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Voltammetric method for determination of Palonosetron Hydrochloride in the presence of its degradant using synergistic effect of ZnO nanoparticles and carbon nanotubes in micellar media

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Abstract

A highly sensitive nano-materials modified electrode was fabricated for the quantification of Palonosetron Hydrochloride (PAL) using anodic differential pulse voltammetry (A-DPV) for the first time. The synergetic effect of Multi-wall Carbon nano-tubes and nano-zinc oxide particles was utilized for the fabrication of the modified electrode (ZnO-NPs/CNTs/CPE). PAL oxidative degradant was prepared and the degradant's identification and degradation pathway illustration were done by NMR and mass spectrometry. PAL quantification in 0.04M B-R buffer electrolyte solution (PH 7.0) using ZnO-NPs/CNTs/CPE showed peak response with linearity in the concentration range 6.50– 38.50 µM. The peak response showed a remarkable increase in micellar media upon the addition of Sodium Lauryl Sulfate (SLS). The minimum detectability (LOD) of PAL was 1.19 µM and the limit of quantification (LOQ) was 3.61 µM. The validation of the proposed method showed good accuracy, precision, and reproducibility compared to the official method. The approached technique which can be described as an environmentally friendly technique is rapid, sensitive, and inexpensive for PAL determination in the presence of its oxidative degradant, in its pharmaceutical vials, and biological fluids.

Keywords:

Palonosetron Hydrochloride, differential Pulse voltammetry, multiwall carbon nanotube, Aloxi® injection vials, electrode, biological fluids

1.Introduction

Palonosetron Hydrochloride (PAL) a drug found in Aloxi® ampoules (3aS)-2-(3S)-1-Azabicyclo[2.2.2]oct-3-yl-2,3,3a,4,5,6-hexahydro-1H-benz[de]isoquinolin-1-one monohydrochloride (fig1) is a serotonin antagonist that is used to treat and prevent chemotherapy-induced nausea and vomiting (CINV) and to control delayed CINV in particular. There is speculative data suggesting that PAL might be more effective than Granisetron.[1,2] PAL is significant for its extended duration of action, due to its prolonged half-life and high receptor-binding.[3,4] In addition to the USP official monograph[5], the literature review of the mentioned drug revealed chromatographic methods; HPLC[6–12], HPTLC[13,14] and MEKC[15], and spectrophotometric methods[16,17]. However, to our knowledge, the cited drug has not been investigated by any electrochemical methods neither as raw material, pharmaceutical dosage form nor applied in serum samples.

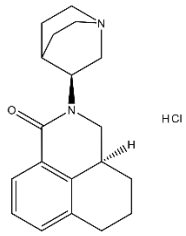

Fig1: Chemical structure of Palonosetron Hydrochloride (PAL).

Thus, the need for drug determination using electrochemical methods is due to the specificity, selectivity, low cost, and short duration of such

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technique. It is also proved that voltammetry is a simple, rapid, accurate, and very sensitive method of analysis used for both qualitative and quantitative analysis.[18,19] It can also be described as an environmentally friendly technique as there is no need for several complex steps, consequently using much fewer chemicals compared to other analytical methods.[20] The investigated redox characteristics of each drug can also be very useful in studying its pharmaceutical activity and metabolic density. Several techniques such as cyclic voltammetry (CV), square wave voltammetry (SWV), and differential pulse voltammetry (DPV) are employed for the study of the redox characteristics of various molecules and drugs using different kinds of electrodes such as Glassy carbon electrode, carbon paste electrode, graphite pencil electrode, and other modified electrodes.[21,22] Carbon paste electrode (CPE) is a carbon-based electrode that is widely used due to the simplicity of its fabrication and preparation, high surface regeneration and renewability and, high sensitivity and low cost.[23] Nanoparticle modified electrodes are highly sensitive and of lower detection limits as nanoparticles possess high electro-catalytic effect and can be modified according to the need thus it is highly used nowadays in sensors and biosensors.[24–26] Carbon nano-tubes modified electrodes (CNT/ME) show high mechanical strength and chemical stability, in addition to its surface that is characterized by its large pores and its insolubility in most solvents. Metal oxides are of high electrical conductivity and therefore better sensitivity especially when used in their nanostructure as they possess higher surface area and electrocatalytic activity and strong adsorbability, therefore electrodes modified with metal oxides nanoparticles such as zinc oxide nano-particles (ZnONP) are widely used nowadays.[27,28]

The study of electrochemical characters in aqueous and micellar media revealed the great impact of adding different surfactants on enhancing the sensitivity of some methods, as they enhance the solution /electrode interface property. The addition of anionic surfactants such as Sodium Lauryl Sulfate (SLS) leads to faster charge transfer, hence better pre-concentration and accumulation on the electrode surface and higher sensitivity.[29,30]

Based on the lack of electrochemical methods and the increased importance of such methods, our main objective is to develop a novel, simple, rapid, accurate, and selective voltammetric method that is capable of the determination of PAL as a single drug and in the presence of its degradation products and that can also be applied to biological fluids and pharmaceutical preparations.

2. Materials and methods

2.1. Chemical Reagents and Materials:

-PAL standard of purity (99.9%) on anhydrous basis, was kindly supplied by EDA.

- Aloxi ® injection was bought from a local market, Each 5-ml vial contains 0.25 mg palonosetron base as hydrochloride, disodium edetate, 207.5 mg mannitol, and citrate buffer in water (PH 4.5 to 5.5), manufactured by Cardinal Health, Albuquerque, NM, USA for Helsinn Healthcare SA, Switzerland.

- Graphite powder (of particle dimension 20 µm), nano-zinc oxide (crystal diameter around 5 nm), multiwalled carbon nanotubes (of purity >95%) were purchased from Sigma-Aldrich.

-Deionized and bi-distilled water utilized throughout the whole work was provided by EDA and referred to as "water".

-0.04 M Britton-Robinson (B-R) buffer was prepared by adding 0.04 M boric acid, 0.04 M orthophosphoric acid, and 0.04 M glacial acetic acid, and adjusted by using 0.2 M sodium hydroxide to obtain the required PH over the range 2-10.[31]

- Cetylpyridinium chloride (CPC), Cetrimonium bromide (CTAB), Tween80, Triton-X 100, Sodium stearate, and Sodium lauryl sulfate (SLS) were provided by EDA.

- Human blank serum was obtained from the holding company for biological products and vaccines (VACSERA, Egypt).

-All reagents, solvents, and chemicals utilized were of analytical grade and were supplied by Sigma-Aldrich.

2.2. Instrumentation

2.2.1. Apparatus: A Computrace electrochemical analyzer with 797VA Computrace software (1.0) from Metrohm, Switzerland was used to perform voltammetric measurements. The apparatus was equipped with a three-electrode cell: working electrode, Reference electrode: Ag/AgCl (3M KCl), and counter electrode: a platinum wire. Electrical contact with the working electrodes was achieved by soldering a copper wire to the contact metallic part of the apparatus. All PH measurements were conducted by a Jenway 3330 Research pH meter. Deionized water and Bi-distilled water were supplied by a Hamilton Aqua-Metric deionized water system. A temperature of 25°C was maintained during the

performance of all experiments. The data processing was done using Microsoft Excel.

2.2.2. Working Electrodes:

Carbon paste electrode (CPE): the carbon paste electrode (CPE) is made by blending conducting graphite powder with a pasting liquid.[32] 250 mg of sigma graphite powder was mixed with 125 mg paraffin oil to prepare CPE. The resulting paste was then loaded into the end of an insulin syringe (internal diameter: 3.0 mm). External electrical contact is set by pushing a copper wire down the syringe.

Multi-wall carbon nano-tubes modified nano-zinc oxide electrode (ZnO-NPs/CNTs/CPE): preparation of the carbon paste was done by mixing 425 mg of graphite powder, 25 mg of Zinc oxide nano-particle powder, and 50 mg of MWCNT powder several times to get a homogenous mixture in a mortar then adding 0.3 mL of paraffin oil to make a homogenous paste. A portion of carbon paste was packed into the hole of the insulin syringe body of diameter 3.0 mm which contained a copper wire that contacted the apparatus. The remaining paste stock was refrigerated at temp 8.0 °C and was valid for use without significant change in sensitivity for 1 month [33]

2.2.3. Stock solution:

An appropriate weight of PAL raw material was dissolved in 50.0ml water and sonicated for 5 mins, then transferred to a 100ml volumetric flask and completed to mark with water to reach 0.001M. The stock solution was freshly prepared on the day of analysis.

To prepare the oxidative degradant, 10 ml of 6.0% (v/v) H_2O_2 was added to PAL and was left to set aside for 45 min. The degradant prepared was evaporated till dry in a rotary evaporator. The degradant's residues were then collected and dissolved in methanol, filtered, and evaporated under vacuum.[34] They were subsequently utilized for the elucidation of their structure and degradation pathway using NMR and Mass spectrometry (illustrated in supplementary data Fig S1 (a), Fig S1 (b), Fig S2).

The same procedure applied to preparing stock standard solutions was used and the degradant's residue was dissolved in 100 ml of water, to obtain a final concentration of 100.0 µg/ml. Water was then used to dilute the solution to reach a working degradant solution concentration of 10.0 µg/ml.s

2.3. Recommended General Procedures:

An appropriate volume of stock solution was added to a 10ml volumetric flask and completed to mark with suitable electrolyte buffer (B-R) of PH 7.0 then transferred into the voltammetric vessel to undergo measurements. The test solutions were purged with nitrogen for 5 sec. The working electrode was retained at the favorable accumulation potential for a certain period of time, whereas the solution was stirred at around 2000 rpm throughout that accumulation period. The stirring was subsequently stopped and the solution was allowed to rest for 5s, a scan was carried out afterward towards positive potentials over the range 0.3 to +1.3 V, and the voltammograms were recorded. The experimental conditions for anodic differential pulse voltammetry (A-DPV) were: sweep rate, 0.050 $V.s^{-1}$; voltage step, 0.005 V; voltage step time, 0.1 s; pulse amplitude, 0.050 V; and pulse time, 0.04 s.

2.4. Construction of calibration curve:

For the calibration curve construction, different aliquots concentrations (from, 50.0 µL to 1.0 mL) of PAL, were separately transferred into the voltammetric cell and completed to 10.0-mL with B-R buffer solution (of suitable PH) employing working electrode and recording the differential pulse voltammograms. The construction of calibration curves was done afterward, and Excel was used to compute the regression equation.

2.5. Analysis of Pharmaceutical Vials:

For the determination of pharmaceutical preparation, an appropriate amount of Aloxi® vial (0.05 mg/ml), was pipetted into a 10mL calibrated flask and was diluted up to the mark with deionized water without any further treatment. Appropriate aliquots were added to 10.0 mL of B_R buffer solutions (pH7.0) and transferred into the voltammetric cell. The linear regression equation attained from the calibration curve was then used to estimate the amount of PAL per vial. The standard addition method was employed by spiking the pharmaceutical preparation with different quantities of the standard drug. The differential pulse voltammograms were recorded according to the mentioned voltammetric procedure. The %RSD and recoveries were calculated afterward.

2.6. Spiked serum analysis

To a volume of 0.3 mL serum, 0.3 mL 5% ZnSO4 and 5 mL ethanol were added, the mixture was then centrifuged for 15 min at 13,000 rpm, and 1 ml of the clear solution was separated and added to 9 mL B-R buffer solution. The mixture was de-aerated for 5 min and spiked with 50 ng/mL of PAL. The procedures then follow as mentioned previously.[35]

3. Results and discussion:

3.1. Analysis of PAL 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. PAL presented a well-defined single anodic peak. The voltammetric response of PAL was studied using CPE and ZnO-NPs/CNTs/CPE. Differential voltammograms of 20 µM of PAL was recorded in B_R buffer solution (pH 7.0) at sweep rate, 0.050 V.s^{-1}; voltage step, 0.005 V; voltage step time, 0.1 s; pulse amplitude, 0.050 V; and pulse time, 0.04 s. presented a well-defined single anodic peak at around 1.20 V (Fig2).

When the voltammograms obtained from the different working electrodes were compared, the peak responses at CPE appeared to be broad and weak; whereas the current intensity of the oxidation peaks from ZnO-NPs/CNTs/CPE was much higher as compared to those from CPE. When surfactant Sodium lauryl sulfate (SLS) (0.001M) was used with electrolyte as illustrated in fig2, the peak height showed an increase underlying the same parameters. As shown in the figure, the current peak height of ZnO-NPs/CNTs/CPE was so much higher than that of CPE; assuming that the electro-activity of the surface area of ZnO-NPs/CNTs/CPE is larger than that of CPE. This significant increase in the peak current can be attained to the porosity of ZnO-NPs/CNTs/CPE,[36] Predicting lower detectability and higher sensitivity. Consequently, the electrode of choice that was used throughout the study was ZnO-NPs/CNTs/CPE.

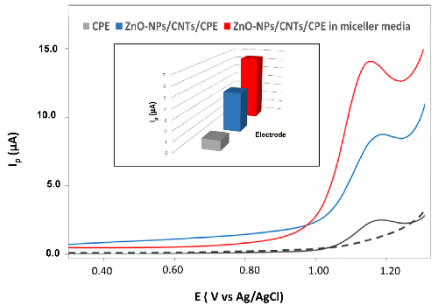

Fig2: Comparative Voltammetric Behavior of PAL at working electrodes.

3.1.1. Morphology of electrode surfaces:

The morphology of electrode surfaces was investigated using a scanning electron-microscope (SEM) as displayed in fig3. AS shown in the figure the electrode ZnO-NPs/CNTs/CPE has a highly porous surface with a flower shape which increases the active surface area compared with CPE so that the electro-activity of the selected electrode (ZnO-NPs/CNTs/CPE) is higher in sensitivity and selectivity for the detection of PAL.

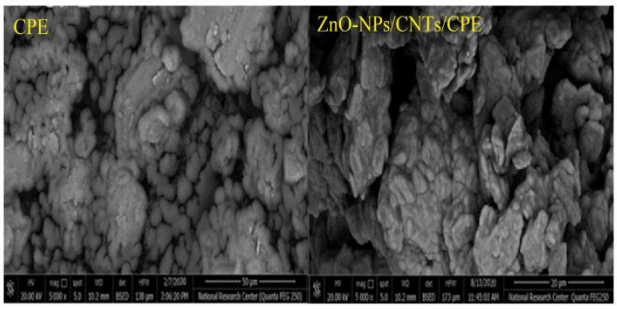

Fig3: SEM images of surface morphology of different electrodes.

3.1.2. The surface area of electrodes:

Randles – Sevcik (R-S) equation was used to calculate the surface area of the modified electrode based on the slope of the I_p vs. $v^{1/2}$ plot using 1.0x10^{-3} M $K_4Fe(CN)6$/0.1 M KCl:[28,29]

$$I_p = 2.69 \times 10^5 \, n^{3/2} \, AD^{1/2} \, Cv^{1/2} \qquad (1)$$

Where I_p refers to the peak current, n refers to the number of electrons transferred, A to electrode area, D to the diffusion coefficient (7.6×10^{-6} cm^{2}s^{-1}), C to the redox concentration, and v to the applied voltammetric scan rate. The electrochemically active areas of the CPE and ZnO-NPs/CNTs/CPE electrodes were found to be 0.025 and 0.174 cm^2 respectively

3.2. Proposed mechanisms:

3.2.1. Drug oxidation mechanism at the electrode surface:

The oxidation mechanism of the drug at the surface of ZnO-NPs/CNTs/CPE was postulated from the data collected from the molecular orbital calculation (Table S1) and the group with the highest electron density was selected to be the tertiary amine in azabicyclo ring[39–42] and it showed that the number of electrons involved in oxidation transfer was equal to 1 electron, indicating that the reaction is irreversible, as presented in the proposed mechanism in fig8.

The Oxidation mechanism was also elucidated using scan rate:

The Linear relation between peak potential and the natural logarithm of scan rate can be expressed as;

$E\ (V) = 1.195 + 0.018 \ln v\ (V\ s^{-1})$

For the totally irreversible electrode process, the relationship between the oxidation peak potential and scan rate is described by Laviron equation[43][44]

$$E_p = E^\circ - \left(\frac{RT}{\alpha nF}\right) \ln\left(\frac{RTk^\circ}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \ln v$$

The slope is 0.018 and α n was calculated to be 1.1. α was calculated to be 0.9 from Tafel plot. Therefore, the

value of n ≈1 was obtained. (Supplementary data Fig S3, Fig S4)

3.2.2. Characterization of the Oxidative-degradant:

The suggested oxidative degradation pathways (fig9) of PAL was characterized by the cleavage of the azabicyclo ring resulting in losing electron density on the amine group thus losing the electro-activity 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 differential pulse anodic peak response of the oxidative degradant. (fig4)

Fig

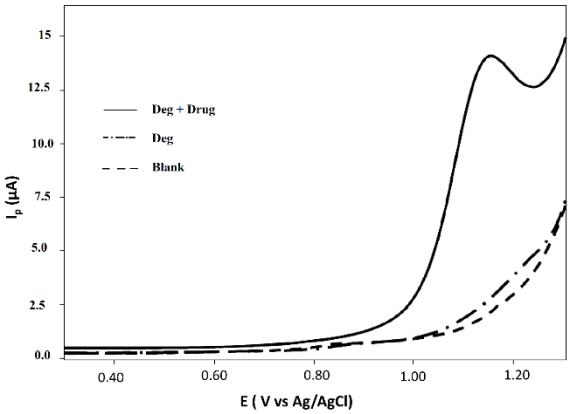

Fig4: DP voltammograms (anodic peak responses) of PAL and its corresponding oxidative degradant at ZnO-NPs/CNTs/CPE at pulse amplitude, 0.050 V; pulse time, 0.04 s; sweep rate, 0.050 V.s^{-1}; voltage step, 0.005 V; and voltage step time, 0.1 s.

3.3. Optimization of Experimental parameters:

3.3.1. 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 PAL. B_R buffer showed the best results concerning sharp peak height and sensitivity. Thus, B_R buffer of pH range between 2.0 and 10.0 was chosen to fulfill the following study at sweep rate, 0.050 V.s^{-1}; voltage step, 0.005 V; voltage step time, 0.1 s; pulse amplitude, 0.050 V; and pulse time, 0.04 s. at ZnO-NPs/CNTs/CPE. A sharp decrease in the anodic peak current was observed upon rising pH in the basic media, (Fig5). Thus, No pH values higher than 10.0 were used in this investigation. A linear dependence of the anodic peak potential (E) and PH was then concluded over the range of 2.0–10.0 as shown in Fig5. This was expressed by the equation:

E (V) = -0.0529pH + 1.5288 (r^2 = 0.9878)

It was found that with the rise in pH, the peak potential shifted towards the negative potential and the peak height showed a great increase making the buffer solution with pH 7.0, the buffer of choice for the subsequent voltammetric experiments.

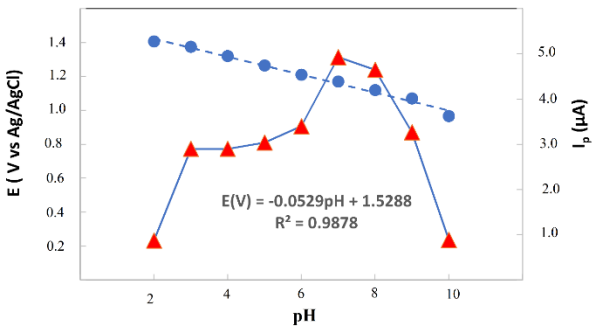

Fig5: The effect of PH on the peak current (I) and potential (E) of 20µM of PAL at ZnO-NPs/CNTs/CPE.

3.3.2. Influence of frequency on peak current and potential:

The influence of frequency on the voltammetric response of PAL was investigated under the mentioned experimental conditions (Fig6). The frequency was increased from 10 to 100 Hz at a specified concentration of 20µM of PAL.

Linear Randles-Sevcik plots for PAL (plot of I_P vs. frequency) were obtained indicating a typical diffusion-controlled mass transport[45,46] as shown in (Fig6) and was expressed in the following equation:

Log I_P (A) = -0.5072log f(Hz) + 6.0773 (r^2 = 0.9888)

Upon increasing the frequency, a positive shift of the anodic peak potentials was noticed along with an increase in currents, confirming the irreversible nature of the oxidation process.[46,47]

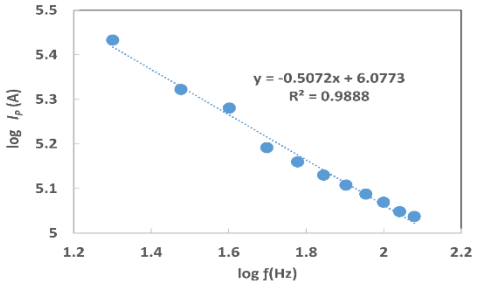

Fig6: Plot of log Frequency (Hz) vs peak current (I_P) of 20µM of PAL at ZnO-NPs/CNTs/CPE.

3.3.3. Influence of Surfactants:

The use of surfactants makes it possible to improve analytical characteristics and, in some cases, to simultaneously determine different analytes.[48]

Six successive additions of different surfactants: cationic surfactants (CPC, CTAB), non-ionic surfactants (Teen80, Triton-X 100), and anionic surfactants (Sodium stearate, SLS), were added to 20µM of PAL in B_R buffer, pH 7.0 at ZnO-NPs/CNTs/CPE and their differential pulse voltammetric responses were studied. No significant change in the peak height nor the peak position was observed upon adding any surfactant other than SLS. There was an increase in peak height and a negative shift of the peak position upon the addition of SLS (as displayed in fig7).It is possible that SLS solves the problem of low currents in the analysis of aqueous solutions. [48]

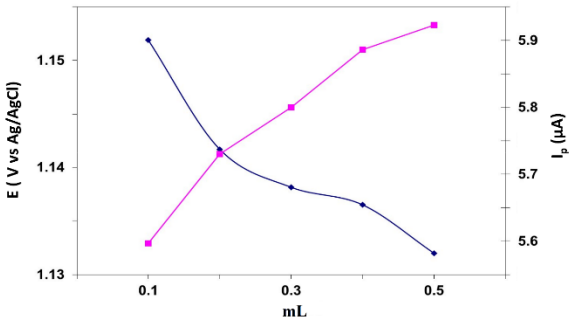

Fig7: The effect of surfactant (MLS added of SLS) on the peak current (I) and potential (E) of 20µM of PAL at ZnO-NPs/CNTs/CPE.

The effect of surfactant can be explained by the fact that the amphiphilic structure of surfactants and their assembly in aqueous solution provides a multifunctional environment for the solubilization and partitioning of aqueous soluble and insoluble compounds [49]

3.4. Method Validation:

In order to validate the proposed method, specificity, precision, accuracy, and linearity of the DPV method were processed according to International Conference on Harmonization (ICH) guidelines.[50]

3.4.1. Linearity:

In this investigation, the quantification of PAL at ZnO-NPs/CNTs/CPE was based on the extent of the dependence of peak current (I) upon its concentration in the analyzed solution under the mentioned optimum experimental parameters, and the calibration curve was made accordingly. A linear relationship was established over was linear over the concentration ranges 6.50-38.50 µM (fig10). The corresponding regression equation was computed in (Table 1).

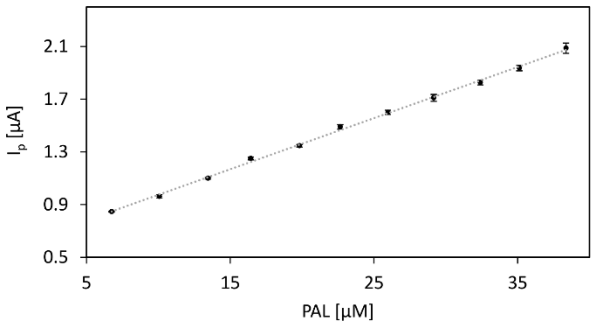

Fig10: Calibration curve of concentration vs peak current of PAL at ZnO-NPs/CNTs/CPE.

Table 1: Characteristics of the calibration curves and validation data of A-DPV method for PAL in a B-R buffer of pH 7 under the optimized conditions at ZnO-NPs/CNTs/CPE

Parameters	PAL
Linearity	
Linearity range(µM)	6.50– 38.50
Slope	0.043
SD of slope	0.0006
Intercept (b)	0.5872
SD of intercept	0.01554
Correlation coefficient (r)	0.9975
LOD (µM)	1.19
LOQ (µM)	3.61
Accuracy[a] (Mean ± SD)	99.97 ±0.02
Specificity[b] (Mean ± %RSD)	100.00 ± 0.056
Precision (%RSD)	
Repeatability	0.55
Intermediate Precision	1.22

[a]n = mean of five determinations.

[b]n = 11.

3.4.2. Limits of Detection and Quantification:

The limit of detection (LOD) and the limit of quantification (LOQ) of PAL at ZnO-NPs/CNTs/CPE were calculated from the following equations:

LOD=3.3 σ/S, LOQ=10 σ/S (where σ is the standard deviation of the peak current in µA, and S is the slope of the calibration curve).

Both LOD and LOQ values in (Table 1) were compared with those calculated by the official HPLC

method[5] confirming the sensitivity of the proposed method.

3.4.3. Accuracy, Repeatability, and Intermediate Precision:

Triplicates of three different concentrations of standard PAL were measured on the same day and for three separate days to examine repeatability and intermediate precision, demonstrating the results' reproducibility of the proposed method. The precision of the proposed method was proved by the fact that the %RSD values of intra- and inter-day experiments were less than 2% (table1). The accuracy of five different concentrations of PAL was calculated, giving satisfying mean recoveries (table1).

Statistical comparison[41,51] using Student's t-test and variance ratio F-test was done to compare the proposed method results with those of the official HPLC method[5] revealing no significant difference between the two methods regarding accuracy and precision (Table 2).

Table 2: Statistical analysis of the results obtained by the proposed and official method for PAL quantification at ZnO-NPs/CNTs/CPE.

Parameters	Proposed method	Official HPLC Method[5]*
Mean[a]	100.048	100.012
SD	0.068	0.0356
Variance	0.00457	0.00127
N	5	5
Student's t-test (2.306)	1.05	
F-test (6.39)	3.388	

[a] Mean of five determinations,

[b] Values in parentheses are the corresponding theoretical values of t and F at P = 0.05.

* PAL HPLC determination on L7 packing column (250 mm× 4.6 mm, 5 µ), Mobile phase: Acetonitrile: water: trifluoroacetic acid (280:720:0.67), flow rate: 1.0 ml/min, at UV 210 nm.[5]

3.4.4. Specificity and Interference Study

Laboratory prepared mixture solutions of PAL and different concentrations (1-50%) of its oxidative degradant were measured, and their results were collected and used to calculate the mean recovery and %RSD (Table1). It indicated the high specificity of the developed method, and this was attributed to the drug oxidation mechanism in relation to the oxidative degradation pathway (presented in fig8 and fig9).

The effect of interfering compounds and excipients present in the pharmaceutical vials was investigated in the interference study. It was achieved by separately adding different concentrations of each substance to a solution of a fixed amount of 20.0 µM of PAL at PH 7.0. It was found that Mannitol, Edetate disodium dihydrate, Trisodium citrate dihydrate, and Citric acid monohydrate did not significantly interfere with the oxidation of PAL at ZnO-NPs/CNTs/CPE. The values of mean percentage recoveries and %RSD based on an average of five replicates, 99.92 ± 0.241 at ZnO-NPs/CNTs/CPE for PAL showed no significant interference from excipients proving the selectivity of the proposed method.

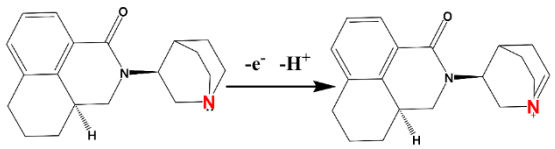

Fig8: The suggested electrochemical oxidation mechanism of PAL.

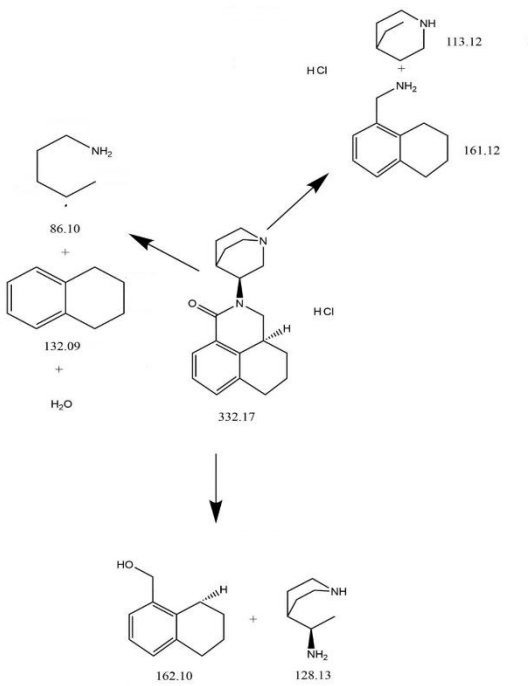

Fig9: General suggested oxidative degradation pathway of PAL.

3.5. Applications:

3.5.1. Application on Pharmaceutical formula (Vials):

The proposed method was successfully used for the determination of PAL in its pharmaceutical preparation (vials). The results were in agreement with the content marked on the label (Table 3). A standard addition technique was used, and the recovery ranged from 99.50% to100.05%, indicating that the proposed method is applicable for PAL determination in its pharmaceutical formula (vial).

Table 3: Determination of PAL at ZnO-NPs/CNTs/CPE in its pharmaceutical vials; application of standard addition technique

Vials	% Found ± SD[a]	Standard addition technique		
		Added (µM)	founded(µM)	% Recovery[a]
aloxi® **Labeled (0.25gm/vial)** B.N.: 38000761	100.04 ±0.311	6.50	6.51	100.10
		16.00	15.92	99.50
		22.00	22.00	100.00
		30.00	30.02	100.05
		35.00	34.98	99.95
		Mean %RSD		**99.92 ± 0.241**

[a] Mean of three determinations.

Table 4: Accuracy and precision of PAL determination at ZnO-NPs/CNTs/CPE in spiked serum samples

PAL	Added conc. (µM)	Found conc. (µM)	Recovery [a] (%)	% RSD
ZnO-NPs/CNTs/CPE	2.0	2.02	101.00	1.820
	2.4	2.36	98.33	0.679
	2.8	2.81	100.36	0.463

[a] Mean of three determinations.

3.5.2. Application on biological fluids:

The proposed method was applied to the determination of PAL in spiked serum samples. Known amounts of PAL were added to spiked serum and the results and recoveries were collected and calculated (table 4). No extra noise peaks nor oxidation compounds appeared in the same potential range where the analytical peak appeared. The recovery experiments ranged from 98.33% to 101.00% assuring the accuracy of the proposed method. The results indicated that the proposed method is applicable for the determination of PAL in spiked serum.

4. Conclusion:

To the best of our knowledge, this paper demonstrates the ability to quantitatively determinate PAL using an electrochemical method for the very first time as a single drug, in its pharmaceutical vials and biological fluids. Differential pulse voltammetry (DPV) was used as its highly sensitive, simple, accurate, and precise. The paper also provides a comparison between CPE and ZnO-NPs/CNTs/CPE at PH 7.0 showing a higher electro-active surface area of ZnO-NPs/CNTs/CPE and even higher when adding SLS surfactant. This method can also quantitatively determine PAL in the presence of its oxidative degradant. The electrodes used offer advantages to the method over any other method as they are Eco-friendly, of low cost, fast surface renewal, and low detection limits. It can also show fast response, good repeatability, and reliability, and high stability. This work also offers further details regarding the electrochemical oxidation mechanism of PAL and oxidative degradation pathways. Thus, this method can replace other expensive or sophisticated methods of PAL determination in quality control laboratories.

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7. Conflicts of interest:

There are no conflicts to declare.

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