MDMA electrochemical determination in aqueous media containing illicit drugs and validation of a voltammetric methodology

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MDMA is the abbreviation for 3,4-methylenedioxymethamphetamine, which is commonly found in “ecstasy” pills. The psychoactive and euphoric effects that MDMA causes make this substance an illicit drug that is constantly seized by police forces. We describe a low-cost and fast voltammetric methodology that requires a carbon paste electrode (working electrode) in aqueous solution containing 0.1 mol L⁻¹ LiClO₄ as the supporting electrolyte. We conducted cyclic and square wave voltammetry and obtained limits of detection of 0.33 µg mL⁻¹ and 0.36 µg mL⁻¹, respectively, as others figures of merit for a complete validation. It includes the analysis main interfering substances, and results for seized samples were compared to those obtained by chromatography, which were close. An extended study of robustness was carried out by Youden’s test, that is inedited to electrochemical techniques when applied to forensic analysis. This test contributes to complete methodology validation and study of electrode cost and efficiency during electrochemical measurements involving a carbon paste electrode. In the end, this work presents a full validated methodology able to be applied in forensic laboratories.

Keywords: forensic chemistry, MDMA, voltammetry, validation.

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Introduction

The need for new sensorial experiences has become the main reason why young people seek the various drugs that are available in the illegal market. Because hallucinogens and amphetamines can distort visual and sensorial perception, they are the most popular options. The hallucinogen amphetamine 3,4-methylenedioxymethamphetamine, commonly known as MDMA, is found in ecstasy tablets, and it is one of the most consumed substances among synthetic illicit drugs [1].

MDMA stimulates the central nervous system: it creates empathogenic euphoria and elevates the user’s energy, but this drug can also cause neurodegeneration. Severe intoxication includes convulsions, coma, and hyperthermia and can thus be fatal [2,3]. As a result of psychological disorders, MDMA users are also potential suicide victims and more susceptible to committing crimes and disturbing social peace. Therefore, these potential risks concern the local authorities and call for efforts within the social, health, and police areas [1].

For law enforcement, the police must properly identify the seized drug. Each country has their standard methods of drug testing, and the recommendations of the Scientific Working Group for the Analyses of Seized Drugs (SWGDRUG) are frequently adopted. Created in 1997 by forensic scientists from the United States, England, Canada, Australia, Japan, Germany, the Netherlands, and several forensic

organizations, this group constantly updates their recommendations to adapt to new equipment and drugs launched in the market [4].

For irrefutable results to be achieved, the SWGDRUG suggests that Infrared Spectroscopy, Mass Spectrometry, Raman Spectroscopy, or Nuclear Magnetic Resonance Spectroscopy (group A) be combined with Liquid or Gas Chromatography or Capillary Electrophoresis (group B) to identify the drug. If none of the group A methods is available, two group B techniques can be combined with a group C test, which encompasses colorimetric tests and tests that verify physicochemical properties, to certify drug identification [4-11].

Despite these recommendations, group A and B techniques are commonly expensive and require specific and highly pure reagents. This situation has encouraged researches to develop devices and methodologies that are cheaper and easier to operate, but which provide the same specificity as the other procedures [12-14].

Voltammetric methodologies can meet such requirements and it might be adopted as routine analysis in forensic laboratories whose structure does not englobe equipment for techniques from class A. These methodologies demand simple apparatus and minimal use of reagents, and they offer similar sensitivity to the sensitivity that is achieved with chromatographic methods. For example, a simple potentiostat may be achieved in small size, for portable analysis with a

third of the budget for a gas chromatography. The analysis does not demand MilliQ water or high purity solvents.

Electroanalytical chemistry presents several methods for analysis of MDMA, cocaine, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), lysergic acid diethylamide (LSD), N-benzyl-substituted phenethylamines (NBOMe), and other drugs [15-29].

Cyclic voltammetry, differential pulse voltammetry, and square wave voltammetry are commonly applied to identify drugs. Carbon paste as working electrode is an asset because it is cheap and easy to handle. Graphite, carbon nanotubes, and carbon black powder can be molded with an agglutinant agent, like mineral oil or paraffin, resulting in an electrode that can detect traces of seized drugs. Addition of a modifier to the mixture can further improve electrode sensitivity and selectivity [30-32].

This work aims to employ both cyclic and square wave voltammetry with a carbon paste electrode to detect and to quantify MDMA. The use of an aqueous medium differentiates this methodology from chromatographic analytical techniques, and the non-modified carbon paste electrode is an alternative to electrodes reported in the literature. This study focuses on analytical validation parameters and on Youden's test as a different approach for voltammetric procedures [2,16,33].

Material and methods

Reagents and solutions

The performance of lithium perchlorate (LiClO_4 , Acros), sodium perchlorate (NaClO_4 , Vetec), potassium perchlorate (KClO_4 , Vetec), and ammonium perchlorate (NH_4ClO_4 , Vetec) as supporting electrolyte was investigated. All the solutions were prepared at a concentration of 0.1 mol L^{-1} in distilled water. The pH variation of these supporting electrolyte solutions was obtained using HCl or KOH, depending on the chosen pH value.

The MDMA analytical standard solution was acquired from Cerilliant and contained 1.0 mg of MDMA in 1.0 mL of methanol. Dilution of this standard was performed by the addition of methanol (JT Barker), and successive aliquots of $2.5 \text{ }\mu\text{L}$ of this standard solution, in concentration 1.0 mg mL^{-1} , were added to the electrochemical cell containing 3.0 mL of $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$ for the analytical curve. A blank solution containing LiClO_4 with $50 \text{ }\mu\text{L}$ of methanol was also analyzed.

For specificity analysis, 1.0 mg of the substances caffeine, procaine, lidocaine, and theobromine (Sigma Aldrich) was dissolved in 1.0 mL of methanol. The cocaine standard were obtained from a scientific partnership between this research group and the laboratory of toxicological analysis – Institute of Criminalistics, Ribeirão Preto city, São Paulo state, Brazil and was also dissolved in 1.0 mL of methanol.

The methamphetamine standard solution was acquired from LGC in a concentration of 0.1 mg mL^{-1} and 3,4-Metilenodioxianfetamine standard solution (LGC) was in a

concentration of 1.0 mg mL^{-1} . For the analysis of these interfering substances solutions were prepared at a concentration of $5.0 \text{ }\mu\text{g mL}^{-1}$ and analyzed in 3.0 mL of $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$.

The four ecstasy seized samples were also obtained from a partnership between this research group and the same laboratory of toxicological analysis. A volume of $10\text{-}20 \text{ }\mu\text{L}$ of a methanolic solution of these samples (1 mg mL^{-1}) was added to 3 mL aqueous solution of $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$ and analyzed by cyclic and square wave voltammetry. The concentration of MDMA in each sample was calculated by the linear equation obtained by the respective analytical curve. The analysis was performed in triplicate.

Equipment

All the measurements were performed on an $\mu\text{Autolab III}$ potentiostat and on an Autolab PGSTAT128N potentiostat/galvanostat operating with NOVA 1.11 software. The last equipment was employed in the robustness test. The following electrodes were used for the voltammetric measurements: Ag/AgCl (containing saturated KCl solution) as the reference electrode, a platinum wire (spiral or square) as the counter electrode, and a carbon paste electrode with 10% paraffin (Sigma-Aldrich or Isogama) and 90% graphite power (Sigma Aldrich or Synth) in its composition as the working electrode. After each measurement, the carbon paste electrode surface was renewed after being lightly slid on a sulfite paper.

Different paraffin brands were tested because they provided paraffin with distinct degrees of purity: Sigma-Aldrich supplies paraffin for laboratory purposes, whereas Isogama supplies paraffin for handicraft purposes, like the production of candles. The objective was to check how these two materials affected the voltammetric response.

Voltammetric measurements were conducted within potentials ranging from -0.1 to 1.5 V . Pre-concentration was accomplished at -0.1 V for 10 s . Other pre-concentration times were also examined (5 s and 20 s). In Cyclic Voltammetry, the scan rates varied from 10 mV s^{-1} to 200 mV s^{-1} , to evaluate the electrochemical process nature.

The Square Wave Voltammetry conditions were optimized in terms of frequency (5 to 40 Hz), amplitude (0.01 to 0.1 V), and step potential (0.001 to 0.01 V). These parameters were set according to the lowest potential peak observed and highest amperometric intensity.

Specificity analysis against caffeine, cocaine, procaine, lidocaine and theobromine was carried out in the same conditions and by using the same methodology as in the case of MDMA analysis.

The four ecstasy samples were also tested by High Performance Liquid Chromatography, using an equipment model Ultimate 3000 (Thermo Scientific) with a C8 column of Nano Science Technologies ($25 \text{ cm} \times 4.6 \text{ mm}$, $5 \text{ }\mu\text{m}$), coupled to a Diode Array Detector. A loop of $10 \text{ }\mu\text{L}$ and flow rate of 1.2 mL min^{-1} was applied in a detector DAD. An isocratic condition was used for the mobile phase, consisted

of 85 % de phosphoric acid (0.5 % v/v) + triethylamine (for pH adjustment 2.35) and 15 % acetonitrile. For the analytical curve was used the range concentration of $5.0 \mu\text{g mL}^{-1}$ to $100 \mu\text{g mL}^{-1}$ of MDMA. The samples solutions were prepared with concentration of 0.1 mg mL^{-1} . The applied methodology is based on the validated method proposed by UNODC [34]. The analysis was performed in triplicate.

Validation and Robustness evaluation (Youden's test)

For the MDMA measurement validation process, the figures of merit such as the Limit of Detection and the Limit of Quantification were calculated as $3\sigma/m$ and $10\sigma/m$ respectively, being σ the standard deviation observed in the linear fit for the linear coefficient and m the voltammetric sensitivity.

In this study, reproducibility was determined as being the deviation observed on different days of analysis, whilst repeatability was calculated on the same day of analysis, with electrode surface renewal: a total of seven assays were performed for each of these parameters, to determine the standard deviation.

The voltammetric method robustness for MDMA quantitation was evaluated by using the method proposed by Youden and Steiner (1975) and it implies in studying the most impacting factor in analysis. The Youden's test is found in recommendations for validation test in guidelines such as AOAC [35,36]. Eight separate experiments were conducted to determine how the seven selected parameters influenced the system. Table 1 lists the applied experimental parameters and the nominal values.

Table 1. Experimental parameters and variations for robustness evaluation.

Parameter		Nominal condition	Variation		
A/a	Potentiostat model	μ Autolab III	A	Autolab 128N	a
B/b	Counter electrode format	Spiral	B	Square	b
C/c	Graphite supplier	Synth (99.0%)	C	Sigma Aldrich (99.99%)	c
D/d	Paraffin supplier	Isogama (unknown purity)	D	Sigma Aldrich (for laboratory purpose)	d
E/e	Light	Absence	E	Presence	e
F/f	N ₂ Flow time (s)	0	F	10	F
G/g	Quality of water	H ₂ O distilled	G	H ₂ O MilliQ	G

The eight voltammetric runs were randomly accomplished. Table 2 summarizes the factorial combination of the seven parameters and their respective variations for Youden's test; the results of the determinations are shown from s to z. Hence,

when combination 1 is tested, the result will be "s"; when combination 2 is tested, the result will be "t"; and so on, until all the eight combinations have been tested.

In each combination, MDMA solution analysis was carried out in triplicate, at the work concentration ($1.99 \mu\text{g mL}^{-1}$). The result observed in each combination was the current (A) in two voltammetric techniques (Cyclic and Square Wave Voltammetry).

To determine the influence of a factor, the four values corresponding to the capital letters (nominal conditions) and the four values corresponding to the lowercase letters (variation) had to be found, and the means of the these two groups had to be compared. For example, to calculate how the potentiostat (A/a) affected the final results, Equation 1 was employed:

$$\text{Effect A/a} = \frac{s+t+u+v}{4} - \frac{w+x+y+z}{4} \quad \text{Eq. 1}$$

All the seven pairs were determined to obtain seven effects, which were ordered to reveal which experimental parameters significantly impacted the result of analyses.

Results and Discussion

First, we investigated the use of perchlorate salts, such as lithium, sodium, potassium, and ammonium perchlorates as supporting electrolyte for MDMA voltammetric detection. We chose the perchlorate anion because supporting electrolytes bearing this anion have been commonly reported in works on the electrochemical detection of synthetic drugs [17,23-25]. The voltammetric response of LiClO_4 exhibited an electric current peak of greater intensity, followed by NaClO_4 , KClO_4 , and NH_4ClO_4 , as indicated in Figure 1. Thus, by comparing the perchlorate salts, we were able to associate the voltammetric response with the cation size: the smaller the cation, the higher the peak intensity. The results were similar for both Cyclic Voltammetry and Square Wave Voltammetry, and the future measurements were taken with LiClO_4 in aqueous medium.

Figure 1. Voltammetric response for MDMA ($1.66 \mu\text{g mL}^{-1}$) in different aqueous supporting electrolyte solutions (for KClO_4 and NaClO_4 : frequency = 25 Hz, amplitude = 0.05 V, and step potential = 0.005 V; for NH_4ClO_4 , LiClO_4 , and LiCl : frequency = 35 Hz, amplitude = 0.07 V, and step potential = 0.005 V; pre-concentration was conducted at -0.1 V for 10 s in all the measurements).

The anodic peak observed for MDMA at 1.24 V for cyclic voltammetry and 1.20 V for square wave voltammetry.

Table 2. Factorial combination of the experimental parameters for robustness evaluation by Youden's test.

Parameter	Experiment number							
	1	2	3	4	5	6	7	8
A/a	A	A	A	A	a	a	a	a
B/b	B	B	b	b	B	B	b	b
C/c	C	c	C	c	C	c	C	c
D/d	D	D	d	d	d	d	D	D
E/e	E	e	E	e	e	E	e	E
F/f	F	f	f	F	F	f	f	F
G/g	G	g	g	G	g	G	G	g
Observed results	S	t	u	v	w	x	y	z

We checked the MDMA oxidation signal behavior in the LiClO_4 solution at pH 2.0, 5.0 and 7.0. As reported before, there is a dependence between oxidation peak and pH, with an increment in pH the potential peaked decreases and the current increase [15]. At pH 2, a low peak current was observed, which increased with increasing pH to 5. Basic medium also becomes easy the oxidation of primary and secondary amines, as in the case of MDA and methamphetamine, that are considered MDMA interfering substances [15]. To avoid this interference, pH 5 was chosen for voltammetric analyzes of MDMA.

The potential scan rate was varied between 10 and 200 mV s^{-1} (Figure 2). Through the relation between scan rate and current values, we can obtain conclusions about the kinetics and mechanism of the reaction involved. The linearity between the peak current and the square root of scan rate characterizes a process of diffusion of the analyte to the electrode surface [38]. The $\log i_p$ vs. $\log v$ curve is linear with slope of 0.36 and also indicates a diffusion-controlled electrode process. A slope close to 0.5 is expected for controlled-diffusion electrode processes and close to 1.0 for controlled-adsorption electrode processes [38].

Figure 2. Voltammetric response of MDMA ($2.59 \mu\text{g mL}^{-1}$) at different scan rates. The supporting electrolyte was LiClO_4 at 0.1 mol L^{-1} (pre-concentration at -0.1 V for 10 s).

As the scan rate increases, it is possible to observe the displacement of the oxidation peaks to more positive potentials, which suggests the irreversible nature of the oxidation process [38]. We decided to employ a scan rate of 50 mV s^{-1} during the cyclic voltammetry experiments.

The dependence of i_p versus $v^{-1/2}$ on v was checked for MDMA oxidation. The current function (i_p versus $v^{-1/2}$) is independent of the scan rate for reversible and irreversible processes [38]. In the case of MDMA oxidation, the decrease of the current function with v indicates that a chemical reaction is coupled to the electrode process and characterized an EC mechanism [38].

The anodic peak observed can be related to an oxidation of the aromatic nucleus of the MDMA molecule. Figure 3 shows a proposed electro-oxidation and formation of a cation radical of MDMA in aqueous media based on literature [7,15,33]. Garrido et al. [15] associated the first anodic peak with that cation radical formation and a second and third oxidation peak to a dimerization process of these radicals followed by the oxidation of the secondary amine present in MDMA molecule. In this studied conditions we observed only the first oxidation peak.

Figure 3. Mechanism for electro-oxidation of MDMA in aqueous media [7,15,33].

Validation

Once we had performed the optimization and verified the experimental conditions, we obtained important information for the validation and the possible quantification of MDMA from the analytical curve. Table 3 presents the Cyclic Voltammetry and Square Wave Voltammetry results obtained after successive additions of the standard solution to the electrochemical cell, as well as the parameters for the

equation, which described the linear relation between the current and the presence of MDMA. We obtained the data from the peak current illustrated in Figure 4.

Table 3. Analytical parameters calculated for MDMA analysis by Cyclic Voltammetry and Square Wave Voltammetry.

Parameter	Cyclic Voltammetry	Square Wave Voltammetry
Limit of Detection	0.33 $\mu\text{g mL}^{-1}$	0.36 $\mu\text{g mL}^{-1}$
Limit of Quantification	1.11 $\mu\text{g mL}^{-1}$	1.22 $\mu\text{g mL}^{-1}$
Linear range	1.11 to 4.97 $\mu\text{g mL}^{-1}$	1.22 to 4.97 $\mu\text{g mL}^{-1}$
Linearity	0.9941	0.9929
Accuracy	98.32%	95.26%
Sensitivity (slope)	3.78 10^{-2} A L mol^{-1}	2.64 10^{-1} A L mol^{-1}
Linear coefficient	-7.45 10^{-8} A	1.75 10^{-6} A
Reproducibility (intra-day precision)	3.06%	3.80%
Repeatability (inter-day precision)	3.23%	3.62%

Figure 4. Voltammograms obtained for successive MDMA concentrations, in LiClO_4 as supporting electrolyte: A) Cyclic Voltammetry (pre-concentration at -0.1 V for 10s, scan rate = 50 mV s $^{-1}$); B) Square Wave Voltammetry (frequency = 35 Hz, amplitude = 0.07 V, step potential = 0.005 V; pre-concentration at -0.1 V for 10 s).

The limit of detection obtained for Cyclic Voltammetry and Square Wave Voltammetry, respectively, corresponded to the presence of 0.33 μg and 0.36 μg of MDMA in a tablet or pill dissolved in 1.0 mL of methanol. This value was lower than the values found in seized samples, more than 50 mg per pill [2].

The techniques afforded close values of linearity, reproducibility, repeatability, and linear range. In general, Cyclic Voltammetry provided more satisfactory accuracy and limits of detection and quantification, as the values were lower than those obtained by Square Wave Voltammetry applied to MDMA analysis in this work.

As explained before, robustness evaluation (Youden's test) fixed the same MDMA concentration, so we were able to determine which factor influenced the analysis the most at the end of the eight assays. Table 4 reports the amperometric responses for each assay.

Table 4. Current values obtained in eight runs performed for Youden's test (MDMA concentration of 1.99 $\mu\text{g mL}^{-1}$).

Experiment	Cyclic Voltammetry (A)	SD (%)	Square wave Voltammetry (A)	SD (%)
1	5.26 10^{-7}	1.42	4.84 10^{-6}	1.34
2	4.88 10^{-7}	1.23	5.15 10^{-6}	0.89
3	4.91 10^{-7}	0.98	5.02 10^{-6}	1.46
4	4.90 10^{-7}	1.67	5.16 10^{-6}	2.66
5	5.17 10^{-7}	1.40	4.82 10^{-6}	0.65
6	5.45 10^{-7}	1.30	5.28 10^{-6}	2.82
7	4.91 10^{-7}	4.00	5.09 10^{-6}	1.42
8	5.26 10^{-7}	1.14	4.79 10^{-6}	2.88

The results in Table 4 show the proximity between the peak current values in all the eight experiments. Table 5 lists the values obtained for each factor after we applied Equation 1. On the basis of these values, we were able to compare the influence of each parameter numerically.

Table 5. Effects of the robustness evaluation proposed by Youden's test.

Effect	Cyclic Voltammetry	Square wave Voltammetry
A/a	-2.111 10^{-8}	4.825 10^{-8}
B/b	1.929 10^{-8}	5.250 10^{-9}
C/c	-5.993 10^{-9}	-1.513 10^{-7}
D/d	-2.692 10^{-9}	-1.023 10^{-7}
E/e	2.549 10^{-8}	-6.875 10^{-8}
F/f	1.129 10^{-8}	-2.340 10^{-7}
G/g	7.907 10^{-9}	1.487 10^{-7}

The presence or absence of light was chosen as one of the factors in the Youden test because MDMA standards are purchased in light-protected ampoules. We want to check if the light affects the voltammetric analysis of MDMA. This factor (E) was the one that most affected the analysis in Cyclic Voltammetry, followed by factor F (nitrogen flow) in Square Wave Voltammetry.

The application of nitrogen flow is a common procedure in voltammetric measurements to remove electroactive oxygen, and it is applied before analysis. Once this step is not necessary, it turns the methodology simpler and faster, as it implies in removing a step in experimental procedure and it also does not imply in acquiring other chemical supply for this nitrogen.

The negative value indicated that factor variance (nitrogen flow time established as 10 s) was the most decisive parameter in Square Wave Voltammetry. However, an additional experimental step pointed to a small difference in peak current. This was based on the average peak current calculated for experiments 2, 3, 6, and 7, where the nitrogen flow was applied: there was a difference of around 0.3×10^{-6} A as compared to the experiments carried out without nitrogen flow (1, 4, 5, and 8).

The most important information obtained during robustness evaluation was the possibility of reducing the costs inherent to this voltammetric analysis: the method did not require high-purity graphite; indeed, a simpler powder provided similar responses. The use of a paraffin from candle decreases the total cost of the analysis, as it is not necessary affording a commercial one with higher purity from chemical industry.

Youden's test reinforced the possibility of using distilled water to prepare the supporting electrolyte solution, which simplified the methodology as MilliQ water production was unnecessary. This is not possible in the case of chromatographic equipment.

This robustness evaluation therefore completely validated the analysis, which requires a cheaper electrode for MDMA detection.

Interfering substance analysis

Because some substances may be added to ecstasy tablets/pills or even substitute MDMA present in them, we also examined the voltammetric response for the standard solution of caffeine, cocaine, procaine, lidocaine, theobromine, methamphetamine and 3,4-Methylenedioxanphetamine (MDA) in the presence or in the absence of MDMA. Only procaine and MDA had an amperometric response, as the MDMA, Figure 5 (A).

The procaine displayed an anodic peak at approximately 1.04 V for Cyclic Voltammetry and 1.02 V for Square Wave Voltammetry [37], therefore, we conducted a study in medium also containing MDMA, as depicted in Figure 5 (B). Nevertheless, MDMA peak (2) shifted to higher potential (1.26 V) for both techniques, but this did not prevent both drugs from being detected in the same matrix. Hence, the present methodology is specific for MDMA analysis even in the presence of its main interfering substances.

The MDA presented a peak at 1.24 V for Cyclic Voltammetry and 1.19 V for Square Wave Voltammetry, very close to MDMA oxidation peak. Because MDA is an MDMA metabolite, they have a very similar structure. The only structural difference between them lies in the amine group,

MDMA has a secondary and MDA a primary amine group [15]. A better differentiation between these two molecules would require the use of a chromatographic technique.

Figure 5. Cyclic voltammograms of A) caffeine, cocaine, lidocaine, theobromine, metamphetamine and MDMA ($5.00 \mu\text{g mL}^{-1}$); B) procaine, MDA and MDMA ($5.00 \mu\text{g mL}^{-1}$). Scan rate: 50 mV s^{-1} and pre-concentration at -0.1 V for 10s.

Analysis of samples

We used the chromatographic and electrochemical methodologies to analyze the four seized ecstasy pills. For both techniques, an analytical curve was used to quantify the samples, and Table 6 compiles these results for MDMA determination in ecstasy pills by HPLC, Cyclic Voltammetry, or Square Wave Voltammetry, performed in triplicate for each sample. The calculated values of MDMA in ecstasy samples 2 and 4 were close to the values achieved with the chromatographic technique. However, the values obtained for samples 1 and 3 in the voltammetric technique showed a considerable difference. Thus, it is suggested that the methodology developed may assist in the presumptive analysis of MDMA. We found between 58 and 93 mg of MDMA in ecstasy pills (considering a total mass of 250 mg) and the typical dosage of MDMA for recreational use varies from 50 mg to 150 mg [39]. Low dose (between 50 and 75 mg) used on single occasion produced the desired effects by

most users like euphoria, well-being, sharpened sensory perception and sociability. At higher doses, undesired effects may appear like headache, nausea, loss of appetite, blurred vision, insomnia, panic attacks, delirium or even brief psychotic episodes [40].

Table 6. MDMA quantification in seized ecstasy pills.

Sample	Cyclic Voltammetry (% m/m)	Square Wave Voltammetry (% m/m)	HPLC (% m/m)
1	25,44 ± 1,65	28,85 ± 1,13	20,46 ± 0,62
2	37,39 ± 3,92	35,53 ± 2,68	34,44 ± 1,30
3	23,22 ± 5,12	28,32 ± 1,39	20,33 ± 1,21
4	28,63 ± 3,72	27,45 ± 5,99	26,06 ± 2,79

Conclusions

We have demonstrated new methodologies for MDMA determination by Cyclic Voltammetry and Square Wave Voltammetry, in aqueous medium. Both techniques provided close values of figures of merit, but Cyclic Voltammetry afforded more satisfactory values of limits of detection and quantification. Robustness evaluation helped to establish the complete validation process and to prove whether it is feasible to apply the methodology to analyze seized samples in forensic laboratories on a routine basis. The methodologies allow for simple, fast, and sensitive MDMA analysis with a cheap working electrode. They are also specific for MDMA analysis even in the presence of its main interfering substances like caffeine, cocaine, procaine, lidocaine, theobromine and methamphetamine. The same methodology can distinguish between MDMA and procaine, which allows for simultaneous drug detection.

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Conflict of interest

The authors declare no conflicts of interest.

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