Rapid screening method for new psychoactive substances of forensic interest: electrochemistry and analytical determination of phenethylamines derivatives (NBOMe) via cyclic and differential pulse voltammetry.

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ABSTRACT: The NBOMe derivatives are phenethylamines derived from the 2C class of hallucinogens. Only a few human pharmacologic studies have been conducted on these drugs and several cases of intoxication and deaths have been reported. Presently, NBOMe are not a part of the routine drugs-of-abuse screening procedure for many police forces and there are no rapid immunoassay screening tests that can detect the presence of those compounds. In this paper, the voltammetric behavior of 25B NBOMe and 25I NBOMe were investigated and their electroanalytical characteristics determined for the first time. A novel, fast and sensitive screening method for the identification of the two most common NBOMes (25B-NBOMe and 25I-NBOMe) in real samples is reported. The method uses the electrochemical oxidation of these molecules to produce an analytical signal that can be related to the NBOMe concentration with an average lower limit of quantitation of 0.01mg/mL for both of them. The method is selective enough to identify the two compounds individually, even given the great similarity in their structure.

INTRODUCTION

The use of Novel Psychoactive Substances (NPS) has rapidly grown in the last years and reports of the availability and manufacture of such substances increased. Those designer drugs are proliferating at an unprecedented rate and posing significant public health challenges [1]. The number of NPS reported worldwide rose from 126 in 2009 to 450 in 2014. In Europe the situation is even worse with a 7-fold increase in NPS seizures from 2008 to 2013 [2,3].

Worldwide, from 2008 to 2013, seventeen percent of the total NPS reported to UNODC by countries were phenethylamines. In Europe phenethylamines represented 20% of the reports of new and existing NPS in 2013 and the second most abundant class of substances. The same happened in South America, Central America and the Caribbean where phenethylamines reports reached 26% of the cases. In USA and Canada phenethylamines posed as the third group of substances most reported [4]. Moreover a recent study exploring the patterns on drug use pointed specifically at NBOMe as the major NPS group reported as the most tried new drug [5]. In the Brazilian Federal District the number of blotter paper seizures raised from 2 in 2012 to 70 in 2015 with NBOMe compounds present in 85,5% of the cases (unpublished data).

The NBOMe compounds are N-benzylmethoxy derivatives of the 2C family of hallucinogens with methoxy substitutions at positions 2 and 5 and a substitution at position 4, often consisting of a halogen (i.e., chlorine, bromine, or iodine) [6–8]. Those compounds are potent agonists of the human 5HT2A receptor, responsible for subjective and behavioral effects [7,8].They were mentioned for the first time in the book PIHKAL (1991)[9]. Although human consumption appears to have begun in 2010, it has increased rapidly as a consequence of the easy availability through the internet [5].

Scientific data regarding NBOMe compounds are very scarce. Only a few pharmacologic studies on humans have been conducted on these drugs. Reports of adverse effects after ingestion began to appear in the scientific literature in 2013, describing prominent neuropsychiatric effects and instability [6,10–16]. In severe cases death can occur even after ingesting a single dose [14]. Despite the fact that they are very similar to one another, 25I-NBOMe (2-(4-iodo-2,5-dimetoxifenil)-N-[(2 metoxifenil)metil]etanamina) appears to be much more commonly reported followed by 25B-NBOMe (2-(4-bromo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl] ethanamine) [17,18].

A routine standardized test on NBOMe compounds is not available in most forensic and clinical toxicology laboratories [19]. Laboratory-based analysis of NBOMEs compounds have been published by a few groups using chromatography, including GC/MS, for seized material and LC/MS, HPLC/MS for blood, urine and serum samples. The first fully validated method to detect NBOMe compounds in seized material was reported by Casale and Hays (2012), using GC/MS, although the mass spectra obtained from 25B-NBOME, 25C-NBOMe and 25I-NBOMe are very similar with the three main ions coincident, being necessary ratios calculation or the use of a complimentary technique such as FTIR for final determination. Zuba (2013) [7] also described the analytical properties of 25C-NBOMe in seized samples using GC/EI/MS with and without derivatization, LC/QTOF/MS, FTIR and NMR. More recently, the use of ATR-FTIR combined with chemometric discriminant analysis were described for detecting illicit substances like NBOMEs compounds and other NPS directly from seized paper

blotters [20]. Polkis (2015) [21] also described the detection of NBOMe compounds in blotter papers by DART-MS, confirmed by HLPC MS-MS.

Toxicology analyses were performed mostly in individual case reports using LC/MS/MS, HLPC/MS/MS and LC/QTOF/MS. Rose et al. (2013) [10] described the first case report with quantification of 25I-NBOMe compound in serum using HPLC/MS/MS. Shortly after, a validated method was described by the same group [22]. Validated methods for quantification of NBOMe compounds including 25B-NBOMe and 25C-NBOMe in body fluids such as serum, urine and blood, were lately described using HPLC/MS/MS, LC/MS/MS and UPLC/MS/MS [6,11,13,16,21,23]. The limit of detection varies, reaching as low as 10 pg/mL [10,21].

Presently, NBOMe are not a part of routine drugs-of-abuse screening and there are no rapid color screening tests or point-of-care devices that can quickly detect them [11]. Just a few commercial reference laboratories offer a qualitative test to identify those compounds [12].

Due to the scenario presented the development and improvement of a specific and appropriate analytical method to rapid detect and quantify NBOMes compounds is urgent. In this article we present an electrochemical method for detection of 25B-NBOMe and 25I-NBOMe that is cost effective, portable and exhibits high sensitivity and selectivity towards the target analytes.

EXPERIMENTAL SECTION

Materials. All reagents used were analytical grade from Sigma Aldrich (Gillingham, UK). Deionised water (resistivity>16 Ωcm) was used throughout all the experiments. 0.2 M PBS buffer was used as supporting electrolyte. Certified standards of 25B NBOMe, 25I NBOMe and 2C-B were also purchased from Sigma-Aldrich (Cerilliant). Blotter papers were seized by the Federal District Civil Police in Brazil between the years 2014 and 2015.

Instruments. GC-MS analysis were performed using Perkin Elmer® Clarus®500 GC interfaced to Clarus®500 GC mass spectrometer using quadrupole mass analyzer. The system was controlled with PerkinElmer® TurboMass™ GC/MS Software version 5.4.2.1617. All electrochemistry experiments and measurements were performed using a portable bipotentiostat/galvanostat μstat 400 (Dropsense, Oviedo, Spain) connected to a personal computer (Dell Vostro, Windows 7, Dell, Round Rock, TX, USA) using Dropview 8400 software (Dropsense). Experiments were performed using screen-printed glassy carbon electrodes (DRP-150 - WE carbon, CE platinum, RE silver) with 3mm working area purchased from Dropsense. Raman analysis were perfomed in a Horiba® LapRAM® HR-UV Spectrometer controlled with LabSpec™ software version 5.93.20.

Methods. Street samples extraction was carried out with no pretreatment and at room temperature as follow. One blotter paper was placed in an Eppendorf tube and 1mL of PBS was added. The tube was then gently mixed to extract th NBOMes. The DPV analysis was carried immediately by adding 50 μL of the resulted mixture to the electrode. GC-MS method was adapted from previously described methods[7,24]. Cyclic Voltametry (CV) was performed using a potential range from -0.2 V to +1.4 V, step potential 0.01 V and scan rate 0.1 V/s. Differential Pulse Voltammetry (DPV) was performed using a potential range from -0.1 V to +1.4 V, step potential 0.01 V, pulse potential 0.02 V, scan rate 0.1 V/s and pulse time 10 ms.

RESULTS AND DISCUSSION

Electrochemical characterization of 25B NBOMe and 25I NBOMe. The investigation of the electrochemical behavior by cyclic voltammetry using a carbon screen-printed electrode showed two oxidative waves observed at +1.04 V and +1.25 V for 25B-NBOMe and +1.02 V and +1.21 V for 25I-NBOMe. A single reduction wave was also observed at +0.06 V for both compounds. It is worth noting that the second anodic wave (peak II in Figure 1) only appeared when the anodic scan was performed to potentials up to or beyond +1.4 V and induced the appearance of the reduction wave (peak III in Figure 1). Moreover, if a second sequential scan was performed using the same electrode, it was also possible to observe a third oxidation peak at lower potential, E_p=+0.12 V (peak IV, Figure 1).

The difference measured between the peak potential E_p (mV) and the potential at half peak height $E_{p/2}$ of the first anodik peak (peak I in Figure 1) was 50 mV. This value for E_p-$E_{p/2}$ is compatible with a monoelectronic electron transfer. In this case it is likely that peak I is a result of the electrochemical oxidation of the secondary amine present in the NBOMe molecule. Moreover the oxidation potentials observed are similar to those previously described in the literature for other similar compounds containing secondary amines [25–27]. The oxidation of the secondary amine (peak I) involves a removal of an electron of the amino-nitrogen atom, leading to the formation of a primary amine which will be attached to the electrode and an aldehyde and likely free primary amine once the latter reached saturation on the SPCE surface (Figure 1).

The second anodic peak (peak II) is a result of the replacement of the halogen present in the NBOMe compounds by an hydroxyl group to further produce a ketone. The generally accepted mechanistic for alkyl iodides and bromides, suggests an initial electron transfer from the highest filled molecular orbital of the organic halide to the electrode. In subsequent steps, the cation radical [RX]+• may undergo fission of the carbon-halogen bond to form carbocations and oxidizable halogen, or a nucleophilic attack (e.g. by the solvent) via SN2 type displacement[28].

Peak III is dependent on peak II occurrence, as previously mentioned. Furthermore, the occurrence of peak IV, present if a second scan is performed in the same electrode, resemble typical quinone/catechol interconversions (Figure 1). The presence of this redox couple suggests interaction of the NBOMe products with the electrode surface at the higher potentials. If further scans are performed in the voltammetric analysis a decrease in the amine oxidation peak (I) and a progressive increase in the current corresponding to redox couple (III/IV) can be noticed. This feature has been previously described during electrochemical grafting experiments and constitutes a clear indication of such a reaction[26]. Also, the apparition of a redox couples by interaction of electroactives compounds at high potentials are typical in carbon electrodes [29].

To verify the hypothesis, that the second oxidation peak (peak II) is due to the halogen oxidation to a hydroxyl group and subsequent oxidation to a ketone (quinone/catechol equilibrium), the electrochemical analysis of 2,5-dimethoxy-4-bromophenethylamine (2C-B) was performed. This compound was chosen as it presents a very similar structure to the 25B NBOMe although it presents a primary amine and therefore an aromatic ring is absent (Figure 2).

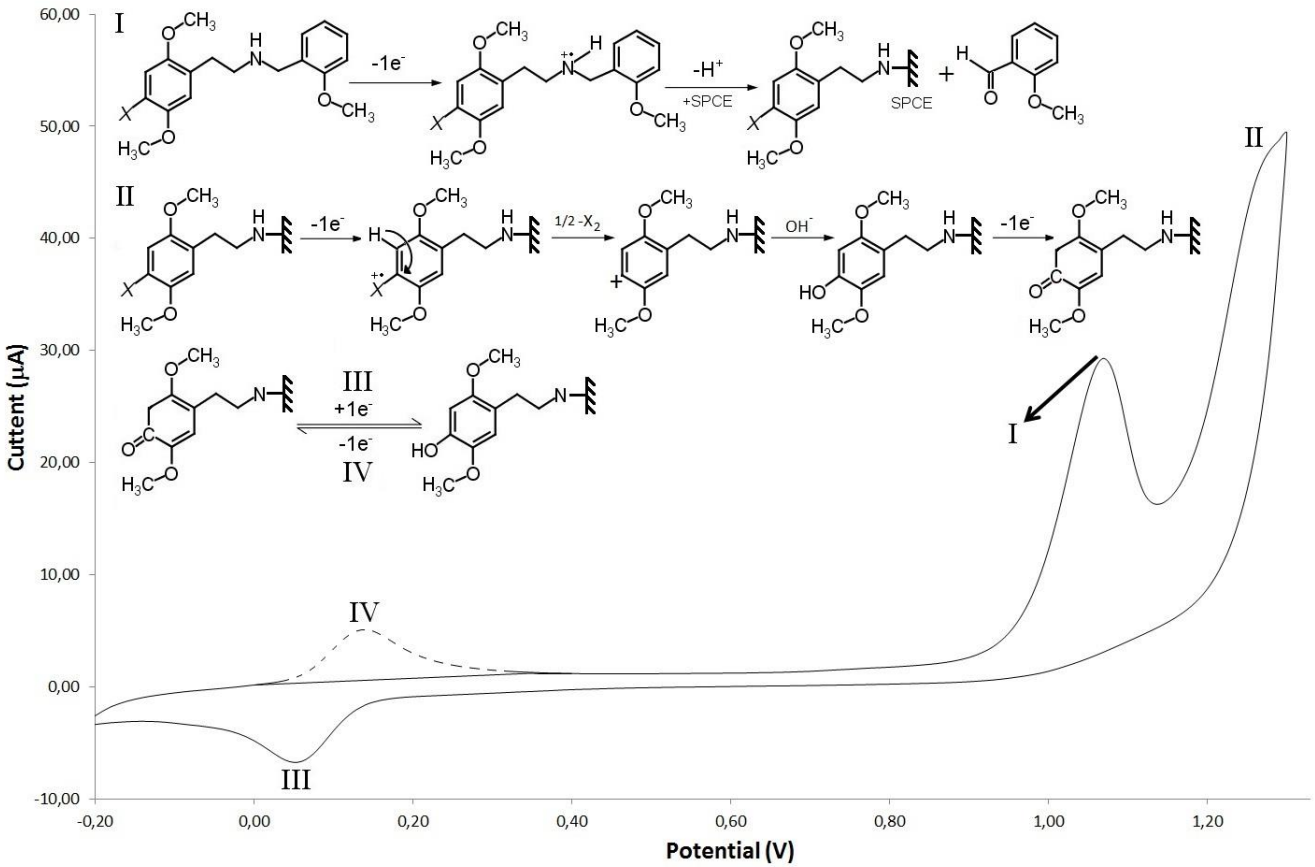

Figure 1. Cyclic voltammetry profiles obtained using a screen-printed carbon electrode in 0.05 mg/mL 25I NBOMe aqueous solution at pH 7 in 0.2 M PBS. Scan rate 0.1 V/s. First scan is represented as a solid line, second scan as a dashed line.

The voltammogram obtained in this analysis showed just one oxidation peak (Ep= +1.21 V) at nearly the same potential of the second oxidation peak (peak II in Figure 1) of the 25B-NBOMe (Figure 3). This corresponds to the fact that 2C-B is similar in structure and just contains as an oxidation site at low potentials the same halogen atom, since the oxidation of the primary amine in 2C-B occurs at much higher potentials than the potential range applied in our experiments [26].

The oxidation of phenethylamines as codeine, MDA and MDMA is complex[27,30,31]. MDA electrochemical studies are controversial as some authors show that MDA can undergo oxidation [30] while others stands the opposite [27]. MDMA oxidation show two anodic peaks and involves the removal of an electron from the amino-nitrogen atom, leading to the formation of a primary amine and an aldehyde [27]. The oxidation study of 2-methoxy amphetamine suggests that the anodic process observed in the second anodic peak in MDMA compounds does not involve the amine-substituted side chain or the aromatic ring rather involve 3,4-methylenedioxy- substituted benzene ring [30], configuring a different process that the one proposed by us.

Figure 2. Chemical structure of 25B-NBOMe, 25I-NBOMe and 2C-B.

In order to confirm that the voltammetric scans were leading to the attachment of an organic group to the surface of the carbon electrode, Raman analyses were performed on the electrode surface after rinsing.

A strong raman signal around 1580 cm^{-1}, suggesting the presence of an aromatic ring attached to the electrode surface (Figure 4) [32] was observed. This seems to indicate that the linkage process initially developed via a one-electron oxidation of the amine group to its corresponding cation radical, which subsequently forms a carbon-nitrogen bond with the carbon surface. The covalent attachment of aliphatic amines is well known in the literature. Just a few aromatic amines demonstrate the ability to link covalently to the carbon surface as the aromatic ring blocks the surface binding sites promoting a low coverage of the modified electrode [26,33–35].

The effect of scan rate (v) on the peak current (i_p) and the peak potential (Ep) upon the electrochemical oxidation of 25B-NBOMe and 25I-NBOMe were examined in a 0.05 mg/mL solution at pH 7. A linear relationship was observed between log i_p and log v corresponding to the equations: log i_p (I) = 0.63±0.01 log v +2.20 ± 0.01 for 25B-NBOMe and log i_p (I) = 0.53 ± 0.03 log v +2.00 ±0.04 for 25I-NBOMe where v is in mVs^{-1}. The slope of 0.63 and 0.53 is close enough to the theoretically expected value of 0.5 for a purely diffusion-controlled current[36]. We have observed only small changes in the peak potential of peak I and never observed a reduction peak in the cathodic direction associate with peak I in the cyclic voltammograms at higher scan rate. This behavior suggests that the process follows a scheme EC with fast chemical reaction. On the other hand, the current intensity corresponding to redox couple III/IV increases with the scan rate in a linear form with v^x where x

is close to 0.75 indicating that the kinetic control is a mixture of diffusion and adsorption processes in a good agreement with the production of covalent attachment and free oxidized forms of NBOMe molecules on carbon electrode.

The effect of the pH versus the electrochemical signal of the 25B-NBOMe and 25I-NBOMe was also studied. DPV voltammograms were obtained in a potential range from -0.2 to +1.4 V in 0.05 mg/mL 25B NBOMe/25I NBOMe solutions in a pH range from 5.0 to 11.0. The linear relationship of E_{p1} vs. pH revealed a slope of −46.0 and -51.0 mV/pH for 25B NBOMe and 25I NBOMe, respectively (Figure 5). This indicates that the proton transfer in the electroactive groups of the NBOMe compounds affects the overall electrode reaction mechanism as it has been indicated in the reactions of the Figure 1.

The linear relationship of E_{p1} vs. pH revealed a slope of −46.0 and -51.0 mV/pH for 25B NBOMe and 25I NBOMe, respectively (Figure 5), indicating an irreversible reaction mechanism, involving the same number of protons and electrons in the process [37]. Those slopes are in the order of magnitude to that expected for a monoelectronic/monoprotonic reaction (59.2 mV/pH at 25 °C) [38].

For values of pH between 5.0 and 11.0, the peak potential E_{p2} is pH independent, for 25B NBOMe and 25I NBOMe indicating that, in this pH range, the peak potential is not affected by the concentration of H+.

Electroanalytical determination of 25B-NBOMe and 25I-NBOMe. Once the electrochemical mechanism and the optimal pH conditions for analysis were defined differential pulse voltammetry was attempted. Figure 6 shows both oxidation waves at the same potentials than those observed in CV for both compounds. The analytical identification for 25B-NBOMe and 25I-NBOMe was performed using the second oxidation wave, although the first oxidation wave was used in the quantification analysis. The method was optimized regarding peak height and reproducibility and optimum instrumental parameters used in DPV were chosen studying variation in step potential, pulse potential, number of scans and pulse time.

To evaluate the analytical performance of the method the main parameters analyzed were stability, linearity, sensitivity, precision and trueness (accuracy), selectivity, robustness and ruddgeness.

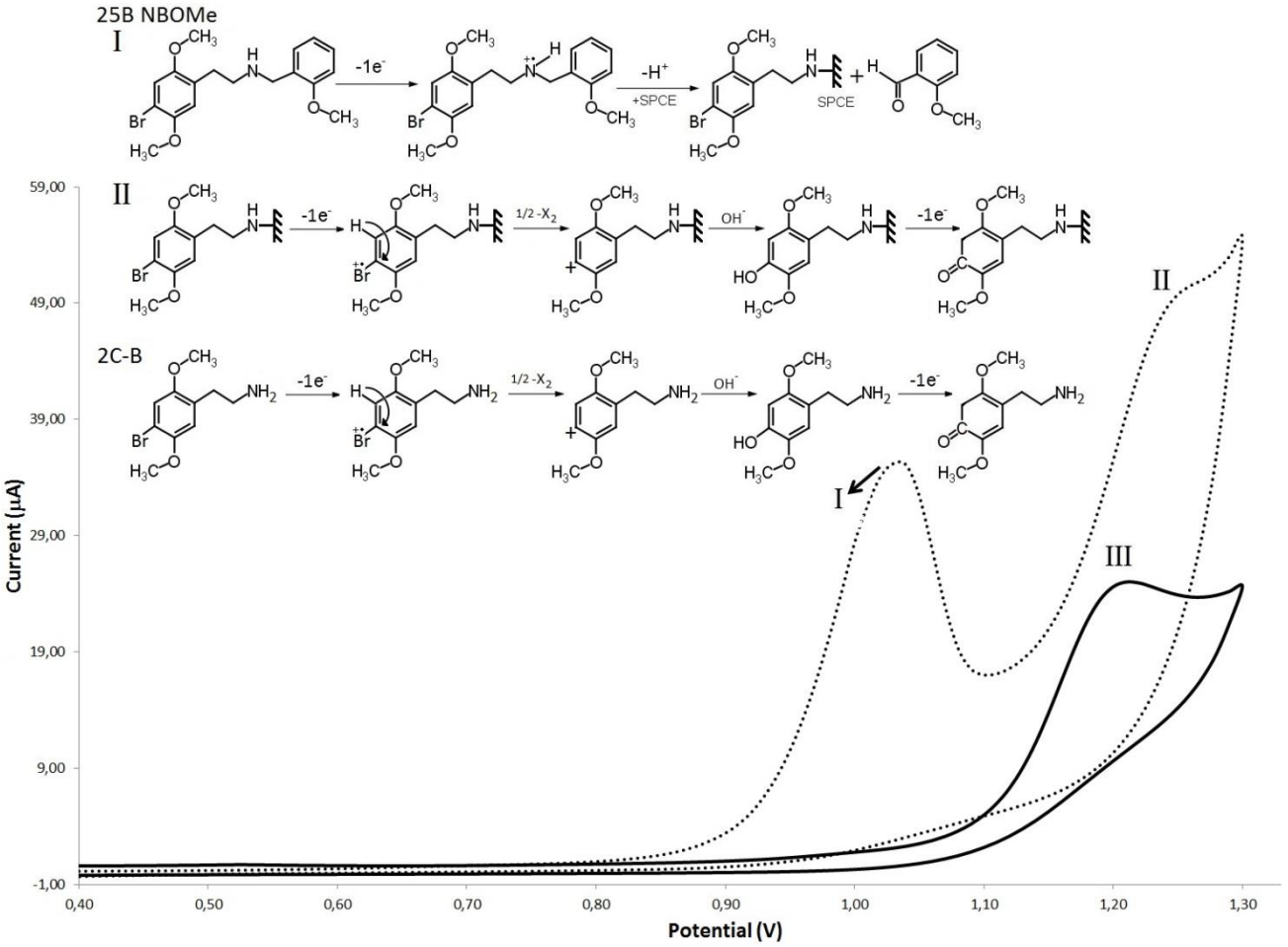

Figure 3. Ciclic voltammetric profiles obtained at a screen-printed carbon electrode in a solution with 0.05 mg/mL of 25B NBOMe (dotted line) and 2C-B (solid line) in a pH7 PBS buffer. Scan rate 0.1 V/s.

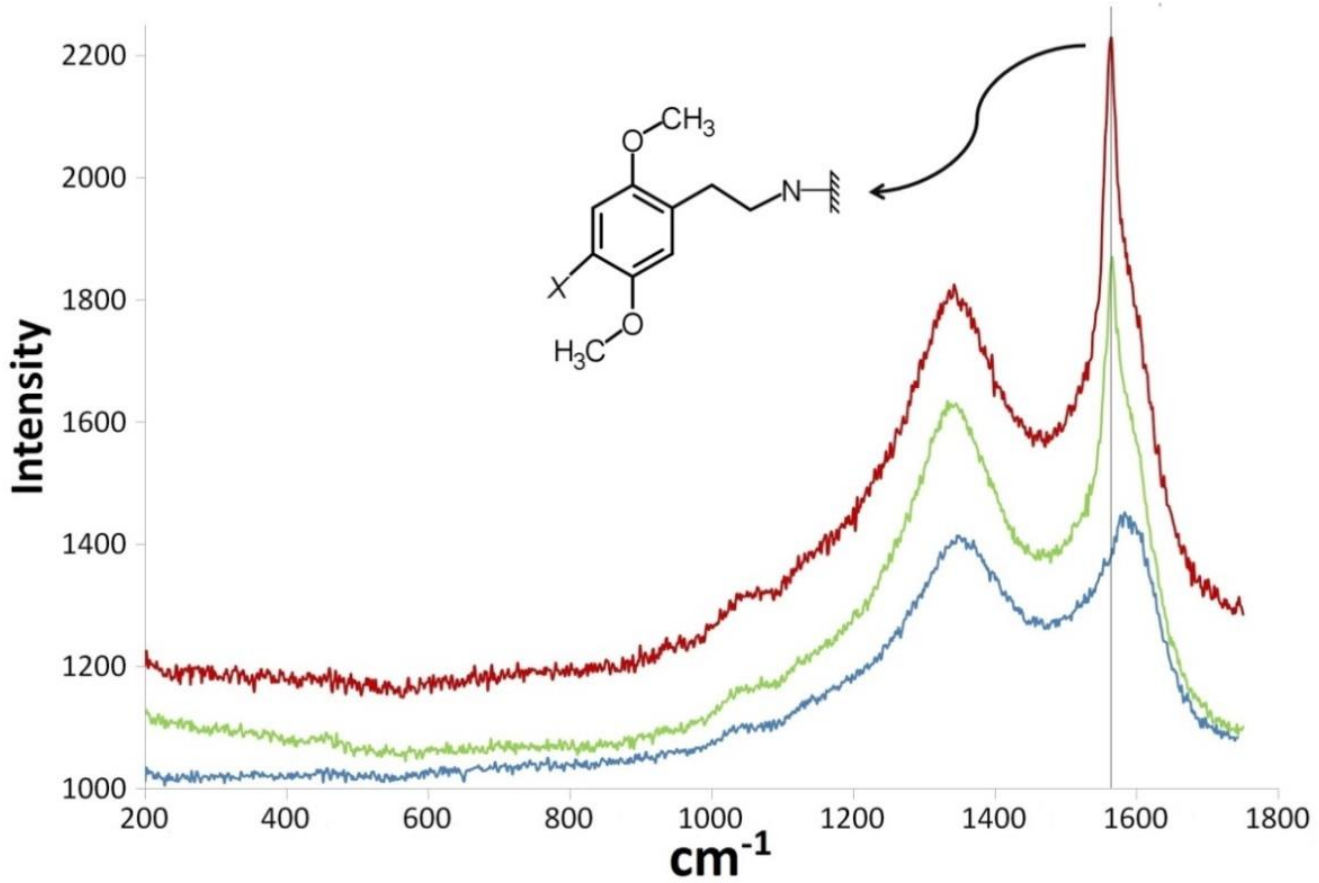

Figure 4. Raman spectra of a blank screen-printed carbon electrode (blue line), after 10 CV scans of a solution containing 25I-NBOMe (green line) and 25B-NBOMe (red line).

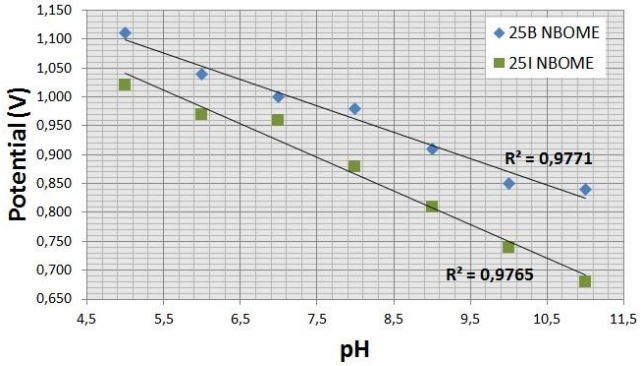

Figure 5. Influence of pH on the peak I potential of a 0.05 mg/mL 25B NBOMe solution (■) and 25I NBOMe (♦) solution dissolved in PBS by DPV. Scan rate 0.1 V/s.

To measure stability, the standard stock solution, stowed in the dark at -4ºC was used in the period over 6 months and no changes were observed in peak potential or peak current. The standards work solutions were prepared daily with PBS at pH 7.0.

An eight-point calibration graph in the range 0.01mg/mL – 0.08 mg/mL, n= 5, were used to verify method linearity and sensitivity. The peak current was found to be directly proportional to the drug concentration in all concentration range analyzed. The detectability of the developed method was checked in terms of limit of detection (LOD) and limit of quantification (LOQ) values. Table 1 summarizes the data from the main calibration curve, statistical evaluation of the regression lines and the analytical characteristics of the method.

The precision and ruggedness of the proposed method was investigated with respect of repeatability and reproducibility considering peak potential and peak current for both oxidation peaks, I and II, as the first peak is important regarding quantification and the second peak is important for identification purposes. The repeatability was evaluated from 5 repeated measurements of the electrochemical signal of 0.05 mg/mL 25B NBOMe and 25I NBOMe solutions under optimal conditions. The mean measured potential for peak I was +1.040 ±0.001 V for 25B NBOMe and +1.020 ±0.010 V for 25I NBOMe and for peak II +1.250 V ±0.006 V for 25B-NBOMe and +1.210 ±0.010 V for 25INBOMe. Intraday and inter-day precisions were evaluated after analysis of three concentrations chosen from low, medium and high concentration levels (0.025 mg/mL, 0.042 mg/mL and 0.072 mg/mL) in the linear range. Every sample in each series was analyzed three times under optimal conditions over 3 consecutive days. Precision was expressed as the relative standard deviation (%RSD) in table 1. Trueness was determined by comparing the calculated mean concentrations and experimental concentrations values and expressed as a percentage relative error (bias %).

The robustness of the method was tested by evaluating peak I potential and current by introducing known changes in the experimental parameters such as pH (7.0 – 7.3) and supporting electrolites (0.18 - 0.2M). Only one parameter was changed at a time. These parameters were evaluated for a 0.05mg/mL solution of 25B NBOMe/25I NBOMe (Table 2). A Friedman test was used for statistical comparison and did not detect any differences across multiple test attempts ($p = 0.13 > p=0.05$). No statistically significant changes were found neither in peak current or peak potential.

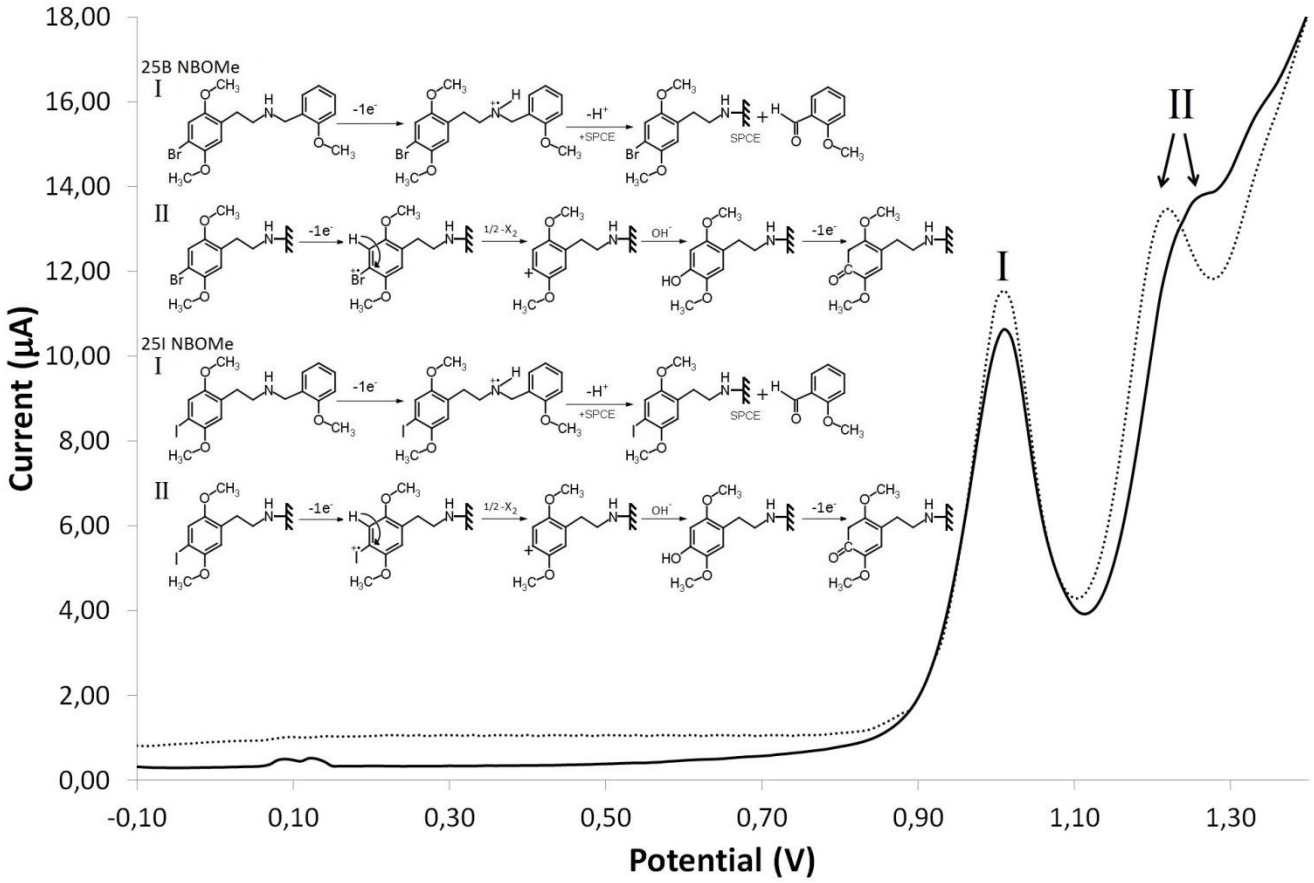

Figure 6. Differential pulse voltammetric profiles obtained at a screen-printed carbon electrode in a solution with 0.05 mg/mL of 25B NBOMe (solid line) and 25I-NBOMe (dotted line) in a pH7 PBS buffer. Scan rate 0.1 V/s.

	25B NBOMe		25I NBOMe	
regression equation [a]	y=(96±4)x+1.4±0.2		y=(122±6)x+3.0±0.3	
correlation coefficient (R^2)	0.991		0.996	
standard error of the slope (mV)	3.57		5.60	
standard error of the intercept (mV)	0.18		0.28	
linearity range (mg/mL)	0.01 - 0.08		0.01 - 0.08	
Calibration graph (n=5)				
LOD (mg/mL)	0.011		0.004	
LOQ (mg/mL)	0.034		0.012	
accuracy (mg/mL) (RSD)	1.85%		0.66%	
	peak I	peak II	peak I	peak II
Intraday precision (n=5)[b]				
peak potential (V)	2.2%	2.0%	1.0%	0.93%
peak current (µA)	4.4%	2.4%	3.5%	1.8%
Inter-day precision (n=5)[b]				
peak potential (V)	2.8%	2.3%	1.3%	1.0%
peak current (µA)	7.9%	5.6%	3.6%	2.3%

Table 1. Analytical parameters for the quantitative determination of 25B NBOMe and 25I NBOMe using the DPV. A $y = bx+a$; x = concentration (mg/mL), y = peak current (V), a = intercept, b=slope.

	25B NBOMe				25I NBOMe			
	Peak Potetial (V)		Concentration found		Peak Potetial (V)		Concentration found	
pH	mean ± SD	RSD%	mg/mL	RSD %	mean ± SD	RSD%	mg/mL	RSD %
7.0	1.040 ± 0.006	0.58	0.049 ± 0.006	0.48	1.02 ± 0.06	0.58	0.049 ± 0.004	0.68
7.1	1.040 ± 0.006	0.59	0.053 ± 0.008	0.81	1.02 ± 0.06	0.56	0.049 ± 0.006	0.78
7.2	1.03 ± 0.01	1.01	0.048 ± 0.009	1.11	1.01 ± 0.01	1.02	0.042 ± 0.008	1.22
Concentration								
0.2M	1.040 ± 0.006	0.57	0.059 ± 0.007	0.75	1.02 ± 0.06	0.58	0.047 ± 0.002	0.37
0.19M	1.03 ± 0.01	1.00	0.06 ± 0.01	1.21	1.03 ± 0.01	1.00	0.045 ± 0.005	0.61
0.18M	1.040 ± 0.006	0.58	0.057 ± 0.009	0.83	1.02 ± 0.06	0.58	0.045 ± 0.004	0.49

Table 2. Robustness data for the DPV method developed in the present study for determination fo 25B NBOMe and 25I NBOMe.

Analytical application. A validation study was performed using GC-MS as the reference method. A nine point concentration set was elaborated from 0.015 to 0.075 mg/mL and the experimental value observed by the voltammetric method was compared against the reference method in triplicate. A linear regression based on peak height (μA) of peak I (Figure 1), showed a correlation coefficient (R^2) of 0.9944 (y = 1.03 ± 0.01 x – 0.0023 ± 0.0009) and the average error was 0.38% for 25B NBOMe and was 0.07% for 25I NBOMe, demonstrating a strength relation between the two methods. Uncertainty, measured as 3 times the standard deviation was 0.15% for low concentration points, 0.04% for mid concentration points and 0.09% for upper concentration points (Figure 7)

Analysis of real street samples. To complete the analytical validation and to prove its usefulness in analyzing real street samples, 20 paper blotters seized by the Federal District Civil Police in Brazil between the years 2014 and 2015, containing 25B NBOMe (n=13) and 25I NBOMe (n=7) were analyzed using the voltammetric method described in this study. DPV results were compared with GC/MS data for both identification and quantification purposes.

DPV was able to correctly identify the presence of the 25B NBOMe or/and 25I NBOMe in 82% of the samples analysed, allowing the rapid identification of the NBOMe compounds present in the paper blotters. Quantification analysis showed an average calculated error of 0.005 mg/mL and RSD% 3.99 for 25B NBOMe and 0.005 mg/mL and RSD% of 5.08 for 25I NBOMe between both methods.

A paired-samples t-test was conducted to test the hypothesis that the results obtained in the analysis using Dropsens and GC-MS were equal. There was no significant difference in the measurements taken by Dropsens (25B NBOMe *M* = 0,0446, *SD* = 0,000417; 25I NBOMe *M* = 0,0436, *SD* = 0,000385) and GC-MS (25B NBOMe *M* = 0,0454, *SD* = 0,000385; 25I NBOMe *M* = 0,0440, *SD* = 0,000363); 25B NBOMe, t (8) = 1.07, p = 0.31; 25I NBOMe t (8) = 1.21, p = 026, demonstrating the suitability of the proposed method as a quick screening method for forensic samples.

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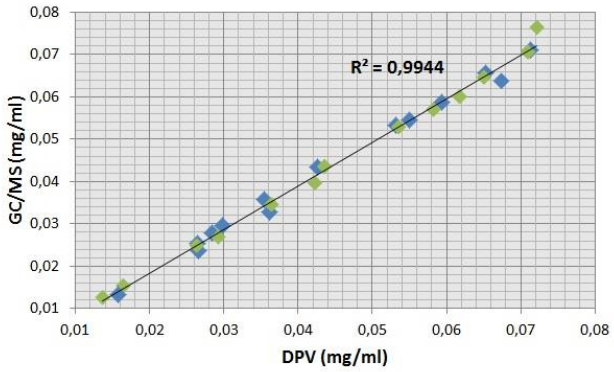

Figure 7. Comparison of concentration values obtain in the experimental set with GC/MS and DPV (n=3 for each concentration). 25B NBOMe (♦) and 25I NBOMe (♦).

CONCLUSIONS

For the first time the voltammetric behavior of phenethylamines such as 25B-NBOMe and 25I-NBOMe was investigated and the mechanism and analytical characteristics determined. The oxidation of the secondary amine present in the NBOMe compounds produced a covalent link to the electrode. This was also confirmed by Raman spectroscopy. The electroanalytical method has good specificity and sensitivity and LOD, LOQ, linear range and precision demonstrated the method to be analytically valuable. The analyses of real forensic samples also proved that the method can be of use as screening method to support a number of others analytical approaches, as it is quick, portable and can be carried out in the field.

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