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Differential Pulse Voltammetric Strategy for Simultaneous Determination of Aceclofenac and Ezogabine in Biological Fluids at Edge-plane Pyrolytic Graphite Electrode

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Abstract- Herein, an eco-friendly and rapid voltammetric method is suggested for simultaneous assay of Aceclofenac (ACL) and Ezogabine (EZO) in biological fluids. Aceclofenac (ACL) and Ezogabine (EZO) are co-administrative drugs which are prescribed for the treatment of epilepsy associated with chronic inflammatory. The voltammetric method is based on the electrochemical oxidation of ACL and EZO at an edge plane pyrolytic graphite electrode using differential pulse voltammetry (DPV). Moreover, the electrochemical oxidation at carbon paste electrode and carbon paste electrodes modified with nanoparticles have been remarked. A number of crucial parameters which affect the electrochemical process at the electrode surface have been optimized. The method is linear over the concentration ranges from 0.2 to 100.0 µM and from 2.0 to 70.0 µM for ACL and EZO, respectively. The average recovery was found to be 100±2% with satisfactory %RSD (<1.5%) for both compounds. The method was applied successfully for simultaneous determination of ACL and EZO in biological samples with satisfactory accuracy and precision.

Keywords- Aceclofenac; Ezogabine; Voltammetry; Edge plane pyrolytic graphite; Biological fluids

1. INTRODUCTION

Aceclofenac (Fig. 1A) is a non-steroidal anti-inflammatory drug used for the treatment of various pain related disorders and inflammatory conditions [1]. It is chemically know as [2-[2-(2, 6-dichloroanilino)phenyl]acetyl]oxy acetic acid, and is structurally related to diclofenac, which is widely prescribed for chronic inflammatory conditions [2-4]. Aceclofenac was developed to address the critical side effects associated with diclofenac, such as ulceration and bleeding. Ezogabine is an anticonvulsant drug that has recently been approved by the FDA for the effective treatment of patients with therapy-resistant epilepsy [5,6]. It works by opening neural KCNQ (Kv7)-potassium channels (KCQN: genes encode family members of the Kv7 potassium channel family), thereby increasing the depolarization threshold of neuronal membranes [7]. Ezogabine (Fig. 1B) is chemically known as N-[2-amino-4-(4-fluorobenzylamino)phenyl] carbamic acid ethyl ester, and is similar in structure to flupirtine, which has been reported with weak anticonvulsant activity [8]. Aceclofenac and ezogabine are co-administrative drugs, and the development of a fast, accurate and precise analytical method for their simultaneous determination is important to control their concentrations at recommended safe levels.

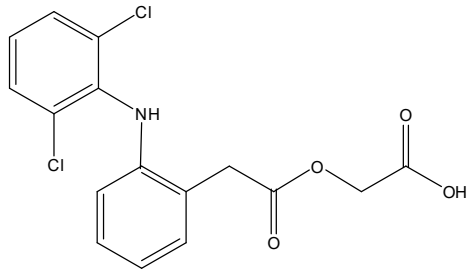
Several analytical methods were developed for the determination of aceclofenac either alone or in combination with other drugs; these methods include HPLC [9-14], spectrophotometry [15,16] and voltammetry [17-20], With regard to ezogabine, the literature survey revealed a few analytical methods including HPLC-UV [21], LC-MS-MS [22,23], and voltammetry [24]. Despite the fact that the chromatographic methods are superior for separation and determination of aceclofenac and ezogabine, the proposed methods use hazardous organic solvents and expensive instrumentations. In addition, none of these methods addressed the simultaneous determination of aceclofenac and ezogabine. Voltammetric techniques are considered to be rapid and inexpensive, and can help measurements with low detection limits [25-27]. Although good results were obtained for ezogabine and aceclofenac using voltammetric techniques, the reported methods may comprise complex and hazardous steps for the preparation and characterization of nanoparticles, which limit their application in laboratories for routine analysis.

Edge-plane pyrolytic graphite electrode is a form of carbon based electrode, at which the rate of electron-transfer of a large variety of redox couples were found to be 10 times faster than that at basal plane carbon based electrodes [28]. Additionally, low background current and better electrocatalytic signals were demonstrated for edge-plane pyrolytic graphite electrodes compared to other carbon based electrodes including boron-doped diamond, glassy carbon, basal-plane pyrolytic graphite and carbon nanotube-modified basal-plane pyrolytic-graphite electrodes.

Herein, we report on the possibility of using edge-plane pyrolytic graphite electrode for simultaneous and sensitive determination of aceclofenac and ezogabine. The electrochemical

response was compared with that obtained using glassy carbon, carbon paste and modified carbon paste electrodes. The effect of several experimental parameters on the electrocatalytic signal was investigated. The method was validated for simultaneous determination of aceclofenac and ezogabine biological fluids.

(A)



(B)

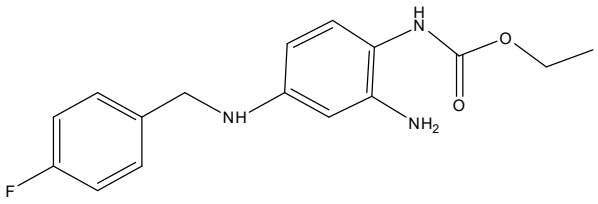


Figure 1. Chemical structure of (A) aceclofenac and (B) ezogabine

2. EXPERIMENTAL

2.1. Instrumentation

The electrochemical measurements were performed using SP-150 Potentiostate which is controlled by EC-lab V11.02 software. The electrochemical cell accommodates three electrodes which are the working, reference, and counter electrodes. The reference electrode is an Ag/AgCl (3M KCl) and the counter electrode is a Pt wire. The pH of the solutions was adjusted using a combined pH glass electrode connected to JENWAY 3510 (England) pH meter.

2.2. Chemicals and reagents

Aceclofenac (ACL) drug substance (99.8% purity, according to the manufacturer certificate) was kindly supplied by Glaxosmithkline, Cairo Egypt. Ezogabine (100.08% purity, according to the manufacturer certificate) was kindly supplied by *Medizen Pharmaceutical* Industries, Alexandria, Egypt. Methanol was purchased from Merck, Darmstadt, Germany. Sodium dodecyl sulphate (SDS), triton X-100 and cetyltrimethyl ammonium bromide (CTAB) surfactants were obtained from Sigma Aldrich, Steinhem, Germany.

Buffers necessary reagents including hydrochloric acid, acetic acid, orthophosphoric acid, boric acid, potassium chloride, potassium dihydrogen orthophosphate, disodium hydrogen

phosphate and sodium hydroxide were purchased from El-Nasr Company, Cairo, Egypt. Double distilled water was obtained from Aquatron A4000 double distillatory (England).

Briton Robinson buffer (B-R) was prepared by mixing solutions of 0.04 mol L^{-1} phosphoric acid, m 0.04 mol L^{-1} acetic acid and 0.04 mol L^{-1} boric acid with the appropriate amount of 0.2 mol L^{-1} NaOH to obtain the desired pH 2.0 - 10.0. Other buffers including Phosphate, Acetate and Buffer solution pH 2.0 were prepared according to the US Pharmacopiea [29].

All chemicals and were used as received without further purification. The solutions were prepared using double distilled water, and all experiments were carried out at ambient temperature (25˚C ± 2).

2.3. Solutions

2.3.1. Standard stock solutions

Standard solution of ACL was prepared in a 10-mL volumetric flask by dissolving 35.42 mg ACL in 10 mL methanol and completing the flask to the mark with methanol (1.0 x 10^{-2} M ACL). EZO standard solution (1.0 x 10^{-2} M) was prepared the same way by dissolving 30.33 mg EZO in 10 mL methanol. The diluted solutions were prepared each in 10-mL measuring flask by serial dilution. The solutions were prepared prior to measurements.

2.4. Procedures

The working electrode was polished on a polishing cloth using 3 µm and 1 µm diamond polish. The electrode was immersed in an electrochemical cell containing acetate buffer pH 2 (10% methanol), and the CV was recorded several times between 0.0 and 1200.0 mV at 100 mV s^{-1} until a stable response was obtained. The electrode was immersed in 10 mL of acetate buffer pH 2 (10% methanol) containing the target analyte and the DPV was recorded at 100 mV/s. Calibration curves were constructed to cover the concentration range from 0.2 µ M to 100.0 µM for ACL and from 2.0 to 70.0 µM for EZO.

2.5. Analytical Applications

The human plasma was obtained from VACSERA, Giza, Egypt. Plasma samples were kept frozen until needed. Plasma samples (1 mL each) were spiked with ACL and EZO, then treated with 3 mL acetonitrile and followed by centrifugation at 5000 rpm for 20 minutes to completely precipitate proteins. The supernatant was cautiously transferred into centrifuge tubes. Afterwards, about 4.5 mL acetate buffer pH 2.0 (10% methanol) was added to 0.5 ml of the supernatant; this supernatant solution was used for preparation more diluted solutions. The calibration curves were constructed using the DPV method to cover the concentration ranges from 2.0-70.0 µM for ACL and from 2.0-70.0 µM for EZO. Method validation was carried out according to EMA guidelines. The accuracy, precision and intermediate precision were investigated at three concentration levels on three consecutive days. Recovery was assessed by

comparing the response obtained from the spiked plasma samples in mixture with that of plasma sample of spiked with pure drug in the same concentration.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behaviour of aceclofenac

The electrochemical oxidation of ACL was studied using cyclic voltammetry at different electrode materials including carbon paste, carbon paste modified with reversed phase silica gel (RPC_8 and RPC_{18}) and edge plane pyrolytic graphite electrodes. Figure 2A shows the CV of ACL at the EPG and bare carbon paste electrodes in the potential range from 0 to 1.2 V at a scan rate of 100 mV/s. It is obvious that ACL is oxidized at about + 0.89V, regardless the electrode material, with a remarkable high current density at the EPG. A reduction peak was observed at 0.6 V in the backward scan, indicating that the oxidation of ACL is quasi-reversible ($\Delta Ep > 59/n$ mV) [30]. The current ratio of the two peaks was found to be less than 1 indicating that α is > 0.5 [30]. Carbon paste modified with different ratio of reversed phase silica gel C_8 and C_{18} (Fig 2 B) were used; however, the maximum oxidation current remained considerably below the oxidation current obtained at EPG electrode. Therefore, the later was employed for all subsequent electrochemical measurements.

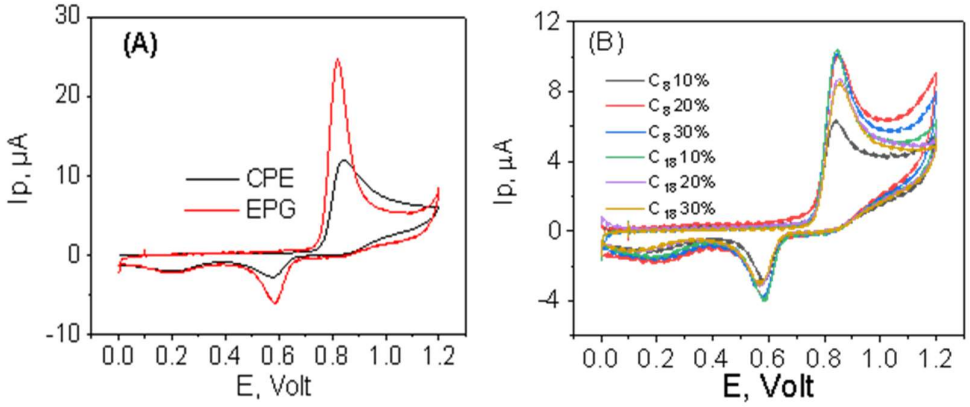


Figure 2. Cyclic voltammograms of 1×10^{-4} M ACL at (A) EPG and carbon paste electrodes and (B) carbon paste electrodes modified with different ratios of reversed phase silica gel C_8 and C_{18}

3.2. Effect of pH of the supporting electrolyte

The oxidation of most organic compounds in aqueous solution at the electrode surface, in particularly, pharmaceutical compounds, is affected by the pH of the medium. Figure 3 shows the CV of ACL in B-R buffer at different pH values (2.0 – 10.0). Looking at the peak potential and peak current, it is becoming clear that the pH is crucial for ACL oxidation at the electrode. When the pH was increased from 2 to 5, the oxidation current dropped dramatically from about

27.0 µA to 5 µA with concomitant shift in the peak potential towards more positive potential. The peak current started to increase again from pH 6 to pH 8 with maximum peak current of 17 µA at pH 8. The current was dropped with deteriorated peak shape at pH 10. Therefore, pH 2 was chosen as optimal pH value for performing all subsequent measurements.

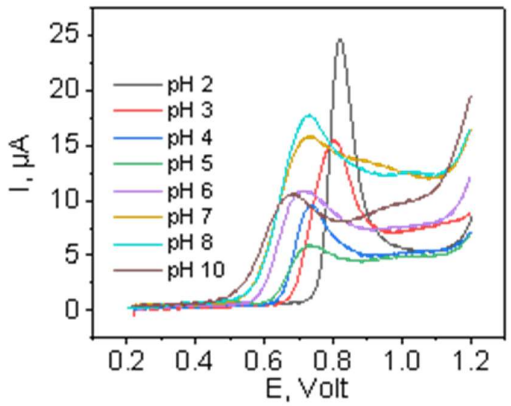


Figure 3. Cyclic voltammograms of 1×10^{-4} M ACL at EPG electrode in acetate buffer of different pH values from pH 2 to 10.

3.3. Influence of the supporting electrolyte

The effect of various types of buffers including Briton Robinson, phosphate, acetate and buffer solutions on the electrochemical oxidation of ACL was investigated. Oxidation current intensity of about 26.6 µA was obtained using acetate buffer compared to16.5, 12.9 and 15.4 µA for phosphate, BR and buffer solution pH 2.0 (Fig. 4). Most probably the acetate ion participates in the electrochemical oxidation of ACL. Thus, acetate buffer was selected as an optimal buffer for further electrochemical studies.

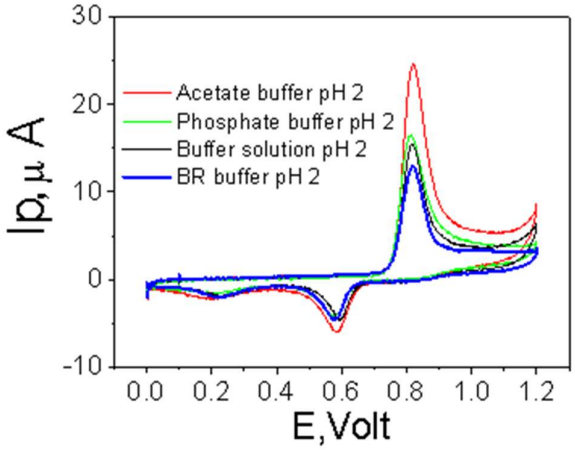


Figure 4. Cyclic voltammograms of $1x10^{-4}$ M ACL at EPG electrode in presence of different types of buffers including BR, acetate, phosphate and buffer solution of pH 2.

3.4. Effect of surfactants

The effect of different surfactants including SDS (ionic surfactant), CTAB (cationic surfactant), and triton X-100 (non-ionic surfactant) on the electrochemical oxidation of ACL was investigated. As it is shown in Figure 5, the peak current is dramatically reduced when surfactants are added to the solution. This would be due to blocking the surface of the electrode with the surfactant, and the more surfactant added to the solution, the less the oxidation current intensity is obtained. Thus, all electrochemical measurements were performed in the absence of surfactants.

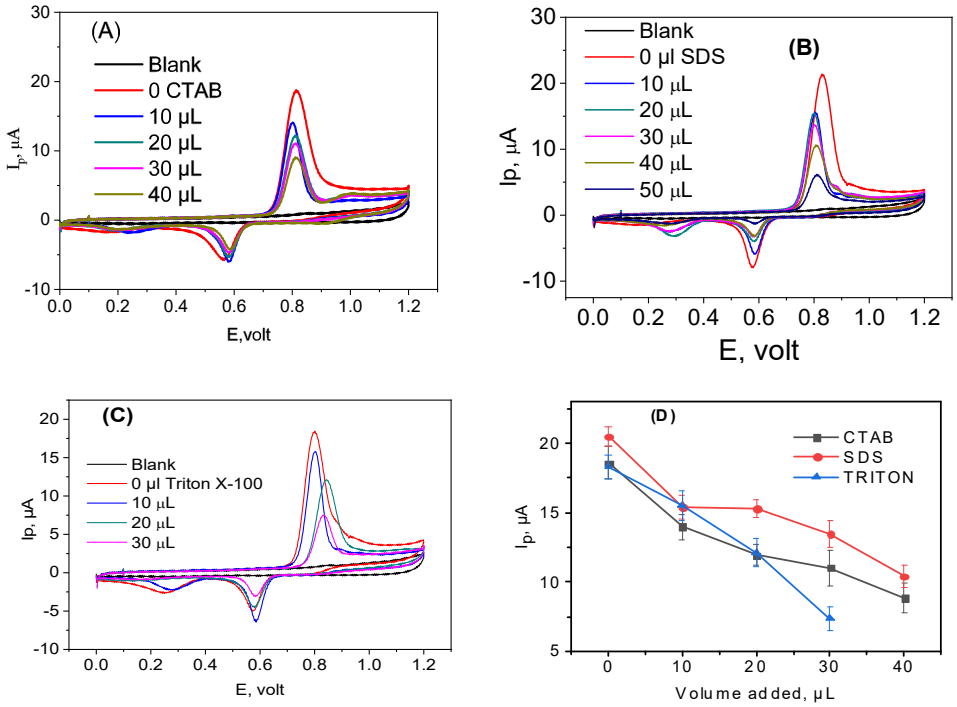


Figure 5. Cyclic voltammograms of 8.8×10^{-5} M ACL at EPG electrode in presence of different types of surfactants including (A) SDS, (B) CTAB and (C) Triton X-100. (D) The relationship between the peak current and the concentration of the surfactant. The experiments were carried out in acetate buffer pH 2 at scan rate of 100 mV/s

3.5. Effect of methanol

ACL is practically insoluble in water and addition of methanol is essential for preparation of aqueous solution of ACL. However, the amount of methanol should be at the limit that does not affect the chemistry of the electroactive species and the mechanism of the electrochemical process at the surface of the electrode. Figure 6 illustrates that the effect of methanol on the peak current is negligible when methanol is added to acetate buffer at a ratio up to 30% (v/v).

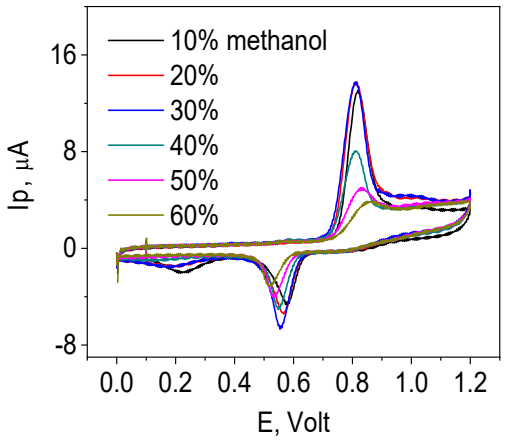


Figure 6. Cyclic voltammograms of 5.5×10^{-5} M ACL recorded at EPG electrode in acetate buffer pH 2 containing different amounts of methanol.

A dramatic decrease in the peak current was observed when the amount of methanol increased from 30 to 40, 50 and 60% (v/v). Therefore, all electrochemical measurements were carried out in acetate buffer containing 10% of methanol.

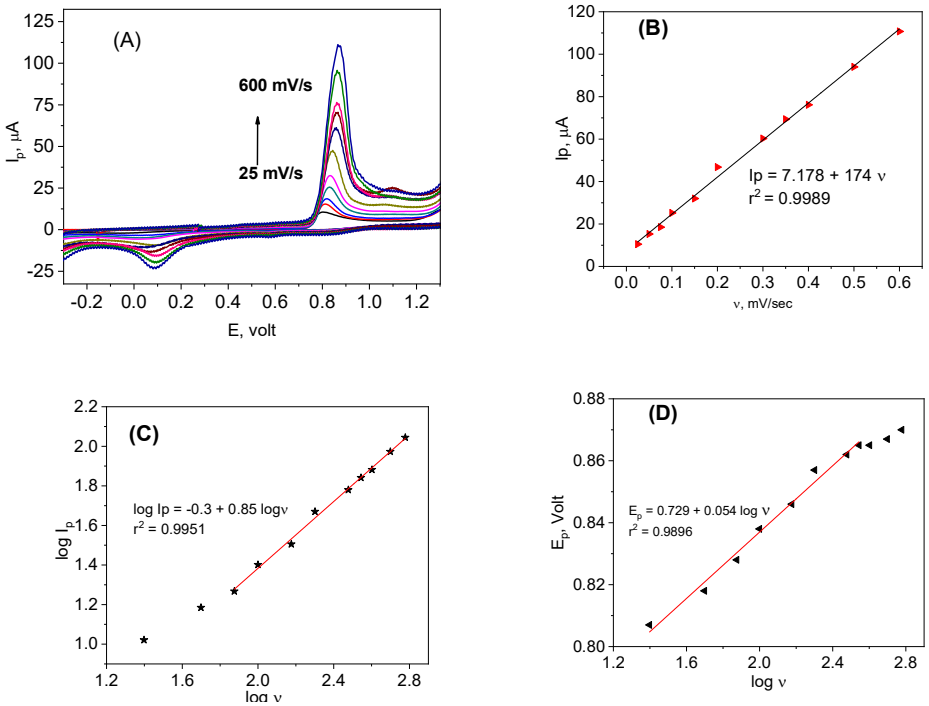


Figure 7. (A) Cyclic voltammograms of 1×10^{-4} M ACL at different scan rates. (B) The relationship between the peak current and the scan rate. (C) The relationship between the logarithem peak current and logarithem scan rate. (D) The relationship between logarithem peak potential and logarithem scan rate

3.6. Effect of the scan rate (υ)

Examining the relationship between the scan rate of the potential (υ) and both the intensity of the peak current (I_p) and the oxidation potential (E_p) can help explain the nature of the electrochemical oxidation of ACL at the surface of electrode. Thus, cyclic voltammograms were recorded at different scan rates from 25.0 up to 600.0 mV sec^{-1}, and the change in peak current and peak potential was recorded for each scan (Fig. 7A). We found that the intensity of the oxidation current (I_p) changes in a linear mode with the rate of potential change; the relationship is controlled by the regression equation $Ip = 0.174\ \nu + 7.178$, $r^2 = 0.9989$, suggesting that the electrochemical process is diffusion controlled (Fig. 7B). Meanwhile, we found that the relationship between the logarithm of the oxidation current and logarithm the rate of potential change is linear and described by the equation $\log I_p = 0.85 \log \nu$ - 0.3, $r^2 = 0.9951$ (Fig. 7C), the value of the slope 0.85 is higher than 0.5 for diffusion controlled, and less than 1 for adsorption controlled process, suggesting that the electrochemical reaction is controlled by both adsorption and diffusion.

Additionally, the number of electrons (n) transferred at the electrode surface as a result of ACL oxidation was calculated by means of Laviron equation [31].

$$E_p = E^\circ + \frac{2.303RT}{\alpha nF}\left(\frac{log\ RTk^\circ}{\alpha nF}\right) + \frac{2.303RT}{\alpha nF} log\ \upsilon \quad (1)$$

where α stands for the electron transfer coefficient, T for the temperature (298 K), R for the gas constant (8.314 J K mol^{-1}), and F for the Faraday constant (96 485 C mol^{-1}). The parameters E° and K° stand for the formal oxidation potential and the standard heterogeneous rate constant of the electrochemical reaction, respectively. αn can be obtained from the slope of the relation between E_p and log υ (Fig. 7D), here, αn was found to be 1.1. For a quasireversible process, α is assumed to be 0. 5 [30]; thus, the value of n is 2.2 (≈2).

3.7. Effect of accumulation time

The accumulation of the drug by the spontaneous adsorption at the electrode surface can help enhance the detection limit. The spontaneous adsorption of ACL at the EPG was studied by CV at different time intervals. The relationship between the peak current and the time of accumulation is shown in Figure 8. It is obvious that the maximum peak current is achieved after 1 min, and remained stable up to 2 min of accumulation, after which the peak decreased dramatically. Therefore, accumulation time for 1 min with stirring was chosen as an optimal pre-concentration time prior to DPV measurements.

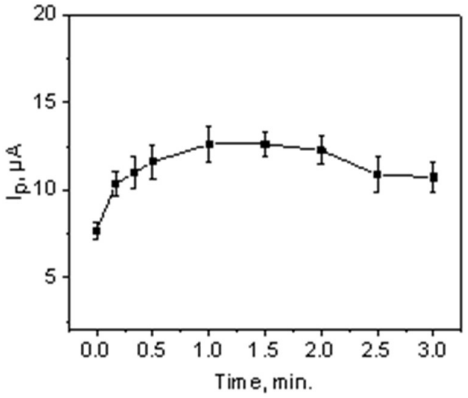


Figure 8. The relationship between the peak current and accumulation time of $5x10^{-5}$ M ACL in acetate buffer pH 2

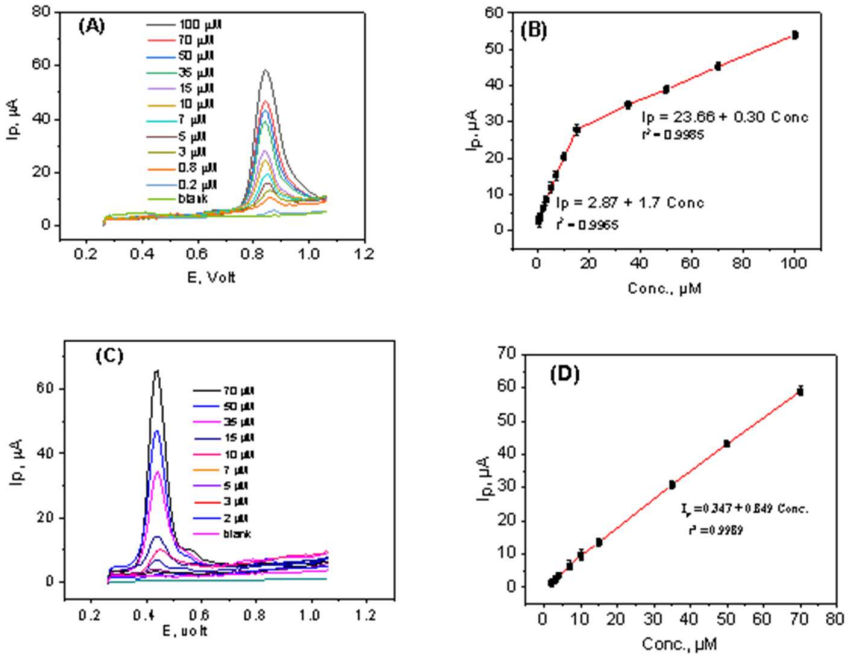


Figure 9. Differential pulse voltammograms recorded using different concentration of (A) ACL and (B) EZO. (C) and (D) represent the relationship between the peak current and the concentration for ACL and EZO, respectively

3.8. Method validation

Method validation was carried out according to the ICH [32] and EMA [33] guidelines. The DPV method was fully validated considering the linear concentration range, the accuracy and precision, selectivity and the quantitation limit.

3.8.1. Linearity and range

The calibration curves were constructed using the DPV by plotting the differential current against the concentration.

The relationship between the peak current I_p and the concentration of ACL (Fig 9 A and B) was found to be linear over the concentration range from 0.2 to 15.0 µM ($r^2 = 0.998$). Another linear line was obtained from 15 to 100.0 µM ($r^2 = 0.9989$) with lower sensitivity. EZO showed an oxidation peak current at about 0.440 mV under the same experimental conditions. Therefore, a calibrated curve was constructed for ezogabine. The DPV voltammogram of EZO at different concentrations and the corresponding calibration graph is shown in Figures 9 C and D. A linear relationship between I_p and the concentration of EZO was obtained over the concentration range from 2.0 to 70.0 µM ($r^2 = 0.9989$). The linearity parameters are summarized in Table 1.

3.8.2. Limit quantification (LOQ)

The limit of quantification was calculated based on the signal to noise ratio by measuring the concentration that gives a peak current which is 10 times higher than the base line noisy. The quantification limits were found to be 0.07 µM (RSD = 4.8%, n =3) and 0.11 µM (RSD = 5.2%, n =3) for ACL and EZO, respectively.

3.8.3. Accuracy and precision

Table 1. Validation parameters for the determination of ACL and EZO in the drug substance using the DPV method.

Parameters	ACL		EZO
Linearity range (µM)	0.2-15.0	15.0-100.0	2.0-70.0
Slope	0.96	0.38	0.998
The standard error of the slope	0.021	0.051	0.010
Intercept	6.4	15.1	4.6
The standard error of intercept	0.651	0.542	0.322
Correlation coefficient (r^2)	0.998	0.9989	0.9989
Accuracy (%recovery, n=3)	100.34	100.03	99.82
Repeatability (%RSD, n=6)	0.48	0.56	1.346
Intermediate precision (%RSD, n=6)	0.214	0.558	1.231
LOQ (µM)	0.07	0.426	0.11

The accuracy and precision of the method was studied for ACL at different concentration levels 2.0, 5.0, 35.0 and 75.0 µM. The average recovery was found to be 100.00 ±2%, and the %RSD was found to be < 1% (n=6). The accuracy and precision of the method were also studied for the quantification of EZO using three concentrations levels 5.0, 15.0 and 35.0 µM; the average recovery was found to be 100.00 ±2% with %RSD < 1.5% (n =9). The validation parameters are summarized in Table 1. These results refer to satisfactory accuracy and precision of the DPV method for quantification of ACL and EZO in the bulk drug substance. The present method was statistically compared to reported HPLC methods for the assay of ACL and EZO in the drug substance [13, 34]. The reported t-value and F-ratio values (Table 2) indicate that there is no significant difference between the proposed DPV and the reported HPLC method.

3.8.4. Robustness

The robustness was examined by recording the DPV for 50 µM ACL and 35 µM EZO in BR buffer pH 2. A deliberate change in the pH of the solution (pH = 2.0 ± 0.02) on the differential pulse current for both ACL and EZO was found to be negligible (%RSD ≤1.7, n=3), indicating to the robustness of the method.

3.8.5. Selectivity

The interference from common excepients and electroactive biological compounds was studied before the analytical applications to real samples. The interfering compounds which were studied include common tablet excepients such as mannitol, ascorbic acid 2-glucoside, magnesium stearate and carboxy methyl cellulose; in addition to ascorbic acid and uric acid. We found that the difference in the intensity of the peak current was less than 3% when using these compounds at concentrations higher that of ACL (>200 times), referring to a negligible interference.

Table 2. Statistical comparison of the experimental data obtained by the DPV method with an official method for ACL [13] and a reported HPLC method for EZO [34].

	Voltammetric method		Reported methods	
Item	ACL	EZO	ACL	EZO
Average (%)	100.32	99.69	99.63	99.06
S.D.	0.827	0.826	1.138	0.729
Variance	0.683	0.708	1.295	0.531
n	9	9	9	9
Student's t-test	1.477 (2.120)	1.704(2.120)		
F-value	1.897 (3.438)	1.333 (3.438)		

Table 3. Analytical parameters for the determination of ACL and EZO in plasma using the DPV method.

Parameters	ACL		EZO
Linearity range (μM)	2.0-15.0	15.0-70.0	2.0-70.0
Slope	0.946	0.386	0.484
The standard error of the slope	0.033	0.006	0.009
Intercept	6.391	14.916	4.026
The standard error of intercept	0.3078	0.285	0.337
Correlation coefficient (r^2)	0.9964	0.9992	0.9984
LOQ	1.586	7.38	2.48

3.9. Analytical applications

3.9.1. Spiked human plasma

3.9.2. Linearity and range in spiked plasma samples

The proposed DPV method was investigated for possible determination ACL and EZO in spiked human plasma (Fig. 10). The method was found to be linear for ACL over the concentration range from 0.2 to 15.0 μM (r^2 = 0.996), a second linear line was obtained from 15.0 to 70.0 μM (r^2 = 0.999). Also, a good linearity for EZO was obtained over the concentration range from 2.0 – 70.0 μM (r^2 = 0.9984). The linearity parameters are summarized in Table 3. The obtained linearity ranges for ACL and EZO prove that the method is useful for simultaneous determination of the two drugs in biological fluids,where the C_{max} is 9.5 μg/mL for a 100 mg dose of ACL [35] and is 0.818 μg/mL for a 200 mg dose of EZO [36].

Table 4. Accuracy and precision results for the determination of ACL and EZO in plasma.

Sample	Conc. (μM)	Found (μM)	±SD	recovery%*	RSD%
ACL (spiked plasma)	5.0	4.319	0.084	86.38	2.661
	15.0	13.71	0.270	91.40	4.001
	50.0	42.560	0.379	85.11	3.433
EZO (spiked plasma)	5.0	4.396	0.156	87.91	3.955
	15.0	13.405	0.312	89.37	3.561
	35.0	31.140	0.809	88.97	3.598

*Each concentration is prepared in triplicate

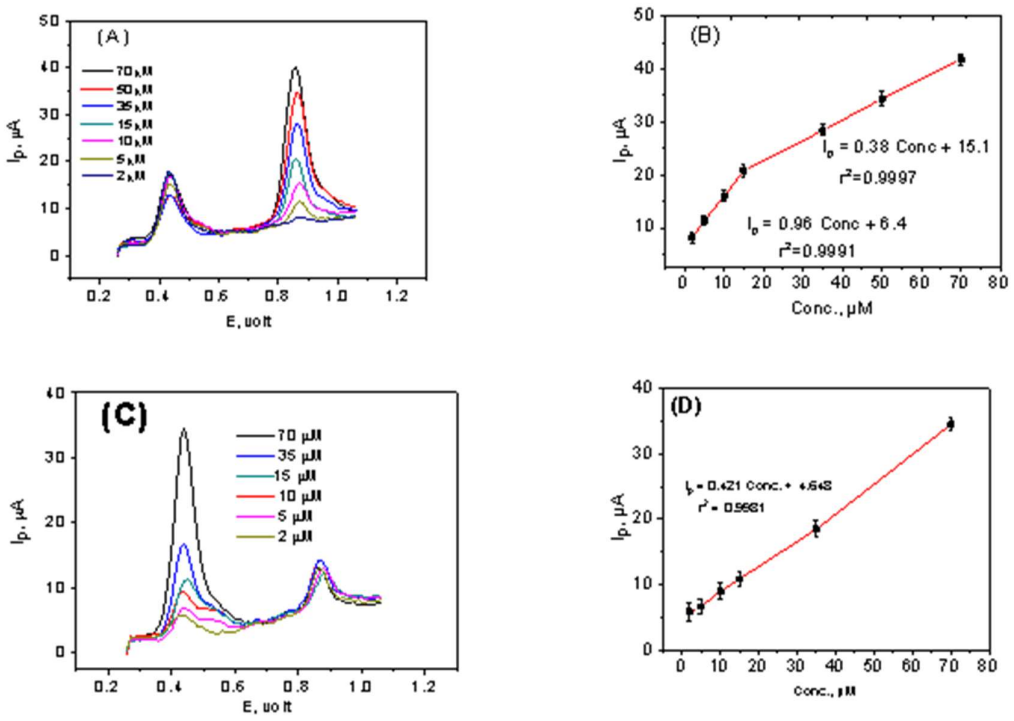


Figure 10. (A) Differential pulse voltammograms recorded for variable concentrations of ACL in presence of 15 µM of EZO and (B) is the corresponding plot of the peak current as a function of ACL concentration. (C) Differential pulse voltammograms recorded for variable concentrations of EZO in presence of 15 µM of ACL and (D) is the corresponding plot of the peak current as a function of EZO concentration.

3.9.3. Accuracy and precision in plasma

The accuracy was studied at three concentrations levels 5.0, 15.0, 50.0 µM for ACL and 5.0, 15.0, 35.0 µM for EZO (Table 4), each concentration was prepared in triplicate. The average recovery (%) for each concentration was found to be 87.63±3.34% for ACL and 88.75±2.59% for EZO, indicating a satisfactory accuracy. The %RSD (n=9, three preparations at each level) was found to be 3.46% for ACL and 3.95 % for EZO, indicating acceptable precision of the method. The intermediate precision was investigated by determination of ACL and EZO over three consecutive days; the %RSD was found to be 3.36% for ACL and 3.71% for EZO, indicating a satisfactory precision of the developed method.

4. CONCLUSION

Herein, we report for the first time a promising differential voltammetric method for simultaneous quantification of ACL and EZO in the biological samples. The best

electrochemical response was obtained at an edge-plane pyroletic graphite electrode in acetate buffer pH 2. A linear relationship between the concentration and the peak intensity was observed over the concentration ranges from 0.2 to 100.0 μM for ACL, and from 2.0 to 70.0 μM for EZO. Limits of quantitations were calculated based on signal to noise ratio and found to be 0.07 μM and 0.11 μM for ACL and EZO, respectively. Statistical comparison showed that the developed DPV method is equivalent to a reported method in terms of accuracy and precision. The method is rapid and used successfully for simultaneous determination of ACL and EZO in biological sample.

REFERENCES

[1] M. Dooley, C. M. Spencer, and C. J. Dunn, Drugs 61 (2001) 1351.
[2] K. Pavelka, Current Medical Research and Opinion 28 (2012) 163.
[3] G. Sharma, J. Singh, D. Anand, M. Kumar, K. Raza, A. Pareek, and O. P. Katare, Current Topics in Medicinal Chemistry 17 (2017) 107.
[4] S. Gajanand, S. Jaspreet, A. Deepika, K. Manish, R. Kaisar, P. Anil, and K. Om Prakash, Current Topics in Medicinal Chemistry 17 (2017) 10.
[5] B. M. Radu, F. B. Epureanu, M. Radu, P. F. Fabene, and G. Bertini, Epilepsy Res 131 (2017) 15.
[6] S. Jankovic, and I. Ilickovic, Expert Opin Drug Discov 8 (2013) 1429.
[7] M. J. Gunthorpe, C. H. Large, and R. Sankar, Epilepsia 53 (2012) 412.
[8] A. D. Wickenden, W. Yu, A. Zou, T. Jegla, and P. K. Wagoner, Mol Pharmacol 58 (2000) 591.
[9] B. Hinz, D. Auge, T. Rau, S. Rietbrock, K. Brune, and U. Werner, Biomed Chromatogr 17 (2003) 268.
[10] A. R. Bhinge, R. V. Kumar, and V. R. Sinha, Journal of Chromatographic Science 46 (2008) 441.
[11] A. Jawale, C. Kothari, M. Patel, and M. Patel, European Journal of Biomedical and Pharmaceutical Sciences 4 (2017) 545.
[12] D. Mohamed, M. A. Hegazy, M. S. Elshahed, S. S. Toubar, and M. I. Helmy, Biomed Chromatogr 32 (2018) e4232.
[13] K. Prema Kumari, V. Murugan, V. Ezhilarasan, and S. R. Joseph, Journal of Chromatography & Separation Techniques 08 (2017)
[14] S. Azhlwar, and T. K. RAVI, International Journal of Pharmacy and Pharmaceutical Sciences 3 (2011) 245.
[15] V. S. Rajmane, S. V. Gandhi, U. P. Patil, and M. R. Sengar, Eurasian Journal ofAnalytical Chemistry 4 (2009) 184.
[16] S. A. Shah, D. R. Shah, R. S. Chauhan, and J. R. Jain, Indian Journal of Pharmaceuticaal Sciences 73 (2011) 296.

[17] P. Manjunatha, Y. Arthoba Nayaka, B. K. Chethana, C. C. Vidyasagar, and R. O. Yathisha, Sensing and Bio-Sensing Research 17 (2018) 7.

[18] J. R. Posac, I. D. Vazquez, l. M. L. Tascon, l. J. A. Aculqa, I. De La Fuente, I. E. Velasco, and P. Sanchez-batanero, Talanta 42 (1995) 293.

[19] J. A. Acuna, C. de la Fuente, M. D. Vazquez, M. L. Tascon, M. I. G. del Rio, and P. Sanchez-Batanero, Quimica Analitica 17 (1998) 105.

[20] M. M. Vinay, Y. A. Nayaka, H. T. Purushothama, R. O. Yathisha, K. V. Basavarajappa, and P. Manjunatha, International Journal of Environmental Analytical Chemistry 99 (2019) 1553.

[21] V. Franco, K. Baruffi, G. Gatti, R. Marchiselli, C. Fattore, M. P. Canevini, F. Crema, and E. Perucca, Biomedical Chromatography 32 (2018) e4168.

[22] S. Deeb, D. A. McKeown, H. J. Torrance, F. M. Wylie, B. K. Logan, and K. S. Scott, Journal of Analytical Toxicology 38 (2014) 485.

[23] E. F. Elkady, A. A. Aboelwafa, and M. A. Fouad, Journal of Advanced Research 22 (2020) 99.

[24] S. A. Alkahtani, M. M. El-Wekil, A. M. Mahmoud, M. H. Mahnashi, and M. Oraby, Journal of the Electrochemical Society 166 (2019) H521.

[25] H. Karimi-Maleh, F. Karimi, Y. Orooji, G. Mansouri, A. Razmjou, A. Aygun, and F. Sen, Scientific Reports 10 (2020) 11699.

[26] H. Karimi-Maleh, F. Karimi, M. Alizadeh, and A. L. Sanati, The Chemical Record 20 (2020) 682.

[27] H. Karimi-Maleh, F. Karimi, S. Malekmohammadi, N. Zakariae, R. Esmaeili, S. Rostamnia, M. Lutfi Yola, N. Atar, SH. Movaghgharnezhad, S. Rajendran, A. Razmjou, Y. Orooji, SH.Agarwal, and V. Gupta, Journal of Molecular Liquids 310 (2020) 113185.

[28] Electroanalytical Chaemistry, McCreery, New York (1990).

[29] USP 40–NF 35, United States Pharmacopeia, MD, USA (2107).

[30] A. A. Gewitrh, Inorganic Electrochemistry: Theory, Practice and Aplications Royal Society of Chemistry, 2003.

[31] E. Laviron, and L. Roullier, Journal of Electroanalytical Chemistry and Interfacial Electrochemistry 115 (1980) 65.

[32] ICH Guideline (2005). Q2 (R1) Validation of analytical procedures: text and methodology

[33] European Medicines Agency (EMA), London, United Kingdom (2011).

[34] V. Franco, K. Baruffi, G. Gatti, R. Marchiselli, C. Fattore, M. P. Canevini, F. Crema, and E. Perucca, Biomed Chromatogr 32 (2018) e4168.

[35] S. Ghosh, and B. B. Barik, Tropical Journal of Pharmaceutical research 9 (2010) 395.

[36] M. Yamada, and T. E. Welty, Ann Pharmacother 46 (2012) 1358.