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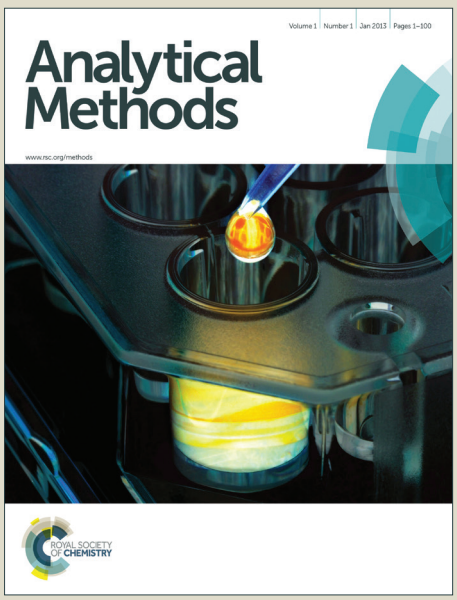

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New voltammetric analysis of Olanzapine analysis in tablets and human urine samples by modified carbon paste sensor electrode incorporating gold nanoparticles and glutamine in a micellar medium

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Abstract

An effective novel electrochemical sensor for selective determination of olanzapine (OLA) was introduced. The prepared sensor was based on carbon paste electrode chemically modified with glutamine (GL) and gold nanoparticles (GN) in presence of sodium dodecyl sulphate (SDS) in the medium. The effect of carbon paste composition and scan rate were tested. Working solution pH was 7 . The analytical method validation parameters were studied. The linear response was obtained for OLA in the ranges of 5×10^{-7} to 1.25×10^{-4} M with correlation coefficient 0.9986. LOD and LOQ were calculated and found to be $3.58\text{x}10^{-9}$ and $1.19\ \text{x}10^{-8}$ M, respectively. The utility of this sensor was examined for the determination of OLA in its pharmaceutical dosage form and human urine. Also the proposed method was applied for simultaneous determination of OLA, Fluoxetine (FLX), ascorbic acid (AA) and uric acid (UA).

Keywords: Modified carbon paste electrode, olanzapine, Glutamine, Gold nanoparticles, Electrochemical sensor.

1. Introduction

Olanzapine (OLA) (2-methyl-4-(4-methyl-1-piperazynyl)-10*H*-thieno-[2,3-*b*][1,5]benzodiazepine) (Figure 1) is a thienobenzodiazepine compound. OLA is one kind of the newer antipsychotic drugs used in the treatment of schizophrenia and other psychotic disorders [1]. Literature review showed several methods have been reported for the analysis of olanzapine in pure form, dosage forms or in combination with other drugs and in biological fluids. These methods include high-performance liquid chromatography (HPLC) [2-10], and liquid chromatography–tandem mass spectroscopy (LC–MS/MS) [11-13]. Also it was analyzed by gas chromatography [14] and titration in non-aqueous media [15] and electrochemical methods [16-18]. Several spectrophotometric methods have been developed for determination of olanzapine in bulk and in formulations. [19-22].

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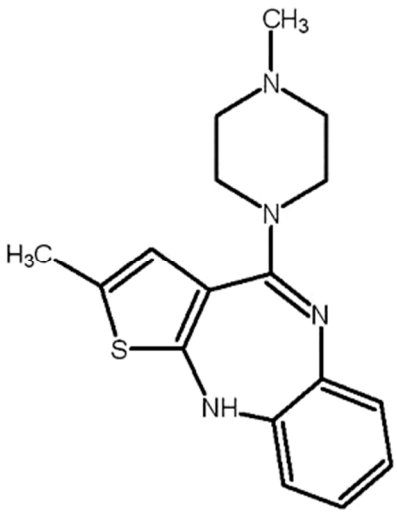

Figure 1: Chemical structure of OLA

Although many of the reported methods are accurate and sensitive, they required the use of sophisticated equipment and expensive reagents. Some are cumbersome, requiring prolonged sample pretreatment, strict control of pH and long reaction times.

The chemically modified electrodes (CMEs) have recently attracted much attention due to their significant advantages such as increasing peak current and decreasing overpotential for redox systems. Modification of electrodes with various modifiers such as transition metal complexes [23], nanostructures [24], molecular sieves [25] and organic compounds [26] have also been reported in recent years.

Carbon paste electrode (CPE), which was made up of carbon particles and organic liquid, has been widely applied in the electroanalytical community due to its low cost, ease of fabrication, high sensitivity for detection and renewable surface [27-29]. Lately, to improve the sensitivity, selectivity, detection limit and other features of CPE, chemically modified carbon paste electrodes (CMCPEs) have been used [30-32]. The operation mechanism of such CMCPEs depends on the properties of the modifier materials used to import selectivity and sensitivity towards the target species [33]. Gold nanoparticles (GN), with large surface area, good biocompatibility, high conductivity and electrocatalysis characteristics, have been used to improve the detection limits in electrochemical studies [34-38]. They are also suitable for many surface immobilization mechanisms and can act as tiny conduction centers and can facilitate the transfer of electrons. Many works had been conducted to construct the immunosensor using CPE modified with gold nanoparticles [28, 29, 39-43].

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In the present study, CPE was modified with glutamine (GL) and gold naoparticles (GN) in presence of sodium dodecyl sulphate (SDS). This modified electrode was examined for the first time for determination of OLA in bulk drug, tablets and urine. Also, since differential pulse voltammetry (DPV) has a much higher current sensitivity and better resolution than cyclic voltammetry (CV), DPV was used for the first time for simultaneous determination of OLA, ascorbic acid (AA) and uric acid (UA) because of their coexistence in human fluids. Also, to the best of our knowledge, there is no voltammetric method reported for simultaneous determination of OLA and the frequently co-formulated drug Fluoxetine (FLX). Therefore, the proposed method was used for that purpose and there was no interference observed by FLX.

2. Experimental

2.1. Materials and reagents

Standard OLA and its pharmaceutical dosage form OLAZINE® were provided by Novartis Pharmaceutical Co., Egypt. OLA stock solution was prepared by dissolving an appropriate amount of OLA powder in methanol to get $1.0x10^{-2}$ M OLA solution and then stored in the dark at low temperature. Standard working solutions were prepared by appropriate dilutions of the stock solution. The stock solution was stable for at least 1 month when kept in a refrigerator.
GL and SDS were purchased from Aldrich, Britton-Robinson buffer (B-R buffer) 4.0×10^{-2} M was prepared by mixing H_3PO_4, acetic acid and boric acid with the appropriate amount of 0.2 M NaOH to obtain the desired pH 2.0 - 9.0. All solutions were prepared from analytical grade chemicals and sterilized Milli-Q deionized water. All materials and reagents were used as received

2.1.1. Preparation of CPE

CPE was prepared by mixing graphite powder (0.5 g) with nujol oil (0.3 mL) in a glassy mortar. The carbon paste was packed into the hole of the electrode body and smoothed on a filter paper until its shiny appearance.

2.1.2. Preparation of Modified CPE

CPE modified with GL was prepared by hand mixing 61% graphite powder, 6% GL and 33% paraffin oil in an agate mortar to get homogeneous carbon paste. Then the electrode was immersed into 6 mM hydrogen-tetrachloroaurate $HAuCl_4$ solution containing 0.1 M KNO_3

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(prepared in doubly distilled water, and deaerated by bubbling with nitrogen), and a constant potential of -0.4 V versus Ag/AgCl was applied for 4 min to form gold nanoparticles/GL modified CPE (GNGLCP). Prepared electrodes were washed with doubly distilled water and dried carefully by a paper without touching the surface and then left to dry in air for ten minutes before being used.

2.2. Instrumental and experimental set-up

All voltammetric measurements were performed using computer-driven, AEW2 Analytical Electrochemical Workstation with ECprog3 electrochemistry software, manufactured by SYCOPEL SCIENTIFIC LIMITED (Tyne & Wear, UK). The one compartment cell with the three electrodes was connected to the electrochemical workstation through a C3-stand from BAS (USA). A platinum wire from BAS (USA) was employed as the auxiliary electrode. All the cell potentials were measured with respect to Ag/AgCl (3 M NaCl) reference electrode from BAS (USA). A Cyberscan 500 digital (EUTECH Instruments, USA) pH-meter with a glass combination electrode served to carry out the pH measurement. All the electrochemical experiments were performed at an ambient temperature of 25 ^{o}C. Scanning electron microscopy (SEM) measurements were carried out using a JSM-6700F scanning electron microscope (Japan Electro Company). SigmaPlot 10 was used for all statistical data.

2.3. Recommended Experimental procedure

Before any voltammetric measurement, the modified electrode GNGLCP was cycled between 100 -700 mV with the scan rate of 100 mV s^{-1} in 4.0 x 10^{-2} M Britton–Robinson buffer solution of pH 7 several times until a reproducible response was achieved. Then, the electrode was transferred into another cell containing 4.0 x 10^{-2} M Britton– Robinson buffer of pH 7 and the proper amount of OLA. Then 10 µl of 1 x 10^{-2} M SDS was added to the solution to enhance the peak current. After accumulating of SDS for 10 s, cyclic voltammograms (CVs) were recorded between 100 -700 mV with the scan rate of 100 mV s^{-1}.

For DPVs procedure, aliquots equivalent to 0.5–125 µM OLA were transferred into a series of 10-mL volumetric flasks using micro pipette. 10 µL of 10-2 M SDS solution were added and the volume was completed to the mark with B-R buffer pH 7. Quantitatively 5 mL was transferred to the electrolytic cell, and DPVs were recorded. The peak current at working GNGLCP/SDS electrode was measured at scan rate of 10 m Vs-1 using DPV method.

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2.4. Analysis of pharmaceutical dosage forms

Ten OLAZINE® tablets of OLA (20 mg/tablet) were accurately weighed and finely powdered. A weighed portion of pharmaceutical powder equivalent to 10^{-2} M of OLA was transferred into a 25 mL calibrated flask then dissolved in methanol (HPLC grade) by sonication for 30 min. Then, final solution was filtered into a 25 mL volume calibrated flask, and the residue washed three times with methanol added to the flask and then diluted to the mark with the same solvent. The amount of OLA per tablet was calculated using the linear regression equation obtained from the calibration curve of pure OLA.

2.5. Application to human urine

OLA urine samples were prepared using a similar method, as reported in Ref [44]. A master solution of $1x10^{-2}$ M of OLA was prepared in methanol. Blank urine samples were collected from 12 subjects, which were then used to prepare urine standards for the method validation. Urine standards were prepared by mixing 500 ml of the$1x10^{-2}$ M stock with 5000 ml of blank urine to produce a concentration of 9.09 $x10^{-4}$ M. DPVs were recorded according to the recommended procedure for OLA. Values of the current (I) versus the corresponding concentration were plotted to obtain the calibration graph. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the institutional committees have approved these experiments

3. Results and discussion

3.1. Morphologies of the different electrodes

The response of the electrochemical sensor was related to its physical morphology. The SEM images of A) CPE, B) GLCP and C) GNGLCP were made, and significant differences in the surface structure were observed (Figure 2). The surface of the CPE was predominated by uniform and smooth shaped graphite flakes and separated layers. On the other hand, the SEM image of GNGLCP shows compact granular shape. Following deposition of gold nanoparticles gives a random distribution of interstices among the nanoparticles in the SEM image of GNGLCP exhibiting a large surface area.

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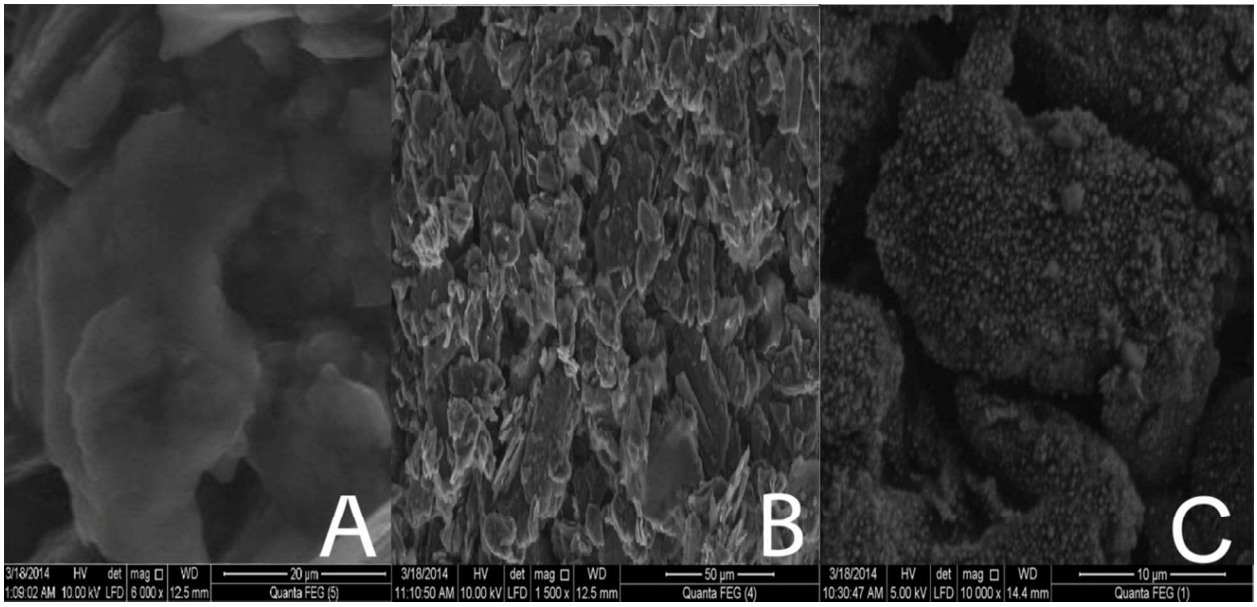

Figure 2: Scanning electron microscope image of A) bare CPE, B) GLCP and C) GNGLCP

3.2. Electrochemical behavior of OLA

The voltammetric behavior of OLA was recorded in the range 100 to -700 mV using CV. Figure 3 showed representative cyclic voltammograms of 1.0 x 10^{-3} M OLA in B-R buffer pH 7, at scan rate 100 mVs^{-1} recorded at four different working electrodes bare CPE, GLCP, GNGLCP and GNGLCP/SDS. For bare CPE the anodic peak current was (ca. 29 µA), which corresponded to the electrochemical oxidation of OLA appeared at 0.312 V. The electrochemical reaction kinetics was improved by the use of GLCP electrode where E_{pa}=0.333 V (with a current value of 40µA) and E_{pc}= 0.131 V, which is higher than the current observed at CPE. This can be attributed to OLA oxidation due to the formation of a hydrogen bond between the diazepine ring of OLA and the carboxamide group glutamate amino acid. As a result, the bond energy between hydrogen and oxygen is weakened and the electron transfer was more likely to occur via N··HO bond. Thus, GL particles can act as a promoter to increase the rate of electron transfer due to its catalytic capability. In the case of GNGLCP, well-defined redox peaks at E_{pa} = 0.350 V and E_{pc} = 0.128 V and a significant increase in the peak currents (with a current value of 56 µA). The increased peak current indicated that the GN contributed to OLA electrocatalysis by increasing the surface area. In addition, GN can assist the direct electron transfer between the drug and the bulk electrode surface. Moreover, when GNGLCP/SDS used, an increase in the current response was observed as the anodic peak current increased to a value of 66 µA.

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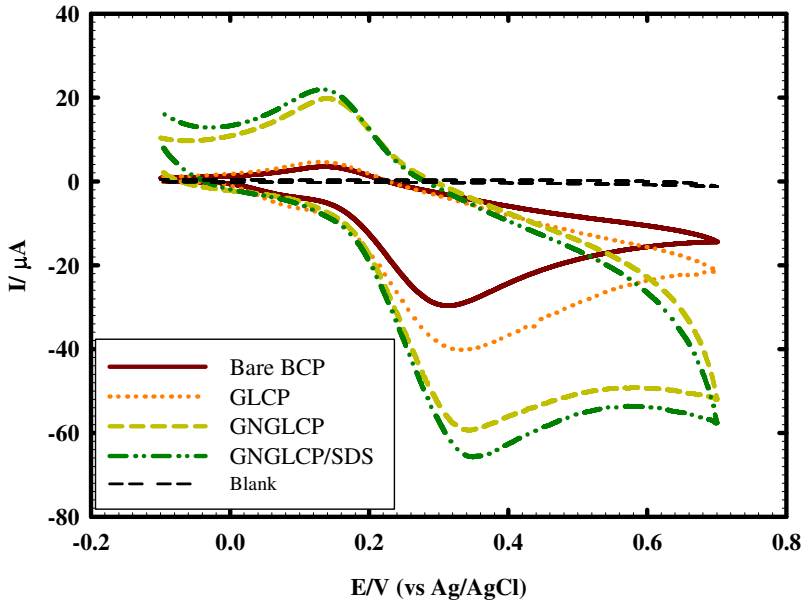
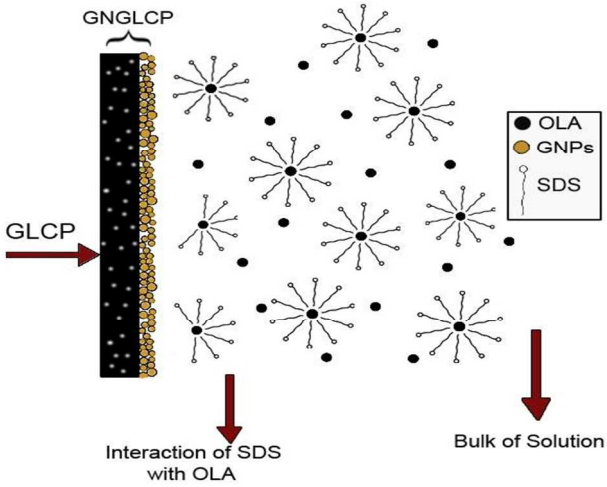


Figure 3: Cyclic voltammograms of 1.0 x 10^{-3} M OLA in B–R buffer pH 7 at a scan rate of 100 mV s^{-1} recorded at four different working electrodes

It was most likely that there was an electrostatic attraction between the cationic OLA and the anionic SDS which enhances the diffusion of OLA through the electrode surface. Also, there was interaction between the positively charged GL and anionic SDS which enhances hydrogen bond formation between OLA and GL and promotes faster electron transfer kinetics. The schematic representation for the interaction of GNGLCP electrode in presence of SDS with OLA was illustrated in the following scheme.



Scheme: GNGLCP interaction with OLA in the presence of SDS.

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3.3. Effect of operational parameters

3.3.1. Effect of solution pH

The effect of solution pH on the oxidation of OLA at the GNGLCP/SDS was studied by the cyclic voltammogram technique using B–R buffers within the pH range of 2–9 (Figure 4). The anodic peak potentials shifted negatively with the increase in the solution pH, indicating that the oxidation of OLA is a pH-dependent reaction showed that protons have taken part in their electrode reaction processes. The relationship between the anodic peak potential and the solution pH value over the pH range of 2–9 could be fit to the linear regression equation of E_{pa}(V) = 0.772–0.061 pH, with a correlation coefficient of r = 0.9951. The slope was 61.1 mV/pH, which is close to the theoretical value of 59 mV. This indicated that the deprotonation step of OLA is prior to the electron transfer step and the number of protons and transferred electrons involved in the oxidation mechanism are equal. OLA anodic current responses gave the highest value at pH 7 and at high pH values the current responses were higher than that at low pH values, this is due to the pK_a value of OLA which is 7.24 [45], therefore, OLA can be attracted by the negative charges of the electrode. The highest oxidation peak current was obtained at pH 7 (around pH medium of the human body pH 7.4). Thus pH 7 was employed for the determination of OLA to achieve higher sensitivity.

3.3.2. Effect of scan rate

The effect of different scan rates (ν ranging from 10 to 100 mVs^{-1}) on the current response of 1.0 $\times 10^{-3}$ M OLA using GNGLCP/SDS in B-R buffer (pH 7) was studied (Figure 5 A). A plot of I versus $\nu^{1/2}$ gave a straight line relationship up to scan rate 100 mVs^{-1} then deviation occurs (Figure 5 B). This indicated that the charge transfer was under diffusion control partially. The reduction and oxidation peak currents increased linearly with the linear regression equations as: I_{pc} (10^{-6} A) = 1.12 $\nu^{1/2}$ $(V\ s^{-1})^{1/2}$ +0.73 (n=5, r= 0.9947), I_{pa} (10^{-6} A) = -5.70 $\nu^{1/2}$ $(V\ s^{-1})^{1/2}$ -3.33 (n=5, r= 0.9964), respectively, suggesting that the reaction is diffusion-controlled electrode reaction.

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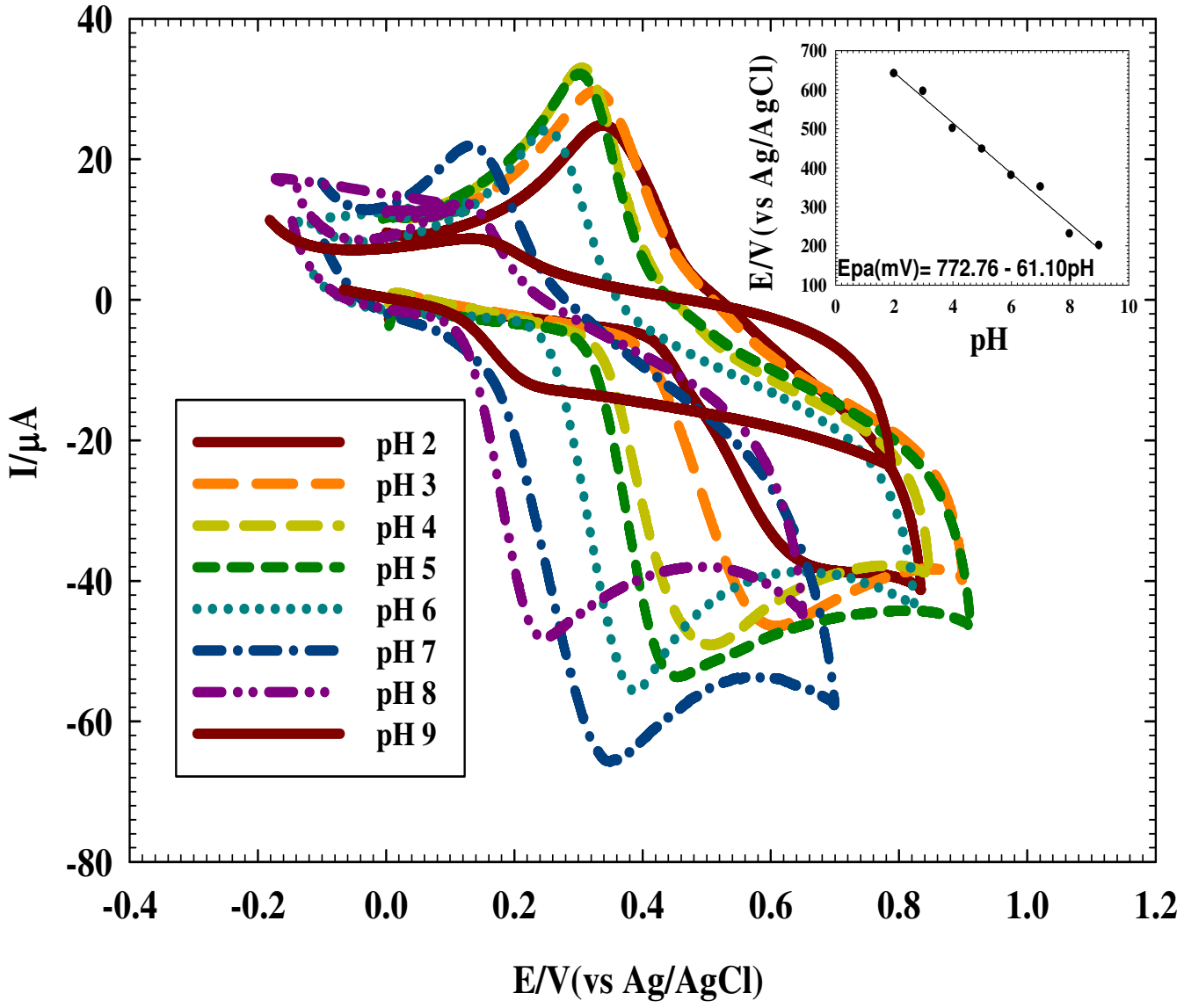

Figure 4: Cyclic voltammetric response of 1.0 x 10^{-3} M OLA at different pH values using 0.04 M B-R buffers using GNGLCP/SDS. The inset: plot of anodic peak potential of OLA versus pH.

In order to obtain information on the rate determining step, the Tafel plot was drawn by using the rising part of the current–voltage curves of OLA monitored at scan rate of 10 mV s^{-1} (Figure

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5C). This part of voltammogram, known as Tafel region, is affected by electron transfer kinetics between substrate (OLA) and GNGLCP/SDS [46].

$$\text{Anodic Tafel slope} = \frac{(1-\alpha)n_{\alpha}F}{2.3RT} \qquad (1)$$

The slope of this plot was 80.1 mV. This slope indicates a transfer coefficient of α= 0.34 for a one electron transfer process [47].

A plot of peak height (I_p) versus the scan rate (υ) in the range of 10–100 mV s^{-1} was constructed (Figure 5D). This plot was found to be linear, corresponding to the following equation: I_{pc}= 0.0932**ν** + 1.061; r=0.9995 and I_{pa}= -0.482**ν** - 7.268; r=0.9993 for the reduction and oxidation peaks, respectively, which confirms that the reaction is diffusion-controlled electrode reaction.

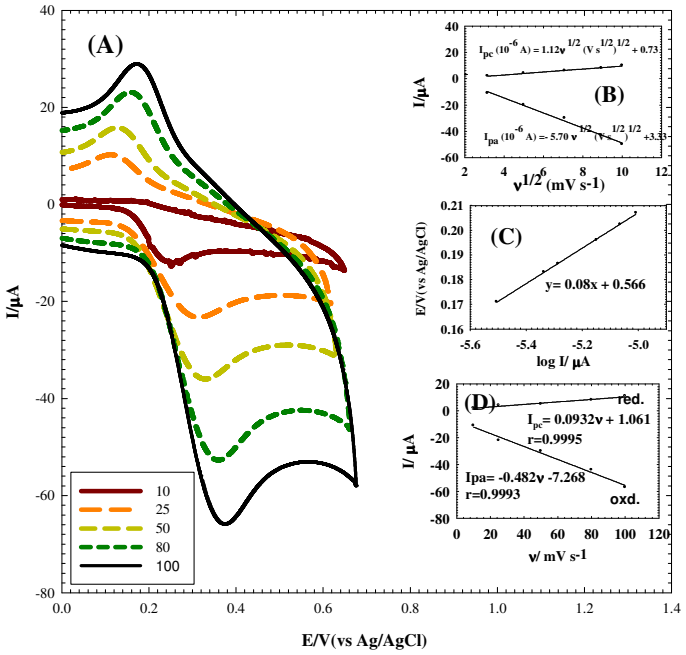

Figure 5: (A) Cyclic voltammograms of 1.0 ×10^{-3} M OLA in B-R buffer (pH 7) at various scan rates: (1) 10, (2) 25, (3) 50, (4) 80 and (5) 100 using GNGLCP/SDS. Insets (B) plot of I_p versus $\upsilon^{1/2}$. (C) Tafel plot derived from the rising part of voltammogram recorded at scan rate 10 mV s^{-1}. (D) Variations of the anodic and cathodic peak currents (I_{pa} and I_{pc}) vs. scan rates.

3.3.3. Effect of deposition time

The choice of the deposition time is related to current response of 1.0 ×10^{-3} M OLA using GNGLCP in B-R buffer pH 7 (Figure 5 A). By increasing the deposition time of GN, the signal

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increases until saturation of the signal (t=4 min). After 4 min deposition time, the current decreased.

3.4. Diffusion coefficients of OLA

The dependence of the anodic peak current density on the scan rate has been used for the estimation of the "apparent" diffusion coefficient, D_{app}, for OLA. D_{app} values were calculated from Randles Sevcik equation [48]

$$\mathbf{I_{pa} = (2.69x10^{5})\ n^{3/2}\ A\ C_o{*}D_o^{1/2}\ \nu^{1/2}} \qquad \mathbf{(2)}$$

where the constant has units (i.e. 2.687 x10^5 C mol^{-1} $V^{-1/2}$).

In these equations: I_p is the peak current density (mA cm^{-2}), n is the number of electrons appearing in half-reaction for the redox couple, v is the rate at which the potential is swept (V s^{-} (1 × 10^{-6} mol cm^{-3}), A is the electrode area (0.0706 cm^2), and D is the electroactive species diffusion coefficient (cm^2 s^{-1}).

D_{app} of OLA on GNGLCP/SDS in B-R buffer (pH 7) was calculated from CV experiments and was found to be 1.21x 10^{-5} cm^2 s^{-1}. This result was compared to those of bare CPE, GLCP and GNGLCP, which were 2.33x 10^{-6} cm^2 s^{-1}, 4.43 x 10^{-6} cm^2 s^{-1} and 8.69x 10^{-6} cm^2 s^{-1}, respectively. This indicated fast electron transfer process of electrochemical oxidation of the analyte molecule at the electrode-solution interface [49, 50]. Furthermore, it also showed that the redox reaction of the analyte species took place at the surface of the electrode under the control of the diffusion of the molecules from solution to the electrode surface. This marked enhancement of peak current at the surface of the modified electrode confirms that GL, GN and SDS facilitated the electrochemical reactions.

3.5. Validation of the proposed method

International Conference on Harmonization (ICH) guidelines [51] for method validation were followed for validation of the suggested method.

3.5.1. Linearity and Range

DPV experiments were performed using GNGLCP/SDS in B-R buffer pH 7 solution containing various individual concentrations of OLA.

The calibration range was established through consideration of the necessary practical range, according to OLA concentration present in the pharmaceutical product, to give accurate, precise

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and linear results. The calibration range of the proposed method is given in Table1. The results showed the peak currents of OLA oxidation at the surface of GNGLCP/SDS were linearly dependent on the OLA concentrations, over the range of 0.5–125 µM with a slope and correlation coefficient of 0.251 µA/µM and 0.9986, respectively (Figure 6).

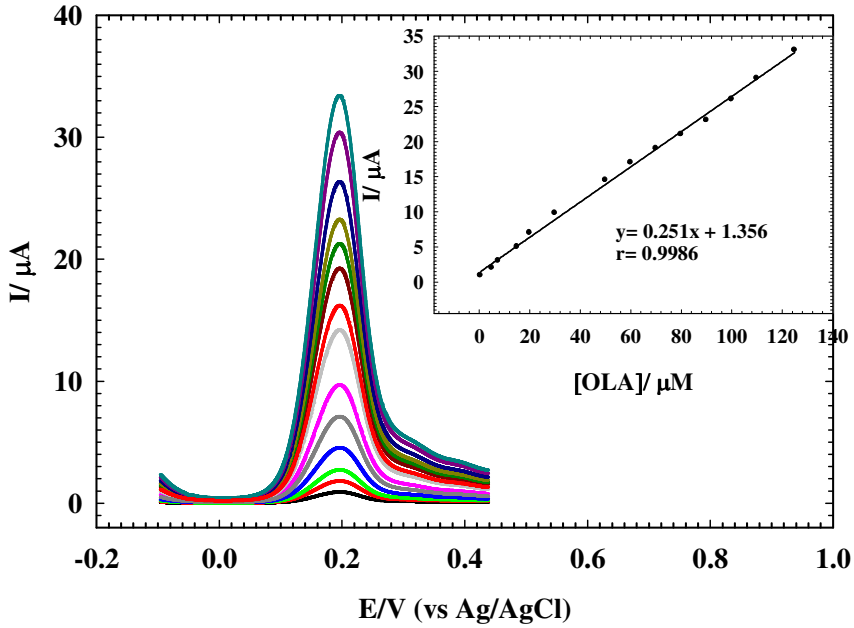

Figure 6: Differential pulse voltammograms of GNGLCP/SDS in a in B–R buffer at a scan rate of 10 mV s^{-1}solution (pH 7) containing different concentrations of OLA correspond to 0.5–125 µM. Inset shows the plot of the peak current as a function of OLA concentration

3.5.2. Detection and quantitation limits

According to ICH recommendations [51], the approach based on both the standard deviation and the slope of the calibration curve, was used for calculating the detection and quantitation limits as presented in Table 1. The LOD was calculated by the equation LOD = *3S/x* where S is the standard deviation of the oxidation peak current (n = 5) and m is the slope of the calibration curve. The calculated LOD was found to be 3.58 $x10^{-9}$ M. The limit of quantitation (LOQ) was estimated by the equation LOQ = *10S/x*, where *S* is the standard deviation of the intercept and x is the slope of the regression line. The calculated LOQ was found to be 1.19 $x10^{-8}$ M. These very low LOD and LOQ values can be attributed to the presence of GN in the structure of the

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modified electrode. These values are compared with values reported by other research groups for the oxidation of OLA at different electrodes by other mediators, Table 2.

3.5.3. Accuracy

The interference of excipients in the pharmaceutical formulations was studied using the proposed method. For this reason, standard addition method was applied to the commercial pharmaceutical formulation containing OLA. In application of standard addition method the mean percentage recoveries and their standard deviation for the proposed methods were calculated (Table 3). According to the obtained results a good precision and accuracy were observed for this method. Consequently, the excipients in pharmaceutical formulations do not interfere in the analysis of OLA in its pharmaceutical formulation.

3.5.4. Precision/reproducibility

The precision of the method was investigated performing three series of measurement (each five runs) with 20, 80, and $100x10^{-6}$ M of OLA solution within one day to evaluate within-day (repeatability) variability. RSD% and bias% values were calculated to check the ruggedness and the precision of the method. To calculate between day fluctuation of the analytical signal (reproducibility), three series of measurements were carried out in two successive days (reproducibility of the same modified electrode) and also over three consecutive new modified electrode (reproducibility of renewed modified electrode), Table 1.

3.5.5. Stability

The stability of the modified electrode has been investigated. The peak current does not change after storage in air for 9 days. The modified electrode retained 98% of its initial response up to 1 month.

3.5.6. Specificity

To test the specificity of the proposed method, it was tested for the voltammetric analysis of OLA in the presence of the frequently co-formulated drug FLX. Figure 7 showed the DPV of a mixture solution of OLA and FLX at GNGLCP/SDS at pH 4 which gave best peaks separation of the two drugs. The voltammetric results showed that the simultaneous determination of these compounds with two well-distinguished peaks at potentials 200 and 354 mV, corresponding to the oxidation of OLZ and FLX, respectively. However, GNGLCP/SDS is specific for OLA at

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pH7, because FLX has no peak at this pH, Figure 7. This means that these compounds can be determined simultaneously using GNGLCP/SDS.

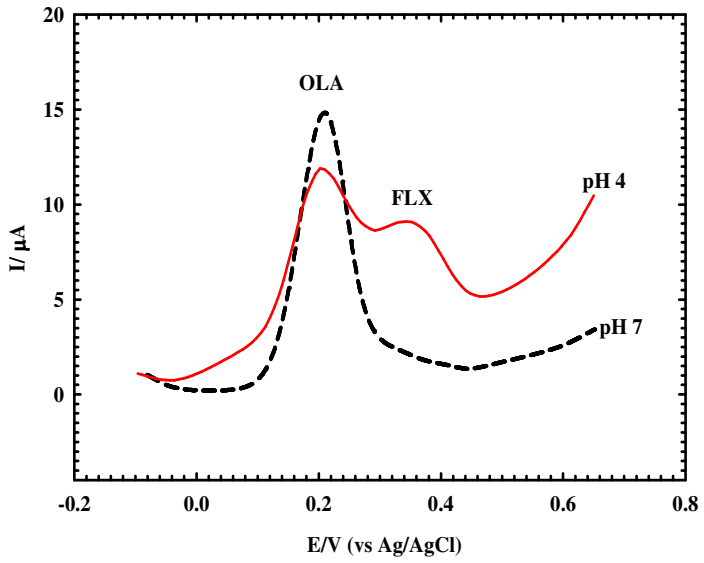

Figure 7: DPVs of GNGLCP/SDS in B-R buffer containing $50x10^{-6}$ M OLA and $1x10^{-3}$ M FLX at pH 4 and 7

3.6. Simultaneous determination of OLA, AA and UA

To the best of our knowledge, there is no report on the simultaneous determination of OLA, AA and UA. Therefore, it's important to study the determination of OLA in presence of AA and UA using GNGLCP/SDS sensor. This was performed by changing the concentration of OLA, and recording the DPVs. The voltammetric results showed well-defined anodic peaks at potentials of 23, 200 and 337 mV, corresponding to the oxidation of AA, OLA and UA, respectively, indicating that simultaneous determination of these compounds is feasible at GNGLCP/SDS sensor as shown in Figure 8. The sensitivity of the modified electrode towards the oxidation of OLA was found to be 0.243 µA/µM. This is very close to the value obtained in the absence of AA and UA (0.251 µA/µM, see Section 3.5.1.), indicating that the oxidation processes of these compounds at the GNGLCP/SDS are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences.

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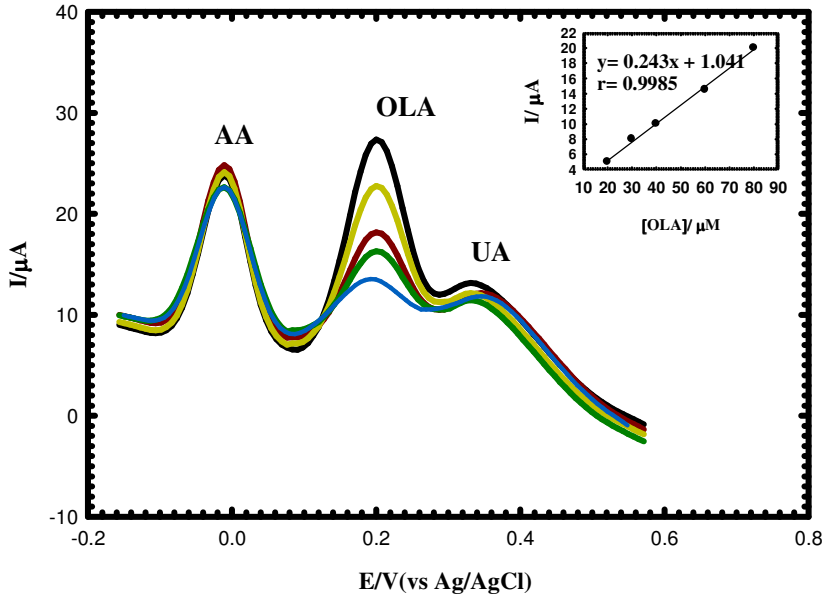

Figure 8: DPVs of GNGLCP/SDS in B-R buffer (pH 7) containing different concentrations of OLA, from inner to outer: 20, 30, 40, 60 and 80 µM in the presence of AA (49 µM) and UA (137 µM) at scan rate 10 mV/s. Inset shows the plot of the peak current as a function of OLA concentration.

3.7. Analytical application

3.7.1. Analysis of OLAZINE® tablets

To demonstrate the applicability of the proposed electrode for the analysis of real samples, it was applied to the determination of OLA in commercial OLAZINE® tablets (nominal contain 10 mg OLA/tablet). The standard addition method was used in these experiments. The OLA content was obtained to be 9.84 mg per tablet with a RSD of 1.27% (n=3) per tablet, which is very close to the labeled amount of 10.0 mg. The validity of the proposed method was assessed by applying the standard addition technique, which showed accurate results and there was no interference from excipients as shown in Table 1.

Statistical calculations were made in order to check the confidence and correlation between the suggested procedures and the reported method[7]. From the calculated t– and F–values at the 95%

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confidence level, it is clear that, the results obtained by the developed method is in good agreement with those obtained by a reported method, Table 4.

3.7.2. Assay of OLA in spiked human urine

The applicability of the proposed technique to the human urine was investigated; the calibration curve was obtained in spiked samples. The obtained regression equation, and related validation parameters are shown in Table 1 as details. Table 3 represents the recovery results of OLA in urine samples, calculated from the related linear regression equation, which was given in Table 1. To determine OLA in spiked urine samples, neither time-consuming extraction and evaporation steps nor sample pretreatment were required. The proposed method gave reproducible results, easy to perform, and sensitive enough for the determination of OLA in human urine samples. Stability of urine samples kept in refrigerator (+4 ^{o}C) was tested by making five consecutive analyses of the sample over a period of approximately 6 h.

4. Conclusions

In this work, the electrochemical behavior of OLA at CPE, GLCP, GNGLCP and GNGLCP/SDS in B-R buffer 4.0 x 10^{-2} M solution (pH 7.0) were compared. GNGLCP/SDS was chosen as a new sensor for the OLA determination in pharmaceutical and human urine samples with good accuracy and precision. The selected electrode showed high reproducibility, sensitivity, selectivity and better stability, not only for OLA determination (one component), but also, for tertiary mixture separation (OLA, AA and UA). The electrochemical investigations of mixture solutions of these three compounds showed three well-defined and well-separated sharp peaks. Also, the prepared voltammetric sensor demonstrated high selectivity, wide linear range, low detection limit, long-time stability and very good response reproducibility together with ease of preparation and surface regeneration. The proposed method enabled the voltammetric analysis of OLA in the presence of the frequently co-formulated drug FLX without interference.

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Table 1

Regression data of the calibration curve for quantitative determination of OLA by DPV method

Parameters	Supporting electrolyte	Urine
Linearity range (M)	$0.5\text{-}125\text{x}10^{-6}$	$0.9\text{-}500\text{x}10^{-6}$
Slope ($\mu A\ M^{-1}$)	0.251	0.093
SE of slope	0.0003	0.0021
Intercept (μA)	1.356	3.808
SE of intercept	0.011	0.370
Correlation coefficient (r)	0.9986	0.9982
LOD (M)	$3.58\text{x}10^{-9}$	$6.77\text{x}10^{-8}$
LOQ (M)	$1.19\ \text{x}10^{-8}$	$2.26\text{x}10^{-7}$
Repeatability of the peak current (RSD%)*	1.32	1.54
Reproducibity of the peak current (RSD%)*	1.45	1.09
Repeatability of the peak potential (RSD%)*	0.98	1.48
Reproducibity of the peak potential (RSD%)*	1.08	1.59

*Obtained from average of five experiments

Table 2

Comparison of the efficiency of some electrodes used in the electrocatalysis of OLA (Method: Voltammetry)

Electrode	Modifier	pH	Scan rate ($mV\ s^{-1}$)	LOD* (M)	linear range* (M)	Reference
Glassy carbon	--------	3.24	20	$6.79\ \text{x}\ 10^{-6}$	$7\text{x}10^{-5}\text{-}1\text{x}10^{-3}$	[7]
Gold electrode	single walled carbon nanotubes (SWCNTs-COOH)	8.5	50	$3.2\ \text{x}\ 10^{-7}$	$0.64\text{-}320\text{x}\ 10^{-6}$	[16]
Carbon paste	Glutamine	7.0	100	$3.58\text{x}10^{-9}$	$0.5\text{-}125\text{x}10^{-6}$	This work

* LOD and linear range were obtained using DPV

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Table 3

Quantitative determination OLA in OLAZINE® tablets and urine by the proposed method and application of standard addition technique

Standard addition technique				
Pure added (μM)	Pure found (μM) OLAZINE®	Recovery%	Pure found (μM) Urine samples	Recovery%
20.00	20.20	101.00	19.61	98.05
80.00	80.26	100.32	78.91	98.64
100.00	100.29	100.29	99.00	99.00
Mean±RSD		100.53±0.40		98.56±0.48

* Average of three determinations

Table 4 Statistical analysis of the results obtained by applying the developed and the reported methods for the analysis of OLA

Parameters		Reported method [7]
Mean	99.47	99.10
S.D.	0.51	0.99
N	5	5
Variance	0.26	0.98
t-test (2.776)	0.74	---------
F-Value (6.390)	3.77	---------

*Values between parentheses are the theoretical values of t and F at confidence 95%.

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