Simple and sensitive determination of sibutramine in slimming tea beverages using a carbon screen-printed electrode with adsorptive stripping voltammetry.

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Abstract: The stimulant sibutramine is an anorexic agent found as an adulterant in natural products and multivitamins supplements used for weight-loss. In this work, a carbon graphite screen-printed electrode (SPE-Gr) with adsorptive stripping pulse differential voltammetry (AdSDPV) is presented for the sensitive and simple detection of sibutramine in slimming tea beverages. The proposed electrochemical method shows a linear working range from 2.0 to 120 μM with a low LOD (0.3 μM) for sibutramine determination in slimming tea samples. The analytical performance of the SPE-Gr with AdSDPV for sibutramine detection suggests its possible application as an easy, fast and low-cost method to analyse adulterated tea samples with this stimulant at low levels (< 0.1%).

Keywords: Adsorptive stripping voltammetry, Adulterated products, Sibutramine, Screen-printed electrode, Tea beverages.

1. Introduction

Sibutramine (Fig. 1) is an anorexic agent used as an appetite suppressant for the treatment of obesity. Due to its side effects such as cardiovascular events or strokes, since 2010, sibutramine has not been recommended in many countries [1-2]. However, several natural products, and multivitamins supplements used for weight-loss have been adulterated with sibutramine (unlabelled) in recent years [3-12], which can lead to serious health risks for customers. One important group amongst these are slimming tea beverages [12]. Accordingly sibutramine determination in various products is great of interest for both forensic and human health fields. Furthermore, sibutramine is considered a stimulant and its intake before or during athletic competitions is prohibited by the World Anti-Doping Agency (WADA) [13]. Some cases have been reported for the positive test of sibutramine in doping control [14-16], so that its determination in food samples is of interest to WADA and professional athletes.

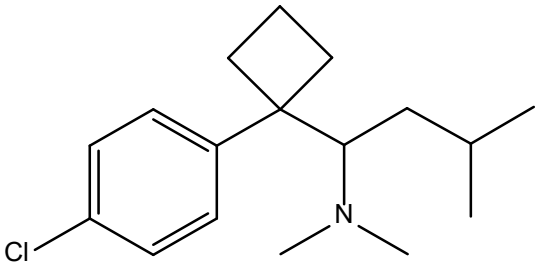

Fig. 1. Molecular structure of sibutramine

Although many analytical techniques have been reported for the determination of sibutramine in natural product samples used for weight-loss [3-12], only a few methods have been applied to slimming tea beverages [11,12]. Fast and low-cost methods using electroanalytical techniques have been used for sibutramine determination in tea samples based on the voltammetric detection at a boron-doped diamond electrode (BDDE) [17] or a hanging mercury drop electrode (HMDE) [18]. Beside these electrodes, a modified glassy carbon electrode (GCE) using microextraction-assisted voltammetry of microparticles with multivariate chemometric techniques was reported for screening

in adulterated tea beverages [19]. Nevertheless, portable and disposable electrochemical sensors such as screen-printed electrodes (SPEs) can provide an even simpler and more attractive method to determinate sibutramine in tea beverages. In addition, the adsorptive stripping voltammetry (AdSV) technique is an excellent approach for increasing the sensitivity of electrochemical detection in which the analyte adsorbs on the electrode surface [20]. Sibutramine has shown an electrochemical oxidation process that is adsorption-controlled at electro-reduced graphene oxide film modified glassy carbon electrode (ERGO-GCE) [21], which was applied for determination of this drug in urine and human serum.

The possible synergy of the AdSV technique with carbon SPEs offers outstanding advantages for electrochemical detection including the simplicity of application and high sensitivity as have been reported in several electroanalytical methods [22-26]. Therefore, in this work we present a simple and sensitive electrochemical procedure using, for the first time, a carbon graphite screen-printed electrode (SPE-Gr) with adsorptive stripping pulse differential voltammetry (AdSDPV) for sibutramine determination in slimming tea beverages.

2. Experimental

2.1. Instrumental and apparatus

Electrochemical measurements were performed using a PGSTAT 128N potentiostat/galvanostat (Eco Chemie, the Netherlands) controlled by GPES software (version 4.9). A Faraday cage thermostatted at 25 ºC was used for all electrochemical measurements. The oxygen from the all studied solutions was removed by bubbling of pure nitrogen gas. Commercial carbon SPEs (DRP-110 model) from Dropsens (Oviedo, Spain) were used for sibutramine determination studies. The working electrode on these SPEs is printed and based on carbon graphite, according to the company literature with a 4-mm diameter carbon disc. Carbon and silver are also printed on the

same SPE base and are used as auxiliary and pseudo-reference electrodes, respectively, in these devices.

Cyclic voltammetry (CV) at a scan rate of 50 mV s^{-1} was used for electrochemical studies of 0.5 mM sibutramine in different pH values (2.0 to 10) using Britton-Robinson buffer (BRB) solution as supporting electrolyte for the carbon graphite screen-printed electrodes SPE-Grs. Prior to each measurement, all SPE-Gr were electrochemically conditioned in 0.1 M sulfuric acid by CV (5 scans) at a scan rate of 100 mV s^{-1} from -1.0 to +1.2V. Phosphate buffer solution pH 7 was also evaluated for sibutramine detection at SPE-Grs. CV technique on SPE-Gr was performed by drop-casting of 100 µL of an electrolyte or diluted tea sample (with or without addition of sibutramine) to physically cover all three electrodes. A scan rate (10 to 200 mV s^{-1}) study was conducted on 0.5 mM sibutramine in BRB solution pH 7.0 at the SPE-Gr.

The amplitude and scan rate were optimized for differential pulse voltammetry (DPV) detection using SPE-Grs. The accumulation time (1.0 to 20 min) for sibutramine detection in 0.1 M BRB pH 7.0 was optimized using AdSDPV with SPE-Gr. The linear range and limit of detection (LOD) were investigated for the determination of sibutramine of 1.0 to 100 µM by AdSDPV at SPE-Gr. The obtained voltammograms by AdSDPV detection were baseline corrected using a polynomial (order 4) to fit the rising background current before subtraction. The charge peaks from the sibutramine oxidation process were calculated after this procedure of baseline correction. This procedure is shown in the Supplementary Information.

2.2. Chemicals and solutions

All reagents used in this work were of analytical grade (purity of > 98%) and solutions were prepared with deionized water, whose resistivity not less than 18.2 MΩ cm at 25 ºC from a Milli-Q system (Elga, UK). Sibutramine ({1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}dimethylamine)

hydrochloride monohydride standard was purchased from Nifty laboratories (Hyderabad, India). Stock solutions of 0.5 mM sibutramine were prepared directly in supporting electrolytes. BRB solutions (pH 2.0 to 10.0) were prepared using boric acid from Vetec (Duque de Caxias, Brazil), phosphoric acid from Dinâmica (Diadema, Brazil) and acetic acid from Merck (Rio de Janeiro, Brazil with different pHs (2 to 10). Sulfuric acid and sodium hydroxide both from Sigma-Aldrich (Lancashire, UK) were used to adjust the pH of 0.1 M BRB and 0.1 M phosphate buffer solutions.

2.3. Saliva sample preparation

The tea beverages labelled to contain "Detox tea" from two different brands were purchased in local pharmacies (Diamantina, MG- Brazil). The composition of these "natural" tea samples were simply declared as *Iles paraguariensis*, *Pnemus boldo* and *Mentha piperita* for "Detox tea". The tea beverages were prepared as recommended, with one tea bag (1.0 g) from each sample extracted in 200 mL of boiling water for 5 min. The analysis in tea sample was carried out according to following steps: (I) an aliquot of the tea beverages (200 mL) with or without addition of sibutramine was diluted ten times in 0.1 M BRB pH 7.0; (II) 100 μL of the diluted sample solution was drop-casted on SPE-Gr using a micropipette; (III) the pre-accumulation and detection steps of AdSDPV technique were performed.

3. Results and discussion

First, in section 3.1 the electrochemical behavior of sibutramine is investigated using a SPE-Gr to evaluate parameters indicating the optimal supporting electrolyte, pH and the possible mass-transport control of the electrochemical reaction. Second, in section 3.2, after the electrochemical oxidation of the sibutramine is identified as adsorption-controlled at the SPE-Gr, the optimum amplitude, scan rate and accumulation time for the determination of sibutramine in 0.1

M BRB pH 7.0 by AdSDPV are investigated. Finally, in section 3.3, the analytical performance of the SPE-Gr using AdSDPV in slimming tea beverages is investigated, showing that the proposed electrochemical sensor is efficient and offers enough sensitivity to detect and quantify the sibutramine in adulterated tea samples.

3.1. Electrochemical behavior of Sibutramine

The solution phase electrochemical behavior of sibutramine was investigated by CV at SPE-Gr (Fig. 2) in different pHs (2.0 to 10) in 0.1 M BRB solutions. As can be seen in Fig 2A, sibutramine showed two oxidation processes with the first well-defined peak current at +0.52 *V* (*vs* (Ag)) and the second as a shoulder at ca. +0.8 *V* (*vs* (Ag)) in BRB solution pH 7.0. Although, this first oxidation process can also be observed in pHs of 2.0 to 9.0 (Fig 2B), the pH 7.0 was chosen due to its better sensitivity and the low anodic peak potential (E_{pa}) for sibutramine determination in tea samples. 0.1 M phosphate pH 7.0 buffer solution was also considered but the BRB solution showed a higher and more defined peak current to determine this stimulant (Fig. S1) and it was selected for all following studies.

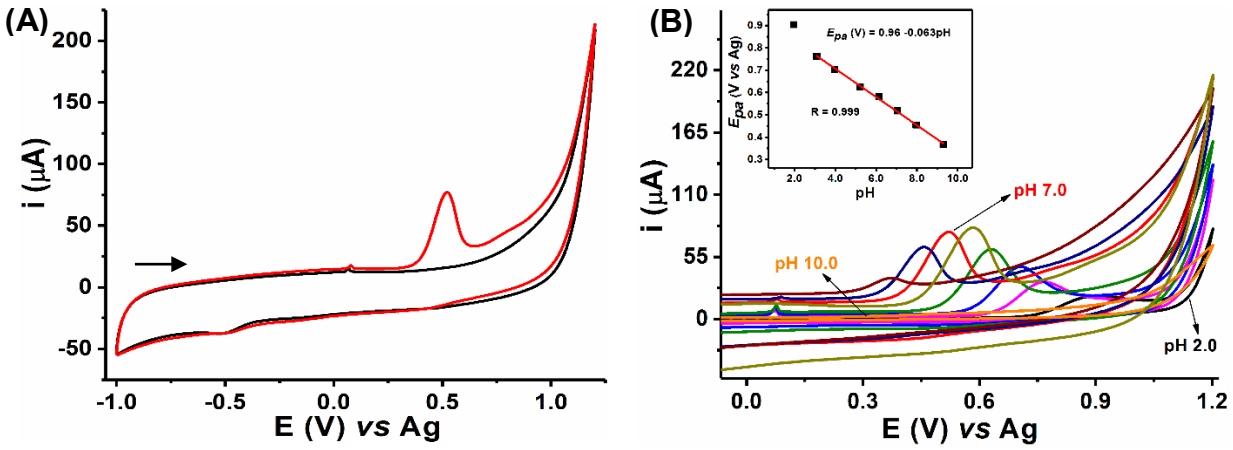

Fig. 2. (A) Voltammograms recorded immediately in 0.1 M BR buffer pH 7.0 at SPE-Gr (black line) with addition of 0.5 mM sibutramine (red line). **(B)** Voltammograms recorded at different pHs (2.0 to 10.0) in 0.1 M BR buffer with addition 0.5 mM sibutramine. For the higher pH values this level of addition exceeds the solubility of sibutramine (see text). Inset is plot of *Epa vs* pH. All potential scans were started at -1.0 V in the positive-going direction with a scan rate of 50 mV s^{-1}.

The oxidation processes shown in Fig. 2A for a sibutramine solution at SPE-Gr have also been reported in pH 7.0 using a bare [21] or modified GCEs [19,21] and BDDE [17]. Note that for pH above 8.0 to 9.0, the sibutramine becomes less soluble in aqueous solutions due to the non-protonation of the amine group (pKa 9.8) [27], which can explain the absence of oxidation peaks for this stimulant in pH > 9.0 (Fig 2B). Freitas and co-workers have suggested that the first oxidation process in 0.1 M H_2SO_4 at BDDE occurs by oxidation of the tertiary amine group with one-electron and one-proton lost in this electrochemical process involving the protonated amine [17]. The peak potential for the first oxidation process of sibutramine was linearly proportional to the pH (inset Fig. 2B) at SPE-Gr in the range of pH 2.0 to 9.0 (E_{pa1} (V) = 0.96 (± 0.01) - 0.063 (± 0.001) *pH*, with r^2 = 0.999). The obtained slope (0.063) by E_{pa1} vs *pH* curve indicates that the same number of proton and electrons is transferred in this electrochemical process, which is consistent with the oxidation mechanism for sibutramine at BDDE [17].

A scan rate study for the first sibutramine oxidation process is shown in Fig. 3. Peak currents from this oxidation (ca. +0.5 *V* (*vs* (Ag)) were linearly proportional to scan rate (inset Fig. 3) with a linear regression coefficient (r^2) of 0.995 for I_{pa} (μA) = 4.6 ± 1.8 + 723.6 ± 19.7 ν (V s^{-1}), indicating that this electrochemical process is adsorption-controlled at the SPE-Gr, which has also been reported for graphene modified electrode [21], but not at BDDE where it is diffusion-controlled [17].

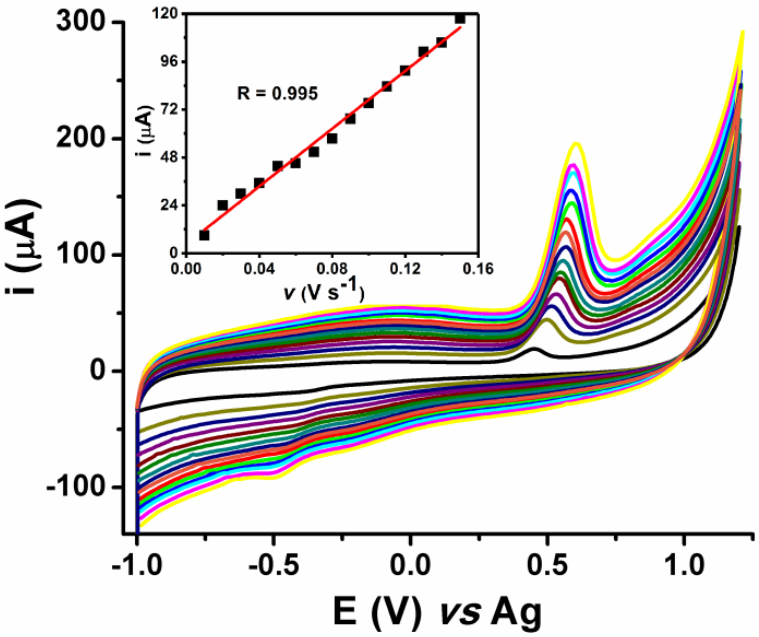

Figure 3: Voltammograms recorded immediately at different scan rate (10 to 150 mV s^{-1}) in 0.5 mM sibutramine in 0.1 M BRB pH 7.0 at SPE-Gr. All potential scans were started at -1.0 V in the positive-going direction.

3.2. Optimization for sibutramine detection by AdSDPV

First, the parameters for sibutramine detection with the DPV technique were optimized to for sensitivity with an amplitude of 100 mV and a scan rate of 80 mV s^{-1}. Next, the pre-accumulation step of sibutramine molecules on SPE-Gr (adsorption) was investigated for detection by the AdSDPV technique. The pre-accumulation was examined between 1.0 and 20.0 min (Fig. 4) for 100 μM sibutramine in 0.1 M BRB pH 7.0 on SPE-Gr. The peak charge of the sibutramine oxidation process attained a constant value after 15 min (Fig. 4), when indicates that the surface area of the working electrode is saturated with sibutramine molecules.

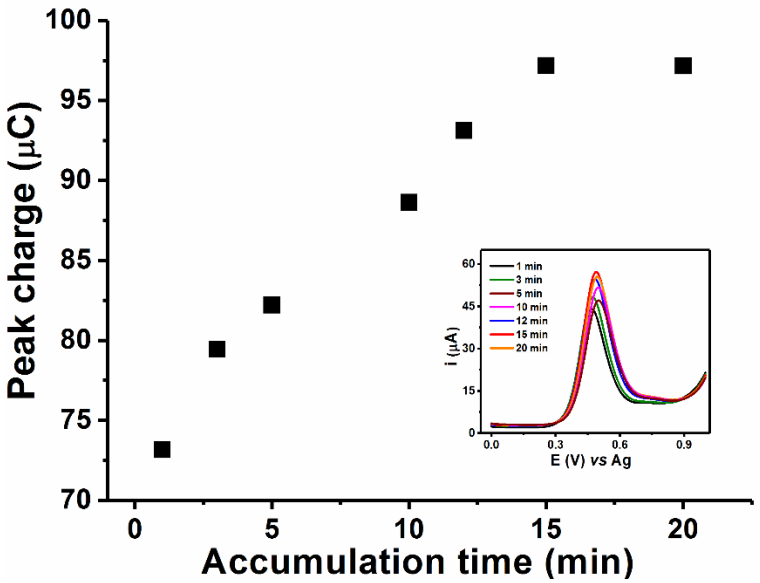

Fig. 4: The responses of oxidation peak charge as a function of accumulation time in 100 μmol L^{-1} sibutramine on SPE-Gr in 0.1 M BRB (pH 7.0). Inset is the AdSDPVs recorded on SPE-Gr at 80 mV s^{-1} and amplitude 100 mV.

The working linear range was evaluated using standard solutions of 2.0 to 120 μM sibutramine in 0.1 M BRB (Fig. 5) and the AdSDPV technique at SPE-Grs. In this range a linear relationship between the sibutramine concentration and its peak charge (from the first oxidation process) was obtained (inset of Fig. 5), with r^2 of 0.996 for the following linear equation: Q (μC) = -2.98 ± 1.53 + 1.10 ± 0.03 (μC / μmol L^{-1}) [sibutramine]. A low "theoretical" limit of detection (LOD) of 0.3 μmol L^{-1} was obtained by $3S_B/m$, where S_B is the standard deviation (N = 10) of the background (blank) response and m is the slope of linear equation. A measurable signal from 2.0 μmol L^{-1} can be seen (red line) in the zoom-in shown inset of Fig. 5 for sibutramine determination by AdSDPV at SPE-Gr.

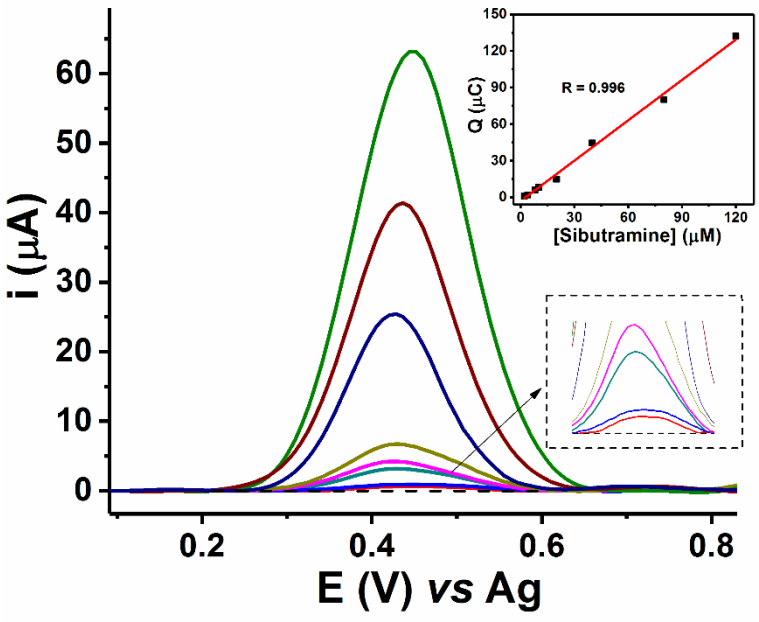

Fig. 5. AdSDPVs recorded (corrected background current) for concentrations from 2.0 to 120 μM sibutramine in 0.1 M BRB (pH 7.0) (blank: black-dash-line) at SPE-Gr. Inset above is the corresponding linear regression and below a zoom-in of the lower mensurable signals, corresponding to a concentration of 2.0 to 10 μM. Accumulation time of 15 min. Amplitude of 100 mV and scan rate 80 mV s^{-1}.

According to the literature [28], for forensic analysis it is required to be able to detect 0.5 % (w/w), or more, of sibutramine in adulterated samples. This corresponds to levels of around 75 μM extracted from a tea bag containing 1.0 g of tea and the tea beverage is made using 200 mL of water. On the other hand, sibutramine has been found in slimming tea at lower levels than 0.5 %. Reports claim 1.8 mg per tea serving (200 mL) [12], corresponding to levels around 30 μmol L^{-1}. Therefore, even using a dilution of 10 times for tea beverages, the proposed method can determinate levels of adulterated tea at the required sensitivity.

The reproducibility of the proposed method was evaluated using three different SPEs-Grs for the determination of 10 μmol sibutramine by the AdSDPV technique (Fig. S2). A standard deviation (RSD) of 6.0 %, for peak charges was found, which can be considered a reasonable RSD for disposable devices.

3.3. Detection of Sibutramine in tea beverages and comparison of electroanalytical methods.

Tea beverages were investigated by AdSDPV at SPE-Gr before and after "spiking" with standard solutions of sibutramine. Slimming tea samples declared as "detox tea" were studied and voltammograms are shown in Fig 6. It can be noticed in Fig. 6, tea sample (black-line) shows an (unknown) oxidation process with a well-defined current peak at ca. 0.1 V at SPE-Gr (vs Ag), but no electrochemical process close to sibutramine oxidation when 50 µM sibutramine (red line) was added ("spiked") in this tea sample. The addition-recovery study was carried out for 2.5 µM sibutramine (Fig. S3) with recovery value for peak charge of 99.4 ± 4.5 % (N=3).

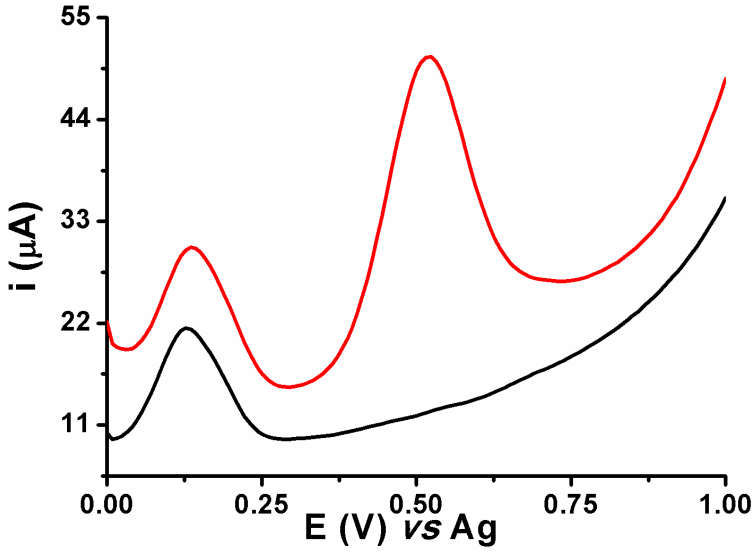

Fig. 6. (**A**) AdSDPVs recorded in 0.1 M BRB at a SPE-Gr in diluted tea sample (black line) without and with addition of 50.0 µM sibutramine (red line). Amplitude of 100 mV and scan rate 80 mV s^{-1}. Accumulation time: 15 min.

In order to compare the performance of the proposed method, Table 1 presents the main analytical parameters obtained for sibutramine determination by other electroanalytical methods in different samples. To the best of our knowledge, only three works have been reported for

sibutramine detection in Tea beverages. One of them [19] is not shown in Table 1 because is related to the screening and authentication method combining chemometrics and voltammetry of sibutramine in tea samples, but with a LOD calculated from current ratios as 2.0 % (w/w). This LOD is higher than that obtained by method developed in this paper (0.3 μmol L^{-1}), which is equivalent to 0.02 % (w/w). Furthermore, as can be seen in Table 1, the method proposed in this paper shows the lowest *measurable* signal for tea samples, which is a more valuable than an estimated LOD as it reveals a more realistic measure of the limit at which the method can be usefully applied. In addition, a disposable device based on an unmodified electrochemical sensor such as SPE-Gr offers a simpler and more attractive application in routine forensic analysis for sibutramine determination.

Table 1: Electroanalytical methods for sibutramine determination.

Technique[a]	**Working electrode**[b]	**Linear range (μmolL^{-1})**	**LOD (μmolL^{-1})**	**Measurable signal**[c] **(μmolL^{-1})**	**Sample**	**Ref.**
AdSDPV	ERGO-GCE	0.25 –20.0	0.05	0.25	Urine and serum	[21]
POT	Selective membrane	4.0-10,000	9.0	4.0	Pharm. Formulations	[29]
POT	Selective membrane	10-10,000	8.0	10.0	Pharm. Formulations	[30]
SWV and BIA-SWV	BDDE	15.0 - 150	0.2 - 5.8	15.0	Tea beverages and vitamins supplements	[17]
POL	HDME	4.2 -100.0	1.2	4.2	Tea beverages and Pharm. Formulations	[18]
AdSDPV	SPE-Gr	2.0 - 120	0.30	2.0	Tea beverages	This work

[a]POT: potentiometry; BIA-SWV: batch injection analysis with square-wave voltammetry; POL: polarography; [b]ERGO-GCE: electro-reduced graphene oxide film modified glassy carbon electrode. [c]The lowest measurable signal reported for the different methods relate to the smallest concentrations used to from a reported calibration curve.

4. Conclusions

A simple and sensitive electrochemical sensing method using a SPE-Gr with AdSDPV has been validated for the detection of sibutramine in slimming tea beverages. The use of the AdSV technique with disposable devices as SPEs offers high sensitivity and simplicity of application for routine forensics analysis of sibutramine (< 0.1%) in adulterated tea beverages and is an easy and inexpensive method for sibutramine determination in slimming tea samples.

5. Acknowledgements

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6. References

[1] EMA, European Medicines Agency recommends suspension of marketing authorisations for sibutramine, Available on https://www.ema.europa.eu/en/medicines/human/referrals/sibutramine (Accessed December, 15 2018).

[2] FDA, Drug Safety and Availability - Questions and Answers: FDA Recommends Against the Continued Use of Meridia (sibutramine), FDA, 2016 Available on (Accessed December, 15 2018) https://www.fda.gov/Drugs/DrugSafety/ucm228746.htm.

[3] J. Jung, M. Hermanns-Clausen, W. Weinmann, *Forensic Sci. Int.* **2006**, *161*, 221.

[4] C. Vidal, S. Quandte, *Ther. Drug Monit.* **2006**, *28*, 690.

[5] S.R. Park, J.G. Lee, S.H. Roh, G. Kim, C.H. Kwon, H.R. Park, K.S. Kwon, D. Kim, S.W. Kwon, *Food Addit. Contam. Part B Surveill.* **2012**, *5,* 29.

[6] M. Martini, L.M. de Carvalho, A. Blasco-Blasco, A. Doménech-Carbó, *Anal. Methods* **2015**, *7* 5740.

[7] C. Mathon, A. Ankli, E. Reich, S. Bieri, P. Christen, *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* **2014**, *31*, 15.

[8] J. Calahan, D. Howard, A. Almalki, M. Gupta, A. Calderón, *Planta Med.* **2016**, *82*, 505.

[9] Y. Liu, F. Lu, *Rev. Anal. Chem.* **2017**, *36*, 2016.

[10] F. V. Hunsel, B. J. Venhuis, P. H. J. Keizers, A. Kant, *Drug Test Analysis* **2016**, *8*, 311.

[11] N. Cebi, M. T. Yilmaz, O. Sagdic, *Food Chemistry* **2017**, *229*, 517.

[12] W. Schänzer, H. Geyer, A. Gotzmann, U Mareck, Sibutramine Found in Chinese Herbal Slimming Tea and Capsules, In: Recent Advances in Doping Analysis (Eds: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck), Sportverlag Strauß, Köln **2007**, pp. 367-370.

[13] The World Anti-Doping Agency (WADA), The 2018 Prohibited List International Standard, Available on https://www.wada-ama.org/sites/default/files/prohibited_list_2018_en.pdf, (Accessed December, 15 2018).

[14] Asian Games gold medallist gets 120-day doping ban - report. Available on https://uk.reuters.com/article/uk-games-wushu-doping/asian-games-gold-medallist-gets-120-day-doping-ban-report-idUKKBN0JP15L20141211, (Accessed December, 15 2018).

[15] (Hockey) Kumar test positive for doping, Available on https://www.nst.com.my/sports/others/2017/12/315843/hockey-kumar-test-positive-doping, (Accessed December, 15 2018).

[16] Adrian Mutu banned nine months for positive drugs test, Available on https://www.telegraph.co.uk/sport/football/european/7607412/Adrian-Mutu-banned-nine-months-for-positive-drugs-test.html, (Accessed December, 15 2018).

[17] J. M. Freitas, T. C. Oliveira, M. H.P. Santana, C. E. Banks, R. A. A. Munoz, E. M. Richter, *Sensors and Actuators B: Chemical* **2019**, *282*, 449.

[18] J. M. Carvalho, A. R. Da Silva, A. L. M. C. da Cunha, R. Q. Aucelio, A. L. M. Alberti and K. C. Leandro, *Quim. Nova* **2012**, *35*, 988.

[19] M. Martini, L.M. de Carvalho, A. Blasco-Blasco, A. Doménech-Carbó, *Anal. Methods* **2015**, *7*, 5740.

[20] W.T.P. dos Santos, H.M.A. Amin, R.G. Compton, *Sensors and Actuators B: Chemical* **2019**, *279,* 433.

[21] N. L. Teradal, P. S. Narayan, S. Jaladappagari, *Anal. Methods* **2013**, *5*, 7090.

[22] K.C. Honeychurch, J. Brooks, J.P. Hart, *Talanta* **2016**, *147*, 510.

[23] P. Fanjul-Bolado, D. H. Santos, V.M. Montoya, A. Costa-García, *Electroanalysis*, **2015**, *27*, 1276.

[24] A.E. Radi, N.A. El-Ghany, T. Wahdan, *Electroanalysis*, **2016**, *28*, 1112.

[25] P. Gan, J. S. Foord, R. G. Compton, *Electroanalysis*, **2014**, *26*, 1886.

[26] H. Ahmar, H. Tabani, M. Hossein Koruni, S.S.H. Davarani, A.R. Fakhari, *Biosensors and Bioelectronics*, **2014**, *54*, 189.

[27] M. Swain, chemicalize.org, *Journal of Chemical Information and Modeling*, **2012**, *52*, 613.

[28] FDA, Merita® label, Available on https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020632s032lbl.pdf, Accessed December, 15 2018).

[29] N. A. El Gohary, R.M. El Nashar, H.Y. Aboul-Enien, *Anal. Lett.* **2011**, *44*, 241.

[30] S. I. M. Zayed, Y. M. Issa, *Anal. Sci.* **2010**, *26*, 45.