fluid. Analysis of simulated oral fluid samples was performed to determine the potential for measurement of fentanyl when present in oral fluid due to the recent implementation of oral fluid testing in some U.S. states. Oral fluid was treated through crashing of the proteins and then spiked for analysis. Both the cell method and drop method were assessed for applicability. The detection of fentanyl was achieved in both methods for the oral fluid samples diluted in buffer. In the higher concentrations of fentanyl, a slight positive shift in the peak potential was observed. The anodic peaks, in these cases, were at 0.81 V and 0.95 V. At lower concentrations, a positive peak shift was observed for the second anodic peak (0.93 V); however, the first anodic peak remained near the expected potential. Increased resistivity due to the presence of the oral fluid matrix could result in the potential shifts and could mask the presence of the second anodic peak. The presence of both anodic peaks in these samples allowed for the identification of fentanyl in the diluted oral fluid samples, suggesting potential future application of this method for oral fluid testing (Fig. 7).

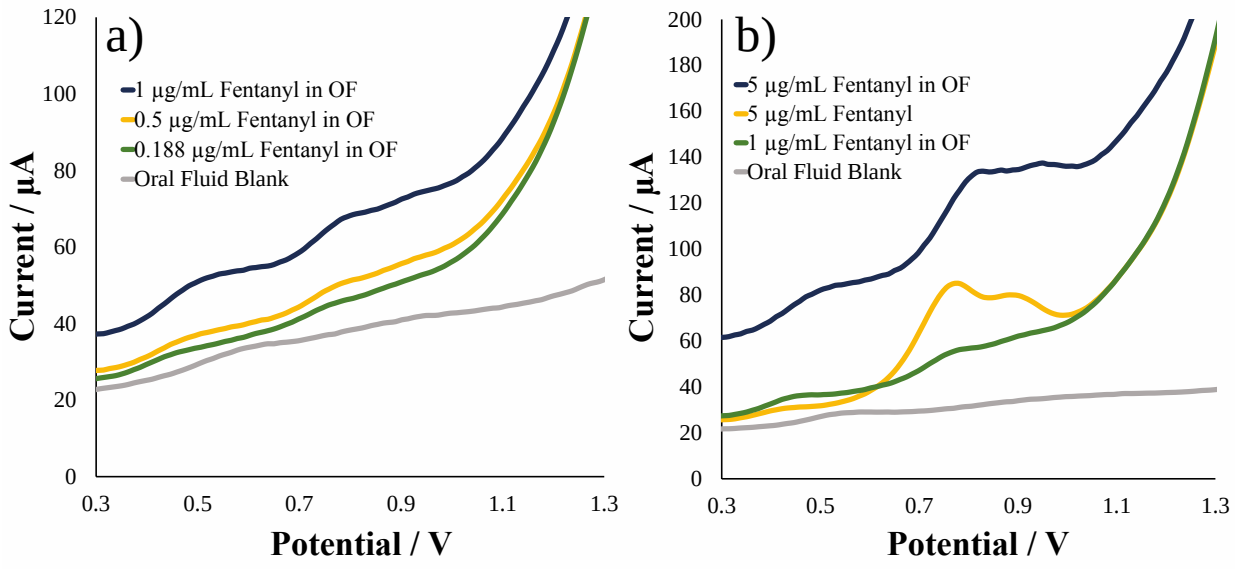

*Fig. 7. SWAdSV for the analysis of fentanyl in oral fluid (OF) using the (a) cell method with Tris-HCl pH 8.5 and (b) the drop method.*

Quantitation of samples was attempted using standard additions of fentanyl in cell and in drop. Standard additions of 10 microliters of 5 µg/mL fentanyl for drop and 15 microliters of 31.8 µg/mL fentanyl for cell was used for analysis. The optimized SWAdSV method was used to measure the diluted oral fluid samples initially, then at each addition of fentanyl standard to the system (Fig. 8). Good accuracy was achieved using the standard addition method for drop (90% for 1 µg/mL and 85% for 0.74 µg/mL diluted concentrations) and for cell (97% for 0.477 µg/mL and 105% for 0.318 µg/mL diluted concentrations). This method demonstrates promise for quantitation of fentanyl samples seized during drug case scenarios and could be utilized for future analysis of oral fluid samples following future research to improve limits of detection. Upon indication of fentanyl, the standard addition method would serve as a simple and time-effective method for quantification.

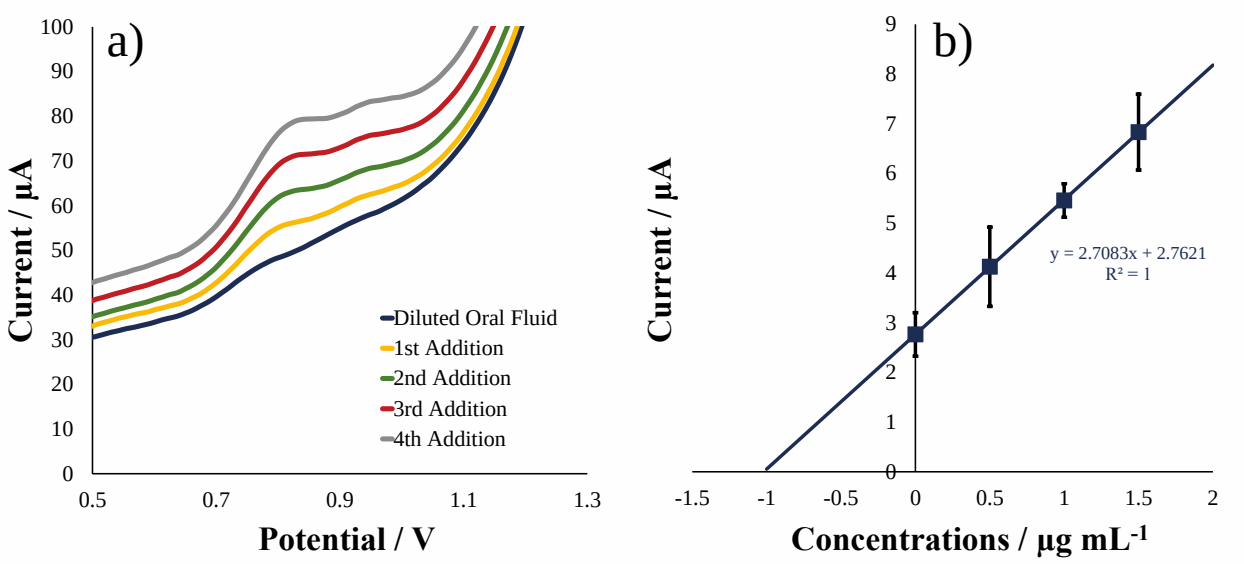


*Fig. 8. Analysis of diluted oral fluid samples in Tris-HCl pH 8.5 (a) SWAdSV measured in drop for 10 microliter standard additions of 5 µg/mL fentanyl and (b) generated standard addition curve for a simulated sample that was 1 µg/mL in drop. Accuracy based on triplicate analysis was 102% with an average accuracy of independent measurements of 90%.*

## 4. Conclusions

The use of a simple SPCE-based sensor approach resulted in a low cost and sensitive detection platform for fentanyl using adsorptive stripping voltammetry. Electrochemical oxidation of fentanyl to norfentanyl

was observed and resulted in two peaks for fentanyl, where peak I was observed at 0.75 V and peak II was observed at 0.88 V. The limit of detection was determined to be 0.037 ± 0.017 μg/mL and 0.233 ± 0.025 μg/mL for the cell and drop methods, respectively. The reproducibility of the method, expressed as RSD of the slopes of the calibration curves, in cell and in drop was 2.6% and 4.0% for peak I and 7.4% and 4.6% for peak II, respectively. Methamphetamine, caffeine, and acetaminophen did not interfere with the identification of fentanyl, while cocaine and quinine were shown to interfere with the detection of peak II at large concentration ratios compared to fentanyl, while peak I remained useful for the identification of fentanyl when present in mixtures. Analysis of fentanyl was demonstrated to be achievable for qualitative identification of fentanyl in seized drug scenarios with semi-quantitative ability when compared to calibration curves as demonstrated by the resulting recoveries and p-values. A possible application of this method exists for analysis of fentanyl in oral fluid samples in potential driving under the influence of drugs (DUID) situations following future research. Analysis of seized drug specimens could be achieved on-site by the use of portable instrumentation and self-contained buffer/electrode cells where the drug sample could be added, dissolved and mixed by shaking, and then analyzed via Bluetooth connection to the instrument or portable laptop connection due to the instrument's small size and versatile performance.

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## References

[1] About the U.S. Opioid Epidemic, U.S. Dep. Heal. Hum. Serv. (2018). https://www.hhs.gov/opioids/about-the-epidemic/index.html#us-epidemic.

[2] Public Health Emergency Declarations, U.S. Dep. Heal. Hum. Serv. (2019). https://www.phe.gov/emergency/news/healthactions/phe/Pages/default.aspx.

[3] Opioid Overdose, Centers Dis. Control Prev. (2017). https://www.cdc.gov/drugoverdose/index.html.

[4] Narcotics (Opioids), Drug Enforc. Adm. (n.d.). https://www.dea.gov/taxonomy/term/331.

[5] H.S. Smith, Opioid metabolism, Mayo Clin. Proc. 84 (2009) 613–624. doi:10.4065/84.7.613.

[6] Opioid painkillers : How they work and why they can be risky, 2014. http://www.nsc.org/RxDrugOverdoseDocuments/opioid-painkillers-how-they-work-and-why-they-are-risky.pdf.

[7] R. Hill, R. Santhakumar, W. Dewey, E. Kelly, G. Henderson, Fentanyl depression of respiration: Comparison with heroin and morphine, Br. J. Pharmacol. 177 (2020) 254–266. doi:10.1111/bph.14860.

[8] Recommended methods for the Identification and Analysis of Fentanyl and its Analogues in Biological Specimens, United Nations Office on Drugs and Crime, Vienna, 2017.

[9] M.J. Menendez, V.W. Weedn, Collaboration in the Fight Against Fentanyl, in: Organized Crime Drug Enforcement Task Forces and George Washington Univeristy, n.d.

[10] R.S. Vardanyan, V.J. Hruby, Fentanyl-related compounds and derivatives: current status and future prospects for pharmaceutical applications, Futur. Med Chem. 6 (2014) 385–412. doi:10.4155/fmc.13.215.Fentanyl-related.

[11] R.C. Baselt, R.H. Cravey, Disposition of Toxic Drugs and Chemicals in Man, 3rd ed., Year Book Medical Publishers, Inc., 1989.

[12] S.R. Bista, M. Lobb, A. Haywood, J. Hardy, A. Tapuni, R. Norris, Development , validation and application of an HPLC – MS / MS method for the determination of fentanyl and nor-fentanyl in human plasma and saliva, J. Chromatogr. B. 960 (2014) 27–33. doi:10.1016/j.jchromb.2014.04.019.

[13] O.A. Farghaly, R.S. Abdel Hameed, A.-A.H. Abu-Nawwas, Analytical application using modern electrochemical techniques, Int. J. Electrochem. Sci. 9 (2014) 3287–3318. doi:10.4172/2155-9872.1000192.

[14] E.R. Lowe, C.E. Banks, R.G. Compton, Indirect detection of substituted phenols and cannabis based on the electrochemical adaptation of the Gibbs reaction, Anal. Bioanal. Chem. 383 (2005) 523–531. doi:10.1007/s00216-005-0043-4.

[15] D. Merli, D. Zamboni, S. Protti, M. Pesavento, A. Profumo, Electrochemistry and analytical determination of lysergic acid diethylamide (LSD) via adsorptive stripping voltammetry, Talanta. 130 (2014) 456–461. doi:10.1016/j.talanta.2014.07.037.

[16] B. Jiang, M. Wang, Y. Chen, J. Xie, Y. Xiang, Highly sensitive electrochemical detection of cocaine on graphene/AuNP modified electrode via catalytic redox-recycling amplification,

Biosens. Bioelectron. 32 (2012) 305–308. doi:10.1016/j.bios.2011.12.010.

[17] L.S. de Oliveira, A.P. dos S. Poles, M.A. Balbino, M.M.T. de Menezes, J.F. de Andrade, E.R. Dockal, H.M. Tristão, M.F. de Oliveira, Voltammetric determination of cocaine in confiscated samples using a carbon paste electrode modified with different [UO2 (X-MeOSalen)(H2O)]·H2O complexes, Sensors. 13 (2013) 7668–7679. doi:10.3390/s130607668.

[18] L. Asturias-Arribas, M.A. Alonso-Lomillo, O. Domínguez-Renedo, M.J. Arcos-Martínez, Electrochemical determination of cocaine using screen-printed cytochrome P450 2B4 based biosensors, Talanta. 105 (2013) 131–134. doi:10.1016/j.talanta.2012.11.078.

[19] J.P. Smith, J.P. Metters, C. Irving, O.B. Sutcliffe, C.E. Banks, Forensic electrochemistry: the electroanalytical sensing of synthetic cathinone-derivatives and their accompanying adulterants in "legal high" products, Analyst. 139 (2014) 389–400. doi:10.1039/c3an01985c.

[20] L. Asturias-Arribas, M. Asunción Alonso-Lomillo, O. Domínguez-Renedo, M. Julia Arcos-Martínez, Cytochrome P450 2D6 based electrochemical sensor for the determination of codeine, Talanta. 129 (2014) 315–319. doi:10.1016/j.talanta.2014.05.053.

[21] J.M.P.J. Garrido, C. Delerue-Matos, F. Borges, T.R.A. Macedo, A.M. Oliveira-Brett, Voltammetric Oxidation of Drugs of Abuse III . Heroin and Metabolites, Electroanalysis. 16 (2004) 1497–1502. doi:10.1002/elan.200302975.

[22] H. Dai, H. Xu, X. Wu, Y. Chi, G. Chen, Fabrication of a new electrochemiluminescent sensor for fentanyl citrate based on glassy carbon microspheres and ionic liquid composite paste electrode, Anal. Chim. Acta. 647 (2009) 60–65. doi:10.1016/j.aca.2009.05.032.

[23] H.M. Elbardisy, C.W. Foster, L. Cumba, L.H. Antonides, N. Gilbert, C.J. Schofield, T.S. Belal, W. Talaat, O.B. Sutcliffe, H.G. Daabees, C.E. Banks, Analytical determination of heroin, fentanyl and fentalogues using high-performance liquid chromatography with diode array and amperometric detection, Anal. Methods. 11 (2019) 1053–1063. doi:10.1039/c9ay00009g.

[24] H. Guo, N. Hu, S. Lin, Adsorptive stripping voltammetric properties of fentanyl at Hg electrode, Talanta. 41 (1994) 1929–1932. doi:10.1016/0039-9140(94)00150-2.

[25] S.A. Goodchild, L.J. Hubble, R.K. Mishra, Z. Li, K.Y. Goud, A. Barfidokht, R. Shah, K.S. Bagot, A.J.S. McIntosh, J. Wang, Ionic Liquid-Modified Disposable Electrochemical Sensor Strip for Analysis of Fentanyl, Anal. Chem. 91 (2019) 3747–3753. doi:10.1021/acs.analchem.9b00176.

[26] A. Barfidokht, R.K. Mishra, R. Seenivasan, S. Liu, L.J. Hubble, J. Wang, D.A. Hall, Wearable electrochemical glove-based sensor for the rapid and on-site detection of fentanyl, Sensors Actuators, B Chem. 296 (2019). doi:10.1016/j.snb.2019.04.053.

[27] H. Cunha-Silva, M.J. Arcos-Martinez, A disposable rhodium nanoparticle-modified screen-printed sensor for direct determination of bromide anions, Sensors Actuators, B Chem. 282 (2019) 603–

608. doi:10.1016/j.snb.2018.11.108.

[28] L. Del Torno-De Román, M.A. Alonso-Lomillo, O. Domínguez-Renedo, M.J. Arcos-Martínez, Gluconic acid determination in wine by electrochemical biosensing, Sensors Actuators, B Chem. 176 (2013) 858–862. doi:10.1016/j.snb.2012.10.053.

[29] J. Wang, M. Pedrero, H. Sakslund, O. Hammerich, J. Pingarron, Electrochemical activation of screen-printed carbon strips, Analyst. 121 (1996) 345–350. doi:10.1039/an9962100345.

[30] L. Cao, M. Skyllas-Kazacos, D.W. Wang, Effects of surface pretreatment of glassy carbon on the electrochemical behavior of V(IV)/V(V) redox reaction, J. Electrochem. Soc. 163 (2016) A1164–A1174. doi:10.1149/2.0261607jes.

[31] H. Wei, J.J. Sun, Y. Xie, C.G. Lin, Y.M. Wang, W.H. Yin, G.N. Chen, Enhanced electrochemical performance at screen-printed carbon electrodes by a new pretreating procedure, Anal. Chim. Acta. 588 (2007) 297–303. doi:10.1016/j.aca.2007.02.006.

[32] D.S.I. Section, 2018 National Drug Threat Assessment, 2018. https://www.dea.gov/sites/default/files/2018-11/DIR-032-18 2018 NDTA final low resolution.pdf.

[33] M. Masui, Anodic oxidation of amines. Part I. Cyclic voltammetry of aliphatic amines at a stationary glassy-carbon electrode, J. Chem. Soc. B. 01 (1968) 973–976.

[34] W.R. Frisell, C.W. Chung, C.G. Mackenzie, Catalysis of Oxidation Coenzymes of Nitrogen Compounds in the Presence of Light, J. Biol. Chem. 234 (1959) 1297–1301.

[35] J.R.L. Smith, Amine oxidation. Part 13. Electrochemical oxidation of some substitued tertiary alkylamines, J. Chem. Soc., Perkin Trans. 2. 01 (1977) 1732–1736.

[36] R.N. Hegde, R.R. Hosamani, S.T. Nandibewoor, Voltammetric oxidation and determination of cinnarizine at glassy carbon electrode modified with multi-walled carbon nanotubes, Colloids Surfaces B Biointerfaces. 72 (2009) 259–265. doi:10.1016/j.colsurfb.2009.04.013.

[37] A. Haddad, M.A. Comanescu, O. Green, T.A. Kubic, J.R. Lombardi, Detection and Quantitation of Trace Fentanyl in Heroin by Surface-Enhanced Raman Spectroscopy, Anal. Chem. 90 (2018) 12678–12685. doi:10.1021/acs.analchem.8b02909.

[38] L.J. Marinetti, B.J. Ehlers, A series of forensic toxicology and drug seizure cases involving illicit fentanyl alone and in combination with heroin, cocaine or heroin and cocaine, J. Anal. Toxicol. 38 (2014) 592–598. doi:10.1093/jat/bku086.

# CRediT author statement

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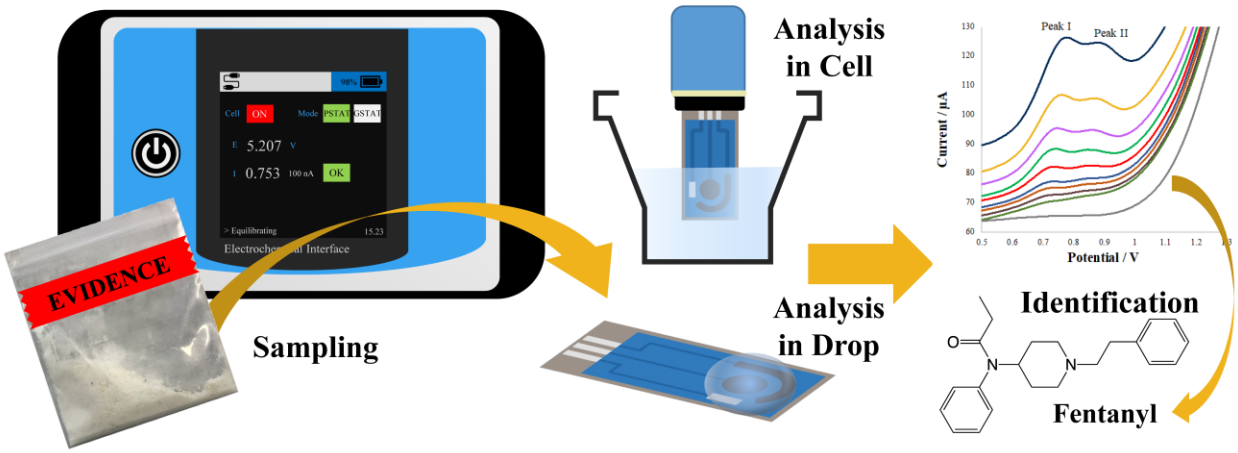
resources, supervision, visualization, Writing- Reviewing and Editing. **Luis E. Arroyo:** Conceptualization, methodology, resources, supervision, project administration, writing- Reviewing and Editing.

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**Graphical abstract**



## Highlights

- Screen-printed electrodes were used for a simple and rapid analysis of fentanyl
- Two anodic peaks were observed for the electro-oxidation of fentanyl
- Electro-oxidation of fentanyl occurs via N-dealkylation to norfentanyl
- Identification of fentanyl was achieved with common interferents and adulterants
- Accuracy of the method was examined using simulated samples