Azhar Int J Pharm Med Sci 2023; Vol 3 (2):133-140
(Research Article)

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Voltammetric Determination of Rebamipide in the Presence of its Degradant, in Bulk, Pharmaceutical Preparation, and Biological Fluids

Ola M. Abdallah [1], Taghreed A. Mohamed [2], Hassan A. M. Hendawy [2], and Hager Elomda [2,*]

[1] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University (Girls), Cairo, Egypt.
[2] Pharmaceutical Chemistry Department, Egyptian Drug Authority, Giza, Egypt.
***** Correspondencehagerelomda@hotmail.com

Article history: Received 2022-09-16 Revised 2022-10-1 Accepted 2022-11-08

Abstract: In this work, we develop, optimize and validate an electrochemical method for determining Rebamipide (REBA) using anodic differential pulse voltammetry (A-DPV) for the first time. The choice of the proper anode was determined through a comparative study between electrodes, and we found the proper one is the carbon nano-tubes that are modified with the nano form of zinc oxide electrode (ZnONP/CNT/ME). The degradant pathway of the Rebamipide was illustrated by FTIR. For the REBA quantification, we have used a 0.04M B-R buffer electrolyte solution (PH = 11.0) using the ZnONP/CNT/ME. The peak showed linearity in the range of (0.09-12.37 µg/mL), limit of detection was 0.028 µg/mL and the limit of quantification was 0.086 µg/mL. Our proposed method proved a good validation profile regarding accuracy, precision, and reproducibility in comparison to the official method. Conclusively, this work suggests the usage of a rapid, sensitive, cheap, and, most importantly, environmentally friendly protocol to determine the REBA either in the presence of its degradant, in its pharmaceutical form, or in human serum.

Keywords: Rebamipide; mifferential pulse voltammetry; multiwall-carbon-nanotube; Mucosta®; biological fluids.

This is an open access article distributed under the CC BY-NC-ND license https://creativecommons.org/licenses/by/4.0/

1. INTRODUCTION

Rebamipide **(Fig. 1)** is used as a gastric mucosal/prostaglandin agonist and gastric agonist; through increasing content of PGE2 in the gastric mucosa and showing a gastrointestinal and cytoprotective effect to prevent mucosal injury[(1)]. Moreover, it can scavange the superoxide production, reduce the hydroxyl radicals and stop the infiltration of the inflammatory cells[(2)]. In addition, REBA plays a vital role as an anti-inflammatory drug and has been used in acute and chronic cases[(3)]. The determination of REBA was reported by numerous methods including the spectrophotometric methods[(4)], chromatographic methods[(5)], spectrofluorimetric methods[(6, 7)] and potentiometric methods[(7)].

Electroanalytical methods are reported to be simple, rapid, sensitive, and cheap method[(8)]. Moreover, the impact on environment hails the electrochemical method as a considerable tool with a high importance to determine and assay the active pharmaceutical substances[(9)]. The electrochemical analysis represents numerous techniques that depend on the chemical reactivity of the sample surface[(10, 11)]. The potentiostat that is connected to electrodes is used to measure the rates of oxidation and reaction rates[(11)].

Carbon paste electrodes (CPEs) have been used to a greater extent in view of their favourable properties such as reproducibility, low price, easy in modifications with many modifiers[9]. The paste blended using two or more nanoparticles could increase the sensitivity as well as stability due to eased electron transfer . carbon nanotubes (CNTs) were found to have exceptional electrical conductivity, high strength, and catalytic behaviour in electrochemical research[9]. The zinc oxide (ZnO) nanoparticles enhance electrical conductivity[9]. Up to date; there is a lack of electrochemical methods

Cite this article: Abdallah O.A., Mohamed T.A., Hendawy H.A., Elomda H. Voltammetric Determination of Rebamipide in the presence of its degradant, in bulk, pharmaceutical preparation, and biological fluids. International Journal of Pharmaceutical and Medical Sciences, 2023; 3(2):133-140. doi: 10.21608/AIJPMS.2023.163295.1171

DOI : 10.21608/AIJPMS.2023.163295.1171

neither less it is importance for supporting the analytical community with a method that can determine the API with high sensitivity and lower cost[12, 13]. In addition, there is no studies measuring the REBA using electrochemical analysis according to our knowledge. Therefore, we aim in this study to investigate the electrochemical method in the assay of REBA among its degredants, in bulk and its pharmaceutical forms.

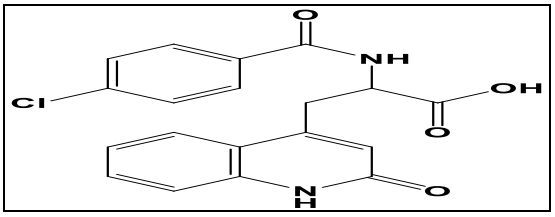

Figure 1. Chemical structure of Rebamipide.

2. METHODS

2.1. Materials and Reagents

The materials, reagents and preparations used throughout this work are mentioned in detail in Supplementary file.

2.2. Instrumentation

2.2.1. Apparatus

Voltammetric measurements were done using Metroham 797 VA Computrace analyzer which is discussed deeply in Supplementary file.

2.2.2. Working electrodes

The composition of the working electrodes is discussed in detail in Supplementary file.

2.2.3. Stock solution

Stock solution of intact drug and degradant are mentioned in Supplementary file.

2.3. Recommended General Procedures

2.3.1. Determination of the pure form of the used Rebamipide

Method development used for determination of REBA is covered under supplementary file.

2.3.2. Analysis of pharmaceutical tablets

Mentioned in detail in Supplementary file.

2.3.3. Analysis of pharmaceutical tablets

REBA is determined in spiked serum; its preparation and procedures are explained in supplementary file.

2.4. Construction of Calibration Curve

2.4.1. Linearity

For the calibration curve construction, different aliquots concentrations of REBA were moved into the voltammetric cell. Then the cell was completed to 10.0-mL with 0.04M B-R buffer solution (of suitable PH) using a working electrode and recording the differential pulse voltammograms. The construction of calibration curves was done afterwards and excel was used to compute the regression equation.

Regression parameters such as slope, intercept, and correlation effect were calculated and presented.

2.4.2. Accuracy

Accuracy of the method was determined through applying the proposed procedure for the determination of 5 different concentrations of REBA, concentrations were measured by the regression equation. The accuracy was presented as recovery (R%).

2.4.3. Precision

Precision was evaluated as both repeatability and intermediate precision.

2.4.3.i. Repeatability

The different concentrations of REBA were analyzed three times for each on the same day (intra-day).

2.4.3.ii. Intermediate Precision

The samples of REBA were analyzed on three successive days by two analysts. The relative standard deviation of the responses was determined as (RSD %).

2.4.4. Robustness

Robustness of the proposed method was evaluated by repeating the mentioned procedure with slight changes to pH or accumulation time. Only one parameter was modified in each case in order to insure robustness.

2.4.5. Limit of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) were measured. Our results are illustrated in Table (1). LOD and LOQ were calculated by the following equations as been mentioned in Miller and Miller (15)

$LOD = 3\ Sy/x / b$

$LOQ = 10\ Sy/x / b$

Where Sy/x = error standard deviation, b = the slope of linearity.

2.4.6. Specificity

Laboratory prepared mixture solutions of REBA and different concentrations (1-50%) of its alkaline degradant were measured.

3. RESULTS AND DISCUSSION

3.1. Analysis of REBA Response at Different Working Electrodes

To comprehend the electrochemical process going on the working electrodes surfaces, differential pulse voltammetric technique was applied using different types of electrodes. As shown in the figure, it was found that the ZnONP/CNT/ME peak was higher than those of GPE and CPE. This result means that the electronegativity of the ZnONP/CNT/ME surface area is larger, which may be dated to the porosity of the ZnONP/CNT/ME. Subsequently, the ZnONP/CNT/ME proved to have lower detectability and higher sensitivity (Fig.2).

Table 1. Regression table for the REBA determination by the proposed A-DPV method in a B-R buffer of pH 11.0 under optimized conditions through ZnO-NPs/CNTs/CPE.

Parameters	The proposed method
Linearity	
Linearity range(µg/mL)	0.09-12.37
Slope	0.305
SD of slope (S_x)	0.003
Intercept (b)	0.306
SD of intercept (S_y)	0.004
Regression SD ($s_{y/x}$)	0.045
Correlation coefficient (r)	0.999
LOD (µg/mL)	0.028
LOQ (µg/mL)	0.086
Accuracy[a] (Mean ± SD)	100.05±0.95
Specificity (Mean ± %RSD)	100.02±1.01
Precision (%RSD)	
Repeatability*	0.140
Intermediate Precision**	0.840

[a] mean of 5 determinations.

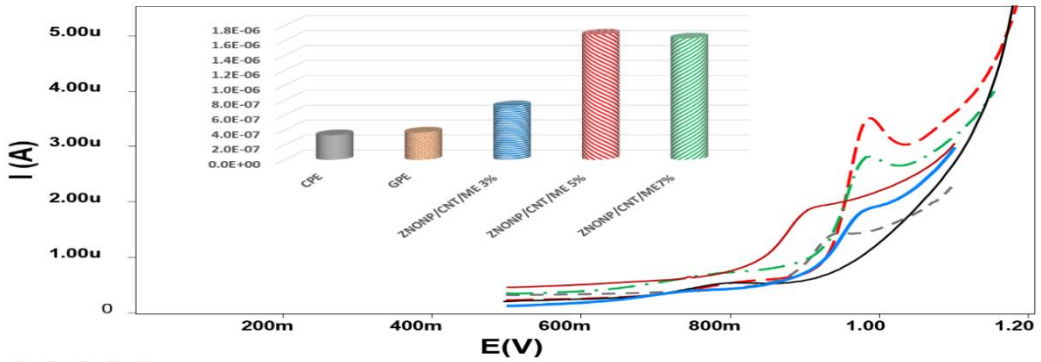

Figure 2. Comparative Behavior of Cyclic Voltammetric of REBA at working electrodes.

3.2. Optimization of Experimental Parameters

Different universal buffer solutions (Britton-Robinson (BR)) at different pH values (from 2 to 12) were prepared by mixing the acid mixture of 0.04M (M) of phosphoric, acetic, and boric acids. The necessary amounts of 0.2 M NaOH was added to reach the needed pH.

The voltammetric behavior of REBA was studied through different electrodes; CPE, PGE, and ZNONP/CNT/ME using 0.04M Britton-Robinson BR buffer solution as supporting electrolyte. We investigated numerous chemical and electrochemical

parameters to visualize the voltammetric behavior of REBA. Applying Cyclic voltammetry technique, REBA showed an anodic peak at a potential around 0.936 V with no peak on the reverse scan. This finding suggest that the reaction was totally irreversible.

3.2.1. Type and pH of the supporting electrolyte

Different types of buffers (borate, citrate, acetate, phosphate, and B-R) were used to study the influence of buffer type on the voltammetric responses of REBA. B_R buffer showed the best results concerning sharp peak height and sensitivity. Thus, B_R buffer of pH range between 2.0 and 12.0 was chosen to fulfill the following study at pulse amplitude, 0.050 V; pulse time, 0.04 s; sweep rate, 0.050 V.s-1; voltage step, 0.005 V; and voltage step time, 0.1s at ZnO-NPs/CNTs/CPE (Fig. 3). The anodic peak height raised from pH=2.0 to pH = 11.0 at four electrodes. Then it decreased at pH=12 and the peak height showed a significant increase making the buffer solution with pH 11.0, the buffer of choice for the subsequent voltammetric experiments. The reported data showed that the increase in pH of the solution shifted the oxidation peak towards the negative direction. A linear relationship was found between the pH ranges of 2.0 to 11.0. (Supplementary file; Figure S2).

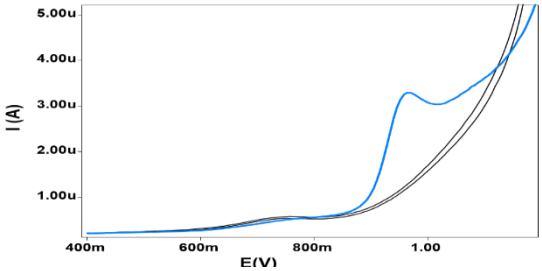

Figure 3. DP voltammograms (anodic peak responses) of REBA and its corresponding oxidative degradant at ZnONP/CNT/ME at pulse amplitude, 0.050 V; pulse time, 0.04 s; sweep rate, 0.050 V.s-1; voltage step, 0.005 V; and voltage step time, 0.1 s.

3.2.2. Impact of frequency on peak current & potential

The impact of frequency on the voltammetric response of REBA was investigated under the mentioned experimental conditions (Supplementary file; Figure S3). The frequency was increased from 10 to 100 Hz at a specified concentration of 20µM of REBA.

Linear Randles-Sevcik plots for REBA (plot of IP vs frequency) were obtained. This indicates a typical diffusion-controlled mass transport as shown in (Supplementary file; Figure S3) and was expressed in the following equation:

Log IP (A) = -0.3508log f(Hz)+ 6.3796

(r^2 = 0.977)

Upon increasing the frequency, a positive shift of the anodic peak potentials was noticed along with a rise in currents that confirm that the oxidation process was irreversible.

The plot of the logarithm of peak current vs logarithm of frequency gave a straight line with a slope of 0.5072 ZNONP/CNT/ME, indicating, that the slope value close to the theoretical value of 0.5, an ideal reaction to the diffusion-controlled electrode.

3.3. Drug Oxidation Mechanism at the Electrode Surface

We concluded the oxidation mechanism of the drug at the surface of ZnONP/CNT/ME from the data collected (Table S1). The group with the highest electron density was selected to be the aliphatic N tertiary amine in side chain. Although all the other nitrogen atoms in the compound contain a higher charge density, there is a tutiorism resonance on the binary bond adjacent to this N atoms, in addition to the spatial steric hindrance caused by the structure of compound. It showed that the number of electrons of oxidation transfer was equal to 2.0 electrons, indicating the irreversible nature of the reaction, as presented in the proposed mechanism in Fig.4.

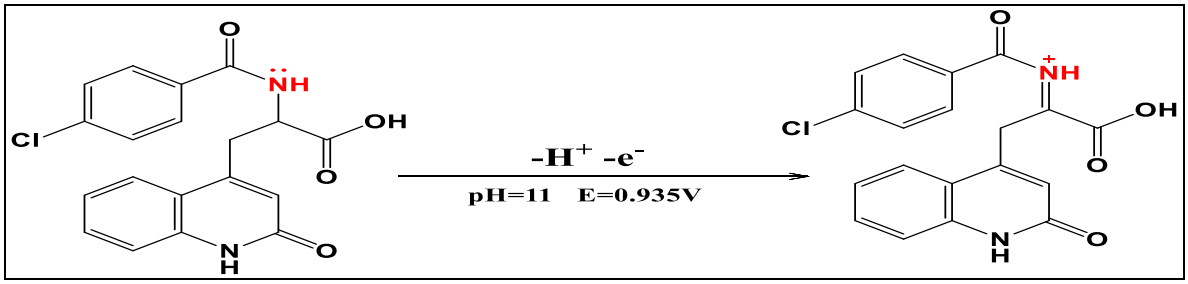

Figure 4. Suggested electrochemical oxidation mechanism of REBA.

3.4. Characterization of the Degradation Products

The suggested oxidative degradation pathways; Fig.5 of REBA were characterized by the cleavage of the azabicyclo ring, resulting in losing electron density on the amine group, thus losing the electroactivity of the studied drug and making it possible to determine the drug in the presence of its oxidative degradant. This was evidenced by the loss of the alkaline degradant's differential pulse anodic peak response; Fig.3

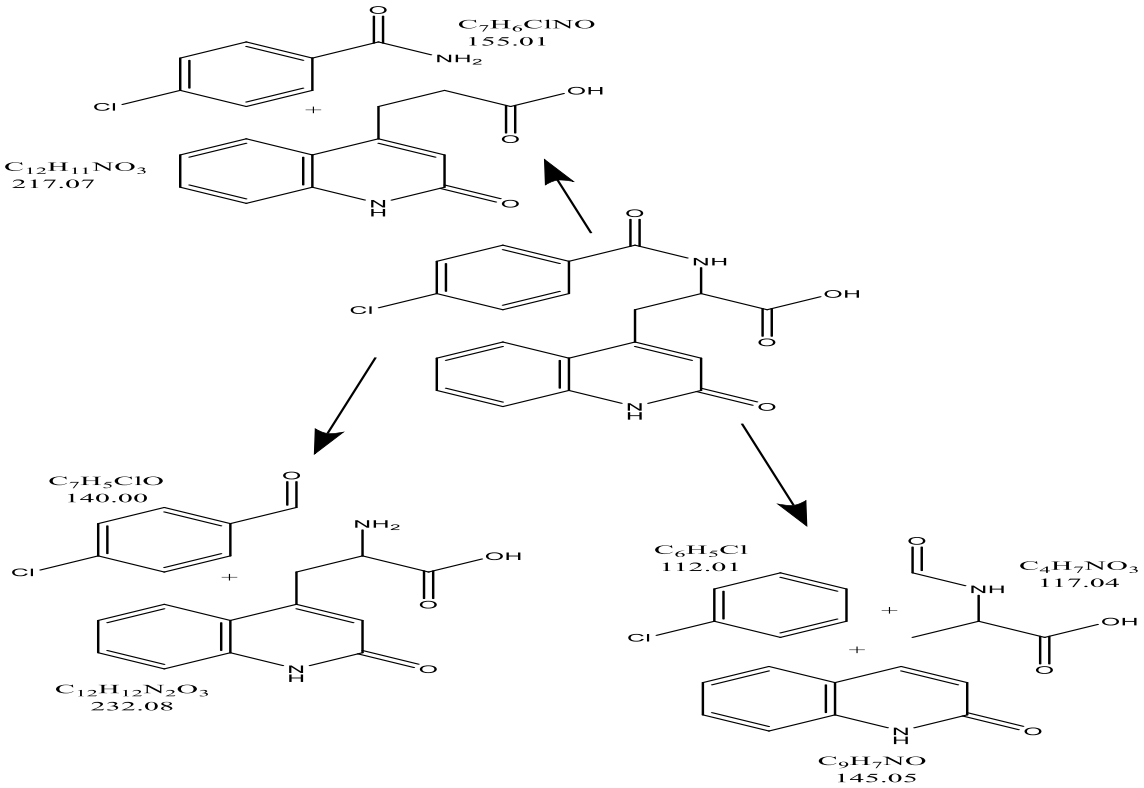

Figure 5. General suggested degradation pathways of REBA.

The prepared alkaline degradant was confirmed by FTIR Figures (S1)(a)(b). The disappearance of N-H medium stretching at 3550 cm^{-1} of IR Spectra of alkaline degradant of REBAwas observed as well as the disappearance of C=O strong stretching at 1750 cm^{-1} of IR Spectra of alkaline degradant of REBA.

3.5. Method Validation

The proposed method was exposed to validation through measuring the specificity, accuracy, precision, and linearity of the DPV method were implemented according to International Conference on Harmonization (ICH) guidelines.[16] Linearity of our method was proved as the voltammograms increases with the increasing amount of REBA. Those results proved considerable linearity with regression parameters calculated, as per Miller and Miller. LOD and LOQ that was reported as 0.242 and 0.801, at the ZNONP/CNT/ME, proved the sensitivity of the proposed method. In order to check the precision; the %RSD values of intra- and inter-day experiments were calculated and found to be less than 2 %. Accuracy was measured by the mean recoveries; and conclusively the proposed method showed considerable accuracy (Table 1, Fig.6). We investigated the interference of excipients and degredants through adding different concentrations of each substance to a fixed amount of 20.0 µM of REBA at PH 11.0. It was found that ascorbic acid, urea and sucrose, at physiological concentrations, did not interfere with the oxidation of REBA at ZnONP/CNT/ME; Table1, Fig.6.

Robustness was checked by studying the effect of changing pH (10.8-11.2), and the accumulation time (8-12 sec), each at a time.

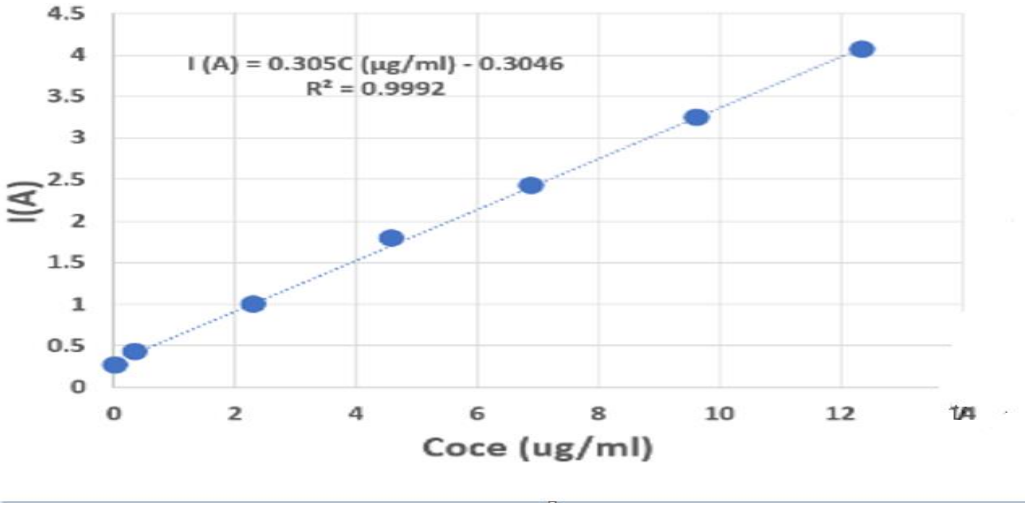

Figure 6. Calibration curve of peak Current of REBA to its Concentration at ZnO-NPs/CNTs/CPE.

It was found that these deliberate variations did not affect the peak response, confirming robustness of the method and the RSD found to be <2% which was also in favor of the developed method, as shown in Table 2.

Table 2. Robustness study for the voltammetric determination of REBA at ZNONP/CNT/ME (DP).

Parameter	ZNONP/CNT/ME	
	pH	**RSD%**
PH	11.2	1.11
	10.8	1.05
	t_{acc}	**RSD%**
Accumulation time	12	1.01
	8	0.12

3.6. Applications

3.6.1. Application to pharmaceutical preparation

Our proposed method managed to determine the REBA in the tablets form (**Mucosta®Tablet**). Our results were in accordance with the concentration labeled on the product as in Table 3. The standard addition technique was used; while the recovery ranged from 99.72% to100.12%, indicating that the proposed method is applicable for REBA determination in its pharmaceutical preparation. The effect of interfering compounds and excipients present was investigated in the interference study. No interference was found from excipients and additives as shown in Table 3. The values of %RSD and mean percentage recoveries were based on an average of three replicates. 99.50±1.10 at ZnO-NPs/CNTs/CPE for REBA showed no significant interference from excipients proving the selectivity of the proposed method. The reported results proved that this method is applicable for REBA determination in its pharmaceutical preparation.

Comparing between the results obtained from the proposed method with those calculated by the reported HPLC method [17] confirmed the sensitivity of the proposed method as shown in table (4).

Table 3. Determination of REBA at ZnO-NPs/CNTs/CPE in Mucosta ®Tablet; application of standard addition technique.

	%Mean± SD[a]	Standard addition technique		
Vials		**Claimed taken (µg/mL)**	**Pure added(µg/mL)**	**% Recovery[a]**
Mucosta®Tablet	99.5±1.10	2.0	3.0	100.12
		2.0	7.0	100.05
		2.0	12.0	99.72
B.N.: A1938		**Mean %RSD**		**99.96 ± 0.214**

[a] Mean of 3 determinations.

Table 4. Statistical analysis of the results of both the proposed and reported method for REBA quantification at ZnO-NPs/CNTs/CPE.

Parameters	Proposed method	Reported HPLC Method[17]*
Mean[a]	100.208	99.95
SD	0.4796	0.5206
Variance	0.230	0.271
N	5	5
Student's t-test (2.306)	0.219	
F-test (6.39)	0.8774	

[a] Mean of five determinations

[b] Values in parentheses are the corresponding theoretical values of t and F at P = 0.05.* REBA HPLC determination on HiQsil C-18 column (250 mm× 4.6 mm, 5 µ), Mobile phase: 0.02M potassium phosphate (pH adjusted to 6.8) : methanol (40:60, v/v), at UV 230 nm. [17]

3.6.2. Application to biological fluids

The current method is successfully employed to determine the REBA in spiked human serum. We added known amounts of REBA to the spiked serum, and we reported all results and recoveries were presented in Table 5. We report no extra noise nor oxidation compounds in the same potential range of the analytical peak. Our recovery records ranged from 99.75% to 100.14% which assures the accuracy of our methods.

Table 5. Accuracy and precision of REBA determination at ZnO-NPs/CNTs/CPE in spiked serum samples.

REBA	Claimed taken (µg/mL)	added (µg/mL)	Recovery [a] (%)	% RSD
ZnO-NPs/CNTs/ CPE	2.5	2.5	99.75	1.17
	2.5	5.0	100.14	1.12
	2.5	10.0	100.05	1.23

[a] Mean of three determinations

4. CONCLUSION

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn. Cost-effective and environmentally friendly method was introduced. This study investigated the application of an electrochemical method for the determination of the concentration of REBA in the presence of its degradant in bulk, pharmaceutical preparation, and biological fluids using the differential pulse voltammetry (DPV). The comparison of the results of three types of electrodes was introduced. The ZnONP/CNT/ME showed the highest electroactivity.

Through this method, we are proposing to the scientific community an eco-friendly, cheap, fast surface renewal, low LOD, repeatable, reliable and high stable method. Therefore, this method can be a replacement for currently existed methods of REBA determination in quality control laboratories. The peak showed linearity in the range of (0.09-12.37 µg/mL), limit of detection was 0.028 µg/mL and the limit of quantification was 0.086 µg/ml. The result demonstrates the need for further research on nanomaterial for determination of drugs in different forms.

Funding: This research was conducted based on no fund or grant from any organization.

Acknowledgments: This work was supported by the Egyptian Drug Authority (EDA).

Conflicts of Interest: No conflict of interests for all the authors.

Author Contribution: All authors contributed substantially to the body of work and have read and approved the final submitted manuscript and no conflict of interest exists.

REFERENCES

1. Arakawa T, Kobayashi K, Yoshikawa T, Tarnawski A. Rebamipide: overview of its mechanisms of action and efficacy in mucosal protection and ulcer healing. Digestive diseases and sciences. 1998;43(9 Suppl):5S-13S.

2. Iijima K, Ichikawa T, Okada S, Ogawa M, Koike T, Ohara S, et al. Rebamipide, a cytoprotective drug, increases gastric mucus secretion in human: evaluations with endoscopic gastrin test. Digestive diseases and sciences. 2009;54(7):1500-7.

3. Naito Y, Yoshikawa T. Rebamipide: a gastrointestinal protective drug with pleiotropic activities. Expert review of gastroenterology & hepatology. 2010;4(3):261-70.

4. Alqarni MA, Moatamed RS, Naguib IA, El Ghobashy MR, Farid NF. Development and Validation of Four Spectrophotometric Methods for Assay of Rebamipide and its Impurity: Application to Tablet Dosage Form. Journal of AOAC International. 2022;105(1):299-308.

5. Sonawane S, Gide P. Optimization of forced degradation using experimental design and development of a stability-indicating liquid chromatographic assay method for rebamipide in bulk and tablet dosage form. Scientia Pharmaceutica. 2011;79(1):85-96.

6. Roy N, Mahato B, Roy D, Das K, Roy MN. Exploring inclusion complexes of cyclodextrins with quinolinone based gastro protective drug for enhancing bioavailability and sustained dischargement. Zeitschrift für Physikalische Chemie. 2021;235(6):723-44.

7. El-Din HAME. Analytical Study of Certain Amide Containing Drugs A Thesis: Cairo University; 2012.

8. Liu Q, Tian J, Cui W, Jiang P, Cheng N, Asiri AM, et al. Carbon nanotubes decorated with CoP nanocrystals: a highly active non-noble-metal nanohybrid electrocatalyst for hydrogen evolution. Angewandte Chemie International Edition. 2014;53(26):6710-4.

9. Malode SJ, Keerthi PK, Shetti NP, Kulkarni RM. Electroanalysis of carbendazim using MWCNT/Ca-ZnO modified electrode. Electroanalysis. 2020;32(7):1590-9.

10. Grieshaber D, MacKenzie R, Vörös J, Reimhult E. Electrochemical biosensors-sensor principles and architectures. Sensors. 2008;8(3):1400-58.

11. Dryden MD, Wheeler AR. DStat: A versatile, open-source potentiostat for electroanalysis and integration. PloS one. 2015;10(10):e0140349.

12. Shah M, Patel N, Tripathi N, Vyas VK. Capillary electrophoresis methods for impurity profiling of drugs: A review of the past decade. Journal of Pharmaceutical Analysis. 2021.

13. Scholz F, Meyer B. Electrochemical solid state analysis: state of the art. Chemical Society Reviews. 1994;23(5):341-7.

14. Shalaby A, Hassan WS, Hendawy HA, Ibrahim A. Electrochemical oxidation behavior of itraconazole at different electrodes and its anodic stripping determination in pharmaceuticals and biological fluids. Journal of Electroanalytical Chemistry. 2016;763:51-62.

15. Mller J, Miller J. Statistics and chemometrics for analytical chemistry Harlow: Pearson Prentice Hall, pp-263. 2005.

16. Branch SK. Guidelines from the international conference on harmonisation (ICH). Journal of pharmaceutical and biomedical analysis. 2005;38(5):798-805.

17. Sonawane S, Gide PJSp. Optimization of forced degradation using experimental design and development of a stability-indicating liquid chromatographic assay method for rebamipide in bulk and tablet dosage form. 2011;79(1):85-96.