Levamisole : a common adulterant in cocaine street samples hindering electrochemical detection of cocaine

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ABSTRACT: *The present work investigates the electrochemical determination of cocaine in the presence of levamisole, one of the most common adulterants found in cocaine street samples. Levamisole misleads cocaine colour tests, giving a blue colour (positive test) even in the absence of cocaine. Moreover, the electrochemical detection of cocaine is also affected by the presence of levamisole, with a suppression of the oxidation signal of cocaine. When levamisole is present in the sample in ratios higher than 1:1, the cocaine signal is no longer detected, thus leading to false negative results. Mass spectrometry and nuclear magnetic resonance were used to investigate if the signal suppression is due to the formation of a complex between cocaine and levamisole in bulk solution. Strategies to eliminate this suppressing effect are further suggested in this manuscript. In a first approach, the increase of the pH of the sample solution from pH 7 to pH 12 allowed the voltammetric determination of cocaine in the presence of levamisole in a concentration range from 10 to 5000 µM at non-modified graphite disposable electrodes with a detection limit of 5 µM. In a second approach, the graphite electrode was cathodically pretreated, resulting in the presence of oxidation peaks of both cocaine and levamisole, with a detection limit for cocaine of 3 µM over the linear range of concentrations from 10 to 2500 µM. Both these strategies have been successfully applied for the simultaneous detection of cocaine and levamisole in three street samples on unmodified graphite disposable electrodes.*

Drug abuse continues to be a major concern for public authorities worldwide, with important health and economic implications. Cocaine (Figure 1) is one of the most consumed illicit drugs in history, stimulating the central nervous system and causing euphoria and dependence. Its short and long term use turns into systemic and central adverse effects, even sudden death due to myocardial ischemia and infarction[1] or ischemic stroke[2]. The development of fast and reliable quantitative methods for the detection of cocaine is of paramount importance to aid public authorities to tackle the problem of drug trafficking and consumption. Currently used on-site screening methods for cocaine in street samples include colour tests, producing only qualitative results, often lacking selectivity and requiring confirmation by gas chromatography coupled to mass spectrometry or flame ionization detection, which are laborious and expensive[3]. Cocaine street samples usually contain adulterants, which mimic or enhance the effects of cocaine, and/or cutting agents, used to "dilute" the sample. Almost 90% of cocaine street samples are adulterated or diluted[4]. Beside their health risks, adulterants and cutting agents can lead to false positive or false negative results in colour tests. Moreover, they can interfere with the electrochemical detection of cocaine due to suppressed or overlapping signals, thus making the analysis of cocaine street samples problematic.

Levamisole (Figure 1), a veterinary anthelmintic drug, is an important adulterant with a detection frequency of 65% in cocaine street samples[4], because it is easy to procure and has similar physicochemical properties and synergistic effects with cocaine[5]. The use of levamisole-laced cocaine poses health risks, such as neutropenia, agranulocytosis, arthralgia, skin necrosis, or leukoencephalopathy[6, 7].

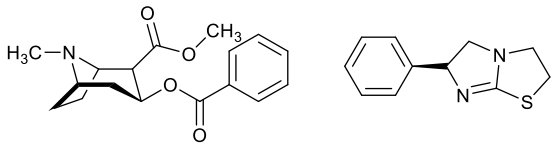

Figure 1. Chemical structures of cocaine (left) and levamisole (right).

Electrochemical techniques have been explored as an alternative for the determination of cocaine in street samples providing fast, low-cost and sensitive detection[8-11]. Freitas et al. [12] reported on a portable electrochemical method based on batch injection analysis coupled with square wave voltammetry for the detection of cocaine and adulterants in street samples using boron doped diamond electrodes in acidic pH with a detection limit for cocaine of 0.76 µM. In most cases, a modification of the electrode is required in order to gain selectivity and to address the issue of interfering adulterants that have a redox potential close to or overlapping with the signal of cocaine. With respect to this, different strategies have been employed. Asturias-Arribas et al.[13] modified the surface of screen printed carbon electrodes with multi-walled carbon nanotubes in order to achieve the detection of cocaine in the concentration range of 10-155 µM, in the presence of paracetamol, caffeine and codeine. Siqueira de Oliveira et al.[14] reported on platinum and glassy carbon electrodes modified with uranyl Schiff base films[14] or carbon paste electrodes modified with salen complexes[15] that allowed the detection of cocaine in the low µM range with no interferences from lidocaine or pro-

caine. Modification of the electrode surface with aptamers has also been widely applied in order to provide selectivity towards cocaine detection[16-19]. However, these modifications can be time-consuming and costly. Only a few electrochemical strategies employing direct detection of cocaine in street samples without further electrode modification have been reported[12, 20], having comparable performances with the modified electrodes.

The present work reports on the influence of the frequently detected adulterant levamisole on the cocaine electrochemical behavior, i.e. a suppressing effect of the cocaine signal, which causes difficulties in the analysis of cocaine street samples containing levamisole. This finding intrigued us to explore whether there is an interaction occurring in bulk solution or at the surface of the electrode and to examine solutions to detect these compounds simultaneously. Detailed electrochemical and spectroscopic studies (mass spectrometry and nuclear magnetic resonance) were performed on cocaine, levamisole and their binary mixture in solution to gain a better understanding of this interference and provide solutions to overcome it. Altering the pH of the sample solution or performing a cathodic pretreatment of the electrode allowed simultaneous voltammetric determination of both cocaine and levamisole in street samples.

Experimental section

Reagents and Samples

Cocaine hydrochloride powder standard was purchased from Lipomed (Arlesheim, Switzerland). Levamisole hydrochloride was purchased from Acros Organics (Geel, Belgium). Three seized street samples were provided by the National Institute of Criminalistics and Criminology (NICC) of Belgium. The street samples were analyzed qualitatively and quantitatively by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID), by NICC in order to establish their chemical composition. Potassium monophosphate, potassium chloride and potassium hydroxide were purchased from Sigma-Aldrich (Overijse, Belgium). A solution of 20 mM phosphate buffer containing 100 mM KCl (PBS) was used as supporting electrolyte and the pH was adjusted to the desired value using a 100 mM KOH solution. All aqueous solutions were prepared using MilliQ water (R > 18 MΩcm). The reagents were of analytical grade and used without supplementary purification.

Instrumentation and apparatus

Electrochemical measurements including square wave voltammetry (SWV), were carried out with an Autolab potentiostat/galvanostat (PGSTAT 302N, ECOCHEMIE, The Netherlands) controlled by NOVA software. Disposable ItalSens graphite screen-printed electrodes (GSPE) containing a carbon working electrode (3 mm diameter), a carbon counter electrode and a (pseudo)silver reference electrode were purchased from PalmSens (Utrecht, The Netherlands). Experiments were performed by introducing 50 µL of solution onto the GSPE. SWV measurements were carried out with a step potential of 5 mV, amplitude of 25 mV and frequency of 10 Hz. All results obtained by SWV were presented after baseline correction using the mathematical algorithm "moving average" (peak width = 1) contained within NOVA software, which improves the visualization and identification of the peaks over the baseline. All electrochemical experiments were performed at room temperature.

Colour tests were performed using commercially available cocaine/crack tests (M.M.C. International B.V, The Netherlands) by adding more than 1 mg sample powder to the test vial, homogenizing with the spatula for 10 seconds and evaluating the colour in the vial visually.

The pH measurements were performed after adjusting the pH of a freshly prepared 20 mM KH_2PO_4 buffer solution containing 100 mM KCl, by adding 100 mM KOH solution, using a Cyber-Scan 510 pH-meter from Eutech Instruments (Landsmeer, The Netherlands) connected to a HI-1131 glass bodied pH electrode from Hanna Instruments (Bedfordshire, United Kingdom).

The liquid chromatography (LC) system was an Agilent 1260 Infinity High Pressure LC (HPLC) system (Santa Clara, California, USA) fitted with a degasser, a binary high-pressure gradient pump, a thermostated column compartment and an autosampler module. The mobile phase was composed of a 50:50 mixture of 0.1% formic acid in ultrapure water and acetonitrile, at a flow rate of 0.3 mL/min. The injection volume was set at 1 µL. No LC column was used but the solution was directly injected in the MS. The LC system was coupled to an Agilent 6410 triple quadrupole mass spectrometer (Santa Clara, California, USA) with an electrospray interface (ESI) operating in positive ionization mode. Source parameters were as follows: gas temperature 350 °C, gas flow 10 L/min, nebulizer 40 psi, capillary voltage 4000 V. Mass spectra were recorded from *m/z* 50 to *m/z* 750 at a scan time of 500 ms and a fragmentor voltage of 135 V.

All nuclear magnetic resonance (NMR) spectra were recorded at 25 °C on a Bruker Avance III spectrometer operating at a ^1H frequency of 500.13 MHz operating under Topspin 3.1pl6 and using a standard 5 mm inverse BBI probe head with a Z-gradient of 50 G/cm. Standard pulse sequences from the Bruker library were used throughout. Samples for reference spectra consisted of 550 µL of 1mM solutions of cocaine and levamisole in D_2O buffered at pH 7 using deuterated PBS buffer. All 1D ^1H spectra were recorded using a spectral width of 11 ppm and consisted of 128 to 256 scans of TD points each preceded by a 2.0s relaxation delay. Processing consisted of one order zero-filling prior to multiplication and Fourier transformation. Chemical shifts are referenced against H_2O, used as secondary reference to external TMS. Additional experiments involved ^1H-{^{13}C} HSQC and HMBC experiments for assignments. Titrations were executed by adding aliquots from a concentrated solution in PBS pH 7 of levamisole:cocaine 20:1 to a 10 mM solution of cocaine, thereby keeping the cocaine concentration constant throughout. Final ratios varied from 0:1 to 10:1. In addition, diffusion ordered spectroscopy (DOSY) was performed. All spectra extended over 10 ppm along the ^1H and 235 ppm along the ^{13}C dimension, respectively.

Results and Discussion

Voltammetric behavior of cocaine-levamisole binary mixtures at pH 7

The analysis of cocaine street samples containing levamisole by voltammetry requires the simultaneous measurements of the oxidation peaks of the illicit drug and the adulterant. To understand the electrochemical behavior of cocaine and levamisole in mixtures, the pure compounds were firstly investigated by SWV at GSPE in PBS at pH 7. It was observed that an oxidation peak appears for cocaine at 1.04 V, due to the irreversible anodic oxidation of a tertiary amine group, which is consistent with previous studies of our group[20], as well as other reported data[13, 21]. Levamisole hydrochloride gives rise to an oxidation peak at 1.24 V in PBS at pH 7. However, when a 1:1 equimolar mixture of cocaine and levamisole was analyzed by SWV at pH 7, the peak of cocaine was suppressed (Figure 2A). With levamisole being one of the most used adulterants in cocaine street samples, this poses difficulties in cocaine detection, leading to false negative results.

The presence of levamisole also results in false positive results for cocaine colour test, leading to a colour change from pink to blue even in the absence of cocaine (inset Figure 2A). To investigate when the complete suppression of the cocaine peak appears, binary mixtures of cocaine-levamisole in different ratios were analyzed by SWV (Figure 2B). The mixtures were obtained by mixing 1 mM cocaine solution with 1 mM levamisole solution in various volume ratios. The oxidation peak current of cocaine decreased dramatically with an increasing levamisole concentration, demonstrating that the suppression effect by levamisole on the cocaine electro-oxidation signal is dependent on the cocaine-levamisole ratio. It can be observed that the oxidation peak of cocaine decreases down to 60:40 (%v/v) ratio and is completely supressed at 50:50 (%v/v) ratio in freshly prepared solutions. At first observation, the chemical integrity of each compound is not affected in the mixture, because, besides the change of the intensity of the peaks, the peak potential does not change significantly.

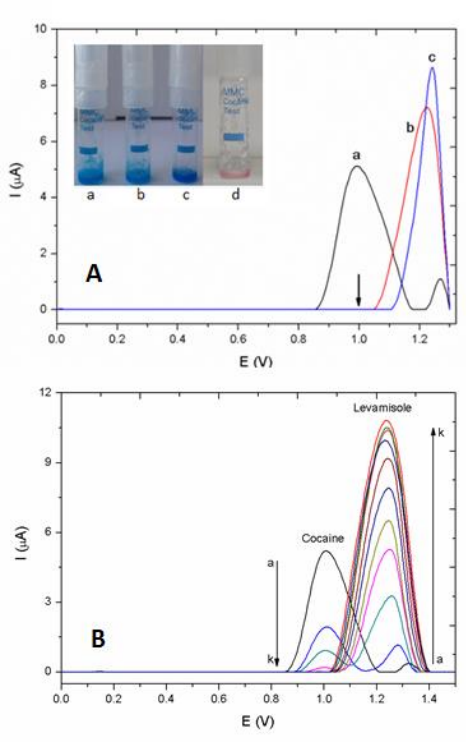

Figure 2. (A) Electrochemical behavior (SWV) of (a) 1 mM cocaine, (b) 1 mM levamisole and (c) their 1:1 binary mixture in PBS at pH 7 at GSPE. Potential range 0-1.3 V, scan rate 0.1 V s^{-1}. Inset: Cobalt thiocyanate based colour test (d) containing (a) cocaine (positive), (b) levamisole (false positive) and (c) their 1:1 binary mixture (positive). (B) Variation of the electrochemical signal of cocaine in the presence of levamisole in different ratios in PBS at pH7. Scan rate 0.1 V s^{-1}, ratios of cocaine 1mM to levamisole 1mM (%v/v): (a) 100/0, (b) 90/10, (c) 80/20, (d) 70/30, (e) 60/40, (f) 50/50, (g) 40/60, (h) 30/70, (i) 20/80, (j) 10/90, (k) 0/100.

The results led to two hypotheses for the mechanisms of cocaine-levamisole interference in PBS at pH 7 resulting in cocaine signal suppression, namely (1) an interaction in bulk solution leading to the formation of a complex or an adduct between cocaine and levamisole and/or (2) an electrode surface reaction suppressing the signal of cocaine.

To better understand the cocaine-levamisole interference at pH 7 and devise a solution for their simultaneous electrochemical determination in street samples, a series of electrochemical and spectroscopic studies were further performed.

As the complete suppression of cocaine oxidation signal appears at a 1:1 ratio cocaine/levamisole, this ratio was selected for further studies. This is also in accordance with the upper limit of levamisole in real street samples that might pose difficulties in their electrochemical analysis, as levamisole was found to be present in mass ratios varying to up to 42% in cocaine street samples seized by Belgian police forces and analyzed by NICC, and 59±22% in cocaine street samples seized in Austria[22].

Mass spectrometry and nuclear magnetic resonance studies on cocaine-levamisole interference at pH 7

To check the occurrence of cocaine-levamisole interaction in bulk solution that would explain signal suppression at pH 7, MS studies were conducted on binary mixtures of cocaine and levamisole at pH 7.

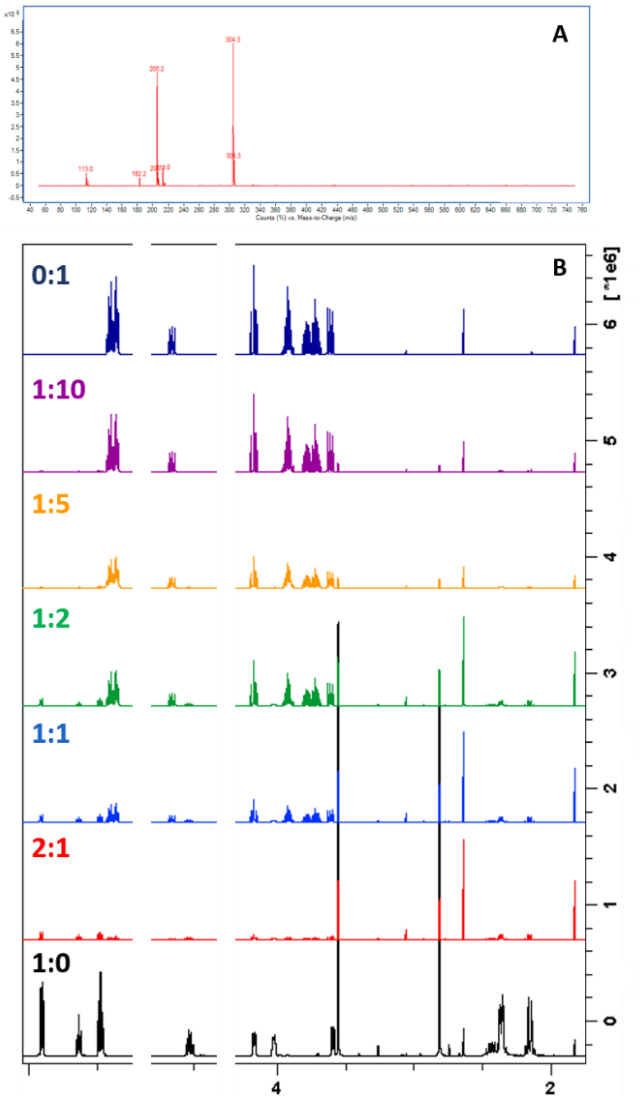

Figure 3. (A) MS spectra of cocaine-levamisole 1:1 mixture at pH 7. (B) Overlay of 5 1D ^{1}H NMR spectra of cocaine-levamisole mixtures at pH 7. The cocaine:levamisole ratio is indicated at the left hand side.

Figure 3A shows the MS spectrum of a cocaine-levamisole 1:1 mixture at pH7. Cocaine gives rise to a peak at *m/z* 304.2, as well as a peak at *m/z* 182 due to fragmentation in the ionization source. Levamisole shows a peak at *m/z* 205.2. The signals at *m/z* 113.0 and *m/z* 213.0 are due to the background ions in the buffer solution. No additional peak is observed that corresponds to a complex formation. However in the case of an adduct formation, in which cocaine and levamisole interact by weak bonds, the experimental conditions used for MS experiments (especially the high temperature in the ionization source) might be too harsh to keep the complex intact. Therefore cocaine, levamisole and their binary mixtures were further investigated by NMR. 1D ^{1}H NMR spectra of cocaine and levamisole are shown in Figure S-1 and Figure S-2 in *supporting information*. Upon interaction, and depending on the exchange kinetics of the interaction, either a concentration dependant perturbation in chemical shift (fast exchange) or the appearance of a second set of signals for the interacting species (slow exchange) with variable relative intensities is expected when mixing increasing amounts of levamisole with cocaine. Figure 3B shows an overlay of a selection of 1D ^{1}H NMR spectra obtained when titrating a 10 mM solution of cocaine stepwise with a levamisole-cocaine mixture in PBS at pH 7 from 1:0 to 0:1. The relative intensities of the resonances specific of cocaine and levamisole change throughout the experiment as expected. However, compared with the spectra of pure cocaine (1:0) and levamisole (0:1), neither a concentration dependant chemical shift nor the appearance of multiple sets of signals is observed.

Using diffusion ordered spectroscopy, the impact of the titration on the translational diffusion coefficient of cocaine and levamisole was also investigated and shown, within error, to be unaffected in the same titration range (Figure S-3 in *supporting information*). This observation supports the outcome of the MS spectra. Thus, NMR provides no support for significant interaction up to mM concentrations of both substances, making it unlikely that an interaction is responsible for the suppression effects in the electrochemical experiments.

Electrochemical studies on cocaine-levamisole interference at pH 7

Since MS and NMR experiments showed no interaction between cocaine and levamisole at pH 7 in bulk solution, a series of electrochemical experiments were performed to investigate if the interference is related to the electrochemical surface processes. To check if adsorption of levamisole or cocaine would occur at pH 7 that might facilitate complex formation at the surface of the electrode, scan rate studies were performed in the range from 5 to 500 mV s^{-1}. For both cocaine and levamisole pH 7 solutions (Figure S-4 in *supporting information*), the peak current varies linearly with the square root of the scan rate indicating a diffusion controlled process. Thus, one can affirm that a cocaine-levamisole interaction is not induced by an adsorption process of the drugs themselves at the electrode surface at pH 7.

Five consecutive SWV scans in the potential range 0-1.3 V of a GSPE in a PBS buffer solution containing 1 mM levamisole showed a decrease of the peak current intensity after each scan demonstrating the formation of oxidation products fouling the electrode surface whilst influencing the oxidation process of levamisole in the next scan (Figure 4A). A similar experiment was performed in a 1 mM cocaine solution in PBS pH 7, showing a stable electrochemical signal (Figure S-5). To check if the electrode fouling would affect the cocaine signal, a SWV scan of 1 mM levamisole solution at pH 7 recorded at the bare GSPE was followed by rinsing of the electrode with PBS at pH 7, prior to a SWV scan in a PBS solution at pH 7 containing 1 mM cocaine. A dramatic decrease of the cocaine peak current intensity from 4.27 µA down to 1.36 µA was observed (Figure 4B), suggesting that the fouling of the electrode by levamisole oxidation products has an effect on the cocaine signal. Furthermore, when two and three SWV scans in a 1 mM levamisole solution are performed prior to the analysis of cocaine solution on the same electrode, the peak current intensity of cocaine further decreases to 0.62 µA and 0.28 µA, respectively. Also the levamisole oxidation potentials slightly shift to the right, proving a hindering effect on cocaine electrochemical oxidation. However, levamisole is oxidized at higher potentials than cocaine. Thus, similar experiments were performed in a 1 mM levamisole solution sweeping the potential up to 1 V and up to 0.6 V, respectively, which is lower than the levamisole oxidation (1.24 V). The electrode was then rinsed with PBS pH 7 and a SWV scan was performed on the same electrode in a 1 mM cocaine solution at pH 7, to check if levamisole is partly oxidized at lower potentials, resulting in cocaine signal suppression. A suppression of cocaine signal was observed also in these cases, however to a lesser extent than the previous experiment when the potential was scanned up to 1.3 V: the decrease in the cocaine peak current intensity was 16.2% (for a scan to 0.6 V) and 50.4% (for a scan to 1 V), compared to 68.2% (for a scan to 1.3 V) (Figure S-6). This may suggest that levamisole may be already partly oxidized before 1.24 V (around 0.95V as shown in the inset of Figure 4A, scan 1), fouling the electrode surface, before cocaine oxidation. The same experiments were performed using PBS solution at pH 7, instead of levamisole, as control experiments. No decrease in cocaine oxidation signal was observed in this case, hence demonstrating the influence of levamisole on the suppression of the cocaine signal.

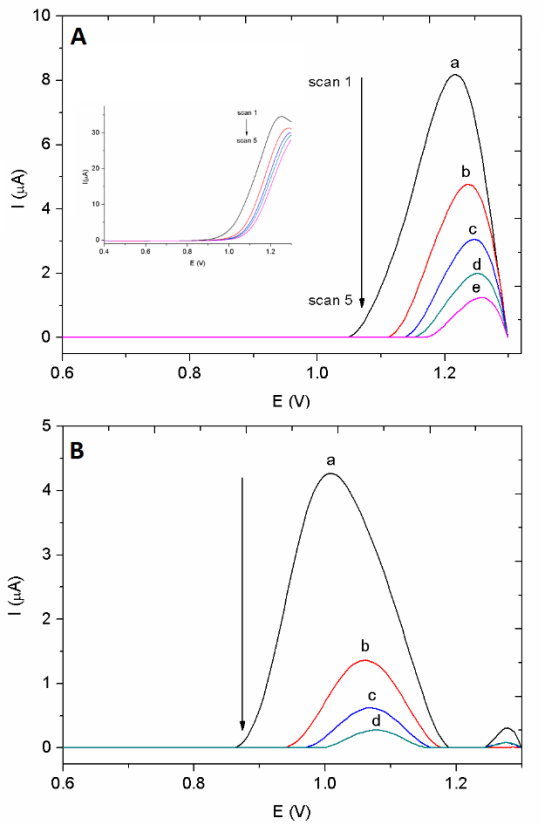

Figure 4. SWV of consecutive scans of 1 mM levamisole solution pH 7 on the same GSPE: 1st scan (a), 2nd scan (b), 3rd scan (c), 4t scan (d), 5th scan (e); inset: raw data before "moving average"

algorithm was applied (A); SWV scan of 1 mM cocaine solution pH 7 on bare GSPE (a) and on GSPE previously tested in 1mM levamisole solution pH 7 for 1 scan (b), 2 scans (c) and 3 scans (d) (B). Potential 0-1.3 V. Scan rate 0.1 V s^{-1}.

Corroborating the experimental findings of MS, NMR and electrochemical measurements, one can state that there is no (strong) interaction occurring between cocaine and levamisole in the bulk solution, but the interference is rather related to the electrochemical surface process. As the amount of levamisole in the sample is increased, the amount of oxidation products that is formed increases as well, fouling the electrode surface and explaining the dependency of the peak intensities on the cocaine-levamisole ratio. With the knowledge gained, we subsequently investigated alternative strategies to simultaneously detect cocaine and levamisole on GSPE.

Firstly, we investigated the effect of altering the pH on the response of GSPE towards cocaine and levamisole. It was previously reported that the pH increase leads to higher oxidation signals for cocaine[13], the experiments being carried out up to pH 10, due to issues related to the precipitation of cocaine base. Since the pH increase results in a more sensitive detection of cocaine, we further extended the pH range to 12, since no precipitation was observed at pH values higher than 10 in the investigated concentration range (0.01-5 mM). Moreover, carbon-based electrodes are prone to variations of pH. The pH alters the surface of electrodes, which is further reflected in their performance in voltammetry[23].

Secondly, the electrocatalytic properties of carbon-based electrodes can also be improved by activation through electrochemical pretreatment, facilitating a fast electron transfer rate of the analyte toward the electrode surface[24-27]. Electrochemical oxidation or reduction is an easy, controllable, and reproducible way to pretreat carbon electrodes[28]. Thus, we also investigated the effect of an electrochemical pretreatment of the electrode surface on the detection of cocaine-levamisole mixtures.

Altering the pH: influence on the electrochemical analysis of cocaine-levamisole binary mixtures

We studied the electrochemical behaviour of cocaine hydrochloride in the pH range 7-12 and observed a six-fold increase in the peak current together with a peak potential negative shift of around 200 mV, as the pH increases from 7 to 12 (Figure 5A). The peak around 1.2 V is attributed to the substrate (GSPE) as also observed in the background. A similar behaviour was observed for levamisole when the pH of the supporting electrolyte was increased to 12, with a two fold increase in the peak current intensity as the pH varied from 7 to 12, together with a shift of potential to less positive values of around 70 mV, as shown in Figure 5B. The pH increase from 7 to 10 results only in a small increase in the peak current intensity (7.7 to 9.3 µA), while the peak potential does not significantly change (1.19 to 1.17 V). A further pH increase to 11 and 12 leads to higher signals (11.4 to 14.8 µA, respectively) at lower potentials (1.12 V).

Since both cocaine and levamisole present a higher signal at lower potential values at pH 12, equimolar mixtures of cocaine and levamisole (1 mM) were analyzed by SWV at GSPE at pH 12 at different times (Figure 6). The oxidation signals of both cocaine and levamisole can be distinguished at pH 12, thus allowing the simultaneous detection of cocaine and levamisole in street samples. However, a decrease in time of the cocaine signal in the mixture was observed, suggesting possible stability issues at pH 12. The oxidation signal of levamisole in the mixture was stable over the investigated time frame. Stability studies on the pure compounds were further carried out at pH 12 and compared to their stability at pH 7, to find the appropriate conditions for handling and analyzing the street samples in case of on-site measurements.

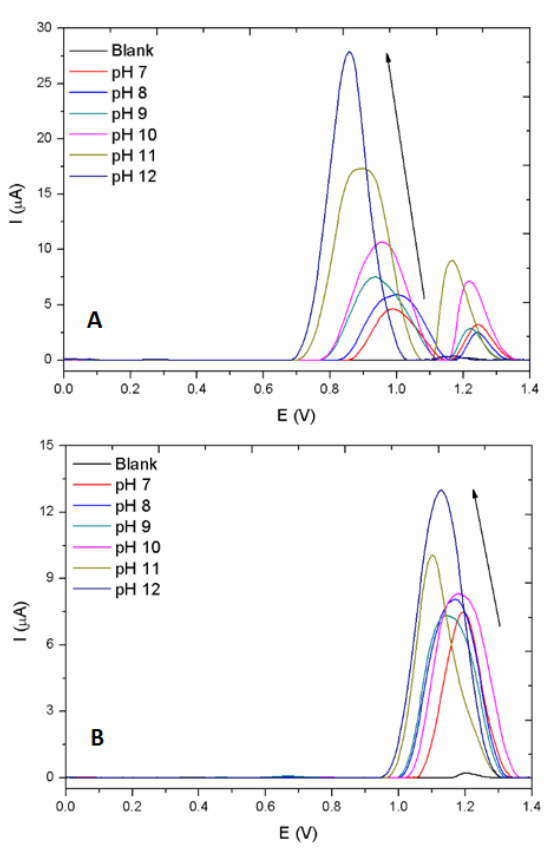

Figure 5. Influence of pH on the redox behaviour of cocaine: SWV of 1 mM cocaine (A) and 1mM levamisole (B) solutions in PBS at pH 7-12.

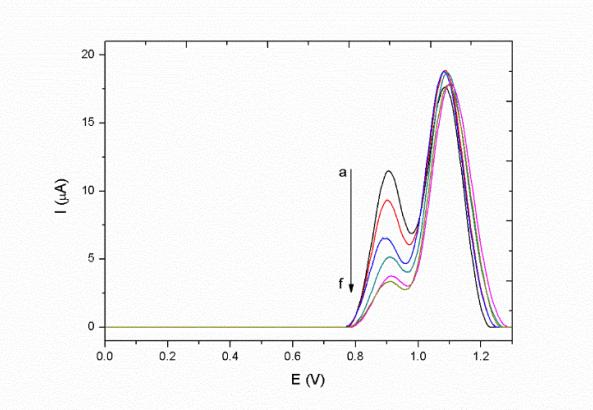

Figure 6. SWV of cocaine-levamisole 1:1 binary mixtures at pH 12 at different times: (a) 0, (b) 5, (c) 15, (d) 30, (e) 45 and (f) 60 min.

Electrochemical stability studies of pure compounds at pH 12 versus pH 7

SWV measurements were performed in fresh and aged samples of cocaine both at pH 7 and pH 12, after 3, 6, 9, 24, 48, 168, 336 h. The intensity of the cocaine peak is quite stable at pH 7 even

after 336 h (14 days), while the oxidation potential is stable for 48 h then slightly decreases (Figure S-7A). Overall the solution of cocaine is stable at pH 7. However, at pH 12 (Figure S-7B), both the intensity and the potential of the cocaine peak are decreasing fast in the first 9 h. After 9 h, the potential has decreased to a value of around 0.76 V and then increases again to values around 0.9 V. The peak current becomes stable after 24 h to around 0.6 µA. The results confirm the instability of cocaine at pH 12 and are in accordance with previously reported data on cocaine hydrolysis in basic media[29].

Mass spectrometry stability studies of pure compounds at pH 12 versus pH 7

MS studies were further performed to evaluate the stability of cocaine and levamisole at pH 12 in comparison with pH 7 and to gain insights in the degradation process of cocaine solutions. The cocaine spectrum at pH 12 is shown in Figure S-8A. As before, cocaine gives an ion at *m/z* 304.2, and at *m/z* 182 due to an in-source fragmentation. The ions at *m/z* 113 and *m/z* 213 are attributed to the background ions in the buffer solution. At pH 12, a decrease of the signal of cocaine *m/z* 304.2 was observed with time together with the appearance and increase of the signal at *m/z* 200.1, which is probably attributed to a hydrolysis product of cocaine (e.g. ecgonine methyl ester[30]). The variation in the peak areas of these two signals in time is also depicted in Figure S-8B. The hydrolysis of cocaine at pH 12 is rather fast, the signal of the hydrolysis product of cocaine appears after the first 2 and increases by six-fold after 30 min and by 11.5 after 2 h. After 60 h, the signal of cocaine completely disappears, while the signal of the product of hydrolysis increases 16-folds with respect to 2 min. This confirms the electrochemical results proving that cocaine undergoes hydrolysis and it is not stable at pH 12.

MS studies further confirmed the stability of both cocaine and levamisole at pH 7. The peak areas of cocaine and levamisole remain constant after 2h ($3.81x10^8$ and $2.54\ x10^8$, respectively) and after 60h ($3.53x10^8$ and $2.54\ x10^8$, respectively)

Stability studies showed that while both cocaine and levamisole solutions are stable at pH 7 for two weeks, a rather fast degradation of cocaine occurs at pH 12, while levamisole solution remains stable at pH 12. This recommends for *ex temporae* preparation of solutions at pH 12 and analysis in a time frame of less than one minute in the case of on-site applications on seized cocaine street samples.

Electrochemical pretreatment of the electrode surface: influence on the analysis of cocaine-levamisole binary mixtures

The effect of a cathodic surface pretreatment on the cocaine detection in the presence of levamisole was evaluated, by applying various potentials for a fixed time (Figure S-9). The potential was investigated for -0.8 V, -0.6 V, -0.5 V, -0.4 V, -0.2 V, while the time was varied from 10 to 600 s. We observed that when a conditioning potential is applied for 10 s and 30 s, the presence of cocaine cannot be detected in a 1:1 binary mixture. However, as the conditioning time is further increased, a peak for cocaine starts to appear. The peak of cocaine in the mixture increases as the time increases and as the potential changes to less negative values. We observed that for a conditioning potential of -0.8 V, the peak of cocaine arises after 60 s and that the current further increases as the time is increased to 360 s, then tends to level. For a conditioning potential of -0.6 V, the peak of cocaine is only visible after 180 s, while for a potential of -0.5 V, the conditioning time needed for the cocaine peak to arise is 360 s. When a potential of -0.4 V or -0.2 V was applied, the peak of cocaine was no longer visible. Since the highest values for the current were obtained for a potential of -0.8 V and a time of 360 s, these were considered as the optimal parameters and were used for further experiments.

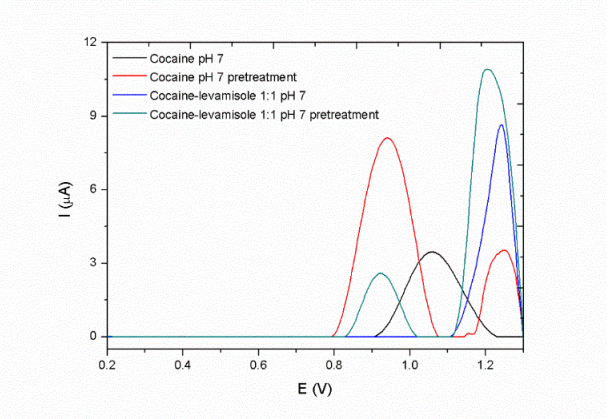

Figure 7. Electrochemical signal of 1 mM cocaine and cocaine-levamisole 1:1 mixture at pH 7 on GSPE without and with electrochemical pretreatment (conditioning potential -0.8 V - 360 s).

Figure 7 shows the effect of cathodic pretreatment on the oxidation peak of cocaine at pH 7. An enhancement in the intensity of the cocaine oxidation signal together with a shift to less positive potentials is observed, proving the electrocatalytic effect of the pretreatment on the cocaine oxidation at GSPE at pH 7. Moreover, after the potentiostatic cathodic pretreatment on cocaine-levamisole 1:1 binary mixtures, the signal of cocaine can be unraveled, with a good peak-to-peak separation, thus allowing a simultaneous detection of cocaine and levamisole in binary mixtures.

The pretreatment procedure is likely to facilitate or inhibit certain reactions occurring in the electrochemical process[27]. It results in electrochemical "cleaning" of the electrode[31], creates defect sites through the removal of carbon material, which can be highly reactive[32] and has an influence on the oxygen-functional groups at the surface of the electrode[27]. Electrochemical reduction decreases the amount of oxygen containing functional groups and largely reduces groups, such as C=O and C-O-C[26], which seems to be beneficial for the electrochemical response towards cocaine and cocaine-levamisole mixtures. The application of negative potentials also helps desorption of organic compounds at the electrode surface[33]. The electrochemical pretreatment is straightforward and less time- and cost-consuming than other strategies for cocaine detection, most of which involving the modification of electrode surface. It also bypasses the use of reagents required for the modification of electrodes that may cause environmental pollution.

Calibration curves for cocaine in the presence of levamisole at pH 12 and at pH 7 with a pretreatment step

Calibration curves for cocaine in the presence of levamisole, in equimolar concentrations, were obtained by SWV using both developed strategies.

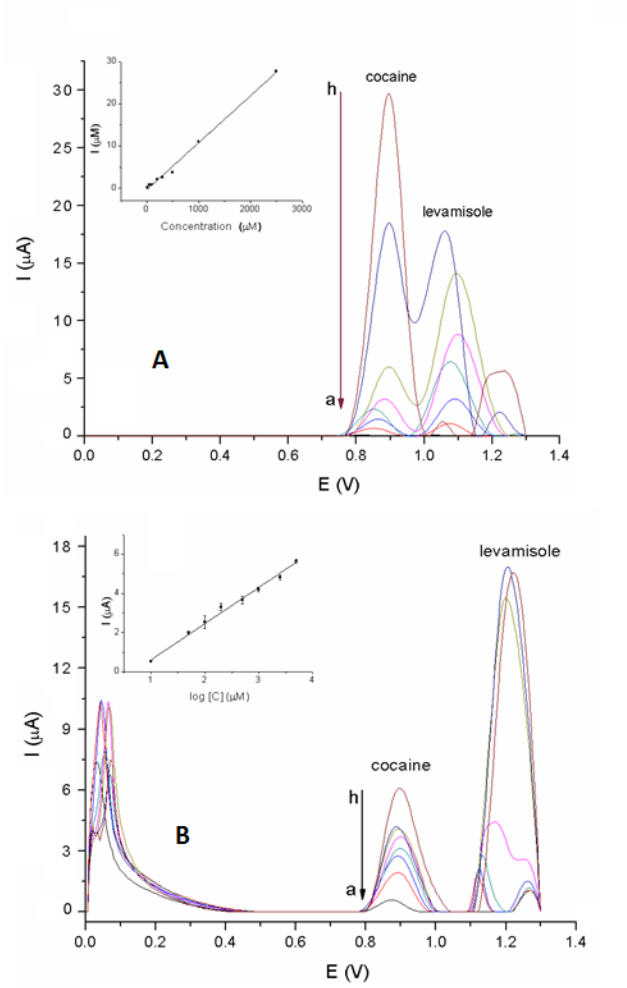

Figure 8. Calibration curves obtained by SWV for cocaine in the presence of levamisole at pH 12 (A) and at pH 7 with a pretreatment step (B). Equimolar concentration of cocaine and levamisole in the mixture (a) 10 µM; (b) 50 µM; (c) 100 µM ; (d) 200 µM; (e) 300 µM; (f) 500 µM; (g) 1000 µM and (h) 2500 µM. The signals around 0V are due to oxidation of silver formed during pretreatment, originating from the Ag/AgCl reference electrode.

SWV recorded at bare GSPE with increasing concentrations of cocaine and levamisole at pH 12, in 1:1 ratios, showed that the peak current of cocaine increased linearly with increasing concentrations of cocaine in the range of 10-2500 µM (Figure 8A). The calibration curve is presented in the inset and was defined by the equation y=0.011x-0.349, R^2= 0.995. The limit of detection based on the minimum distinguishable signal for lower concentrations of analyte was 5 µM with an RSD of 2.5% (n=5).

Figure 8B shows the square wave voltammograms of different concentrations of cocaine in the presence of levamisole (1:1 molar ratio) under the optimized pretreatment conditions at pH 7 with inset representing the corresponding calibration plot. The oxidation peak currents of cocaine were found to be linear with the logarithm of the concentration over the range from 10-5000 µM. The equation obtained in this case for the calibration curve for cocaine was y=1.798log(x)-1.116, R^2=0.991. The lowest concentration that could be experimentally detected using the pretreatment strategy at pH 7 was 3 µM with an RSD of 3.1% (n=5).

Analysis of cocaine in real samples

To assess the usefulness of the developed strategies for the determination of cocaine in the presence of levamisole, three street samples were further analyzed with the proposed methodologies and compared with those obtained by GC-MS. For this purpose, 1 mg street sample was dissolved in 10 mL PBS at pH 7 and pH 12, respectively, and the measurements were carried out as previously described. The quantification of cocaine was achieved by applying the standard addition method and the results are presented in Table 1. The results demonstrate that the developed strategies can enable the detection of cocaine samples adulterated with levamisole and provide a useful tool for selective on-site determination of cocaine in street samples, with a better reproducibility for the strategy involving electrode pretreatment.

Table 1. Analysis of street samples acquired from NICC with the developed SWV strategies. SWV measurements at pH 7 were performed with electrode pretreatment.

No	Sample Composition *	GC-MS wt%	SWV pH 12 wt%	RSD %	SWV pH 7 wt%	RSD %
1	Cocaine Levamisole	70 23	78	3.7	76	3.0
2	Cocaine Levamisole	57 41	61	6.4	62	1.2
3	Cocaine Levamisole	55 42	59	5.7	58	4.6

*No other compounds were identified by GC-MS.

Conclusion

The suppression of the electrochemical oxidation signal of cocaine in the presence of the popular adulterant levamisole at pH 7 at GSPE represents an inconvenience for the analysis of cocaine in street samples containing levamisole, leading to false negative results. Electrochemical and spectroscopic experiments carried out on cocaine-levamisole binary mixtures at pH 7 showed that this interference is related to the electrochemical processes at the electrode surface, i.e. adsorption of oxidation products and electrode fouling, rather than an interaction in bulk solution. We demonstrated the usefulness of two electrochemical strategies to overcome this problem and to achieve selective detection of cocaine in street samples in the presence of levamisole: (1) adjusting the pH of the detection solution to pH 12 and (2) employing a pretreatment step by applying a potential of -0.8 V for 360 s prior to measurements at pH 7. These simple strategies offer the advantage of selective detection of cocaine without further modification of the electrodes, thus avoiding cost- and time-consuming steps and the use of polluting reagents. Calibration curves for the analysis of cocaine in the presence of levamisole were obtained for both developed strategies with detection limits as low as 3 and 5 µM, at pH 7 and pH 12, respectively. The electrochemical strategies were successfully applied for the detection of cocaine in street samples.

ASSOCIATED CONTENT

Supporting Information

1D ^{1}H NMR spectrum of cocaine and of levamisole; Diffusion coefficients of both cocaine and levamisole in binary mixtures in

different ratios**;** Scan rate studies for cocaine and levamisole at pH7; Consecutive SWV scans of 1 mM cocaine at pH 7 at GSPE; SWV scans of cocaine after SWV scans of levamisole up to 1 V and 0.6 V; Stability SWV studies of 1 mM cocaine solution at pH 7 and pH 12; Mass spectrometry spectra of cocaine and peak area variation at pH 12; The effect of pretreatment potential and time on cocaine SWV signal in 1:1 binary mixtures at pH 7. This material is available free of charge via the Internet at http://pubs.acs.org

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Author Contributions

All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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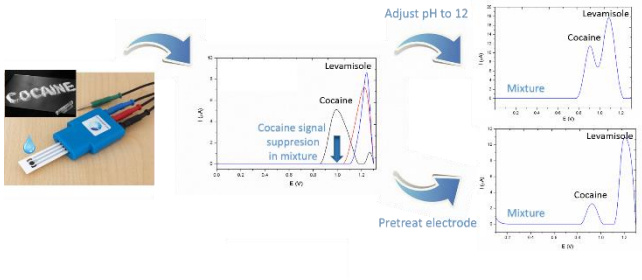
For TOC only


COCAINE
Levamisole
Cocaine
Cocaine signal
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in mixture
Adjust pH to 12
Pretreat electrode
Levamisole
Cocaine
Mixture
Levamisole
Cocaine
Mixture

Supporting information

Levamisole: a common adulterant in cocaine street samples hindering electrochemical detection of cocaine

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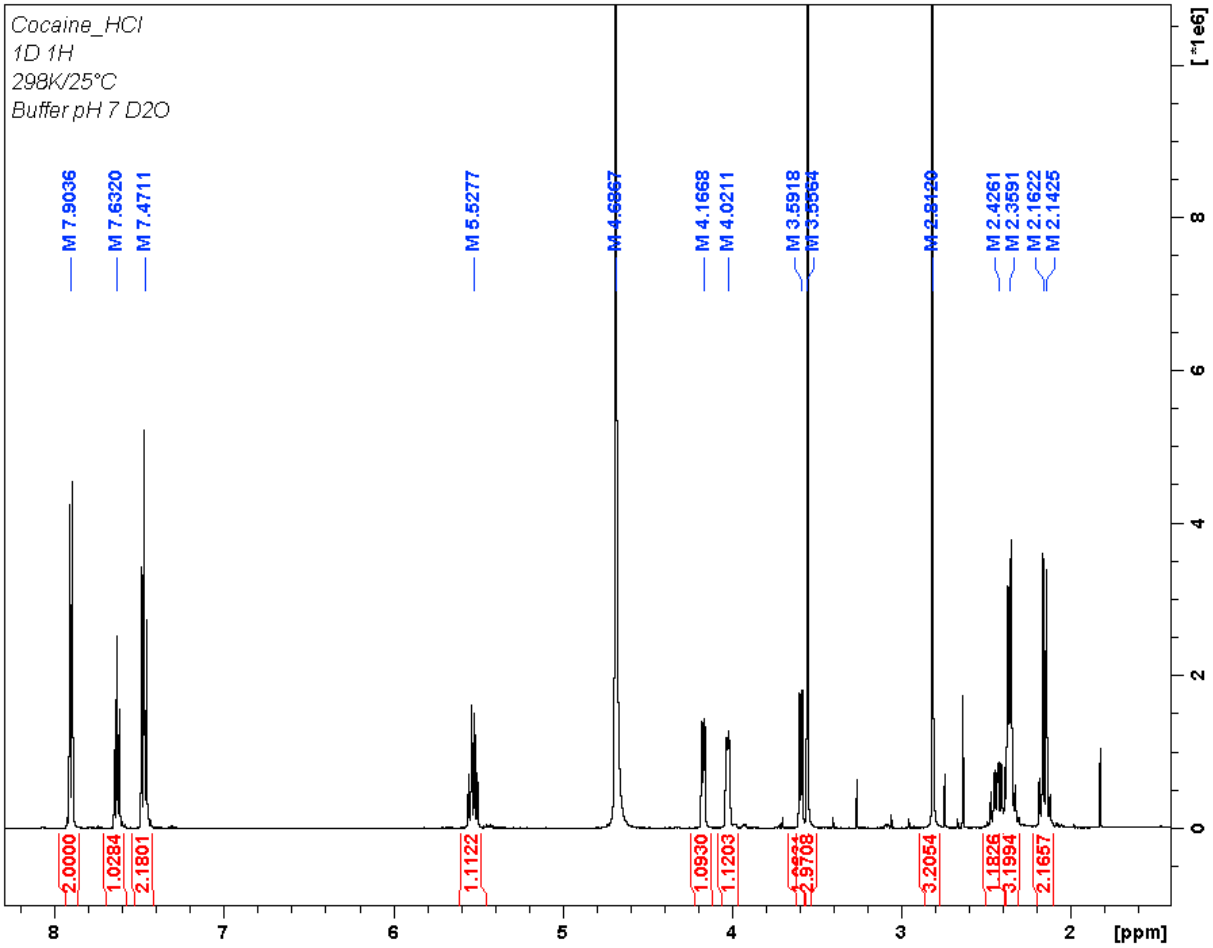
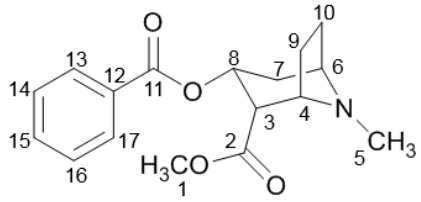


Figure S-1. 1D ^{1}H spectrum of cocaine with integrals for the amount of protons (red), chemical shifts (blue).

Cocaine



^{1}H NMR (500 MHz): δ 7.90 (2x d, 2x 1H, 13 and 17), 7.63 (t, 1H, 15), 7.47 (t, 2x 1H, 14 and 16), 5.53 (q, 1H, 8), 4.69 (s, residual water), 4.17 (d, 1H, 4), 4.02 (t, 1H, 6), 3.59 (dd, 1H, 3), 3.56 (s, 3H, 1), 2.81 (s, 3H, 5), 2.43 (m, 2H, 9 or 10), 2.36 (dd, 2H, 7), 2.16 (q, 2H, 9 or 10).

Scheme S-1. Chemical structure and chemical shifts (NMR) for cocaine.

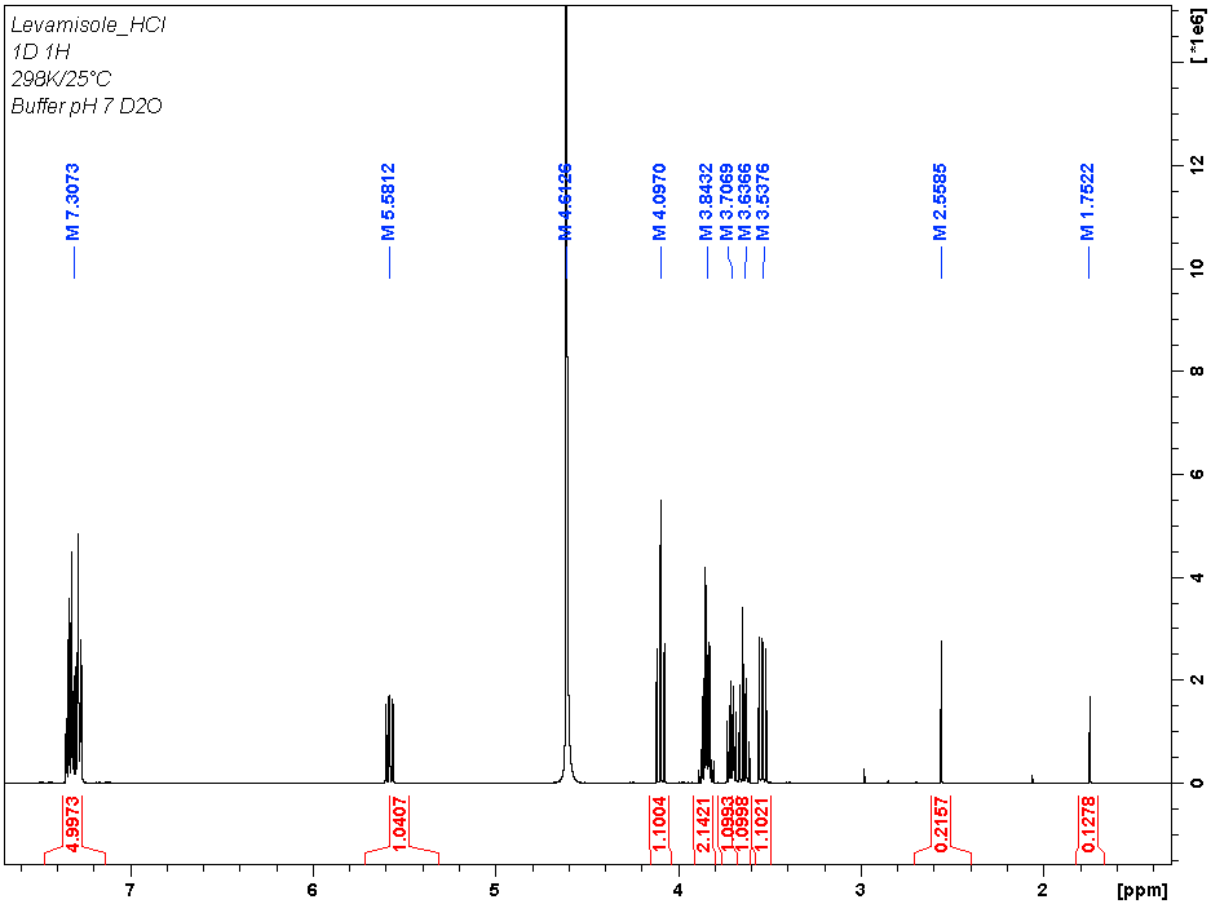
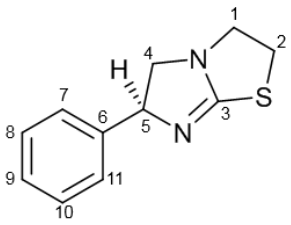


Figure S-2. 1D ^{1}H spectrum of levamisole with integrals for the amount of protons (red), chemical shifts (blue).

Levamisole



^{1}H NMR (500 MHz): δ 7.31 (m, 5x 1H, 7-11), 5.58 (t, 1H, 5), 4.61 (s, residual water), 4.10 (t, 1H, 1), 3.84 (m, 2H, 4), 3.71 (m, 2H, 1), 3.64 and 3.54 (m, 2H, 2).

Scheme S-2. Chemical structure and chemical shifts (NMR) for levamisole.

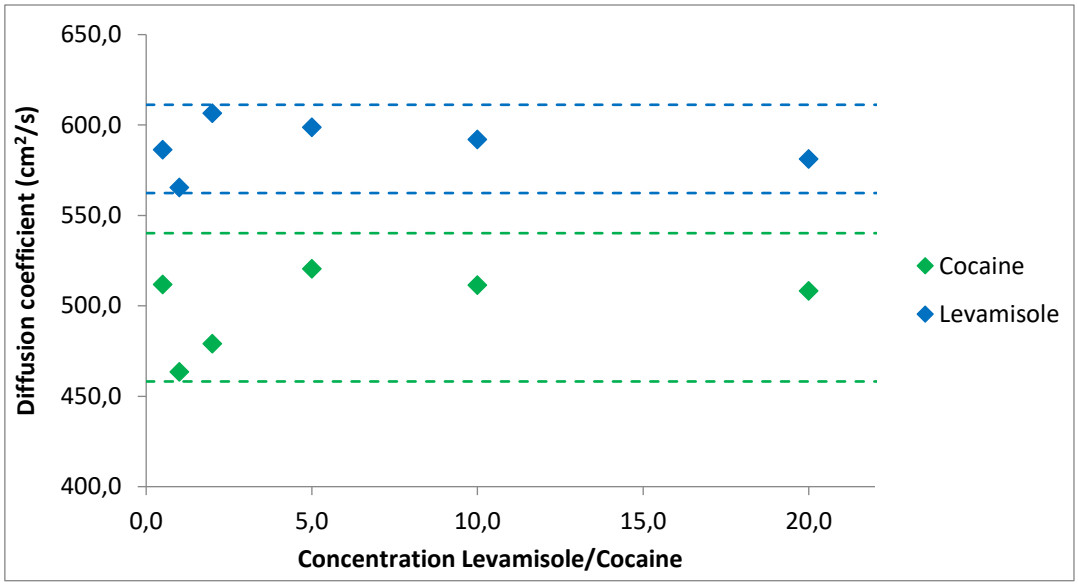


Figure S-3. Diffusion coefficients (D), as determined by DOSY spectroscopy, of cocaine (green) and levamisole (blue). The dotted lines represent the experimental error of D for cocaine (green) and levamisole, respectively.

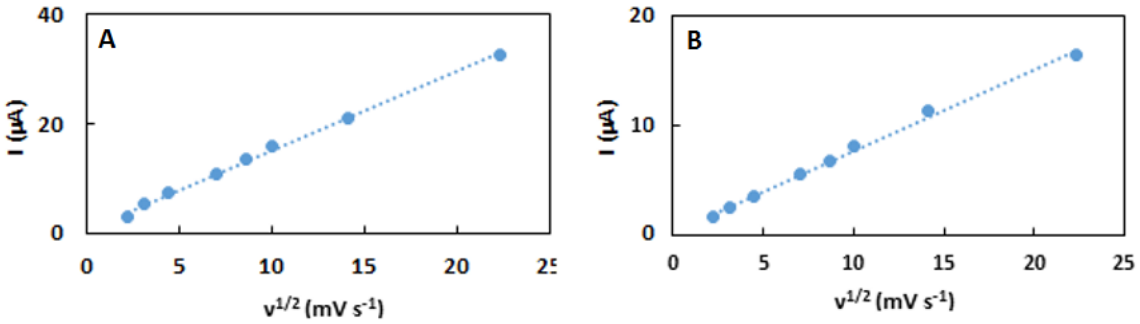


Figure S-4. Scan rate studies for cocaine (A) and levamisole (B) at pH 7.

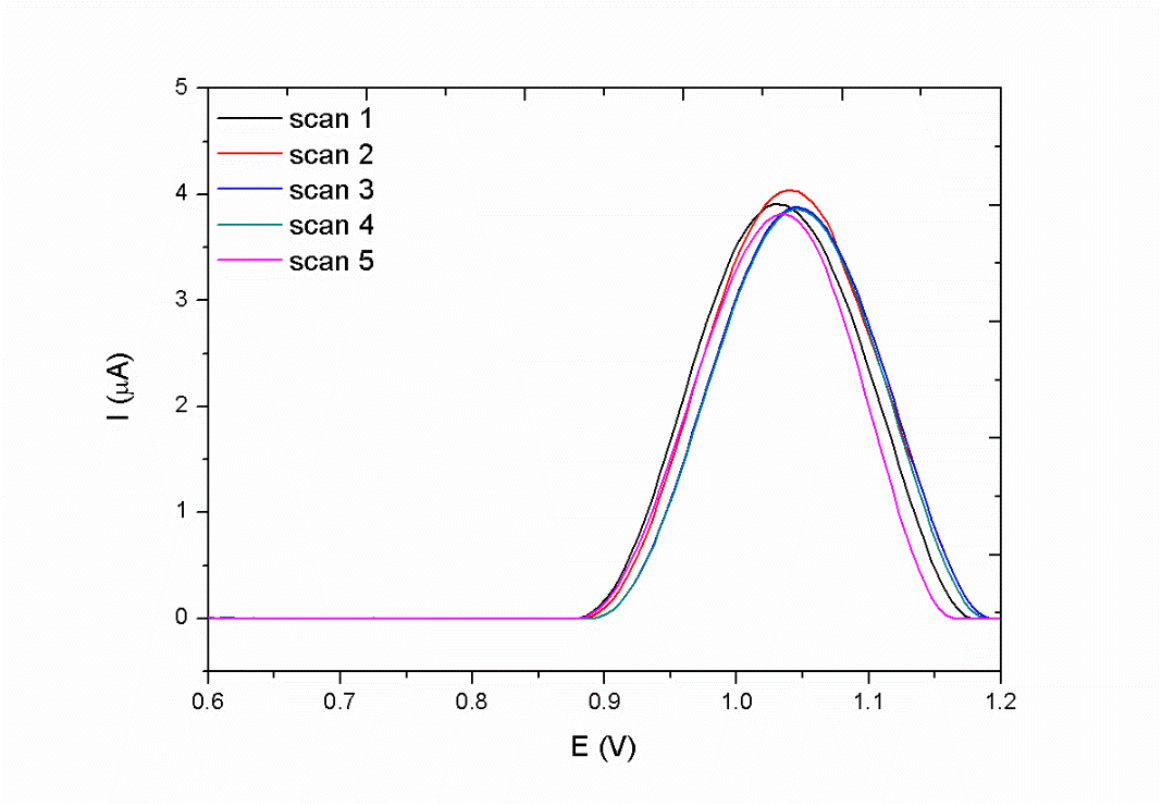


Figure S-5. Consecutive SWV scans of 1 mM cocaine at pH 7 at GSPE.

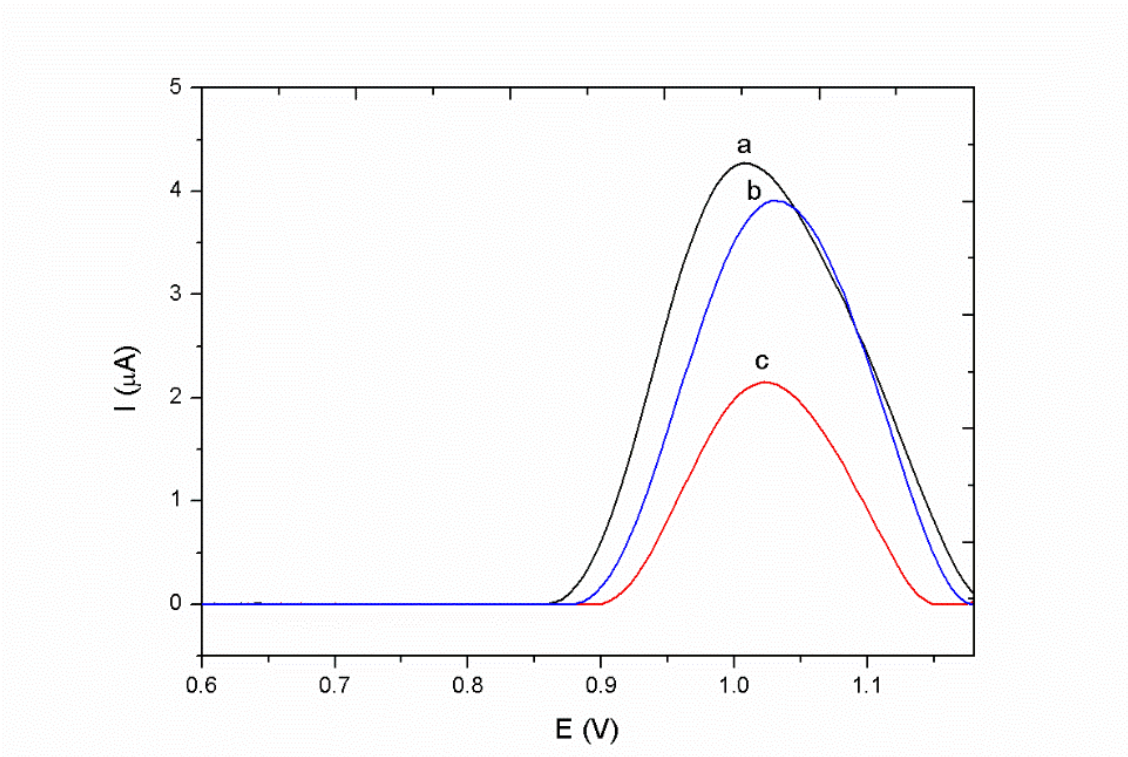


Figure S-6. SWV scan of 1mM cocaine on bare GSPE (a) and after a SWV scan of 1 mM levamisole up to 1 V (b) and 0.6 V (c)

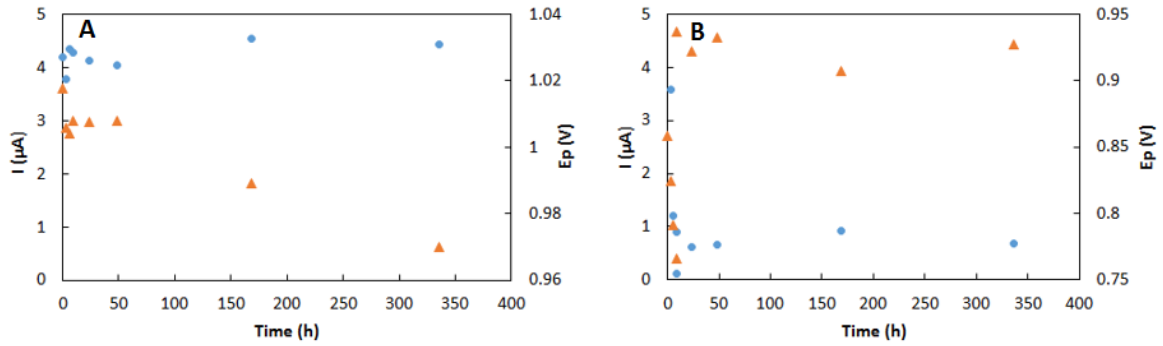


Figure S-7. Stability SWV studies of 1mM cocaine solution at pH 7 (A) and pH 12 (B) over time; peak current (●) peak potential (▲).

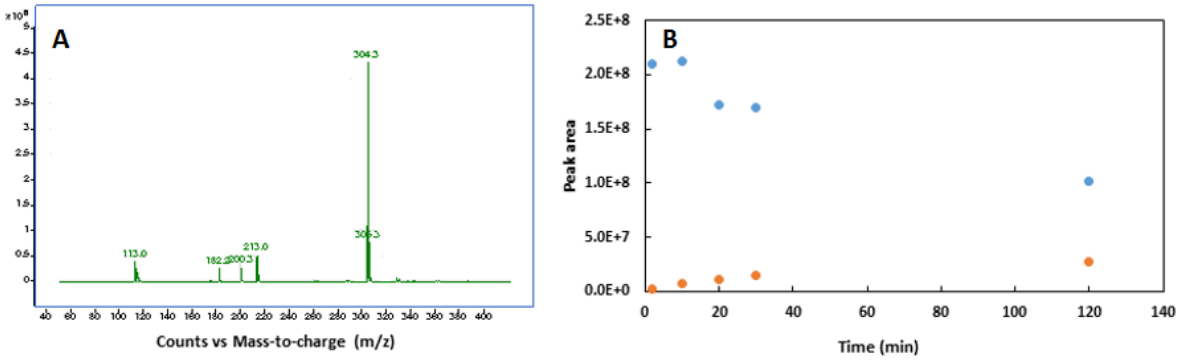


Figure S-8. MS spectrum of cocaine solution 0.01 mM at pH 12 (A). MS peak area variation in time for cocaine (●) and its product of hydrolysis (●) at pH 12 (B).

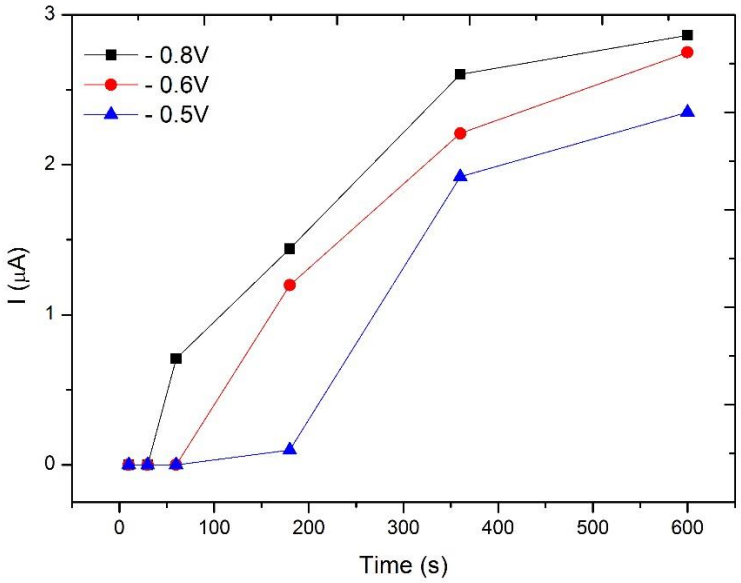


Figure S-9. The effect of pretreatment potential and time on cocaine SWV signal in 1:1 binary mixtures at pH 7.