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A validated micro-sensitive stability-indicating adsorptive stripping voltammetric determination of Alverine citrate utilizing G

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Abstract

An inexpensive stability indicating anodic voltammetric method for rapid determination of ALV has been developed and validated. The method was based on the enhancement of voltammetric response at a nano zinc oxide and graphene oxide modified electrode (ZGE). Square wave voltammetry (SWV) method was developed for quantification of drug in BR buffer solution (pH 6.0) at ZGE. The ZGE displayed very good voltammetric behavior with significant enhancement of the peak current compared to carbon paste electrode (CPE). Stress stability studies were performed using 0.1 M of HCl and NaOH. Mass and infrared spectroscopy were used for identification of degradants and their pathways were illustrated. Under optimal conditions, the peak currents showed a linear dependence with drug concentrations. The achieved limits of detection (LOD) were 0.640 and 1.940 for first peak and second peak respectively of ALV oxidation waves. The developed voltammetric method was successfully applied for direct determination of ALV in drug substances, pharmaceutical vials and in presence of either their corresponding hydrolytic, oxidative-degradants or interfering substances with no potential interferences. The SWV voltammograms were highly advantageous and applicable in QC laboratories for rapid, selective micro-determination of ALV.
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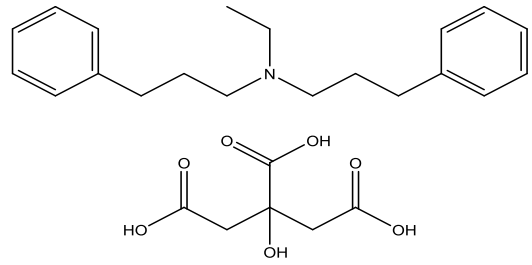
1. Introduction

Alverine citrate (ALV) N-Ethyl-3-phenyl-N-(3-phenylpropyl) propan-1-amine dihydrogen 2-hydroxypropane- 1, 2, 3-tricarboxylate (Scheme 1) is a white crystalline powder, slightly soluble in water. Alverine in a medicine form is used for functional gastrointestinal disorders. Alverine is used to promote relaxation or reduce tension of smooth muscle. Smooth muscle is a nature of muscle that is not underneath voluntary controller and present in body organs such as the gut and uterus. Collected review study shown that estimation of ALV was done by using visible spectrophotometric method established on dye formation binary complex with drug in acidic media has been reported in literature[1], UV spectrophotometer determination, Quantitative Analytical of FTIR Spectroscopy[2], number of methods for the determination of ALV were reported e.g. high performance liquid chromatographic Stability indicating method combined with UV detection had been studied for the estimation of ALV in bulk and human plasma [3], LC-MS/MS method for the quantification of ALV in human plasma for a bioequivalence was studied[4,5] and voltammetry method[6].

In these studies; the influence of different type of supporting electrolytes and pH values were examined. These procedures were described for drug metabolites determination and pharmaceutical dosage formulas. Later the progress of recent electroanalytical computer-based voltammetric procedures as square wave voltammetry (SWV) has been applied for precise and sensitive determination of a varied range of drug compounds, because of their availability, simplicity, relatively short determination time and environmental friendly. It is advantageous to be less sensitive to the effects of complex matrix over other analytical effects; so that they facilitate simple determination of the studied drugs without time-consuming extraction procedures prior the voltammetric measurement. The use of low cost graphite pencil electrodes in electroanalysis has

The Extensive literature survey hadn't reveal any stability indicating determination technique for the estimation of ALV in biological and Pharmaceutical dosage formula which provides info about the products of degradation as well as separation of degradation products.



N-ethyl-3-phenyl-*N*-(3-phenylpropyl)propan-1-amine 2-hydroxypropane-1,2,3-tricarboxyl
Chemical Formula: $C_{26}H_{35}NO_7$
Exact Mass: 473.24
Molecular Weight: 473.57
m/z: 473.24 (100.0%), 474.24 (28.1%), 475.25 (2.7%), 475.25 (1.4%), 475.25 (1.1%)

Scheme 1. Structural formula of Alverine citrate

2. Experimental

2.1. Instrumentation

Voltammetric measurements were achieved using a potentiostat a Metrohm Computrace electroanalyzers Model 797VA Computrace. The measurements outcome signal were verified via VA Computrace version 1.3.1. The three-electrode system consisted of an Ag/AgCl (3 M KCl) electrode as the reference electrode and a platinum wire as the auxiliary electrode while the working electrode was a lab-made electrode assembled from carbon-fiber micro-wire. A JENWAY 3510 pH meter with a combined electrode (glass-reference electrodes) with an accuracy of ±0.05 pH was used as to adjust the pH values. All the solutions were prepared with bidistilled water, all experiments were carried at an ambient temperature of 25 ± 0.1°C.

2.2. Chemicals and reagents

All reagents were of analytical-reagent grade and used without further purification. High purity nitrogen was used for desertion, Alverine citrate (ALV), kindly supplied by NODCAR, was prepared standard Stock solution of 1.0×10^{-3} M by dissolving an appropriate weight in bidistilled water and stored in refrigerator. A 0.04 M of Britton–Robinson (BR) buffer prepared as explained in a previous our paper[7], was used as the supporting electrolyte and the pH was adjusted to the desired value by adding appropriate amounts of 0.2 M NaOH stock solution

2.3. Preparation of the working electrode

Modified of carbon paste electrode, 0.225 g graphite powder, 0.025 g ZnO nanoparticles and 0.03g graphene oxide was added to mix to develop homogeneous uniform mixture. After that 0.13 g paraffin oil was added to the mixture then it was mixed will by using mortar and pestle to get a soft homogeneous paste. This paste was packed into the melting Point Tube 0.9-1.1mm and was compressed by a copper wire from the tube other side. The copper wire was connected to the instrument. To gain fresh surface of modified electrode, a further paste pushed into tube and polished surface with weighing paper to obtained Smooth surface. Additionally, the unmodified electrode was mad with the similar method without addition both modifier (CPE).

2.4. Electrochemical working procedure

The voltammetric behavior of ALV was verified through SWV in BR buffer, pH 6.0. suitable ALV concentration and volume of electrolyte solutions were transferred into the voltammetric vessel and the optimization parameters of the voltammetric procedure for SWV was done subsequent a efficient study of the experimental factors, for instance pH of the electrolyte buffer solutions, the pulse potential frequency (f), pulse amplitude (a) and the elevation of the voltage step (ΔEs) or scan increment, for a window of potential ranging (0.6 to +1.5 V). All parameters were adjusted properly since their values had a significant influence on the sensitivity of voltammetric measurements, the optimization is associated with maximum value of peak current and maximum selectivity (half-peak position). Beforehand every testing, N_2 gas was purged into the electrolyte solution for 60sec to renew the surface of working electrode by eliminating the adsorbed compounds, after parameters optimization 10.0-mL of electrolyte buffer solution having a quantified concentration of the ALV was transported to the measurement cell followed by dipping of working electrode. The solution of ALV was stirred for 20s (accumulation time) at potential (0.0 V) after the accumulation time, the stirrer was stopped and equilibrium time for 5 s was allowed for the electrolyte to become quiescent. Formerly, the voltammograms were resisted at Voltage step (V) 0.006, Amplitude (V) 0.020, Frequency (Hz) 20.0 Sweep rate (V/s) 0.060 using applied potential in the range from +0.6V to +1.5 V against Ag/ AgCl/KCl reference electrode. The Assembly of calibration curve was plotted and the analytical validations were figured.

2.5. Method Validation

So as to validate the suggested technique, specificity, linearity, accuracy and precision of SWV method were studied as stated by USP procedures [2].

2.6. Analysis of Pharmaceutical capsule

Three hard capsules of ALV (60 or 120 mg) were accurately mixed separately then complete the volume to 10ml of bidistilled water followed by magnetic striding for 10min and filtrate by syringe filter paper 0.2µm. Suitable

amounts equivalent to 2mg/ml transferred into separate 10.0mL volumetric flasks, then dissolved in water. Appropriate aliquots equivalent to 20µg/mL of ALV, were added to 10.0-mL of0.04M BR buffer solutions (pH 6.0) employing ZGE and quantitatively transported to the amusement cell. The SWV voltammograms were recorded succeeding the previously outlined voltammetric process. The RSD% and recovery were computed. The standard addition method was used by spiking the medicine formula with different quantities of the standard drug stock solution. The SWV were recorded and the recoveries were studied

3. Results and Discussion

3.1. Characterization of ZGE and CPE Electrodes

The active surface areas of the compared CPE and ZGE were investigated by applying cyclic voltammetry in electrolyte containing 1.0 mM K4Fe (CN)6 at different scan rates. The subsequent Randles-Sevcik equation[8,9] was applied:

$Ipa = (2{:}69 \times 10^5)\, n^{3/2}\, A_o\, D_o^{1/2}\, C_o^{*}\, \upsilon^{1/2}$

Where Ipa denotes to the anodic peak current, A_0 is the active surface area of the electrode, n is the number of electrons transferred, D0 is the diffusion coefficient, υ is the scan rate (V.s1) and C0 is the concentration of K4Fe (CN)6. For 1.0 mM K4Fe (CN)6, n=1, $Do=7.6 \times 10^{-6} cm^2 s^{-1}$ then by calculating the slope from the plot relation of Ipa vs. $\upsilon^{1/2}$ the electro-active surface area was computed[7]. In our experimentation, surface areas of CPE and ZGE were found to be 0.092 and 0.211 cm^2, the active surface area of ZGE was found to be greater than that of CPE, and hence it had greater response of peak current was observed for ZGE towards redox of ALV which rises the sensitivity of ZGE.

3.2. Square-wave voltammetry parameters Optimization

3.2.1. Supporting Electrolyte Type and pH effect

The effect of different Supporting Electrolyte buffers type (phosphate, borate, acetate, citrate and BR) on the redox responses of ALV was measured. The promising results taken with respect to sharp peak height and sensitivity, which obtained in case with BR buffer. So, the subsequent studying was achieved in 0.04M BR buffer in pH range 3.0 to 9.0 at ZGE with ALV concentration 12µgm/ml; with Voltage step (V) 0.006, Amplitude (V) 0.020, Frequency (Hz) 20.0, Sweep rate (V/s) 0.060. The height of anodic peak current decreased with rising pH in the alkaline medium, So, pH values greater than 9.0 were not used in this studying. The obtained redox peak current came to be maximum values at pH 5 at ZGE which was selected as the optimum working pH values for the determination of ALV.

A linear relation of the anodic potential (Ep) peak with pH values was established through the pH range of 2.0–9.0 at a signal wave for ALV as represented in Fig.(1). This was introduced by the resulting two equations from two peaks:

E (V) = -0.050 pH + 1.2189 $R^2 = 0.9904$

E (V) = -0.0575 pH + 1.4507 $R^2 = 0.9936$

Linear pH dependency of anodic peak potential for two waves over the full pH range was observed. With the rise in pH, the peak potential of both waves shifted towards the negative potential. So, a proton involved in ALV oxidation.

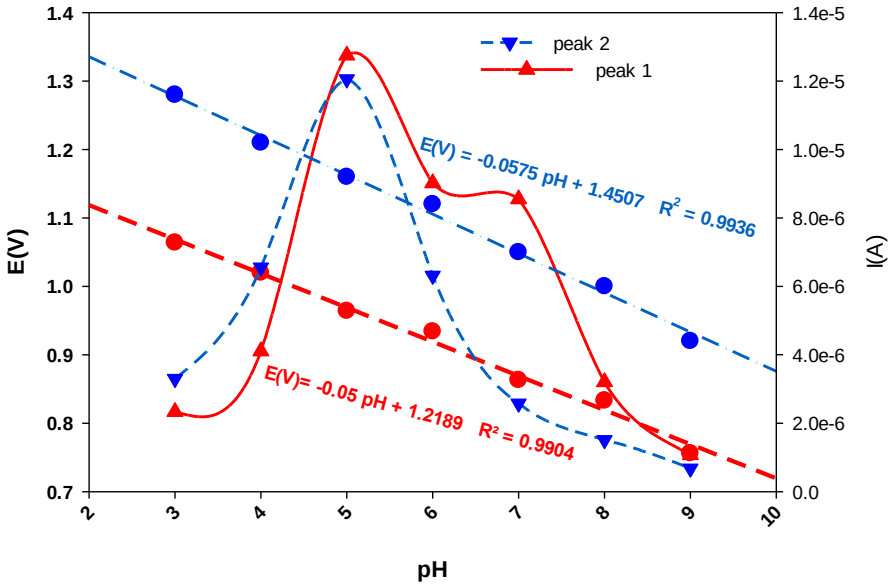
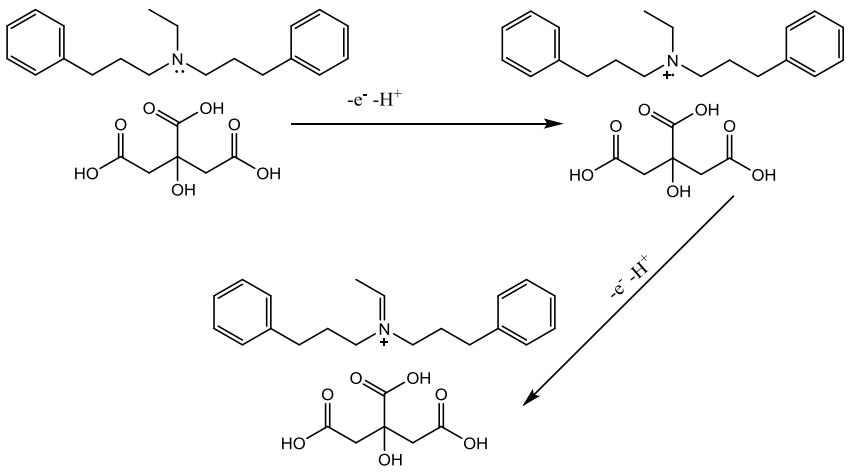


Fig (1): SWV voltammograms peak current (▲▲) and potential (●●) in two peaks, dependence of pH; analyte, ALV concentration 12µgm/ml; supporting electrolyte, 0.04M BR buffer solution; method, SWV with voltage step 0.006(V) , amplitude 0.020 (V), frequency (Hz) 20.0, sweep rate 0.060 (V/s).



(Scheme 2): The proposed mechanism of the voltammetric anodic oxidation reaction of ALV Drug

3.2.2. Mechanism

The oxidation mechanism of ALV at the ZGE surface was assumed that the data combination from Nernst equation relations and frequency with potential exposed that numbers of electrons involved in redox transferred were equal to 1 for both wave (1) and wave (2) in ALV, and that the reaction is completely irreversible, as designated above. Therefore, the oxidation mechanism was presented in (Scheme 2). The designated position of redox related to wave (1) was assumed to be the lone pair of electrons on the nitrogen of tertiary amine in center position which simplifies the loss of electrons in two subsequent anodic redox process to be happen.

Moreover, the effect of (*a)* on Ip was measured for the ALV redox process for from rang 5 to 50 mV, the voltammograms responses showed that an increase in Ip occurred as long as *a* values were increased. However, only linear increased happened for a less than 30 mV; overhead this value, the deviation occurred because of it is approximately difficult to complete steady state settings at high amplitude values. Also, Ep values shifted to positive values as (*a*) values increased, which is characteristic for redox processes including intensive adsorption from reactants and products on the surface of electrode[10,11] as displayed in first measurements using cyclic voltammetry. So, (a) value of 30 mV was chosen as optimum for analytical applications,

Intended for ΔEs, the increase of its value will also increase the signal and hence sensitivity of the procedure. But, for greater values of this factor, broaden of the peaks may be happen, thus detraction the determination of the analysis. Consequently, a valuation was recognized and the voltammograms responses presented that ΔEs confirmed a rise in peak current till 6 mV. However, no linear correlation between ΔEs and Ip was achieved, also characteristic of the voltammetric responses in redox containing products and reactants adsorbed at the surface of electrode.

3.4. Influence of Different Surfactants

The SWV voltammetric response of 12µgm/ml ALV in 0.04 M in BR buffer (pH 6.0) at ZGE with six sequential added extras of different surfactants e.g. cationic surfactants (CTAB, CPC) and non-ionic surfactants (Tween 80, Triton-X 100) had been examined. But in case of anionic surfactants (SDS), it was displayed that inhibited and improved methodology on the anodic peaks currents of drug were recognized as in illustrated above in fig.2 with concentration rang $1.3x10^{-5}$- 8 $x10^{-5}$M of SDS therfor calbiration ubdergo with 8 x10-5M of SDS.

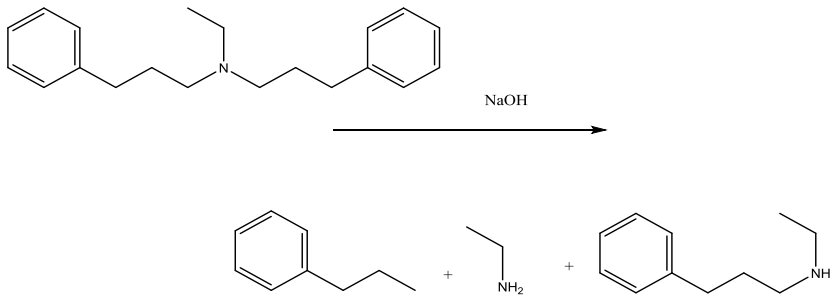
3.4.1. Diffusion Coefficients of LAV

the apparent diffusion coefficient of ALV at ZGE have been also computed from the slope of the linear relation of the anodic peak current on the square root of the potential scan rate using the Randles–Sevcik equation for totally irreversible electron-transfer process [12,13]. Where A is the electrode area, α is the charge transfer coefficient, Do is the diffusion coefficient of the electroactive species and Co is the bulk concentration of the solution. Electro active surface areas of ZGE is 0.211, the diffusion coefficients of the electro-active species are 3.78 $x10^{-4}$ cm2.s1, at a concentration of the solution is 0.5 mM.

$Ip = (2.99x10^5)n\alpha^{1/2}AC^*D_o^{1/2*}\upsilon^{1/2}$

3.5. Characterization of the Prepared Hydrolytic and Oxidative-degradants

The forced hydrolytic-degradation of ALV [4,14] drug was uniformly described by cleavage of tertiary amine group, resulting in the formation of an different degradants in acidic medium and a sodium salt degradants in basic medium. While, for the oxidative-degradation (Scheme 3) is characterized by the formation of two different degradants. First major degradant exhibited the persistence of the characteristic b-lactam carbonyl group with the formation of amine group (O=N=O) and the second one was the same as the hydrolytic degradant. Scheme 2 (a & b) is the suggested pathways for the hydrolytic and oxidative degradation of the studied drugs [27].



Scheme 3 :hydrolytic- degradation of ALV

3.6. Validation of the Proposed Method

3.6. 1Linearity and Range

In the present work, quantification valuation of ALV at ZGE was established the extent of the dependence of peak current (Ip) upon its concentration in the studied electrolyte solution under the above optimum conditions as stated The current density (CD) is the peak height of current produced divided by the geometric active surface area of the working electrode; the created linearity correlation curves were linear above the concentration ranges 0.53 - 5.20 ug/ml and 2.65 - 9.88 ug/ml corresponding to two different anodic peaks for ALV. The corresponding regression equations were computed in (Table 1).

CD (µA/cm) = 0.4794 C (ug/ml) + 0.3135 (R^2 = 0.9924) peak1

CD (µA/cm) = 0.2791 C (ug/ml) - 0.1111 (R^2 = 0.9904) peak2

Limits of Detection and Quantification

Limit of detection (LOD) and limit of quantification (LOQ) [15-17]of ALV were estimated at ZGE from the following equations: LOD=3.3s/S, LOQ=10 s/S (where s is the standard deviation of the peak current in mA, and S is the slope of the calibration graph). Both LOD and LOQ values in (Table 1) confirmed the sensitivity of the proposed method compared with those calculated by HPLC official or reported methods.

Repeatability and Intermediate Precision

Repeatability and intermediate precision[18] were examined by performing triplicate measurements for three different concentrations on the same day and for three separate days of standard ALV demonstrated the reproducibility of the results obtained by the proposed procedure under linearity. The RSD% values of intra- and inter-day studies were less than 2%, indicating that the developed methods were precise with confidence, as presented in (Table 1). Accuracy of five different concentrations in triplicate of both drugs was calculated from the corresponding regression equations and satisfactory mean percentage recoveries were illustrated in (Table 1).

Table (1): Accuracy of five different concentrations in triplicate of both drugs.

Parameters	Peak 1	Peak 2
pH	6.0	6.0
Concentration (µg/ml)	0.53 - 5.2	2.65 - 9.88
SD	0.39	0.313
RSD %	1.34	1.466
Slope of regression line (a)	0.4794	0.2791
$S_{y/x}$ (µA cm^{-2} µg/ml)	0.097	0.036
Intercept of regression line (b)	0.3135	0.1111
S_b (µA cm−2 µg/ml)	0.289	0.241
Correlation coefficient (r^2)	0.9924	0.9904
LOD (µg/ml)	0.640	0.796
LOQ (µg/ml)	1.940	2.4

4. Conclusion

In the present work, the electrochemical behavior of ALV at the surface of ZGE were explored using cyclic voltammetry at pH 6.0. The electro-redox was studied and displayed well- defined irreversible anodic response with

adsorption controlled mass transport. The proposed SWV voltammetric technique at ZGE was considered a stability-indicating method for determination of drug in presence of their hydrolytic and oxidative-degadants at micro-concentrations. To the best of our knowledge, no report of a stability-indicating voltammetric method of non-classical ALV was studied before. The proposed method at ZGE was accurate, precise, and specific and can be used for the routine quality control in pure forms and pharmaceutical vials with no potential interferences from excipients. The results showed that the quantity of drug substances in drug products were in a good agreement with given labeled quantity. When we take into consideration the properties of ZGE, it has higher electro-active area than CPE, allows for disposable.

Acknowledgment
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