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Simultaneous Determination of Valsartan and Amlodipine Besylate in Human Serum and Pharmaceutical Dosage Forms by Voltammetry

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Anodic behavior of binary mixture of amlodipine besylate (AMD) and valsartan (VAL) was studied on glassy carbon electrode based on the irreversible oxidation signal of AMD at 0.95 V and that of VAL at 1.15 V versus Ag/AgCl at pH 5.0 in Britton-Robinson buffer. Differential pulse voltammetric method was proposed to direct determination of AMD and VAL in pharmaceuticals and spiked human serum. Linearity for AMD was in the range from 1.0 µM to 35.0 µM and that for VAL was in the range from 1.5 µM to 32.0 µM when concentrations of AMD and VAL are increased simultaneously. Limit of detection and limit of quantification were found to be 0.31 µM and 1.03 µM, for AMD and 0.36 µM and 1.21 µM for VAL, respectively. The method was successfully applied with good recoveries between 90.8 % and 100.4 % with relative standard deviation less than 10 % for tablet samples and around 15 % for serum samples.

Keywords: Amlodipine besylate, valsartan, binary mixture, electrochemical behavior, simultaneous determination

1. INTRODUCTION

Hypertension is a leading risk factor for coronary heart disease, stroke, congestive heart failure, renal insufficiency, and peripheral vascular disease and still seems to be a major health problem in most countries [1]. Many classes of drugs such as thiazide diuretics, the ACE inhibitors, the calcium channel blockers, the beta blockers and the angiotensin II receptor antagonists are being used for treatment of hypertension [2,3].

Amlodipine besylate (AMD) chemically known as 3-ethyl-5-methyl (4R,S)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine 3,5-dicarboxylate

benzenesulphonate (Fig. 1a) is a calcium channel blocker inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle and is used in the treatment of hypertension and angina [4-7].

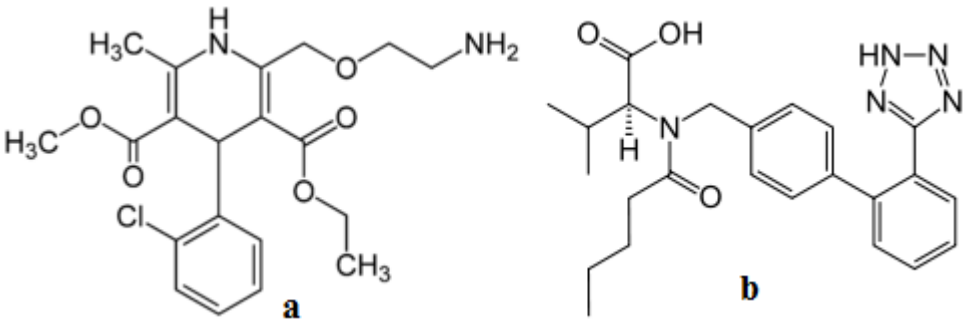

Figure 1. Chemical structure of (a) AMD (b) VAL

Various analytical techniques including HPLC [8, 9], LC-MS-MS [10], voltammetry [11-14], spectrophotometry [15-22], capillary electrophoresis [23], spectrofluorometry [24], and titrimetry [25] have been reported for the determination of AMD individually, in pure, pharmaceutical dosage forms and/or biological fluids.

Valsartan (VAL), (N-valeryl-N[[2'-(1H-tetrazole-5-yl)biphenyl-4-yl]methyl]valine) (Fig.1b) is a potent, highly selective and orally active antihypertensive drug belonging to the family of angiotensin II receptor antagonists acting at the ATI receptor which mediates all known effects of angiotensin II on the cardiovascular system. By blocking the action of angiotensin II receptors, VAL dilates blood vessels and causes reduction in blood pressure. It is also available in combination with other antihypertensive drugs.

Different analytical methods such as HPLC [26-31], reverse phase ultra-performance liquid chromatographic (RP-UPLC) [32], LC-MS/MS [33], spectrophotometry [26, 34, 35], voltammetry [4, 36], and potentiometry [37], have been reported for the individual assay of VAL.

A new combination dosage form of AMD and VAL is purposed for the treatment of hypertension to patients whose blood pressure is not adequately controlled on either component mono therapy.

Fast and reliable simultaneous determination of AMD or VAL in biological fluids and pharmaceutical dosage forms is required due to the therapeutic importance. There are a few methods including spectroflourimetry [38], potentiometry [39], thin-layer chromatography [40], HPLC [40-42], and capillary electrophoresis [43] reported for the determination of the assay of this combination. However, these methods are usually laborious, expensive, time-consuming and complex to be operated. Therefore, a fast, simple, low cost, accurate, precise and sensitive method is very important especially for routine simultaneous determination of pharmaceuticals containing both AMD and VAL.

Electroanalytical techniques such as cyclic voltammetry (CV), linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), square-wave voltammetry (SWV), bulk electrolysis (BE) have many advantages, in mechanistic studies and pharmaceutical analysis due to instrumental

simplicity, short analysis time, low cost, high sensitivity, portability and they have been used for determination of a wide range of pharmaceuticals [44-50]. Since VAL and AMD are electrochemically active, several methods have been reported for the individual analysis of them and to our present knowledge, no voltammetric method is reported for the simultaneous determination of these substances in dosage forms. This study reports for the first simultaneous determination of AMD and VAL by voltammetry in human serum and in pharmaceutical dosage forms.

2. EXPERIMENTAL SECTION

2.1. Reagents and solutions

Voltammetric measurements such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were carried out using a CH-instrument electrochemical analyzer (CHI 760). A three electrode cell system incorporating the glassy carbon electrode (GCE BAS MF 2012) as working electrode, platinum wire as an auxiliary electrode (BAS MW 1034) and an Ag/AgCl reference electrode (MF 2052) were used in all experiments whereas pH measurements were made with Thermo Orion Model 720A pH ion meter by using combined Orion glass pH electrode (912600) and double-distilled deionized water was supplied from Elga, Ultra-Pure Water System in which all the experiments were performed at room temperature.

Stock solutions of AMD and VAL were prepared daily by dissolving known amounts of AMD and VAL (both supplied from Novartis, Drug Company in Turkey) in ethanol. Calibration solutions were prepared by diluting the stock solutions with Britton–Robinson buffer (BR) and pH value of these solutions were adjusted by using 0.1 M NaOH solution. Ultra-pure deionized water was used in preparations of all the solutions.

2.2. Preparation and analysis of samples

Exforge tablets (product of Novartis, from local pharmacy in Ankara, Turkey) were used as pharmaceutical dosage form which contains 160 mg of VAL and 6.94 g salt of AMD which is equivalent for 5 mg AMD per tablet. Tablet solutions and human serum samples were prepared and analyzed in the same manner given in our early studies [44-47].

2.3. Voltammetric procedure

In all voltammetric studies (CV, DPV, and SWV) 10.0 mL of mixture solution (i.e. AMD and VAL) in BR was placed into the electrochemical cell. After adjusting of electrode connections cell content was deoxygenated with purified argon (99.99 % purity) for 30 min before the first running and 30s between all individual successful runnings. After 5s equilibration time voltammograms were recorded by applying a positive-going potential scan.

3. RESULTS AND DISCUSSION

3.1. Anodic behavior of AMD-VAL mixture

Anodic behavior of AMD and VAL mixture was investigated by using CV and SWV. As could be seen in Fig.2 AMD-VAL mixture exhibited two distinct oxidation peaks at 0.95 V and 1.15 V (vs. Ag/AgCl). When the concentration of AMD is increased while the concentration of VAL held constant increasing in the current of peak at 0.95 V was recognized. When VAL concentration was increased by keeping the concentration of AMD constant increasing for the current of peak located at 1.15 V was noticed. As a result oxidation peak at 0.95 V might belong to the oxidation of AMD and oxidation peak at 1.15 V might belong to the oxidation of VAL. Both oxidation peaks have no reduction peak at reverse scan indicating the irreversibility of the oxidation processes for AMD and VAL.

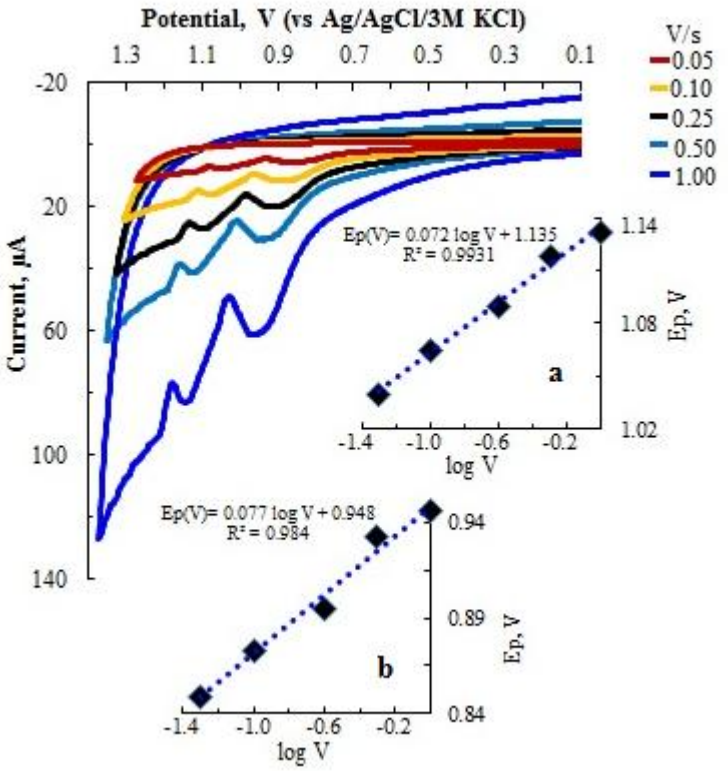

Figure 2. Effect of scan rate on peak parameters in BR buffer of pH 5.0, C_{AMD} = 0.2 mM, C_{VAL} = 0.25 mM (inset: graph of peak potential vs. logarithm of scan rate for (a) VAL, (b) AMD)

More detailed studies were carried out about characteristic of the oxidation of mixture. First of all, effect of scan rate on peak potential and peak current for both species in mixture were studied. As could be seen in Fig.2, peak potential shifts to more anodic values with increasing scan rate confirming and supporting the irreversible characteristic of oxidations. For this kind of mechanism, the relationship between the peak potential (E_p) and logarithm of scan rate (ln V) is expressed as follows [46-48]:

$$E_p = k + \frac{RT}{(n\beta)F}\ln V \qquad (3.1)$$

Here, E_p is peak potential in V, R is ideal gas constant, T is absolute temperature, F is Faraday's constant, n is number of electrons transferred in mechanism, β is anodic charge transfer coefficient, and V is scan rate in Vs^{-1}.

Straight lines were observed when E_p was plotted against log V for the both component of the mixture at a particular concentration of both and at pH 5.0. As could be seen in Fig.2 insets (a and b), these lines could be expressed as: E_p (V) = 0.072 log V + 1.135 with R^2 = 0.9931 for VAL and E_p (V) = 0.077 log V + 0.948 with R^2 = 0.984 for AMD. Using the slope values of these lines and eq.3.1, the βn value was calculated as 0.36 for AMD and 0.33 for VAL.

Effect of frequency (f) in SWV like effect of scan rate in CV on peak parameters was also investigated. As could be seen in Fig.3, oxidation potentials of both AMD and VAL shift to more positive (more anodic) values with increasing frequency (parallel to scan rate). In such studies, peak potentials for AMD and VAL were found to change linearly with logarithm of frequency (Fig.3 inset).

Using the slope values of E_p vs. log f and modified version of eq.3.1, the βn value was calculated as 0.33 for AMD and 0.27 for VAL.

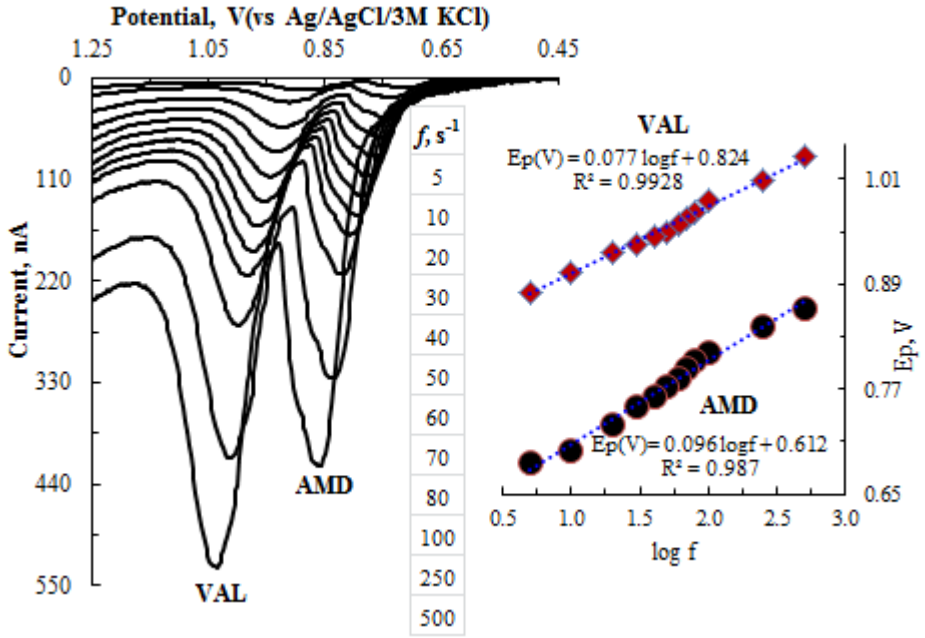

Figure 3. Effect of frequency on peak parameters of AMD and VAL in BR buffer of pH 5.0, C_{AMD} = 5.0 µM, C_{VAL} = 6.0 µM (inset: graphs of peak potential vs. logarithm of frequency for both AMD and VAL)

In order to confirm these values for βn, same parameter (βn) was calculated from Eq.3.2 which express the difference between peak potential and half-peak potential [51, 52], in cyclic voltammograms and it was calculated to be 0.38 for AMD and 0.30 for VAL.

$$| E_p - E_{p,h} | = \frac{1.857RT}{n\beta F}, \text{and at } 25°C \; | E_p - E_{p,h} | = \frac{47.7}{n\beta} \qquad (3.2)$$

In this equation, $E_{p,h}$ is half peak potential in V and other abbreviations have the same meaning as in Eq.3.1.

Effects of scan rate on peak current for both peak were also studied. As scan rate increased from 0.05 Vs^{-1} to 1.00 Vs^{-1} at fixed concentration of AMD and VAL, peak current for both AMD and VAL changed linearly with scan rate indicating the effect of adsorption on electrode mechanism [49-52]. Logarithm of peak current changed linearly with the logarithm of scan rate for both AMD and VAL and slope value for this linear line is 0.76 for AMD and 0.72 for VAL. These slopes are in the midway between 0.5 and 1.0 for ideal diffusion controlled and adsorption-controlled charge transfer mechanism, respectively [44-47]. Relation between peak current and square root of scan rate is linear for AMD and it is not linear for VAL indicating the efficiency of diffusion to electrode mechanism for AMD and inefficient effect for VAL oxidation on GCE.

The following equation for diffusion coefficient which expresses adsorption phenomena validated by Garrido [53], was used to calculate the diffusion coefficient of AMD and VAL:

$$i_p = 1.06 \times 10^6 n^2 A C \vartheta \sqrt{D t_p} \qquad (3.3)$$

Where, A is the area of electrode surface in cm^2, C is the analytical concentration of diffuses species in $molcm^{-3}$, D is diffusion coefficient in cm^2s^{-1}, t_p is time required to reach peak potential from the beginning of potential scan, and others are known from early equations. The mean of the diffusion coefficient calculated from this equation was obtained as $(4.2 \pm 0.09) \times 10^{-8}$ cm^2s^{-1} for AMD and $(1.1 \pm 0.02) \times 10^{-8}$ cm^2s^{-1} for VAL. All these results pointed out the co-contribution of adsorption and diffusion on electrode mechanism.

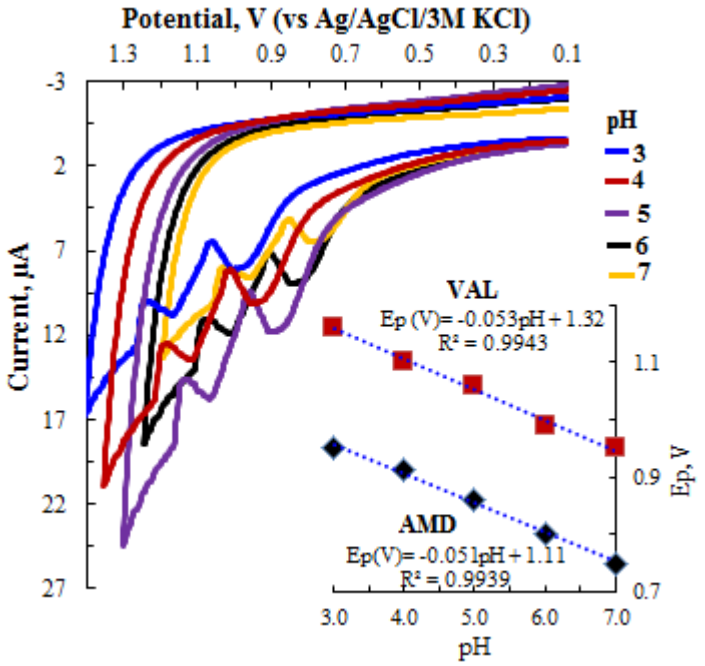

Figure 4. Effect of pH on peak parameters in BR buffer, scan rate 0.100 Vs^{-1}; C_{AMD} = 0.2 mM, C_{VAL} =0.25 mM (inset: graph of peak potential vs. pH for AMD and VAL)

Effect of pH on peak parameters was also analyzed. As shown in Fig.4, peak potentials for both AMD and VAL shift to smaller values (less anodic values) with increasing pH from 3.0 to 7.0, and peaks were distorted and less resolved at higher pH values after 7.0 hence at higher pH was not studied.

Shifting in peak potentials with pH was evaluated as existence of protons in charge transfer mechanisms. As could be seen in inset graph in Fig.4, linear relation between peak potential and pH for AMD could be expressed as: E_p (V) = -0.051 pH + 1.11 (with R^2 = 0.9939) and for VAL: E_p (V) = -0.053 pH + 1.32 (with R^2 = 0.9943). When the slopes of these relations were evaluated in Eq.3.4, [49] the ratio of proton to electron participated in mechanism was calculated as to be 0.86 for AMD and 0.89 for VAL.

$$E_p = E^o - \frac{RT}{nF}\ln\frac{[\mathrm{Ox}]}{[\mathrm{Red}]} \pm \frac{2.303\partial RT}{nF}\mathrm{pH} \qquad (3.4)$$

Here, $E°$ is standard peak potential in V; [Ox] and [Red] are equilibrium concentrations of oxidized and reduced species, respectively, and ∂ is number of proton participated in mechanism and others are common abbreviations.

Shifting in peak potential to less anodic potential with increasing pH (oxidation is easier at higher pH in studied pH range) may be concluded as the deprotonation step before electron transfer step.

Although the exact electrode mechanism was not aimed and determined, some conclusions about the electroactive centers under the working conditions could be predicted. Taking into consideration all the experimental studies, it could be thought that the electrode reaction is the oxidation of tetrazole moiety in VAL molecule and oxidation of amino (NH_2) moiety in the AMD molecule.

3.2. Simultaneous voltammetric determination of AMD and VAL

3.2.1. Optimization of experimental conditions

Different kind of electroanalytical method such as LSV, DPV and SWV were examined for quantitative determination and compared with other electrochemical techniques, DPV gave the highest oxidation peak couple for AMD and VAL. The oxidation peaks in DPV was at least 2 times higher than the corresponding peaks of other methods, DPV has lower residual current, and DPV has more symmetric and resolved peaks for AMD and VAL. DPV was therefore used to optimize a rapid and sensitive electroanalytical procedure for simultaneous determination of AMD and VAL.

Voltammetric response depends markedly on instrumental and experimental variables. To obtain a much more sensitive peak current, the optimum instrumental conditions, such as, pulse amplitude, E_a, scan increment, E_s, pulse period, t_p, were studied for 10.0 µM AMD and VAL solution. Peak current change nearly linearly with increasing E_s between 1-10 mV but higher E_s than 5 mV residual current also increases and peak shape was distorted. When E_a was varied in the range 5–100 mV, the peak current increased linearly with increasing E_a. When E_a was greater than 65 mV the peak

width increased at the same time. Hence, the best peak definition was recorded when using 65 mV pulse amplitude, 5 mV scan increment and 50 ms for pulse period.

3.2.2. Validation of proposed methods

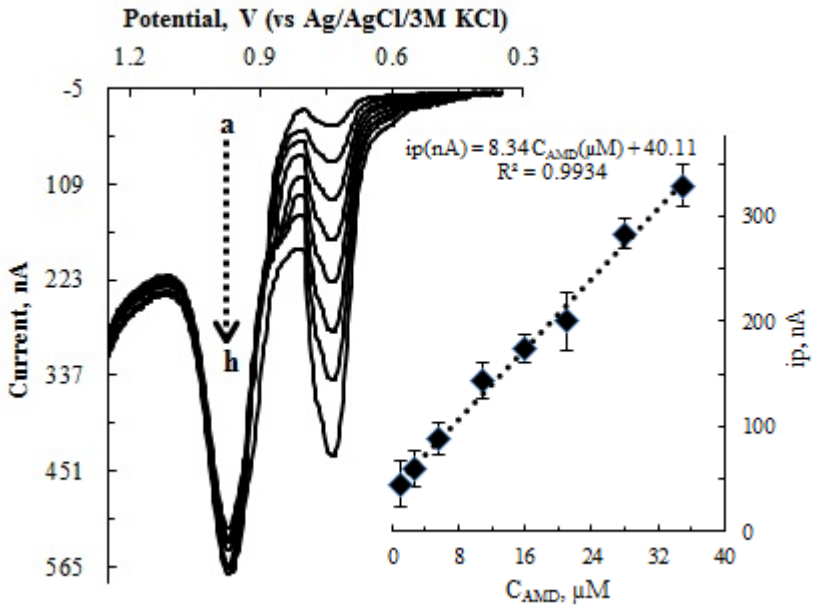

Figure 5. Calibration voltammograms and calibration graph for AMD with fixed concentration of VAL at 32.0 µM in BR buffer of pH 5.0. Concentrations of AMD (a=1.0, b=2.8, c=5.5, d=11.0, e=16.0, f=21.0, g=28.0 and h=35.0 µM)

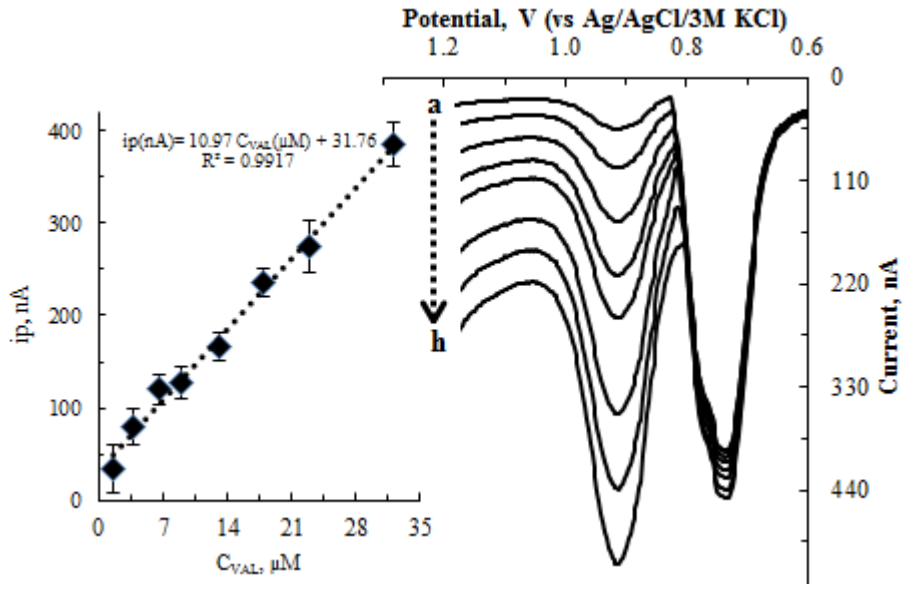

Figure 6. Calibration voltammograms and calibration graph for VAL with fixed concentration of AMD at 35.0 µM in BR buffer of pH 5.0. Concentrations of VAL (a=1.5, b=3.7, c=6.5, d=9.0, e=13.0, f=18.0, g=23.0 and h=32.0 µM)

When all variables that could affect the performance of proposed method optimized, variation of the peak current with the bulk drugs concentration was investigated by recording voltammograms using proposed method for serial solutions of (*i*) constant VAL concentration with increasing AMD concentration (Fig.5) (*ii*) constant AMD concentration with increasing VAL concentration (Fig.6) and (*iii*) simultaneous increasing of the concentration of both AMD and VAL (Fig.7)

In Fig.5 the response of the oxidation peak for AMD was linear in the concentration range from 1.0 μM to 35.0 μM for AMD by obeying the calibration equation: i_p(nA) = 8.34 C_{AMD}(μM) + 40.11 (R^2 = 0.9934) when concentration of VAL is held constant at 32.0 μM.

As could be seen in Fig.6, the response of the oxidation peak for VAL was linear in the concentration range from 1.5 μM to 32.0 μM for VAL by obeying the calibration equation: i_p(nA) = 10.97 C_{VAL}(μM) + 31.76 (R^2 = 0.9917) when concentration of AMD is held constant at 35.0 μM.

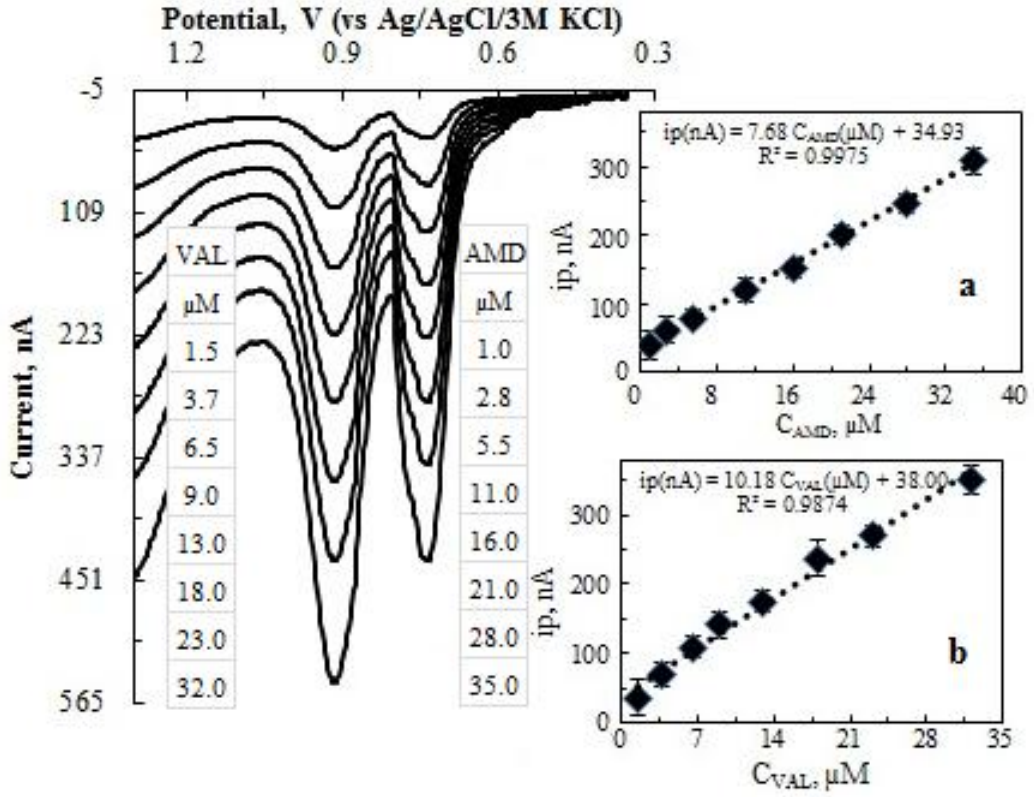

Figure 7. Calibration voltammograms and calibration graph for (a) AMD and (b) VAL with simultaneous increase in their concentration in BR buffer of pH 5.0.

Calibration characteristic for the mixture in which concentration of both AMD and VAL is increased simultaneously is depicted in Fig.7. As could be seen from Fig.7 the response of the oxidation peak for AMD was linear in the concentration range 1.0 μM to 35.0 μM for AMD by obeying the calibration equation: i_p(nA) = 7.68 C_{AMD}(μM) + 34.93 (R^2 = 0.9975) (inset a) and the response of the oxidation peak for VAL was linear in the concentration range 1.5 μM to 32.0 μM for

VAL by obeying the calibration equation: i_p(nA) = 10.18 C_{VAL}(μM) + 38.00 (R^2 = 0.9874) (inset b). Calibration characteristics and the related validation data are given in Table 1.

Table 1. Calibration parameters and validation data for AMD and VAL at the same solution

Parameter	AMD	VAL
Linearity range, μM	1.00 – 35.00	1.50 – 32.00
Slope of calibration curve, ALmol^{-1},(m)	0.0077	0.0102
Intercept, nA, (b)	34.9	38.0
Standard deviation (SD) of regression, nA,(s_r)	2.75	8.82
SD of slope, mALmol^{-1} (s_m)	0.07	0.05
SD of intercept, nA, (s_b)	0.77	1.22
Limit of Detection (LOD), μM	0.31	0.36
Limit of Quantification (LOQ), μM	1.03	1.21
Regression coefficient, R^2	0.9975	0.9874

Limits of detection and quantification of procedure are also shown in Table 1; they were calculated from the calibration plots using the equations: LOD=3s_b/m and LOQ=10s_b/m (where s_b is the standard deviation of the intercept and m is the slope of the calibration plot) [47].

The precision of the proposed method was determined by performing five replicate measurements of 5.0 and 10.0 μM for AMD and of 3.0 and 20.0 μM for VAL in solutions. Relative standard deviation ranged from 4.23 % to 5.68 % for peak current in intra-day measurements and ranged from 8.97 % to 12.47 % in inter-day measurements. Same parameter for peak potential was evaluated as ranged from 1.87 % to 3.98 % for intra-day measurements and ranged from 2.96 % to 5.83 % for inter-day measurements.

3.2.3. Determination of AMD and VAL from pharmaceuticals and biological samples

To check the applicability of the proposed method, a commercial tablet formulation containing both AMD and VAL was analyzed. The AMD and VAL content of commercially available tablets was determined by estimating recoveries at five different concentrations using the corresponding calibration equation. Recoveries of the drug from this type of matrix ranged from 96.24 % to 100.35 % in proposed method with relative standard deviations less than 9.0 % (Table 2.).

Table 2. Result of tablet analysis by proposed method

Sample	Tablet value, mg	Found, mg	Recovery, %*	RSD, %
AMD	5.0	4.32, 4.51, 4.76, 5.15, 5.32	96.24	8.74
VAL	160.0	152.56, 155.65, 158.68, 163.25, 172.65	100.35	4.87

*average value of recovery values of found amounts ± ts/√N, at 95 % confidence level

To control the applicability of the proposed methods for biological samples, spiked human serum samples were used. In such applications, known volume of mixture of standard solutions of AMD-VAL were spiked to human serum samples in order to have AMD and VAL concentration in linear range, then voltammetric measurements were performed and recovery values of proposed methods were calculated by using related calibration parameters at five different concentrations in three groups using the corresponding calibration equation. Recoveries of the drug from this type of matrix ranged from 90.79 % to 96.93 % with RSD values around 15 %. To discover whether any possible organic and inorganic species in serum interfered with the analysis, voltammetric base line of biological samples were measured and no extra voltammetric signals in the peak potential ranges of AMD and VAL was recognized and mean recovery values (Table 3.) for such samples are good enough indicating the absence of any interfering effect of biological samples.

Table 3. Results of proposed method to spiked human serum

Sample	Spiked, µg	Found, µg	Recovery, % *	RSD,%
AMD	15	12.02, 12.11, 12.83, 15.00, 16.07	90.79	13.43
VAL	32	25.88, 27.82, 28.40, 33.91, 36.86	95.55	15.07
AMD	25	19.90, 21.00, 21.58, 26.78, 27.89	93.72	15.51
VAL	50	42.96, 44.19, 46.43, 54.26, 54.49	96.93	11.42
AMD	40	32.16, 34.60, 35.39, 42.27, 44.99	94.70	14.43
VAL	80	66.15, 70.94, 71.45, 83.26, 91.24	95.76	13.48

*average value of recovery values of found amounts ± ts/√N, at 95 % confidence level

4. CONCLUSION

Electrochemical behavior of the mixture of AMD and VAL, antihypertensive drugs, on GCE was investigated. Results of electrochemical studies may be used in investigating the adsorption, distribution, and many other pharmacokinetic and physicochemical parameters of biologically and technological important molecule under investigation. It also could be significant to investigate further studies regarding its side effect, target, related organs, form and way of excreting of drug molecules.

A high percentage of recovery and low RSD values indicate that the proposed method could be used to quantify AMD and VAL simultaneously without interference from other ingredients. Furthermore, proposed voltammetric method has distinct advantageous over other existing methods regarding sensitivity, minimum detectability, applicability to biological samples without any pretreatment and time saving. Moreover, no sophisticated instrumentation is required. Consequently, proposed voltammetric methods have the potential for being a good alternative for simultaneous determination AMD and VAL.

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