Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
ISSN 1984-6428

| Vol 9 | | No. 4 | | July-September 2017 |

Full Paper

Simultaneous Voltammetric Determination of Amlodipine and Atorvastatin on Anodically Pretreated Boron-Doped Diamond Electrode

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Article history: Received: 089 March 2017; accepted: 13 July 2017. Available online: 24 September 2017. DOI: http://dx.doi.org/10.17807/orbital.v9i4.973

Abstract: An innovative electroanalytical method has been described for the simultaneous determination of amlodipine besylate (AML) and atorvastatin calcium (ATOR) using the square-wave voltammetry and an anodically pretreated boron-doped diamond electrode. Two very well-resolved and reproducible oxidation peaks for AML and ATOR were obtained in Britton-Robinson buffer solution (pH 4.0). Under the optimum analytical experimental conditions, the method exhibits linear responses to AML and ATOR in the concentration ranges 2.0 – 28 and 1.0 – 50 µmol L^{-1}, respectively, with detection limits of 0.028 and 0.38 µmol L^{-1}, respectively. The proposed novel method was applied in the simultaneous determination of AML and ATOR content in combined dosage forms and the accuracy was attested by means of comparison with those data obtained from high performance liquid chromatography at a 95% confidence level (paired t-test).

Keywords: amlodipine determination; anodic pretreatment; atorvastatin determination; BDD electrode; square-wave voltammetry

1. INTRODUCTION

Combination drugs consisting of amlodipine besylate (AML) and atorvastatin calcium (ATOR) provides a more integrated approach to the treatment of cardiovascular risks. The combination of both substances in one dosage form is used to treat two different conditions, high blood pressure and high cholesterol. AML (2[(2-aminoethoxy)methyl]-4-(2-chloro-phenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid, 3-ethyl, 5 methylester besylate) is a dihydropyridine derivative with calcium antagonist activity which is used in the management of hypertension, cardiac arrhythmias, and coronary heart failure. ATOR ([R-(R∗, R∗)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid calcium) is a member of the class of lipid-lowering agents called statins and it is potent inhibitor of HMG-CoA reductase that has been demonstrated to be effective in reducing cholesterol and triglyceride. Chemical structures of AML and ATOR are shown in Fig. 1(A) and (B), respectively. An overdose of these combinated drugs leads to unwanted effects, such as headache, nausea, and improper use of drugs can lead to side effects such as headaches, dizziness, insomnia, and gastrointestinal discomfort [1]. Hence, their determination in pharmaceuticals is of great importance.

Reviewing the literature revealed that all the reported methods for the simultaneous determination of AML and ATOR in tablets and biological fluids relies on the use of chromatographic [2–9], spectrophotometric [10–12] and capillary electrophoresis [13] techniques. These methods face the drawbacks of being laborious and requiring pretreatment of samples, need mathematical approaches, need sample clean-up, toxic organic solvents and relatively heavy instrumentation. A simultaneous method of both drugs is not official in any pharmacopoeia.

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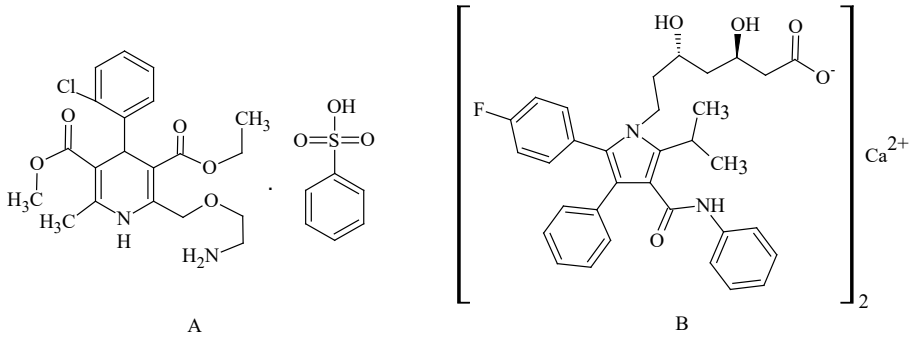

A B

Figure 1. Chemical structures of (A) AML and (B) ATOR.

Voltammetric techniques can be an alternative to these methods. It has been utilized in development of analytical procedures for the individual and/or simultaneous determination of a wide range of compounds of pharmaceutical interest [14–17], due to rapidity, sensitivity, precision, accuracy and simplicity [18]. There are relatively few voltammetric methods for simultaneous determination of AML and ATOR [19,20], underscoring the importance of this work for publication. A first derivative of ratio-differential pulse voltammetric method has been described for the simultaneous determination of AML and ATOR in binary mixture and pharmaceutical formulations employing a glassy carbon electrode (GCE) [19]. A multi-walled carbon nanotubes graphite (MWCNP:G) paste electrode was developed for the simultaneous determination of AML and ATOR in commercial tablets using differential pulse voltammetry (DPV) [20].

Boron-doped diamond (BDD) electrode is widely used for the development of analytical procedures for different analytes, due to very wide working potential window, very low and stable background current, long term stability, low sensitivity to dissolved oxygen, and an extreme electrochemical stability in both alkaline and acidic media [21–24]. This electrode has been used to individual and simultaneous quantification of several antihypertensives in pharmaceuticals samples, mainly in our group [25–32]. To the best of our knowledge, BDD electrode has not been used for simultaneous determination of an antihypertensive and a statin in commercial tablets.

In view of this, the present work aims to the development of a reliable, low cost and selective method for the simultaneous determination of AML and ATOR in pharmaceutical formulations based on SWV using an anodically pretreated BDD electrode. Utilizing the developed method, simultaneous determination of the both drugs has been carried out in pharmaceutical formulations. Moreover, the proposed voltammetric method was validated by HPLC technique [2].

2. MATERIAL AND METHODS

2.1 Chemicals and solutions

All chemicals were analytical grade, and the solutions were prepared using ultra-purified water (resistivity > 18.2 MΩ cm) supplied by a Milli-Q system (Millipore®). AML and ATOR were obtained from Sigma-Aldrich. Acetic acid, boric acid, citric acid, lactic acid, ortophosphoric acid, and sodium hydroxide were obtained from Synth. Commercial pharmaceutical samples used in these studies were: AML:ATOR tablets (labeled 5:10 and 10:10 mg). These samples were purchased from local drugstore in city of Londrina in Brazil.

Britton-Robinson (BR) buffer solution (pH 4.0) containing 10 % methanol (v/v) was chosen as supporting electrolyte (as reported further below). Methanol was added in this solution because of lower solubility of ATOR in aqueous medium. BR buffer solutions were prepared by 0.04 mol L^{-1} in acetic, orthophosphoric, and boric acids, with pH adjusted with 2.0 mol L^{-1} NaOH solution.

Standard solutions 10 mmol L^{-1} of AML and ATOR were prepared before the use in a BR buffer solution (pH 4.0) containing 10% methanol (v/v). Both AML and ATOR working solutions were prepared by appropriated dilution of these stock solutions with the BR buffer solution (pH 4.0).

2.2 Apparatus

All the electrochemical experiments were conducted in a three-electrode single-compartment

glass cell, including a BDD electrode as working electrode, a platinum plate as auxiliary electrode, and an Ag/AgCl (3.0 mol L^{-1} KCl) as reference electrode. The voltammetric measurements were carried out using a Palmsens potentiostat/galvanostat controlled by the Palmsens PC software.

BDD film (8000 ppm; 0.28 cm^2 exposed area) was obtained from Adamant, Switzerland. Prior to the experiments, the BDD electrode was electrochemically pretreated in a 0.5 mol L^{-1} H_2SO_4 solution. For anodic pretreatment was applied a current of 0.5 A cm^{-2} during 30 s, and for the cathodic one was applied a current of –0.5 A cm^{-2}, during 120 s. Both anodic and cathodic pretreatments were carried out using a Microquímica potentiostat/galvanostat.

The pH was measured at 25.0 ± 0.5 ºC using a pH-meter (Hanna Instruments), model HI-221, employing a combined glass electrode with an Ag/AgCl (3.0 mol L^{-1} KCl) external reference electrode.

The determinations of AML and ATOR by HPLC were carried out using a LC Shimadzu coupled to a system with a LC-20AT pump, SIL-20AC automatic injector and SPD-M20A PDA detector. The chromatographic separation conditions were carried out in according to previous work.[2] The separation of AML and ATOR was accomplished on an ACE 5 C18 column (250 mm x 4.6 mm i.d., particle size: 5 µm) at 24.0 (± 0.1) ºC.

2.3 Measurements procedures

Cyclic voltammetry (CV) and DPV were employed for preliminary studies on electrochemical behavior of AML and ATOR. SWV and DPV were used for the development of electroanalytical methodologies for simultaneous determination of AML and ATOR in commercial pharmaceutical samples.

With the utilization of optimal parameters, calibration curves were obtained by successive addition of aliquots of AML and ATOR stock solutions into the measurement cell containing 10 mL of supporting electrolyte. Square-wave and differential pulse voltammograms were obtained after each aliquot addition of both analytes. The detection limit (LOD) value was calculated as three times the standard deviation of the blank solution divided by slope of the analytical curve according to IUPAC recommendation [33].

2.4 Sample preparation

The proposed method was carried out for the simultaneous determination of AML and ATOR in commercial pharmaceutical samples.

As for samples preparation, ten tablets of each dosage were reduced to a homogeneous fine powder in a mortar with a pestle. These powders were weighed and a mass corresponding to one tablet was transferred to 25 ml calibrated volumetric flask containing 10 mL methanol. After sonication for 5 min, the volumes of the flasks were supplemented with methanol. Then, an adequate aliquot of each sample was directly transferred to the electrochemical cell containing 10 mL of the supporting electrolyte, after which the voltammograms were obtained. The AML and ATOR concentrations in each sample solution were determined directly by interpolation in the previously obtained analytical curves.

2.5 Comparative method

The results obtained using the proposed SWV method was compared with those from chromatographic method [2]. For such, ten tablets of each pharmaceutical product were reduced to a homogeneous fine powder in a mortar with a pestle. These powders were weighed and a mass corresponding to one tablet was transferred to 25 ml calibrated volumetric flask and was dissolved in the mobile phase. The mobile phase consisted in a mixture of 50 mmol L^{-1} phosphate buffer (pH 3.5), acetonitrile and methanol (30:50:20, v/v/v). The sample solutions and mobile phase were filtered using a PTFE 0.20 µm and nylon 0.45 µm membrane filters (Millipore®). After appropriate dilution with the mobile phase, chromatograms were obtained for both analytes. The flow rate was 1.0 mL min^{-1} and the injection volume was 20 µL. The detector was set in 240 nm

3. RESULTS AND DISCUSSION

3.1 Electrochemical behavior of AML and ATOR on BDD electrode

The voltammetric behavior of AML and ATOR was obtained by CV. Fig. 2 shows the cyclic voltammograms for 20 µmol L^{-1} AML and 50 µmol L^{-1} ATOR in BR buffer solution (pH 4.0) on the

anodically pretreated BDD electrode at the scan rate of 40 mV s^{-1}. Both analytes presented a well-defined irreversible oxidation peak, AML at 0.776 V and ATOR at 1.03 V. A good separation of peak potential (ΔE_{ap}) of about 0.254 V was observed, which indicate that the simultaneous determination of AML and ATOR is feasible at these conditions.

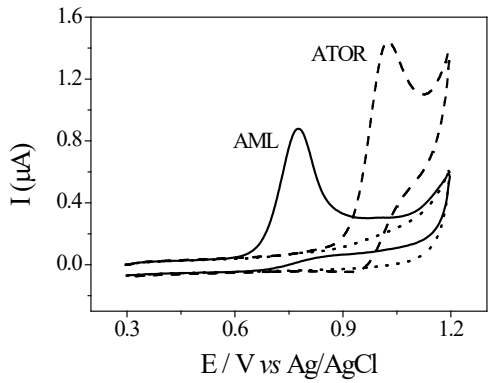

Figure 2. Cyclic voltammograms at 40 mV s^{-1} of (dotted line) blank solution, (solid line) 20 μmol L^{-1} AML and (dashed line) 50 μmol L^{-1} ATOR in BR buffer solution (pH 4.0).

The number of electrons (n) was determined applying the following equation: $E_{ap} - E_{ap/2} = 47.7$ mV/αn [34], which α is the transfer coefficient. For AML, E_p is 0.776 V and $E_{p/2}$ is 0.722 V and for ATOR, E_p is 1.02 V and $E_{p/2}$ is 0.967 V. Assuming α value as 0.5, commonly employed for totally irreversible systems [35], the calculated number of electrons is 2 (1.77) for AML and 2 (1.80) for ATOR. According to previously studies of AML and ATOR oxidation [36–38], the electrochemical oxidation of AML is believed to occurs in 1,4-dihydropyridine ring with two electrons and two protons [36] and the oxidation of ATOR can be occurs in the heterocyclic amine (pyrrole ring) involving two electrons and one proton [37,38].

The effect of surface termination of BDD electrode on a mixture containing AML and ATOR as investigated using 30 μmol L^{-1} AML and 50 μmol L^{-1} ATOR in BR buffer solution (pH 3.0). The BDD electrode was either anodically (0.5 A cm^{-2} during 30 s) or cathodically (0.5 A cm^{-2} during 120 s) pretreated. The anodic pretreatment implies in oxygen predominantly terminated surface while the cathodic one implies in hydrogen predominantly terminated surface [39]. The respective voltammograms is shown in Fig. 3. When the anodically pretreated BDD electrode is used, two well-defined oxidation peaks in distinct potential values can be observed. On the other hand, a considerable overlap of the peaks is clearly evident when the cathodically pretreated BDD electrode was used. Thus, the following experiments were carried out using an anodically pretreated BDD electrode.

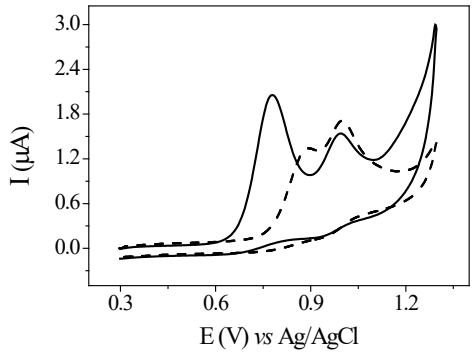

Figure 3. Cyclic voltammograms at 40 mV s^{-1} of 30 μmol L^{-1} AML and 50 μmol L^{-1} ATOR in BR buffer solution (pH 3.0) using (solid line) anodically and (dashed line) cathodically pretreated BDD electrode.

3.2 Study of pH, supporting electrolyte and scan rate

The effect of pH in the voltammetric response for of 20 μmol L^{-1} AML and 50 μmol L^{-1} ATOR on the anodically pretreated BDD electrode was investigated in the pH range 2.0 – 6.0, using a BR buffer solution. Table 1 presents the values of I_{ap} of AML and ATOR in BR buffer at different pH values, as well as ΔE_{ap} obtained by DPV experiments (a = 50 mV, v = 40 mV s^{-1} and t = 5 mV). The peak potential shifted slightly toward less positive values for AML and ATOR with increasing pH. The obtained results showed that I_{ap} has a maximum value at pH 5.0 for AML and for ATOR, the I_{ap} decreases with increasing pH with a maximum value at pH 2.0. In the pH 6.0 no oxidation peak was observed for ATOR. A better repeatability of current values (RSD_{AML}: 2.30 %, for N = 5; RSD_{ATOR}: 1.56 %, for N = 5) for both analytes was obtained when pH 4.0 was employed. In this pH value a ΔE_{ap} of about 0.194 V between both oxidation peaks clearly allows the simultaneous determination of AML and ATOR on anodically pretreated BDD electrode. Thus, pH 4.0 was chosen as the supporting electrolyte for further experiments.

Additionally, the effect of different supporting electrolytes at this pH value (pH 4.0), such as BR, citrate, Mc'Ilvaine, acetate and lactate buffer solutions were investigated in the simultaneous determination

of AML and ATOR. BR buffer solution presents the higher current values for AML and ATOR and it was chosen as the optimal medium for the sequential analysis.

Table 1. pH influence on peak separation and peak currents of AML and ATOR using an anodically pretreated BDD electrode.

pH values	ΔE_{ap} (V)	I_{ap} (µA)	
		AML	ATOR
2.0	0.249	1.07	9.47
3.0	0.206	1.41	6.42
4.0	0.194	0.87	3.40
5.0	0.184	1.55	1.38
6.0	–	1.38	0.0

The scan rate study was carried out using CV of 20 µmol L^{-1} AML and 50 µmol L^{-1} ATOR in BR buffer solution (pH 4.0). The cyclic voltammograms revealed that peak currents increase and peak potential shift for more positive values as the scan rate increase for these analytes, a typical characteristic of irreversible electrochemical reactions [35]. It was observed a linear dependence when peak current was plotted against square root of scan rate for both analytes (R_{AML} = 0.983; R_{ATOR} = 0.992), indicating that both analytes are subjected to diffusion controlled mass transport in the slow step of oxidation process. In addition, the plot log I_{pa} versus log v result in a linear dependence, according to the following equations: $\log(I_{AML}) = -0.932 + 0.57 \log(v)$ (R = 0.996) and $\log(I_{ATOR}) = -0.625 + 0.47 \log(v)$ (R = 0.995), which both slope values in close agreement with theoretical value 0.5 for diffusion controlled mechanism [35].

3.3 Chronoamperometry study

Diffusion coefficients of AML and ATOR were determined on anodically pretreated BDD electrode in BR buffer solution (pH 4.0) by chronoamperometry using Cottrell equation [34]. The fixed anodic potentials were 0.78 V and 1.01 V and the measurement as carried out using a range of concentration from 1.0 to 7.0 µmol L^{-1} and from 1.0 to 7.0 µmol L^{-1} for AML and ATOR, respectively. The slope of plots of I_{ap} vs. $t^{-1/2}$ were used to calculate the diffusion coefficients (D_o) for each molecule (data not shown). For AML, D_o was found to be 1.2×10^{-5} cm^2 s^{-1}, which is in closed agreement with the reported elsewhere (3.1×10^{-5} cm^2 s^{-1} using phosphate buffer solution (pH 7.0) [32] and 5.4×10^{-5} cm^2 s^{-1} using BR buffer solution (pH 5.0) [30]. For ATOR, D_o was found to be 3.0×10^{-6} cm^2 s^{-1}.

3.4 Simultaneous determination of AML and ATOR using a BDD electrode

The simultaneous determination of AML and ATOR was carried out by SWV and DPV techniques using an anodically pretreated BDD electrode. Before, for both techniques, the experimental parameters were optimized using 20 µmol L^{-1} AML and 50 µmol L^{-1} ATOR in BR buffer solution (pH 4.0) in order to obtain current responses for the electrochemical oxidation of AML and ATOR with highest magnitude and best peak shape. Table 2 shows the studied ranges and the optimum values.

Table 2. Instrumental parameters of SWV and DPV for determination of AML and ATOR in BR buffer solution (pH 4.0).

Technique	Parameters	Studied range	Optimum value
SWV	Square wave frequency (f; s^{-1})	5 – 70	10
	Pulse amplitude (a; mV)	10 – 50	40
	Scan increment (ΔE_S; mV)	0.5 – 3	1
DPV	a (mV)	10 – 75	50
	Scan rate (v; mV s^{-1})	5 – 25	10
	Modulation time (t; ms)	3 – 10	5

The previously optimized experimental parameters were used to record the simultaneous analytical curves by adding small volumes of concentrated standard solutions of both analytes (in triplicate) in BR buffer solution (pH 4.0) using an anodically pretreated BDD electrode. Table 3 summarizes the analytical parameters obtained to simultaneous determination of AML and ATOR. For

both techniques, the linear ranges of concentrations observed were 1.99 – 27.5 μmol L^{-1} for AML and 1.00 – 55.0 μmol L^{-1} for ATOR. As can be seen, a better sensibility was obtained using DPV technique; however, a considerable overlap of the peaks is clearly evident using DPV technique (Fig. 4), invalidating the simultaneous determination of both analytes. Thus, the simultaneous determination of AML and ATOR were carried out using SWV technique. Square-wave voltammograms obtained after successive additions of the AML and ATOR standard solutions are shown in Fig. 5.

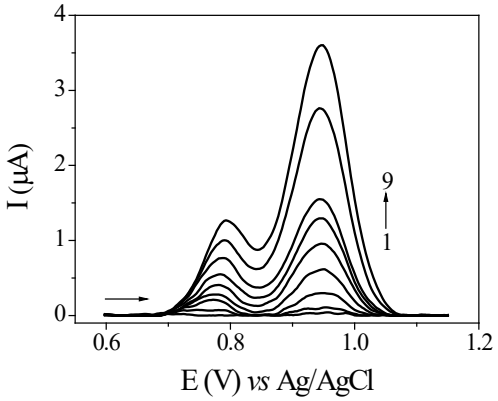

Fig. 4. Differential pulse voltammograms obtained for the oxidation of AML and ATOR in BR buffer solution (pH 4.0) employing an anodically pretreated BDD electrode. The concentrations of both analytes were changed simultaneous (1): blank solution, (2 – 9): 1.99 – 27.5 μmol L^{-1} AML and 1.00 – 55.0 μmol L^{-1} of ATOR.

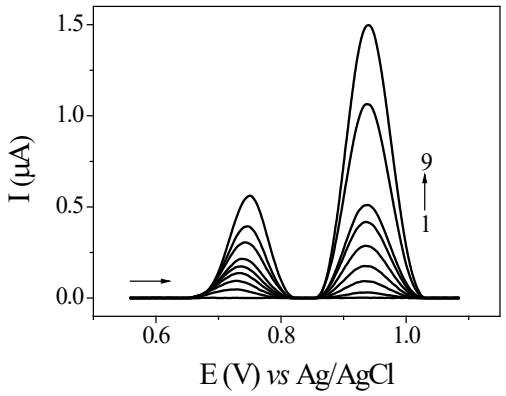

Fig. 5. Square-wave voltammograms obtained for the oxidation of AML and ATOR in BR buffer solution (pH 4.0) employing an anodically pretreated BDD electrode. The concentrations of both analytes were changed simultaneous (1): blank solution, (2 – 9): 1.99 – 27.5 μmol L^{-1} AML and 1.00 – 55.0 μmol L^{-1} of ATOR.

The intra-day repeatability of the magnitude of peak currents was determined by successive measurements (N = 10) of AML and ATOR solutions at 6.0 μmol L^{-1} concentration using SWV, obtaining RSD of 2.3 and 1.6 %, respectively, for AML and ATOR. The inter-day repeatability of magnitude of the peak currents was obtained by measuring the peak current for similar fresh solutions over a period of 5 days which RSD values were 3.4 and 2.8 % for AML and ATOR, respectively.

Table 3. Analytical parameters for the voltammetric determination of AML and ATOR in BR buffer solution (pH 4.0) using an anodically pretreated BDD electrode.

	AML		ATOR	
	SWV	**DPV**	**SWV**	**DPV**
Peak potential (V)	0.743	0.772	0.938	0.946
Linear range (μmol L^{-1})	1.99 – 27.5		1.00 – 55.0	
Slope (μA mol^{-1} L)	2.0×10^4	4.4×10^4	2.7×10^4	6.2×10^4
Intercept (μA)	0.02	0.05	0.01	0.23
Correlation coefficient	0.999	0.971	0.999	0.991
LOD (μmol L^{-1})	0.028	0.078	0.383	0.904

After these studies, the interference of each analyte in the simultaneous determination of its pairs was performed by changing one analyte concentration and keeping the other unchanged, in the BR buffer solution (pH 4.0) by SWV. The separate determination of AML in the concentration range 1.99 – 27.5 μmol L^{-1} was accomplished in solutions containing ATOR at the fixed concentration of 20 μmol L^{-1} (its peak oxidation current remained constant – RSD = 9.7 %), according to the analytical equations are $I_{AML}/\mu A = 0.001 + 2.05 \times 10^4$ [c/(mol L^{-1})] (R = 0.998). On the other hand, the separate determination of ATOR in the concentration range 1.0

– 55.0 µmol L^{-1} was accomplished in solutions containing AML at the fixed concentration of 30 µmol L^{-1} (its peak oxidation current remained constant – RSD = 4.1 %), according to the analytical equation $I_{ATOR}/\mu A = -0.09 + 2.97 \times 10^4$ [c/(mol L^{-1})] (R = 0.998). It should be concluded that the change of concentration of one studied analyte did not have the significant influence on the peak current and peak potential of the other one. It is very important to note that the oxidation processes of AML and ATOR on anodically pretreated BDD electrode are independent.

3.5 Comparison with other voltammetric methods

The analytical characteristics of the present method and previous voltammetric methods for simultaneous determination of AML and ATOR were compared and the data are resumed in Table 4. The results reveal that the anodically pretreated BDD electrode associated with the SWV technique showed better response for a simultaneous determination of AML and ATOR when compared with GC [19] and MWCNP:G [20] electrodes, with lower LOD values. Moreover, BDD electrode provided simplicity of use and very high stability, which can be used as an alternative method for the simultaneous determination of AML and ATOR in commercial pharmaceutical formulations.

Table 4. Comparison of the analytical parameters obtained using different electrode and/or technique for the determination of AML and ATOR.

Analyte	Technique	Electrode	Linear concentration range (µmol L^{-1})	LOD (µmol L^{-1})	Reference
AML	DPV	GC	4.00 – 100	0.80	[19]
	SWV	GC	4.00 – 100	0.85	[19]
	DPV	MWCNP:G	4.41 – 176	1.76	[20]
	SWV	BDD	1.99 – 27.5	0.028	This work
ATOR	DPV	GC	2.00 – 100	0.59	[19]
	SWV	GC	2.00 – 100	0.47	[19]
	DPV	MWCNP:G	4.47 – 179	1.79	[20]
	SWV	BDD	1.00 – 55.0	0.38	This work

3.6 Application of the proposed method in the simultaneous determination of AML and ATOR

Prior to the analysis of samples, the selectivity of the proposed method was evaluated. The addition of possible interferents (commonly present in the analyzed pharmaceutical formulations), such as starch, povidone, microcrystalline cellulose, titanium dioxide, iron(III) oxide, magnesium carbonate and magnesium stearate was investigated in a standard solution containing 20 µmol L^{-1} AML and 20 µmol L^{-1} ATOR in BR buffer solution (pH 4.0), in the concentrations ratios (standard solution:interferent compound) of 1:1, 1:10, and 10:1 (molar/molar). The corresponding oxidation peak currents were compared with those obtained in the absence of each interferent. The analysis of the obtained responses allowed concluding that these compounds do not significantly interfere (< 4.1 %) in the determination of AML and ATOR under the used working conditions.

After, two different commercial samples (tablets) containing AML and ATOR in a combined formulation were analyzed. The results of the analyses of all samples are summarized in Table 5, where the nominal content of antihypertensives and the data obtained by SWV and HPLC comparative method are presented. No significant difference was observed between the obtained values for the contents of AML and ATOR in the commercial pharmaceutical samples using the proposed method and the comparative one. Besides, considering that the paired t-test [40] was applied to these results and the calculated t values (1.66 (AML) and 2.00 (ATOR)) are smaller than the critical one (12.7, α = 0.05); it may conclude that the results obtained with either methods are not statistically different, at a 95 % confidence level.

Recovery experiments were carried out to evaluate matrix effects after standard solution additions yielding excellent recovery averages for both substances (101 ± 3 % for AML and 98 ± 2 % for ATOR), indicating that there was no important matrix interference for the samples analyzed by the proposed method.

Table 5. Results obtained in the simultaneous determination of AML and ATOR in combined dosage forms using the proposed method compared with HPLC.

Samples	Analyte	Amount (mg tablet^{-1})[a]			E (%)[b]
		Label	HPLC	SWV	
A	AML	5	5.1 ± 0.4	5.5 ± 0.2	7.8
	ATOR	10	10.3 ± 0.3	10.1 ± 0.1	–1.9
B	AML	10	10.3 ± 0.1	10.2 ± 0.2	–1.0
	ATOR	10	9.9 ± 0.2	10.5 ± 0.3	6.0

[a]Average of 3 measurements. [b]100 × (SWV method – comparative method / comparative method).

4. CONCLUSION

The obtained results showed that the anodically pretreated BDD electrode can be used in conjunction with a SWV technique for electrochemical behavior study and simultaneous determination of AML and ATOR. The anodic pretreatment of this electrode promoted the satisfactory separation and simultaneous determination of both analytes. Compared with GC and MWCNP:G electrodes, the BDD electrode used for the first time as sensor for the simultaneous determination of AML and ATOR, showed a better response presenting a lower LOD. Under optimized conditions, the anodically pretreated BDD electrode showed a wide linear range from 2.0 – 27.5 µmol L^{-1} for AML and 1.0 – 55.0 µmol L^{-1} for ATOR in BR buffer solution (pH 4.0), with lower LOD values of 0.028 and 0.38 µmol L^{-1}, respectively. Practical applicability of the proposed method was demonstrated on the determination of the AML and ATOR in commercial pharmaceutical samples, with satisfying results. Furthermore, the proposed method is simple, rapid, selective, sensitive, and inexpensive for the simultaneous determination of AML and ATOR in combined dosage forms.

5. ACKNOWLEDMENTS

The authors gratefully acknowledge financial support and scholarships from the Brazilian funding agencies CNPq (grant numbers 445841/2014-1, 303902/2015-9), CAPES and Fundação Araucária do Paraná. Special thanks to Prof. Dr. Ieda Spacino Scarmínio for her kind helps to chromatography measurements and to DIA laboratory for the availability of use of chromatograph.

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