SIMULTANEOUS DETERMINATION OF L-DOPA AND BENSERAZIDE IN BINARY MIXTURES USING FIRST DERIVATIVE OF THE RATIO-VOLTAMMETRIC METHODS BASED ON THEIR OXIDATION ON SOLID ELECTRODE

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Received June 29, 2011
Accepted July 18, 2011
Published online January 11, 2012

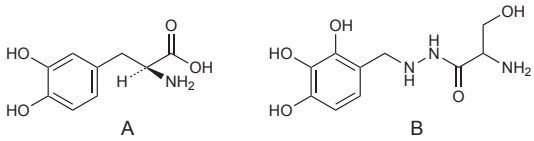
The electrochemical behavior of L-dopa and benserazide at a solid electrode has been investigated using different voltammetric techniques. The anodic voltammetric characteristics of the selected compounds have been studied in aqueous media as a function of pH by cyclic, differential pulse and square wave voltammetric techniques. First derivative of the ratio voltammetric methods for determination of these compounds in their dosage forms in the presence of each other has been described. This technique depends on the measuring of the first derivative of ratio voltammograms of each concentration as a function of the increased concentrations. DP and SW voltammetric methods depend on the measuring of first derivative of the ratio voltammetry by measurements of the selected potentials corresponding to either maximums or minimums for both drugs. The linear response was within the range of 1×10^{-5}–8×10^{-5} and 1×10^{-5}–2×10^{-4} M for L-dopa and benserazide, respectively. The validity of the proposed methods was successfully assessed for analyses of both drugs in laboratory-prepared mixtures and in commercial tablet formulations. The proposed methods have successfully applied to the simultaneous determination of these compounds in the presence of each other.

Keywords: L-Dopa; Benserazide; Ratio derivative; Voltammetry; Glassy carbon electrode; Pharmaceuticals.

Parkinson's disease is a progressive, neurodegenerative disorder of the extra-pyramidal nervous system affecting the mobility and control of the skeletal muscular system. Its characteristic features include resting tremor, rigidity, and bradykinetic movements. L-Dopa (LD), a naturally occurring amino acid, is the immediate precursor of the neurotransmitter dopamine. It is chemically described as L-3,4-dihydroxyphenylalanine (Scheme 1). In the situation of alone usage of LD, so rapidly decarboxylation occurs so

doi:10.1135/cccc2011136

that very little unchanged drug is available to cross the blood–brain barrier for central conversion into dopamine[1–3]. Consequently, LD is usually given together with a peripheral decarboxylase inhibitor such as Benserazide (BEN) thus permitting a considerably higher proportion of LD to enter the brain. The dosage forms of LD combine with BEN, which improves the action of LD and reduces some of its side effects, particularly nausea. BEN is described chemically as 2-amino-3-hydroxy-*N*′-(2,3,4-trihydroxybenzyl) propanehydrazide (Scheme 1). It is an inhibitor of the decarboxylation of peripheral LD to dopamine, having actions similar to those of carbidopa.



SCHEME 1
The chemical structures of LD (A) and BEN (B)

Several techniques have been reported in the literature for the simultaneous determination of LD and BEN in pharmaceutical dosage forms. Different methods appeared in the literature for the simultaneous determination of LD and BEN in their binary mixtures based on capillary electrophoresis[4–7], LC-MS/MS [8], different spectrophotometric techniques such as derivative, ratio derivative[9–11] chemometric methods[12–14] etc., FIA with chemometric methods[15,16], FIA with spectral analyze[17] and electrochemical methods with chemometric calculations[18,19] and sequential voltammetric determination using chloranil as a mediator[20]. Most of the reported methods required time-consuming preparations, extraction steps or equations and calculation steps, which are not economically feasible for routine use[12–16,18,19]. To our knowledge, there is no official method for the simultaneous determination of these drugs in any pharmacopoeia.

One of the classic analytical problems of multi-component analysis is that the analyte of interest is often accompanied by other compounds effecting in the same working region. LD and BEN have similar chemical structures and thus, their physical and chemical properties are also similar. Consequently, their voltammograms seriously overlap, and it is difficult to determine the drugs individually from their mixture without a preseparation. Hence, direct voltammetric determination methods are not effective in BEN and LD binary mixtures because they are subjected to interferences by each other. In old strategies, for resolving overlapping

voltammograms were to use chemical reagents for extracting or complexing one component in the mixture, in order to shift or to dispose of it further apart the peak potentials or to mask one component completely. Also, these problems can very often be overcome by using various chemical procedures such as altering the pH of supporting electrolyte, which shift one signal relative to the other, suppress one of them or make the other sharper. This kind of process usually involves time-consuming manipulation of the sample in order to obtain a specific and selective signal.

Electrochemical techniques offer high sensitivity, selectivity and require no large sample volumes. In recent years, voltammetric determination of mixtures has been assessed using different techniques such as derivative, ratio derivative and chemometric methods. Ratio derivative spectrophotometric method for resolving binary mixtures was developed by Salinas et al.[21]. The absorption spectrum of the mixture is obtained and divided (amplitudes at each wavelength) by the absorption spectrum of a standard solution of one of the components, and first derivative of the ratio spectrum is obtained[21–26]. Ratio derivative method[21–23] involves calculating and plotting one of the mathematical derivatives of a curve, which offers an alternative approach to drug analysis. Hence, we have applied the theory of this method to our overlapping differential pulse and square wave voltammograms for removing these binary mixture interferences on their voltammograms. First derivative of the ratio-voltammetric methods have proved advantageous in eliminating voltammetric interferences. In order to resolve complex voltammograms and to avoid time-consuming separation procedures, we attempted for simultaneous determinations using ratio derivative voltammetric techniques. This method permits the determination of a component in their binary mixture at the potentials corresponding to a maximum or minimum and also the use of the peak-to peak between consecutive maximum and minimum. For applying this method, we calculated all necessary equations and parameters using Microsoft Excel® Programme.

To the best of our knowledge, no simple simultaneous determination method like first derivative of the ratio voltammetry has been performed on LD and BEN in their binary mixtures using glassy carbon electrode, either in bulk form or in pharmaceuticals. Also, there have been no studies published related to the electrochemical oxidation details on the glassy carbon electrode of these compounds in the presence of each other. That is why, we aimed to study the voltammetric behavior of LD and BEN and their simultaneous determination at a glassy carbon electrode using different electrochemical techniques. This study described a fully validated, simple, rapid and more sensitive procedure for the simultaneous determination

of LD and BEN in binary mixtures and in their pharmaceutical form employing first derivative of the ratio-differential pulse and first derivative of the ratio-square wave voltammetric methods at the glassy carbon electrode. As an advantage, the determination procedure did not require sample pre-treatment or any time-consuming extraction step prior to the drug assay. As a comparison method, first derivative of the ratio spectrophotometric method[10] has been used for the determination of both drugs in the presence of each other.

EXPERIMENTAL

Instrumentation

All voltammetric measurements were performed using a BAS 100 W (Bioanalytical System Inc., USA) electrochemical analyzer. A glassy carbon (GC) working electrode (BAS; Φ: 3 mm diameter), an Ag|AgCl reference electrode (BAS; 3 M KCl) and platinum wire counter electrode (BAS) and a standard one-compartment three-electrode cell of 10 ml capacity were used in all experiments. The working electrode was polished manually with aqueous slurry of alumina powder (Φ: 0.01 μm) on a damp smooth polishing cloth (BAS velvet polishing pad), before each measurement. All measurements were realized at room temperature. DPV conditions were: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate, 20 mV/s and SWV conditions were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step, 4 mV. All measurements were carried out at ambient temperature of the laboratory (23–27 °C).

The pH value of the solutions was measured by Model 538 pH meter (WTW, Austria) using a combined electrode (glass electrode-reference electrode) with an accuracy of ±0.05 pH and calibrated with standard buffers (FIXANAL, Riedel–de Haen, Germany).

Ratio derivative spectrophotometric studies[10] were used as the comparison method. A Shimadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) coupled an IBM-PC computer running spectrophotometric software Shimadzu UVPC software was used.

Reagents and Solutions

LD and BEN and their dosage form were kindly provided by Deva Pharm. Ind. (Istanbul, Turkey). Each film-coated tablet (Madopar®) was contained 100 mg LD and 25 mg BEN and the inactive ingredients. All chemicals were of reagent grade quality (Merck, Sigma or Riedel) and they were employed without further purification.

The standard stock solutions of LD and BEN (1×10^{-3} M) were prepared daily by direct dissolution in water and kept in the dark in refrigerator. Working solutions under voltammetric investigation were prepared by dilution of the stock solution with the selected supporting electrolyte. 0.2 M phosphate buffer at pH 1.6–8.0, 0.04 M Britton–Robinson buffer at pH 2.00–12.0 and 0.2 M acetate buffer at pH 3.65–5.75 were used for supporting electrolyte.

Procedure

Standard solutions were prepared by dilution of the stock solution with selected supporting electrolyte to give solutions containing LD in the concentration range from 1×10^{-5} to 8×10^{-5} M and BEN in the concentration range from 1×10^{-5} to 2×10^{-4} M.

The DP and SW voltammograms of the separate and binary mixtures prepared at different concentrations of LD were recorded and stored in the computer. According to the theory of the ratio derivative method[21–26], the stored voltammograms of the mixtures was divided, potential-by-potential, by a standard DP or SW voltammograms of BEN solution (6×10^{-5} M in Britton–Robinson buffer at pH 5.0) using Microsoft Excel® Programme. Then, the first derivative of the ratio voltammograms were calculated and drawn. The values of these first derivative peaks were measured at suitably selected potentials in the range of 0–500 mV for DPV, 200–550 mV for SWV and plotting against the corresponding concentration to obtain the calibration graph.

The similar procedure was followed for the different concentrations of BEN when LD 8×10^{-5} M in Britton–Robinson buffer at pH 5.0 used as a divisor. The stored voltammograms of the mixtures were divided, potential-by-potential, by a standard DP or SW voltammograms of LD solution (8×10^{-5} M in Britton–Robinson buffer at pH 5.0) using Microsoft Excel® Programme. Then, the first derivative of the ratio voltammograms were calculated and drawn. The calibration curve was obtained by plotting the drug concentration against the signal in the first derivative of ratio voltammogram between 0 and 500 mV for DPV, 200 and 550 mV for SWV.

Validation of the Methods

In method validation, the quantitative characteristics of interest relate to the accuracy and precision of the result likely to be supplied. The precision was checked in the same day (n = 5) and three different days (n = 5) over a week period. Relative standard deviations were calculated to check the precision of the method[27–30]. The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (Bias %). One example of relative error is the accuracy, which describes the deviation from the expected results[27–30]. All solutions were kept in the dark and were used within 24 h to avoid decomposition. However, voltammograms of the sample solutions recorded a week after preparation did not show any appreciable change in assay values.

Pharmaceutical Dosage Forms Assay Procedure

For all methods, ten tablets labeled to contain 100.0 mg LD and 25 mg BEN and excipients were accurately weighed and finely powdered by pestle in a mortar. An adequate amount of this powder, corresponding to one tablet content was accurately weighed, transferred into a 50-ml calibrated flask and completed to the volume with water. The content of the flask were sonicated for 10 min for complete dissolution. The sample from the clear supernatant liquor was withdrawn and quantitatively diluted with the selected supporting electrolyte.

This solution was then transferred to a voltammetric cell and DP and SW voltammograms were recorded. For the calculation of compounds amount in pharmaceutical dosage form, the same procedure was used as described in section 2.3. LD and BEN contents in each tablet were determined referring to the related regression equations.

Recovery Studies from Tablets

If no matrix blank is available, standard addition technique should be chosen for the accuracy test. To study the accuracy, reproducibility and to check the interference from the excipients used in the formulations of these techniques, recovery experiments were carried out using the standard addition method. Because other components of the tablet dosage forms may interfere with the analysis or accurate quantitation of the analyte, potential effects from matrix components must be investigated. Recovery experiments are performed in the presence of the matrix[27–30]. In this method, the known amounts of LD and BEN are spiked into a sample matrix that already contains some amount of the investigated compound. The difference between the spiked and unspiked sample results is the recovered part of the added analyte, which can be compared with the known amount added.

The mixtures were calculated using the same way described in Procedure by both proposed techniques. The recovery results obtained after five repeated experiments for both techniques.

RESULTS AND DISCUSSION

Optimization of Voltammetric Responses of LD and BEN

Voltammetric detection is a promising means of determining electroactive drug active compounds in pharmaceutical formulations[31–33]. Both LD and BEN contain electroactive phenolic hydroxyl groups on their rings, both analytes are electroactive, which makes them suitable for electrochemical detection.

LD and BEN were electrochemically oxidized in a broad pH range using a carbon based electrode such as GC, producing one or more complex signals at the anodic potentials depending on pH and buffer type. Therefore, several measurements with different electrochemical techniques were performed using various supporting electrolytes, buffers and pH values in order to obtain such information. The cyclic, linear sweep, DPV and SWV voltammetric behavior of 1×10^{-4} M LD and 1×10^{-4} M BEN were examined individually with varying pH over a wide range of values from acidic (pH 1.6) to alkaline (pH 12.00) in acid solutions and different buffer aqueous media. As a first step, LD and BEN were subjected to CV and LSV studies with the aim of the detailed characterizing its electrochemical oxidation behavior, on GC electrode and to rapid, sensitive, selective and validated simultaneous voltammetric studies in DPV and SWV modes.

CV and LSV curves showed that LD exhibited one distinct, well defined anodic peak and/or an ill defined wave at different potential values in different supporting electrolyte compositions and at all pH values between pH 1.6 and 12.00 (Fig. 1a–1d). However, using all voltammetric techniques,

BEN showed similar behavior with LD because of its similar chemical structure and oxidizable groups nearly in all investigated pH values. CV measurements of LD showed a reversible or quasi-reversible nature of the oxidation process depending on buffer type and pH values (Fig. 1a–1d). The scanning was started at 0.0 V in the positive direction. The main anodic oxidation of LD occurred at about +0.50 V in acidic media on GC electrode (Fig. 1a). By reversing at +1.60 V, the reduction signal corresponding to the anodic response was observed at about +0.35 V on the cathodic branch in acidic media.

Voltammograms of BEN showed one distinct and well defined anodic peak and one additional ill-defined anodic wave at different potentials depending on pH values and supporting electrolyte compositions (Fig. 1a–1d).

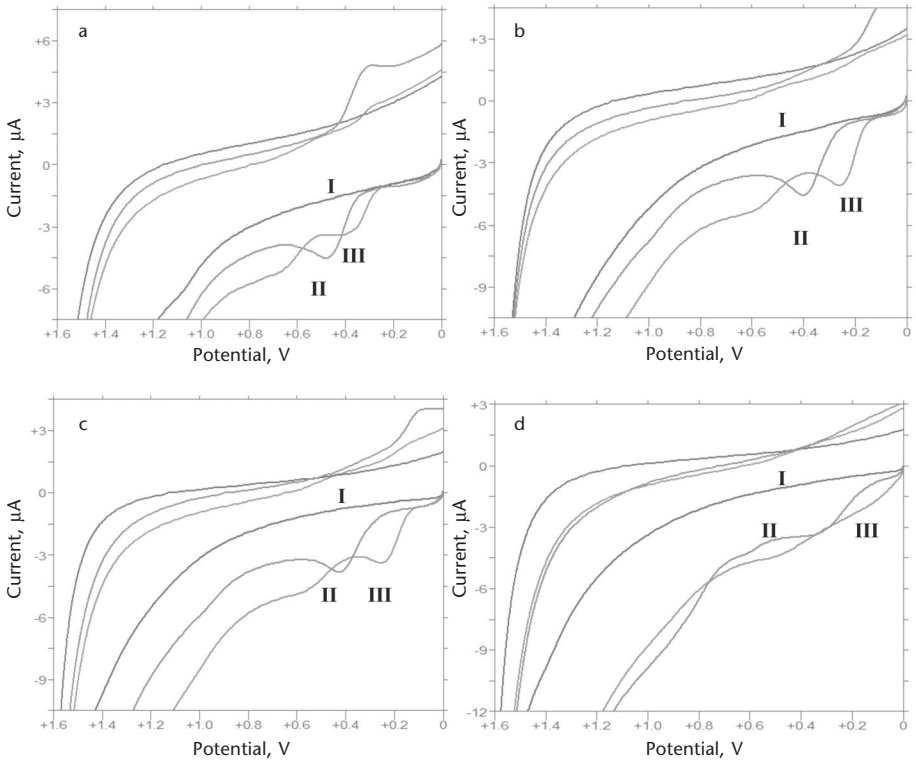


FIG. 1
Cyclic voltammograms obtained for a glassy carbon electrode (a) in pH 2.75 phosphate buffer, (b) in pH 4.64 acetate buffer, (c) in pH 5.00 Britton–Robinson buffer, (d) in pH 7.00 Britton–Robinson buffer (I), containing 1×10^{-4} M BEN (II), 1×10^{-4} M LD (III). Scan rate 100 mV/s

After pH 6.0, only an ill-defined wave was observed in all supporting electrolytes (Fig. 1d). Figure 1 shows a well-defined oxidation peak and an ill-defined wave which are observed at about +0.35 and +0.70 V for BEN, respectively, with GC electrode using by CV and LSV techniques. By reversing at +1.60 V, no reduction signal corresponding to the second anodic response which are obtained as second wave, was observed. However, an ill-defined reduction wave was obtained at about +0.25 V on the cathodic branch in acidic media corresponding to the first peak of BEN (Fig. 1b).

The electrochemical oxidation of LD and BEN in Britton–Robinson buffer at pH 5.0 was explored next. As shown in Fig. 1c, GC electrode exhibits an extensive oxidation wave at about +0.40 V for LD and +0.30 V for BEN. A considerable overlap of the peaks is clearly evident in Fig. 1 in the studied pHs and media. According to Fig. 1, their binary mixtures were affected between each compound current. Determination studies were realized using Britton–Robinson buffer at pH 5.0 since these conditions provided minimal interaction between compounds. Also the obtained peaks from both compounds were not overlapped in this pH value. Hence, the first derivative of the ratio method can be applied.

The responses of pH on the voltammetric waves were explored for both compounds. Over the pH range of 1.60 to 9.0, a linear variation of peak potentials (E_p) and peak currents (I_p) with pH were observed for BEN and LD, as shown in Fig. 2a, 2b and 2c, 2d, respectively.

For the DPV response in all working media, the relationship between the peak potential and pH can be expressed by the following equations.

For LD:

$$E_p = 526.91 - 46.99 \text{ pH}; \; r = 0.994 \text{ (between pH 2.0 and 9.0).}$$

For BEN:

$$E_p = 466.7 - 61.51 \text{ pH}; \; r = 0.995 \text{ (between pH 1.6 and 7.0).}$$

For both compounds, potential values remain pH-dependent. The potential values shift to less positive values with increasing pH (Fig. 2a and 2c). The slope of these equations was 46.99 and 61.51 mV/pH for LD and BEN, respectively. According to the obtained slope values of these equations, equal amounts of electrons and protons are involved in the rate-determining steps[24,25].

The experimental results showed that shapes of the curves, maximum peak currents and resolution of the peaks were better in acidic pH values for BEN and LD (Fig. 2b, 2d and Fig. 3a, 3b). Nevertheless, the best separation between LD and BEN and sharp responses was obtained in Britton–Robinson buffer at pH 5.0 (Fig. 1). Hence, for the simultaneous determination of both compounds, Britton–Robinson buffer at pH 5.0 was selected as the supporting electrolyte. The peaks of both compounds shift to less positive potentials and separate from each other as the pH increases. However, their responses become an ill-defined wave above pH 7.0 (Fig. 1).

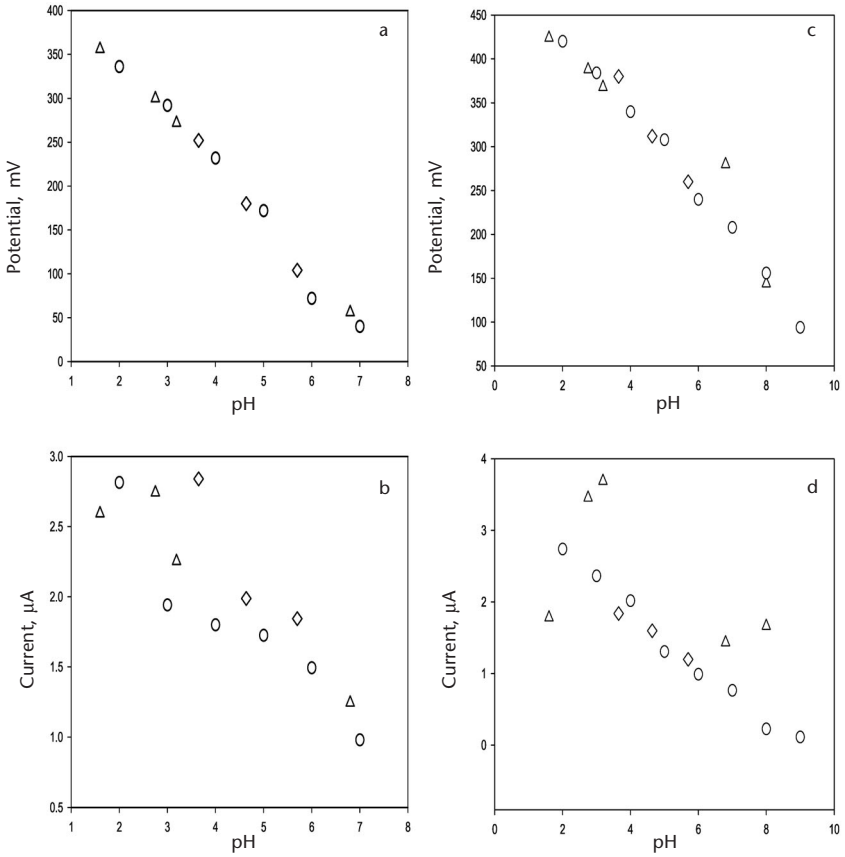


FIG. 2
Effect of pH on BEN peak potential (a) and peak current (b), on LD peak potential (c) and peak current (d); BEN and LD concentration 1×10^{-4} M. ◇ Acetate (0.2 M), △ phosphate (0.2 M), ○ Britton–Robinson buffer (0.04 M)

Scan rate studies were carried out to assess at LD and BEN, under diffusion or adsorption control. Using the concentration of 1.0×10^{-4} M for LD and BEN, separately, in Britton–Robinson buffer at pH 5.0, the voltammetric peak currents were observed as the scan rate over the range of 5–200 mV/s for both compounds (currents are in μA in these studies). The linear responses relationship existing between the peak current and the square root of the scan rate ($r > 0.99$) showed that the oxidation process is predominantly diffusion-controlled in whole scan rate range studies. These equations are given below.

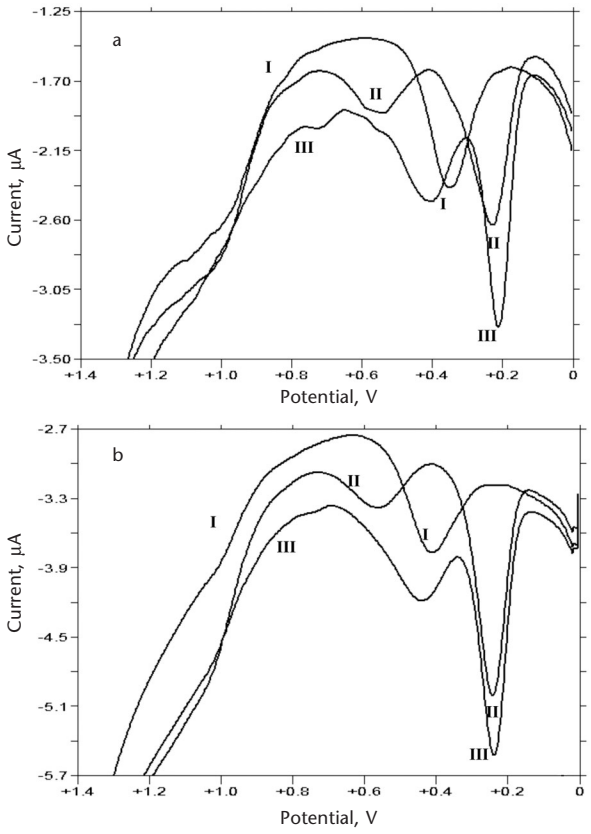


FIG. 3
DPV (a) and SWV (b) curves obtained in Britton–Robinson buffer pH 5.00, containing 8×10^{-5} M LD (I), 6×10^{-5} M BEN (II) and their binary mixture (III)

For LD:

$$I_p = 0.17\ v^{1/2} + 0.26;\ r = 0.993.$$

For BEN:

$$I_p = 0.35\ v^{1/2} - 0.20;\ r = 0.996.$$

Also a plot of the logarithm of the peak current versus the logarithm of the scan rate for LD and BEN gave a straight line with a slope of 0.42 and 0.52, respectively, close to the theoretical value of 0.5, which is expected for an ideal reaction of solution species[34]. Such dependence also indicated that the oxidation of the working compounds was indeed diffusion controlled. It is expected for an ideal reaction of solution species[34].

The equations obtained are given below.

For LD:

$$\log I_p = 0.42 \log v - 0.55\ (r = 0.998)$$

For BEN:

$$\log I_p = 0.52 \log v - 0.54\ (r = 0.995).$$

Voltammetric methods, especially cyclic voltammetry are most suitable for investigating the redox behavior of the pharmaceutically active compounds which can give insights into its metabolic fate[32,35,36]. The electrochemical oxidation of LD and BEN appears to be a complex process and different reaction pathways might be possible. Cyclic voltammetric measurements on the anodic way showed an irreversible nature of the oxidation process of BEN (Fig. 1a–1d). However, LD showed a reversible or quasi-reversible nature of the oxidation process on the anodic way (Fig. 1a–1d) depending on the pH value. Actually catecholamine oxidation processes has already been known and reported process[35,37–39]. The anodic oxidation of oxygen-containing compounds such as catecholamine still continues to be an active area of research. The course of anodic oxidation of phenolic compounds is remarkably complex[35,37–39]. In general, the oxidation of phenol in a solution at high pH will generate the phenoxy radical giving additional oxidation and reduction process. The many species involved are related to one another by a series of electron and proton trans-

fers that may occur as the result of biomolecular interactions. Voltammetric studies show the expected reversible and irreversible electron process leading to formation quinonic structure for LD and BEN, respectively. LD and BEN has the different structures, hence their responses showed this differentiation.

Simultaneous Determination of LD and BEN Using Electroanalytical Methods

The aim of this work was to develop a rapid, simple, selective and sensitive simultaneous determination of LD and BEN in their binary mixtures and dosage forms. Various electrolytes, such as Britton–Robinson, acetate and phosphate buffer were examined. In order to develop a voltammetric procedure for the simultaneous determination of the drug, DPV and SWV techniques were selected for LD and BEN because of their sharper and better-defined responses than those obtained by CV and LSV. pH is one of the variables that influence voltammograms most strongly and hence the resolution of mixtures as it affects different analytes in different forms. The best results with respect to signal enhancement and peak shape accompanied by sharper response was obtained with Britton–Robinson buffer at pH 5.0 for both compounds. These supporting electrolytes were chosen for the subsequent experiments. Figures 3a and 3b show the voltammograms given by each of the individual and mixture of LD and BEN (Fig. 3a and 3b).

The zero order DP and SW voltammograms of LD and BEN between 0.0 and +1.60 V potential ranges are shown in Fig. 3a and 3b, respectively. The DP and SW voltammograms of LD and BEN were found to be overlapped, making simultaneous determination difficult as shown in Fig. 3. Hence, the determination of these two drugs was not possible for reliable direct current measurements. Ratio derivative voltammetry can be suitable to obviate this problem. The main advantage of the ratio derivative voltammetric methods like ratio derivative spectrophotometric methods[21–26,40] may be the option of doing measurements in correspondence of peaks, hence a potential higher sensitivity and accuracy. While the main disadvantages of the zero crossing method in derivative voltammetry for resolving of the binary mixtures of components with overlapped voltammograms are the risk of small drifts of the working potentials and circumstance that the working potentials generally do not fall in correspondence of peaks of the derivative voltammograms. The ratio derivative method permits the use of the different concentrations as the divisor to obtain the different calibration graphs. An accurate choice of divisor standard concentration is fundamental for

several reasons. Hence we tested the methods with various divisor concentrations. We carried out preliminary investigations, to randomly select the standard solutions as divisor at an appropriate concentration of LD and BEN in their linearity range. The results of all tests are not shown for the sake of brevity and because these do not add to the scientific value of the work. A concentration of 8×10^{-5} M of LD and 6×10^{-5} M BEN as a divisor gave best results in term of signal-to-noise ratio and highest correlation coefficient values, being and indication of the quality of fitting of the data to the straight line.

The ratio voltammograms and the first derivative of these ratio voltammograms of different LD standards at increasing concentrations in Britton–Robinson buffer at pH 5 obtained by dividing each of these voltam-

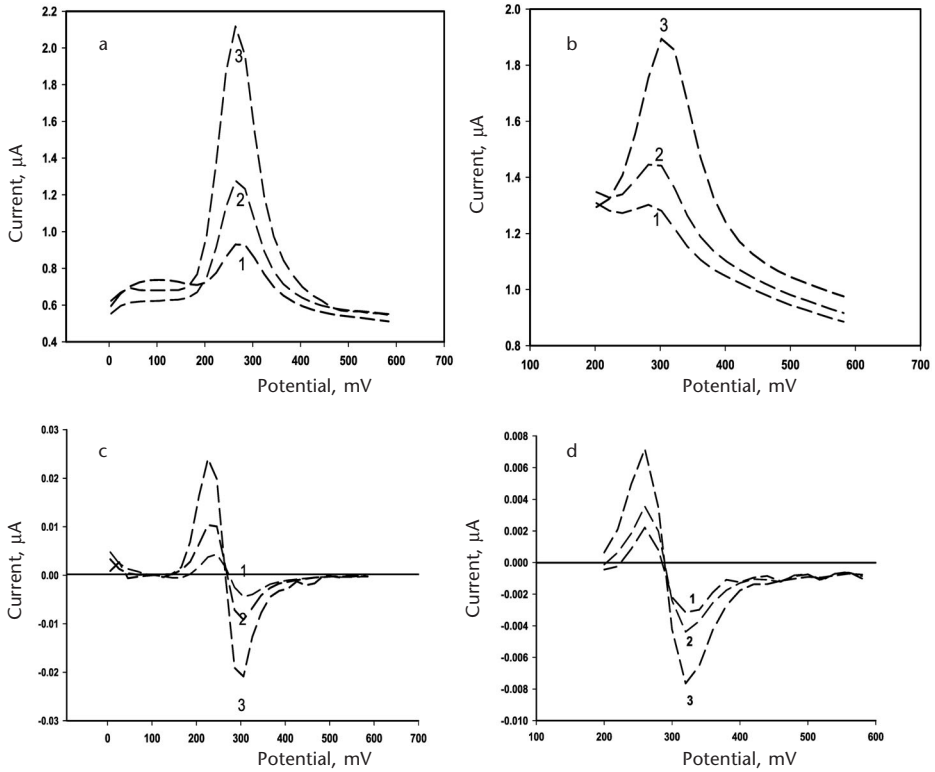


FIG. 4
Ratio voltammogram (a, b) and first derivative of the ratio voltammogram (c, d) of 1×10^{-5} (1), 2×10^{-5} (2) and 6×10^{-5} M (3) LD using 6×10^{-5} M BEN as a divisor for DPV and SWV, respectively

mograms with the voltammograms of the standard solution of BEN 6×10^{-5} M are shown in Fig. 4. In Fig. 4a and 4b, the ratio voltammograms of LD/BEN with DPV and SWV, respectively, are shown. In Fig. 4c and 4d, the corresponding first derivative of the ratio voltammograms of Fig. 4a and 4b, respectively, are shown. For calibration graph, the potentials were selected which exhibited the best linear response to the analyte concentration, i.e., in the first derivative mode 0.304 and 0.340 V for LD, with DPV and SWV methods, respectively.

For determining the other component, BEN, an analogous procedure followed. Figure 5 shows the ratio spectra (Fig. 5a and 5b) and their first derivatives (Fig. 5c and 5d) of the ratio voltammograms of different standard solutions of BEN in Britton–Robinson buffer at pH 5, using the standard

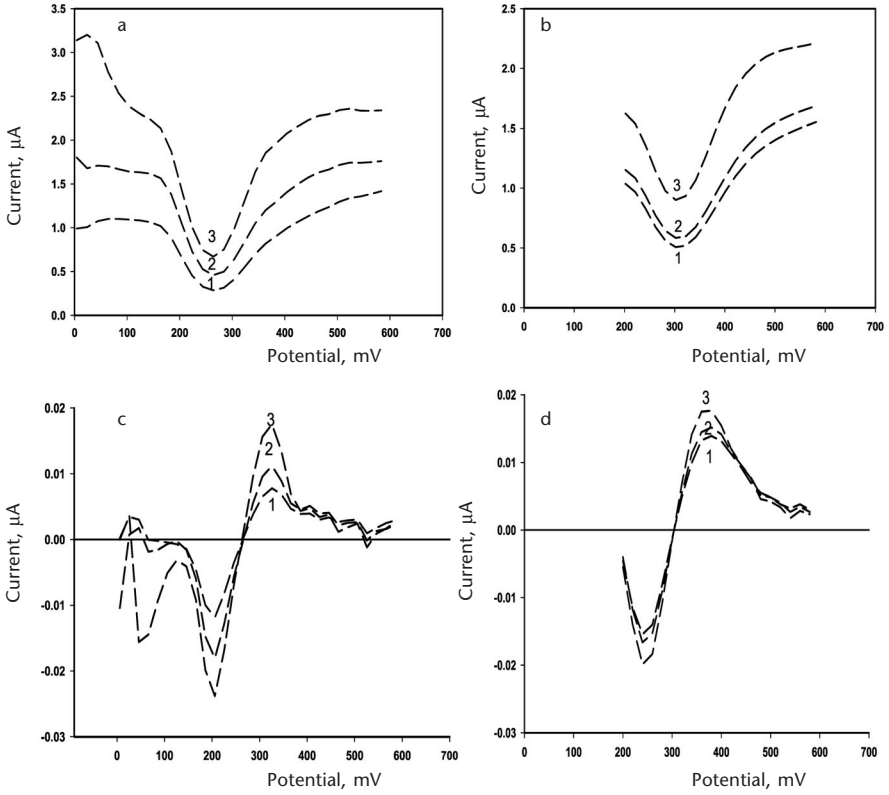


FIG. 5
Ratio voltammogram (a, b) and first derivative of the ratio voltammogram (c, d) of 1×10^{-5} (1), 6×10^{-5} (2) and 2×10^{-4} M (3) BEN using 2×10^{-5} M LD as a divisor for DPV and SWV, respectively

DPV or SWV curves of a 8×10^{-5} M of LD solution as a divisor. As seen from Fig. 5c and 5d, there obtain more than one maxima and minima and it was found that the maximum at 0.326 V for DPV and at 0.362 V for SWV is suitable for the assay of BEN in binary mixture with LD for both voltammetric methods.

Once the optimum working conditions have been established, calibration graphs were obtained using selected potentials for each compound with DPV and SWV methods. The results showed that the proposed methods are applicable over the ranges of 1×10^{-5}–8×10^{-5} M for LD and 1×10^{-5}–2×10^{-4} M for BEN. The characteristic parameters of the regression equations are summarized in Table I. The calibration graphs of each drug at selected potentials were achieved by plotting the values of the first derivative of the ratio voltammetric response of LD/BEN and BEN/LD, with variable concentrations of LD and BEN. The linearity ranges, limits of detection (LOD), limits of quantification (LOQ), repeatability, reproducibility, precision, recovery, bias % and selectivity were evaluated for both methods. The characteristic parameters and necessary statistical data of the regression equations are reported in Table I. The LOD and LOQ values were calculated using the following equations[27–30].

$$\text{LOD} = 3.3 \times \text{SD/m} \quad \text{and} \quad \text{LOQ} = 10 \times \text{SD/m}$$

Repeatability and reproducibility results were characterized by RSD % and by the difference between theoretical and measured concentrations. There was no significant difference for the assay, which was tested within day (repeatability) and between days (reproducibility).

Analysis of LD and BEN in Pharmaceutical Dosage Form

When working on the standard compounds, results encourage the use of the proposed methods described for the simultaneous determination of LD and BEN in pharmaceutical dosage forms. The proposed first derivative of the ratio DPV and SWV methods could be used for the simultaneous determination of LD and BEN in the presence of each other and without prior separation of the excipients. Each Madopar® tablet contains 100 mg of LD and 25 mg of BEN and the inactive ingredients. The adequacy of the developed methods was evaluated by quantifying LD and BEN in commercial tablet dosage form. Pretreatment was not required for samples either time-consuming extraction or evaporation steps prior to the analysis. The utility of all of the proposed methods was verified by means of replicate estima-

TABLE I
Statistical data for the calibration graphs of BEN and LEV using the first derivative of the ratio DPV and SWV techniques

Parameter	BEN		LD	
	DPV	OSW	DPV	OSW
Measured potential, V	0.326	0.362	0.304	0.340
Linearity range, M	1×10^{-5}–2×10^{-4}	1×10^{-5}–2×10^{-4}	1×10^{-5}–8×10^{-5}	1×10^{-5}–8×10^{-5}
Slope	45.62	48.77	339.40	109.00
Intercept	6.5×10^{-3}	1.24×10^{-2}	-3.1×10^{-3}	1.9×10^{-3}
Correlation coefficient	0.996	0.998	0.999	0.996
SE of slope	1.94	1.12	3.17	6.09
SE of intercept	1.83×10^{-4}	1.06×10^{-4}	1.56×10^{-4}	2.99×10^{-4}
LOD, M	2.99×10^{-6}	2.62×10^{-6}	2.52×10^{-6}	2.89×10^{-6}
LOQ, M	9.05×10^{-6}	7.93×10^{-6}	7.64×10^{-6}	8.77×10^{-6}
Repeatability of peak current (RSD, %)[a]	1.56	1.00	1.21	0.99
Reproducibility of peak current (RSD, %)[a]	1.64	1.19	1.80	1.26

[a] Each value is obtained from five experiments.

TABLE II
Results of the assay and recovery analyses of BEN and LD in tablet dosage forms using the first derivative of the ratio DPV and SWV techniques

Parameter	BEN			LD		
	DPV	SWV	Spectrophotometric method[10]	DPV	SWV	Spectrophotometric method[10]
Labeled claim, mg per tablets	25.00	25.00	25.00	100.00	100.00	100.00
Found, mg[a]	24.33	24.33	24.73	98.67	99.01	98.50
RSD, %	1.71	1.29	0.71	0.72	0.39	0.38
Bias, %	2.68	2.68	1.08	2.33	0.99	1.50
$t_{calculated}$	0.081	0.038	$t_{theoretical}$ 2.31	0.33	0.061	$t_{theoretical}$ 2.31
$F_{calculated}$	0.12	0.29	$F_{theoretical}$ 5.05	0.24	0.95	$F_{theoretical}$ 5.05
Added, mg	25.00	25.00	–	100.00	100.00	–
Found, mg[a]	23.98	24.39	–	100.57	101.18	–
Recovery, %	95.90	97.54	–	100.57	101.18	–
Bias, %	4.10	2.64	–	–0.57	–1.18	–
RSD, % of recovery	0.63	1.34	–	0.69	0.42	–

[a] Mean value of five determinations.

tions of pharmaceutical preparations and results obtained were evaluated by statistically. Table II shows the results obtained in the analysis of tablets for proposed first derivative of the ratio voltammetric methods. Results obtained from proposed methods of the analyses of both drugs in tablets indicate that the proposed techniques can be used for simultaneous quantitation and routine quality control analysis of this binary mixture in pharmaceuticals.

A comparison with an official reference simultaneous determination method has not been possible in any pharmacopoeias, because so far no other procedure for the simultaneous quantitation of LD and BEN from tablets has been reported. For this reason, proposed methods were compared with the literature method which is related with the first derivative of the ratio spectrophotometric method[10]. Table II compares the results of the analyses of LD and BEN between proposed and literature methods. The amounts of LD and BEN are fairly close to the labeled amounts for both techniques. However, the proposed method is sensitive, selective and more precise than the first derivative of the ratio spectrophotometric assay. The results obtained from the two methods were statistically compared with each other at the 95% confidence level with the aid of *t*- and *F*-tests. The *F*- and Student *t*-tests were carried out on the data and statistically examined the validity of the obtained results by first derivative of the ratio spectrophotometric method. According to the Student *t*- and variance ratio *F*-test, the calculated *t* and *F* values were less than the theoretical values in either test at the 95% confidence level. This indicates that there are no significant differences between the performances of the proposed and spectrophotometric methods with regards to accuracy and precision (Table II).

The accuracy of the proposed methods was determined by the recovery experiments. The recovery of the procedure was carried out by spiking the already analyzed samples of tablets with the known amounts of standard solutions of LD and BEN. The results of the recovery analysis for the proposed techniques are tabulated also in Table II. It is concluded that the proposed methods are sufficiently accurate and precise in order to be applied to tablet dosage forms. High percentage recovery data shows also that the proposed methods are free from the interferences of the excipients used in the formulations.

CONCLUSION

The electrochemical behavior of LD and BEN on glassy carbon electrode was established and studied as alone and in the presence of each other. LD

and BEN are reversibly and/or irreversibly oxidized at high positive potentials in different supporting electrolytes.

The proposed first derivative of ratio DP and SW voltammetric techniques can be used for simultaneous determination of LD and BEN in tablets. The principal advantages of first derivative of ratio DP and SW voltammetric techniques over the other techniques are that they may be applied directly to the analysis of tablet dosage forms without the need for separation or complex sample preparation, since there was no interference from the excipients. The other advantages of the proposed first derivative of the ratio voltammetric methods over the published methods, is the possibility of performing measurements in correspondence of peaks, hence a potentially greater sensitivity and accuracy. Also the proposed assay are easy measurement on the separate peaks, higher values of analytical signals and no need to work only at zero-crossing point in comparison with the derivative methods.

The results obtained show the above described methods are useful not only for simultaneous determination of LD and BEN in supporting electrolytes, but also in more complex matrices such as dosage forms. The proposed first derivative of ratio DP and SW voltammetric methods enable the quantitation or mixtures of LD and BEN with good accuracy and precision, either in laboratory made samples or in pharmaceutical dosage forms. These are suitable for quality control laboratories, where economy and time are essential. High percentage recovery shows that the methods are free from the interferences of the commonly used excipients and additives in the formulations of drugs.

This work is produced from Ph.D. Thesis of Pharm. B. T. Demircigil (Ankara University, Health Science Institute).

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