Electrochemical behavior of almotriptan and its determination at multiwalled carbon nanotubes film modified electrode

ABSTRACT

Multi-walled carbon nanotubes (MWCNTs) film was used for modification of GCE to investigate the electrooxidative behavior of an antimigraine drug, almotriptan malate (ALM) employing cyclic and differential pulse voltammetric techniques. ALM was found to undergo irreversible oxidation at a peak potential of 738 mV (versus SCE). It was observed that the multiwalled carbon nanotubes modified GCE (MWCNT-GCE) significantly enhanced the oxidation peak current of ALM. This property was exploited to develop a simple, sensitive and time-saving differential pulse voltammetric method for the determination of ALM in bulk pharmaceutical samples and biological fluids. Linear relationship was observed between the peak current and concentration with a correlation coefficient of 0.9915 in the range of 0.25-37.5 μM ALM.

The results of this section are published in

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INTRODUCTION

Electrochemical techniques have found vast applications in the analysis of pharmaceuticals, environmental samples, food analysis and energy storage. The electrode surface is a key factor for the successful application of any electrochemical technique for analysis. By controlling the electrode potential, the electrode can be used as a variable free energy source (or sink) of electrons. Further, the electrons that cross the electrode-solution boundary can be determined with a greater sensitivity by measuring the current responses. The applicability of most of the electrodes to analytical schemes encounters specific phenomena that reduce their applicability for particular applications. Primary phenomenon among these are slow electrochemical reaction rates of some analytes or slow heterogeneous electron transfer rates and fouling of the electrode by adsorption processes or unwanted precipitation. Few of these problems can be tackled by manipulating the chemical nature of the electrode surface.

If an appropriate reagent was chosen for modification of the electrode, desirable properties such as reagent-based control of electron transfer rates, selectivity of electrochemical reactions and freedom from fouling and interferents might be achieved. This tempting concept of rational design of electrode surfaces has attracted considerable interest and success in electroanalytical chemistry research since its inception.

Since its discovery in 1991 by Iijima [1], carbon nanotubes (CNTs) including single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs) have attracted much attention due to their unique structures and extraordinary properties [2]. CNT possesses fine electronic properties, huge surface area, efficient catalytic activity and strong adsorption ability, high chemical and thermal stability, high elasticity, high tensile strength and in some instances metallic conductivity [3,4]. The modification of electrode surfaces with MWCNTs for use in analytical sensing is well documented.
Many attempts have been made to utilize functionalized carbon nanotubes in the field of electroanalytical chemistry, medicinal chemistry, materials science and technology including photovoltaics [5-8]. The advancement of sensors based on MWCNTs has been the target of a large variety of applications, predominantly for solid-state chemical and biological sensors [9,10]. The modification of electrode surfaces with MWCNTs for use in analytical sensing has been well documented. They were claimed to show advantages such as increased peak currents and heterogeneous electron-transfer rates, resistance to surface fouling of CNT-based electrodes and catalytic effect towards the oxidation/reduction of a wide variety of compounds. Thus, leading to low detection limit, high sensitivity and reduction of overpotential. Further, carbon nanotube-modified electrodes have been reported to give super performance in the study of a number of biological and pharmaceutical species [11-16]. Modification of carbon electrodes is particularly easy and can be achieved either through physical adsorption of the modifier onto the carbon surface or by covalent attachment of the modifier [17]. Generally, the improvement in the peak current observed at the MWCNT-GCE reflects the enhancement of the electron transfer between the analyte molecule and the modified electrode. As expected for these electrodes, these can be ascribed to surface defects and metal impurities that remained even after treatment with hot mineral acid, strong adsorptive ability and electrocatalytic surface properties of MWCNTs [18].

Almotriptan malate, (Drug profile is shown on page 26) is a selective serotonin (5-HT1) agonist with actions similar to those of sumatriptan. It is used for the treatment of the headache phase of migraine attacks. Almotriptan malate (ALM) is available commercially in the market as conventional tablet, Axert.

A few HPLC [19-24], HPTLC [25], HPLC-MS/MS [26], LC-ESIMS/MS [27], UV spectrophotometric [28-32], fluorometric and colorimetric methods [33] have been reported for the determination of ALM in
pharmaceutical formulations or biological fluids. The reported chromatographic methods are expensive and are time-consuming while the spectrophotometric/colorimetric methods are either less sensitive or involve heating. Further, the utilization of these chromatographic methods generated considerable waste. In view of this, we have developed a simple, rapid and cost effective analytical method for routine analysis. Attempts have not been made so far to investigate the electrochemical behavior of ALM and further to develop an electro-analytical method for the determination of ALM in dosage forms. Hence, we have investigated the electrochemical behavior of ALM and developed a convenient and sensitive DPV method for the determination of ALM in bulk sample and pharmaceutical formulations employing MWCNTs modified electrode.

**EXPERIMENTAL**

**Apparatus**

Electrochemical studies were carried out on a CHI-1110a electrochemical Analyzer (CH Instruments Ltd. Co., USA, version 4.01) consisting of a GCE (3 mm diameter) or MWCNT-GCE as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode as the reference electrode.

**Working solutions**

- A stock solution of ALM (0.5 mM) was prepared in milli-pore water and stored in a refrigerator at 4 °C.
- Working solutions were prepared by diluting the stock solution with phosphate buffer (0.2 M) of required pH. Phosphate buffer (pH 3.0-10.0) was used as the supporting electrolyte in the present study.

**Preparation of MWCNT-GCE**

MWCNTs were refluxed in a mixture of HNO₃ and H₂SO₄ (1:3) for 12 h to promote functionalization. They were then filtered through a 0.45 µm milli-pore nylon filter membrane. The resulting MWCNTs were continuously
washed with milli-pore water until the pH of the filtrate was neutral and then
dried in an oven at 120 °C [14]. The MWCNTs suspension was prepared by
dispersing 2 mg of MWCNTs in 10 mL acetonitrile using ultrasonic agitation
to obtain a relatively stable suspension. Before modification, the GCE was
carefully polished with 0.05 μm α-alumina on a smooth polishing cloth, and
then washed with milli-pore water. The cleaned GCE was coated by casting
25 μL (5×5 μL) of MWCNTs suspension and dried in air (Scheme 1). After
modification, the electrode was rinsed with water to remove the loosely
adsorbed MWCNTs.

The microscopic areas of MWCNT-modified GCE and the bare GCE
were obtained by CV using 1 mM K₃[Fe(CN)₆] as a probe at different scan
rates. For a reversible process, the Randles–Sevcik formula was employed:

\[ I_{pa} = (2.69 \times 10^5) A n^{3/2} D_0^{1/2} C_0^{1/2} v^{1/2} \]  

where \( I_{pa} \) is the anodic peak current, \( n \) is the number of electrons transferred, \( A \)
is the surface area of the electrode (cm²), \( D_0 \) is the diffusion coefficient (cm²/s),
\( C_0 \) is the concentration (mol/cm³) of K₃[Fe(CN)₆], and \( v \) is the scan rate (mV/s).
For 1 mM K₃[Fe(CN)₆] in 0.1 M KCl electrolyte, \( n=1 \) and \( D_0=7.6\times10^{-6} \) cm²/s.
From the slope of the \( I_{pa}v^{1/2} \) relation, the microscopic areas were calculated.
The electrode surface area of bare GCE and MWCNT-modified GCE was
found to be 0.0584 and 0.1635 cm², respectively.

Working procedure

The MWCNT-GCE was first activated in phosphate buffer of pH 7.0 by
CV sweeps between 0 and 1400 mV until stable cyclic voltammograms were
obtained. The modified electrode was then transferred into another 10 mL of
phosphate buffer (pH 7.0) containing suitable aliquots of ALM and
accumulation was carried out under open circuit potential for a time period of
150 s. After this accumulation time, voltammograms (either cyclic
voltammogram or differential pulse voltammogram) were recorded.
All the electrochemical experiments were carried out at 25±1 °C. After every measurement, new MWCNT-GCE was prepared for reproducible results.

**Analysis of tablets**

Ten tablets containing ALM were powdered in a mortar. A portion of the powder equivalent to 0.5 mM ALM was transferred into a 100 mL volumetric flask and completed to volume with milli-pore water. The contents of the flask were sonicated for 10 min to effect complete dissolution. Recovery experiments were carried out by the method of standard addition. The content of the drug in the tablet was determined from the calibration graph or regression equation.

**Determination of ALM in spiked human urine and plasma samples**

Spiked urine sample was obtained by treating 0.9 mL aliquot of urine with 100 μL standard ALM solution (2.5 mM) to obtain 250 μM ALM. A suitable aliquot of spiked urine was diluted with phosphate buffer, without any pre-treatment, to prepare appropriate sample solutions and differential pulse voltammogram was recorded under optimized conditions.

Serum samples, obtained from healthy individuals (after having obtained their written consent), were stored frozen until use. For the determination of ALM in plasma, 500 μL ALM solutions (2.5 mM) were added to 500 μL of untreated plasma. The mixture was vortexed for 30 s. To precipitate the plasma proteins, the plasma samples were treated with 250 μL perchloric acid (15 %). After that, the mixture was vortexed for further 30 s and then centrifuged at 5000 rpm for 5 min. Appropriate volume of supernatant liquor was transferred in the voltammetric cell containing phosphate buffer of pH 7.0 and voltammograms were recorded. Voltammograms of blank samples (without ALM) did not show any signal that could interfere with the direct determination. The content of the drug in plasma was determined referring to the calibration graph or regression equation.
RESULTS AND DISCUSSION

Electrochemical behavior of ALM

Cyclic voltammograms of blank and 25 μM ALM were recorded in phosphate buffer of pH 7.0 at bare GCE and MWCNT-GCE to investigate the electrochemical behavior and for the assay of ALM. The corresponding voltammograms are shown in Figure 1. ALM showed one oxidation peak (a₁) at 683 mV at bare GCE. No reduction peak was observed in the reverse scan indicating that the oxidation of ALM at GCE was irreversible. The oxidation peak appeared at 681 mV with considerable enhancement in peak current at MWCNT-GCE.

Multisweep cyclic voltammograms were recorded in order to check the adsorption property of the oxidation product of ALM on MWCNT-GCE. The oxidation peak current of ALM was observed to be decreased during the successive voltammetric sweeps and finally disappeared after few cycles. This was observed from the fact that the MWCNT-GCE was blocked by the adsorption of oxidation products of ALM thereby reducing the effective reaction sites at the modified electrode surface [34].

Effect of amount of MWCNTs suspension

Different amounts of MWCNT suspension were transferred by casting on the polished GCE surface and voltammograms of 25 μM ALM were recorded to study the influence of amount of MWCNTs suspension on electrooxidation of ALM. The oxidation peak current of ALM was observed to be increased by 5.2 times (Figure not shown) with increase in the volume of MWCNTs suspension from 0 to 25 μL, probably due to the increased surface concentration of ALM at MWCNT-GCE. The oxidation peak current remained almost constant with further increase in the volume. Considering the peak current as well as the time needed for evaporation of acetonitrile, 25 μL (5×5 μL) of MWCNTs suspension was used to modify the GCE surface.
Effect of accumulation time

When the adsorption property of the molecule is under consideration, it is important to optimize the accumulation time. So, we have examined the influence of accumulation time on the oxidation peak current of ALM at the MWCNT-GCE under open circuit potential. While extending the accumulation time from 0 to 150 s, the oxidation peak current increased by 4.0-fold (Figure 2). However, the oxidation peak current decreased slightly with further increase in the accumulation time indicating that the amount of adsorbed analyte tends to a limiting value at the MWCNT film [35]. In other words, accumulation of ALM (saturation amount) at the electrode surface occurred beyond an accumulation time of 150 s. Another possible reason for the decreased peak current could be attributed to the fact that long time accumulation reduced the stability of the film [36]. So an accumulation time of 150 s was maintained by considering sensitivity and working efficiency.

Effect of pH

Electrochemical properties like involvement of protons and electrons in the electrode process can be ascertained by studying the electrochemical behavior of the molecule under different pH conditions. For this, cyclic voltammograms of ALM in phosphate buffer of different pH were recorded (Figure 3). One oxidation peak was observed in the initial scan while no reduction peak was observed in the studied pH range. Like in other triptan members, the peak potential of ALM was found to be pH dependent indicating the involvement of proton in the oxidation process [37-39]. With increase in pH from 3.0 to 6.0, the oxidation peak currents of peak ai gradually increased at GCE. But, it remained almost constant in the pH range of 3.0-5.0 and then increased up to pH 7.0 on MWCNT-GCE. Further, increase in pH to 9.1 resulted in broader and ill-defined peaks (Figure 3). On further increase in pH to 10.0, peak ai disappeared on both GCE and MWCNT-GCE. The plot of peak current against pH of ALM on bare and MWCNT-GCE is shown in Figure 4.
Upon treatment of MWCNTs with nitric acid, oxygen-containing groups like carboxylic groups are introduced at the surface of CNTs [18]. Therefore, protonated ALM did not interact with the working electrode surface and hence, its oxidation peak current was relatively low. In conditions wherein ALM molecules are positively charged and carboxylic groups of MWCNTs are deprotonated (at pH<7.0) and negatively charged, a better interaction between ALM molecules and nanotube film was noticed. This was evident at pH 7.0 as its electro-oxidation peak current was found to be the highest (Figure 4). Therefore, buffer of pH 7.0 was selected for the determination of ALM.

Further, the effect of pH on oxidation peak potential was examined. Peak potentials varied linearly with pH over the entire tested pH range with two breaks at pH 4.2 and 8.0 (Figure 5), probably due to change in the protonation–deprotonation equilibrium of electroactive component. The intersection point observed at pH 8.0 was found to be close to pKa of ALM (8.77) [40] while the intersection point noticed at pH 4.2 was attributed to pKa of the indolyl radical moiety, which is known to be 4.3. When the pH of the supporting electrolyte was increased from 3.0 to 9.1, the oxidation peak potential gradually shifted to negative potential suggesting that the protons were involved in the oxidation of ALM. In acidic medium, the tertiary amine group of ALM was protonated to give a cationic species. However, at pH greater than 8.0, deprotonation of the tertiary amine group occurred. Further, the slope of 55.46 mV/pH obtained in the plot of \( E_{pa} \) versus pH was found to be close to that noticed for other reported triptans [37-39] and the theoretical value of 59 mV/pH expected for the participation of equal number of electrons and protons in the electrode process. Thus, the electrode process confirmed the participation of equal number of electrons and protons. The corresponding equations are shown below:

\[
E_{pa} \text{ (mV)} = -55.46 \text{ pH} + 1079.2 \quad (r^2=0.9947) \quad \text{[pH range of 4.2-8.0]}
\]

\[
E_{pa} \text{ (mV)} = -57.16 \text{ pH} + 1116.4 \quad (r^2=0.9827) \quad \text{[pH range of 8.0-10.0]}
\]
The average surface coverage (Γ, mol/cm²) of ALM on bare and MWCNT-GCE was estimated [41] from the area under the anodic voltammetric peak which was calculated according to the Faraday’s law, Γ=Q/nFA. Here Q is the charge (C), which was ascertained by integrating the anodic peak of the cyclic voltammogram of IPH, n is the number of electrons transferred (n=2), F is the Faraday constant (96486 s A/mol), and A is the electroactive surface area of the electrode (cm²). The surface coverage (Γ) of ALM was found to be 3.035×10⁻¹⁰ and 1.0479×10⁻¹⁰ mol/cm² on MWCNT-GCE and bare GCE, respectively. Higher concentration of ALM accumulated at MWCNT-GCE indicated that higher adsorption characteristics of MWCNT greatly enhanced the surface concentration of ALM on MWCNT. This enabled to achieve better sensitivity.

**Effect of scan rate**

Useful information with regard to electrochemical mechanism could be obtained by recording cyclic voltammograms at different potential sweep rates. Therefore, the electrochemical behavior of 25 μM ALM was investigated on the surface of MWCNT-GCE in phosphate buffer solution of pH 7.0 in the scan rate range of 5-150 mV/s (Figure 6). It could be seen that the oxidation peak potential was shifted positively with increase in scan rate. Further, linear relationship between Epa and logarithm of scan rate was noticed implying that the electron transfer was not very fast [42]. The corresponding equation is shown below:

\[ E_{pa} \text{ (mV)} = 0.0667 \log v + 6.842 \quad (r^2 = 0.9958) \]

The linear relationship between scan rate and peak current indicated that the electrode process was controlled by adsorption on the surface of the modified electrode. The corresponding regression equation is shown below:

\[ I_{pa} \text{ (μA)} = 0.138 v \text{ (mV/s)} + 1.139 \quad (r^2 = 0.9914) \]

The slope of 0.9096 observed in the plot of log Ipa versus log v (Figure 7) supported the above conclusion with regard to electrode process. The corresponding regression equation is shown below:
Probable reaction mechanism for electrochemical oxidation of ALM

Based on the above experimental results, we have proposed the probable mechanism for oxidation of ALM (Scheme 2a and 2b). The observed anodic peak could be attributed to irreversible one electron oxidation of N-heterocyclic nitrogen of the indole moiety to yield a radical cation at pH above 4.2. The radical cation has further undergone one electron oxidation besides the loss of a proton. This yielded quinine imine that was vulnerable to nucleophilic attack. Further, one-electron abstraction by radical-radical or radical-substrate coupling resulted in the formation of a dimerized product. This was further supported by the recent report on electrooxidation of a similar molecule, naratriptan [37].

ANALYTICAL APPLICATIONS

Based on enhanced electrochemical response of ALM at MWCNT-GCE, a new electroanalytical method was proposed for its determination. For this, differential pulse voltammetric method was employed with the following parameters: sweep rate-20 mV/s, pulse amplitude-50 mV, pulse width-30 ms and pulse period-500 ms. Under optimized conditions, the oxidation peak currents were found to be proportional to the concentration of ALM over the range of 0.25-37.5 μM in phosphate buffer of pH 7.0 (Table 1). The RSD values for slope and intercept of calibration curve are shown in Table 1. The lower values of RSD indicated the good precision of the calibration curve. The corresponding results are shown in Figure 8 and the corresponding regression equation is shown below:

\[ \log I_{pa} = 0.9096 \log v - 0.6248 \quad (r^2 = 0.9946) \]

The values of LOD and LOQ were calculated and these were observed to be 69.83 nM and 232.79 nM (for 5 replicates) with RSD values of 0.611 and 0.601 (five runs) respectively. Lower values of LOD and LOQ indicated the sensitivity of the proposed method.
Further, the inter-day and intra-day assay precision of the method was examined by recording voltammograms of 5 replicates of 5, 10 and 20 μM ALM. These analyses yielded the RSD values of 1.54, 1.35 and 1.58 % for inter-day assay and 1.10, 1.28 and 1.23 % for intra-day assay, respectively. RSD values lower than 1.58 % highlighted good precision of the proposed method for the assay of ALM.

**Sensitivity, stability and reproducibility of MWCNT-GCE**

In order to examine the stability and repeatability of the MWCNT-GCE, differential pulse voltammograms of 25 μM ALM were recorded using five different MWCNT-GCEs. The corresponding RSD value of the peak current was noticed to be 3.8 % thereby revealing good reproducibility. Since, we have recorded voltammograms at a new modified electrode every time, it is important to know the reproducibility of peak currents between different modified electrodes. So, reproducibility studies were carried out with modified electrodes on different days (3 consecutive days). The corresponding RSD value of the peak current was found to be 4.1 % indicating good reproducibility.

To check the stability of the modified electrode, we have stored MWCNT-GCE in a refrigerator (at 4 °C) for three weeks and used to record voltammograms. It was noticed that 95 % of its initial oxidation current was retained thereby indicating that the MWCNT-GCE has long-term stability. Sensitivity of the MWCNT-GCE was obtained from the slope of the calibration plot and found to be 0.7498 μA/mM cm² for ALM.

**Effects of Interferents**

The selectivity of the proposed method was investigated by studying the effects of interferents viz., glucose, sucrose, magnesium stearate, talc, cellulose and starch on electrochemical determination of ALM. For this, we have recorded differential pulse voltammograms of 5 μM ALM in the presence of different concentrations of each interferent, separately. The results of effects of interferents on peak currents of ALM are shown in Table 2. It was noticed that the magnesium stearate did not interfere up to 50-fold excess, glucose and sucrose exerted no effect up to 80-fold excess while talc, cellulose and starch
did not interfere up to 100-fold excess. Therefore, the proposed method offered good selectivity for the determination of ALM.

**Analysis of tablets and statistical comparison of the results with the reported method**[^28]

The utility of the proposed method for the assay of pharmaceutical preparations was examined. The results of assay of tablets containing ALM are summarized in Table 3. The results were found to be satisfactory. The accuracy of the proposed method was also evaluated by performing a recovery test after spiking known amounts of the samples. Higher recovery and lower % bias values revealed the accuracy of the proposed method.

The results of analysis were compared statistically by Student t-test and by the variance ratio F-test with those obtained by reported spectrophotometric method (using Folin Ciocalteu reagent with $\lambda_{\text{max}}$ of 770 nm; Beer's law range of 4-12 µg/mL) [28]. The Student t-value at 95% confidence level did not exceed the tabulated value of 2.132 indicating that there was no significant difference between the accuracy of the proposed and reported methods. It was also observed that the variance ratio F-value calculated for $p=0.05$ did not exceed the tabulated value of 6.26 indicating that there was no significant difference between the precision of the proposed and reported methods.

**Determination of ALM in spiked urine and plasma samples**

Recovery from urine is the preferred method for analyzing and estimating bioavailability of ALM in humans because it is not metabolized completely and is excreted unchanged in urine to about 69.6 % [25,28]. Further, the proposed method involved the determination of ALM in spiked human urine samples without any preliminary treatment. The calibration graph was used to determine the concentration of ALM in urine samples. The results of analysis are listed in Table 4. Higher recovery values (>99.2 %) and low RSD values (<1.62 %) indicated accuracy and precision respectively, of the proposed method.

The applicability of the proposed method was further demonstrated by analyzing ALM in plasma samples. Spiked serum samples were diluted with
the supporting electrolyte and their differential pulse voltammograms were recorded. The amount of ALM in serum samples was determined by referring to the calibration plot. The respective results are incorporated in Table 4. Recovery values higher than 96.72% indicated the accuracy of the proposed method.

CONCLUSIONS

The MWCNT-GCE, that offered a diametric improvement in peak current responses of ALM, was used as an electrochemical sensor for electrochemical investigation and determination of ALM. Due to larger surface area and higher accumulation efficiency, the MWCNT film exhibited stronger enhancement effect on the oxidation of ALM, and greatly increased the oxidation current signal. This sensing system for ALM was found to be convenient and showed excellent analytical characteristics such as low detection limit and higher sensitivity.
REFERENCES

Figure 1. Cyclic voltammograms of blank (1) and 25 µM ALM in phosphate buffer of pH 7.0 at bare GCE (2) and MWCNT-GCE (3).

Figure 2. Effect of accumulation time on electrooxidation of ALM
Figure 3. Cyclic voltammograms of 25 μM ALM in phosphate buffer of different pH at a scan rate of 100 mV/s [pH 3.0 (1), 4.2 (2), 5.0 (3), 6.0 (4), 7.0 (5), 8.0 (6), 9.1 (7) and 10.0 (8)].

Figure 4. The plot of peak current of ALM against pH at bare GCE (△) and MWCNT-GCE (■) in phosphate buffer.
Figure 5. Effect of pH on the peak potential of ALM.

Figure 6. Effect of scan rate on cyclic voltammograms of ALM at 10 (1), 20 (2), 30 (3), 40 (4), 60 (5), 80 (6), 100 (7), 120 (8) and 150 (9) mV/s.
Figure 7. Plot of logarithm of peak current versus logarithm of scan rate for electrooxidation of 25 μM ALM in phosphate buffer of pH 7.0.

Figure 8. The relationship between the oxidation peak current and the concentration of ALM in the range of 0.25 (1), 1.25 (2), 2.5 (3), 3.75 (4), 6.25 (5), 10 (6), 15 (7), 20 (8), 25 (9) and 37.5 (10) μM.
MWCNT suspension was drop casted

Cleaned GCE MWCNT-GCE

Scheme 1. Schematic description of preparation of MWCNT-GCE.
Scheme 2. Probable reaction mechanism for electrooxidation of ALM at MWCNT-GCE in the pH range of 4.2-8.0 (a) and in the pH range of 8.0-10.0 (b).
Table 1. Characteristics of calibration plot for ALM.

<table>
<thead>
<tr>
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<th>DPV</th>
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<tr>
<td>Linearity range (μM)</td>
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<tr>
<td>Slope (μA/μM)</td>
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<tr>
<td>Intercept (μA)</td>
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<td>RSD (Intercept)* %</td>
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<tr>
<td>LOQ (nM)</td>
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<td>Inter-day assay RSD*,%</td>
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<tr>
<td>Intra-day assay RSD*,%</td>
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</table>

*Average of 5 determinations

Table 2. Effects of interferents in the determination of 5 μM ALM at MWCNT-GCE.

<table>
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<tr>
<th>Interferent</th>
<th>Concentration (μM)</th>
<th>Current (μA)</th>
<th>Signal change (μA)</th>
<th>RSD*,%</th>
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<tr>
<td>ALM only</td>
<td>5</td>
<td>2.751</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>250</td>
<td>2.650</td>
<td>0.101</td>
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<td>Glucose</td>
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<td>2.718</td>
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<td>Sucrose</td>
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<td>2.702</td>
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<td>Talc</td>
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<td>500</td>
<td>2.621</td>
<td>0.130</td>
<td>2.35</td>
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</table>

* Average of 5 determinations
Table 3. Results of analysis of ALM in pharmaceutical samples.

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Axert (^a)</th>
<th>Found by reported method (^#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labeled claim (mg)</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Amount found (mg)</td>
<td>6.18 ± 0.105</td>
<td>6.15 ± 0.073</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>98.88</td>
<td>98.46</td>
</tr>
<tr>
<td>Bias (^b) (%)</td>
<td>1.12</td>
<td>1.54</td>
</tr>
<tr>
<td>RSD (^b) (%)</td>
<td>1.71</td>
<td>1.18</td>
</tr>
<tr>
<td>t-value at 95% confidence level</td>
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<td>1.02</td>
</tr>
<tr>
<td>F-value at 95% confidence level</td>
<td>2.09</td>
<td>4.67</td>
</tr>
<tr>
<td>Pure ALM added to tablet solution (mg)</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Amount found (mg)</td>
<td>19.75</td>
<td>9.85</td>
</tr>
<tr>
<td>Recovery (^b) (%)</td>
<td>98.75</td>
<td>98.46</td>
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<tr>
<td>RSD (^b) (%)</td>
<td>1.32</td>
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<tr>
<td>Bias (^b) (%)</td>
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<td>1.54</td>
</tr>
</tbody>
</table>

\(^a\)Marketed by Janssen Pharmaceuticals

\(^b\) Average of 5 determinations


\(^#\) Reported spectrophotometric method [24] using Folin Ciocalteu reagent (2N) at \(\lambda_{\text{max}}=770\) nm.

Table 4. Results of analysis of ALM in spiked urine and serum samples at MWCNT-GCE.

<p>| Urine samples | | | | |
|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>ALM added (µM)</th>
<th>n</th>
<th>Amount found (µM)</th>
<th>Average recovery (%)</th>
<th>RSD (%)</th>
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<td>99.2</td>
<td>1.62</td>
</tr>
<tr>
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<td>4</td>
<td>9.92</td>
<td>99.2</td>
<td>1.12</td>
</tr>
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<td>25</td>
<td>4</td>
<td>25.1</td>
<td>100.4</td>
<td>0.53</td>
</tr>
</tbody>
</table>

| Serum samples | | | | |
|----------------|----------------|----------------|----------------|
| 5 | 4 | 4.93 | 98.6 | 2.32 |
| 10 | 4 | 9.83 | 98.3 | 1.39 |
| 25 | 4 | 24.18 | 96.72 | 2.12 |
Electrochemical oxidation and determination of isothipendyl hydrochloride at an electrochemical sensing film constructed by multiwalled carbon nanotubes

ABSTRACT

The electrochemical behavior of an antihistamine, isothipendyl hydrochloride (IPH) was studied at multiwalled carbon nanotube modified glassy carbon electrode (MWCNT-GCE). IPH showed two oxidation peaks in Britton-Robinson (BR) buffer of pH 7.0. The oxidation process of IPH was observed to be irreversible over the pH range of 2.5-9.0. The influence of pH, scan rate and concentration of the drug on anodic peak was investigated. A differential pulse voltammetric method with good precision and accuracy was developed for the determination of IPH in pure and biological fluids. A linear relationship was observed between peak current and concentration of IPH in the range of 1.25-55 μM. The values of LOD and LOQ were noticed to be 0.284 and 0.949 μM, respectively.

The results of this section are published in

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INTRODUCTION

When used as electrode material, carbon nanotubes (CNTs) have the ability to mediate electron-transfer reactions with an electroactive species in solution, owing to its extraordinary electronic properties [1-3]. These excellent properties suggest that CNT is a fascinating electrode material, and hence, it is widely used in electrochemistry and electroanalytical chemistry [4-6]. CNT modified electrodes are widely used for the determination of various compounds of biological interest [7-11].

General drug profile of IPH has been given in Chapter I under “drugs selected in the present study”.

Several methods have been reported for the determination of IPH in forensic samples, pharmaceutical formulations and body fluids [12-15]. Because of its pharmacological importance and lack of reports on its electrochemical behavior and analysis by voltammetry, we thought of investigating the electrochemical behavior of IPH at MWCNT-GCE in detail. Further, we have developed a differential pulse voltamometric method for sensitive determination of IPH in pure and biological samples.

EXPERIMENTAL

Apparatus

Electrochemical studies were carried out on a CHI-1103A electrochemical analyzer (CH Instruments Ltd. Co., USA, version 9.03). A conventional three electrode system consisting of a GCE or MWCNT-GCE as the working electrode, a platinum wire as a counter electrode and an Ag/AgCl (3 M KCl) as reference electrode was employed.

Working solutions

- A stock solution of IPH (2.5 mM) was prepared in milli-pore water and stored in a refrigerator at 4 °C.
- Working solutions were prepared daily by diluting the stock solution as required with BR buffer (0.04 M) of required pH.
• BR buffer (pH 2.5-10.6) was used as the supporting electrolyte.

**Preparation of MWCNT-GCE**

MWCNTs were refluxed in concentrated nitric acid for about 5 h, filtered and washed with milli-pore water till the filtrate became neutral and finally dried [16]. The MWCNTs suspension was prepared by dispersing 2 mg of MWCNTs in 10 mL acetonitrile using ultrasonic agitation to achieve a fairly stable suspension. For reproducible results, improved sensitivity and resolution of voltammetric peaks, the working electrode was polished with 0.05 micron alumina powder on a polishing cloth. Then, it was thoroughly rinsed with milli-pore water. The cleaned GCE was coated by casting 20 µL of the black suspension of MWCNTs and dried in air. After modification, the electrode was rinsed with water for about 5 min to remove the loosely adsorbed nanotubes, if any.

**Working procedure**

The MWCNT-GCE was first activated in BR buffer of pH 7.0 by cyclic voltammetric sweeps between 0 and 1.4 V till clear cyclic voltammograms were obtained. The modified electrode was then transferred into 10 mL BR buffer (pH 7.0) containing IPH (55 µM) and an accumulation time of 240 s was given. After this accumulation time, the electrode was used to record the cyclic voltammogram/differential pulse voltammogram. After every measurement, new MWCNT-GCE was prepared.

For DPV, the following parameters were maintained: sweep rate-20 mV/s, pulse amplitude-50 mV, pulse width-30 ms and pulse period-500 ms. For analytical applications, oxidation peak a₁ was selected. All electrochemical experiments were carried out at 25 °C.

**Determination of IPH in human urine and plasma samples**

Spiked urine samples were obtained by treating 0.45 mL aliquots of urine with 50 µL IPH standard solution (2.5 mM) to obtain 250 µM IPH. A suitable aliquot of spiked urine was diluted with BR buffer, without any pre-
treatment, to prepare appropriate sample solution and differential pulse voltammogram was recorded under optimized conditions.

Spiked serum samples were prepared by following the procedure reported earlier [17]. Serum samples, obtained from healthy volunteers (upon collecting their written consent), were stored frozen until assay. For the determination of IPH in plasma, 500 μL of IPH (2.5 mM) was added to 500 μL of untreated plasma. The mixture was vortexed for 40 s. The plasma samples were then treated with 250 μL perchloric acid (15%). After that, the mixture was vortexed for further 30 s and then centrifuged at 5000 rpm for 5 min. An appropriate volume of supernatant liquor was transferred in the voltammetric cell containing BR buffer of pH 7.0 and voltammograms were recorded. The voltammogram of blank sample did not show any signal that could interfere with the direct determination. The content of the drug in plasma was determined referring to the calibration graph or regression equation.

**RESULTS AND DISCUSSION**

**Cyclic voltammogram of IPH at MWCNT-GCE**

Figure 1 depicts the cyclic voltammograms of 55 μM IPH at bare GCE and MWCNT-GCE in BR buffer of pH 7.0 along with that of the blank. Two oxidation peaks of IPH at 721 mV (a₁) and 958 mV (a₂) were observed at the bare GCE (Figure 1). At MWCNT-GCE, a considerable enhancement in the peak current was noticed. Further, the oxidation peaks were appeared at 696 mV and 912 mV, respectively. Thus, the negative shifts in peak potentials were 25 mV and 46 mV for peak a₁ and a₂ respectively, suggesting that MWCNT exhibited catalytic effect towards electrooxidation of IPH. No reduction peak was observed in the reverse scan suggesting that the electrochemical oxidation of IPH was an irreversible process. The oxidation peak of IPH at the bare GCE was observed to be weak owing to slow electron transfer. However, the peak current response was considerably improved with shift towards less positive potentials at the MWCNT-GCE. This was attributed to the electrocatalytic effect of MWCNTs [1,18]. The basis for better
performance of the MWCNT-GCE might be due to the nanometer dimensions of MWCNTs, the electronic structure and topological defects present on MWCNTs surfaces [1].

Successive cyclic voltammograms were recorded to check the adsorption property of IPH or its oxidation product on MWCNT-GCE. It was clear that the oxidation peak current of IPH decreased significantly during the successive voltammetric sweeps and finally remained unchanged. This was attributed to the adsorption of oxidative product of IPH on the modified electrode surface.

**Influence of the amount of MWCNT suspension**

The amount of MWCNT suspension on GCE is an important parameter that may influence the electrooxidation of IPH. With increase in the volume of MWCNT suspension from 0 to 20 µL, the oxidation peak current of IPH was observed to be increased remarkably (Figure 2) as the surface concentration of IPH at MWCNT-GCE increased. With further increase in the volume, the oxidation peak current remained almost constant. Considering the peak current as well as the time needed for evaporation of acetonitrile, 20 µL of MWCNT suspension was used as optimum volume for the modification of GCE.

**Effect of accumulation time**

We have investigated the influence of accumulation time on oxidation peak currents of IPH at MWCNT-GCE. When extending the accumulation time from 0 to 240 s, the oxidation peak currents remarkably increased (Figure not shown). Further increase in accumulation time led to slight decrease in peak currents suggesting that the amount of IPH tends to a limiting value at the MWCNT film. Considering sensitivity and working efficiency, an accumulation time of 240 s was maintained.

**Effect of pH value**

The electrochemical behavior of IPH was investigated in BR buffer of different pH. At pH 3.5, the voltammogram of IPH was almost similar to that of promethazine (PMZ) as expected, owing to the close similarity in structure
[19]. IPH is different from PMZ only in one benzene ring, which is replaced by pyridine ring in IPH. Two oxidation waves were seen on the initial scan and no reduction peak was observed. Like in the case of another phenothiazine, ethopropazine, the peak potential of $a_1$ (of IPH) was pH dependent indicating the involvement of proton in the oxidation process [20]. With increase in pH from 2.5 to 7.0, the oxidation peak currents of $a_1$ and $a_2$ gradually increased at MWCNT film-modified GCE (Figure 3). Further increase in pH to 9.0, the oxidation peak currents of $a_1$ gradually decreased with broader and ill-defined peaks. Apparently, the oxidation signals of IPH were seem to be sensitive in the buffer of pH 7.0. Besides, the effect of pH on the oxidation peak potential was examined. When the value of pH increased from 2.5 to 7.0, the oxidation peak potential gradually shifted to negative potential suggesting that protons were involved in the oxidation of IPH. The plot of peak potential of $a_1$ versus pH showed a linear segment at pH 8.0. This intersection point of the curve was found to be close to the pKa value of IPH (8.6) [21]. This could be attributed to changes in protonation of acid-base properties of the molecule. The slope was found to be 29.6 mV/pH, which is close to that reported for PMZ [22,23] and the theoretical value for a two-electron and one-proton transfer reaction. Thus, it could be concluded that the electrode reaction mechanisms of IPH and PMZ are identical at least over the pH range of 2.5-7.0.

The average surface coverage ($\Gamma$, mol/cm$^2$), of IPH on bare and MWCNT-GCE was calculated from the area under the anodic voltammetric peak as described in Chapter III, Section A. The $\Gamma$ of IPH was found to be $1.1635\times10^{-9}$ and $6.7633\times10^{-10}$ mol/cm$^2$ on the MWCNT-GCE and bare GCE, respectively. Higher concentration of IPH accumulated at MWCNT-GCE revealed that higher adsorption characteristics of MWCNT significantly increased the surface concentration of IPH on MWCNT. This helped in achieving higher sensitivity.
Effect of scan rate

Information on electrochemical mechanism can be acquired from the relationship between the peak current and scan rate. Therefore, the electrochemical behavior of IPH at different scan rates from 10 to 100 mV/s was investigated. The effect of scan rate on the anodic oxidation of 55 μM IPH in BR buffer of pH 7.0 is illustrated in Figure 4. The peak current was observed to be proportional to the scan rate indicating that the electrode process was adsorption controlled [24]. A linear relationship was observed between log $I_{pa}$ and log $v$ as per the equation shown below:

$$\log I_{pa} = 0.8255 \log v - 0.0591$$

The slope of 0.83 (obtained from the plot of log $I_{pa}$ versus log $v$) was noticed to be close to the theoretical value of 1.0 for an adsorption-controlled process [25,26]. The $E_{pa}$ of the oxidation peak was also noticed to be dependent on the scan rate. Further, the peak potential was shifted to more positive values with increase in the scan rate. Linear relationship was observed between $E_{pa}$ and scan rate indicating the irreversibility of the oxidation process with a correlation coefficient of 0.99 according to the equation shown below:

$$E_{pa} (mV) = 1.127 v + 651.7$$

ANALYTICAL APPLICATIONS

Effect of concentration on oxidation of IPH

DPV was used as a tool for the determination of IPH because of its higher sensitivity compared to CV. Sharper and well resolved curves were obtained in BR buffer of pH 7.0. Under the optimized conditions, a linear relation between the peak current and concentration of the drug was observed in the range of 1.25-55 μM IPH ($r^2=0.993$). Beyond 55 μM IPH, the linearity was lost. Differential pulse voltammograms of different concentrations of IPH are shown in Figure 5. The plot of $I_{pa}$ versus concentration of IPH is shown in Figure 6. The linear relation expressing the dependence of $I_{pa}$ on concentration is shown below:
I_{pa} (\mu A) = 1.0527 (\mu M) + 3.1423 \quad (r^2=0.9930)

Characteristics of the calibration graph are recorded in Table 1. The values of LOD and LOQ were calculated (n=5) based on the peak current and found to be 0.28 \mu M and 0.94 \mu M, respectively. The inter-day reproducibility of the method was examined by recording voltammograms of 5 replicates of 5, 20 and 50 \mu M IPH. This assay yielded the RSD values of 1.28, 1.42 and 1.53 % respectively. Further, the RSD values for intra-day assay at 5, 20 and 50 \mu M solutions (n=5) were found to be respectively, 1.12, 1.36 and 1.15 %. The corresponding results are shown in Table 1. Low values of both LOD and LOQ confirmed good sensitivity of the proposed method. Further, low values of RSD revealed the reproducibility of results by the proposed method for the assay of IPH.

Effects of interferents

The selectivity of the proposed method was examined by studying the effects of various interferents viz., glucose, sucrose, starch, acacia powder, ascorbic acid and talc on electrochemical determination of IPH. For this, we have recorded differential pulse voltammograms of 2.5 \mu M IPH in the presence of different concentrations of each interferent separately. The results of effects of interferents on peak currents of IPH are shown in Table 2. It was noticed that the ascorbic acid did not interfere with the peak current of IPH up to 12-fold excess while the acacia powder, talc and starch showed no effect on the peak current up to 20-fold excess. Further, glucose and sucrose did not exhibit any interference up to 32-fold excess. These results indicated that the proposed method has good selectivity for the determination of IPH. Hence, IPH could be easily determined in the presence of above interferents.

Determination of IPH in urine and plasma samples

The practical application of the proposed method was further established by determining IPH in human urine samples without any preliminary treatment. The recoveries from urine samples were examined by spiking drug free urine with known amounts of IPH. The calibration graph was used to determine the
concentration of IPH in urine samples. The results of analysis are listed in Table 3. The average recovery was observed to be higher than 99.37 % and the RSD values were less than 1.51 %. These values indicated good recovery and reproducibility of results.

The applicability of the proposed method was examined by analyzing IPH in plasma samples employing MWCNT-GCE. Suitable amounts of IPH spiked serum samples were diluted with supporting electrolyte. The amount of IPH in serum samples was determined by referring to the calibration plot. The results incorporated in Table 3 indicated good recovery of IPH. The proposed method is simple, easy to perform and sensitive enough for the assay of IPH in human serum samples.

CONCLUSIONS

Present method utilizes the enhanced peak current responses of IPH oxidation at MWCNT-GCE for its assay by differential pulse voltammetric method. The effects of amount of MWCNT, accumulation time, pH and scan rate on electrochemical response of IPH have been investigated. This novel sensing system for IPH was found to be convenient and showed excellent analytical characteristics such as significant lowering of the detection limit, higher sensitivity and satisfactory selectivity. The method provides a simple approach for the determination of IPH in spiked human urine and serum samples.
REFERENCES


Figure 1. Voltammograms of blank buffer (1); 55 μM IPH at bare GCE (2) and MWCNT-GCE (3) in BR buffer of pH 7.0.

Figure 2. Effect of volume of MWCNT on electrooxidation of IPH.
Figure 3. Plot of $I_{pa}$ versus pH for electrooxidation of IPH on MWCNT-GCE in BR buffer at a scan rate of 50 mV/s.

Figure 4. Voltammograms of 55 μM IPH in BR buffer of pH 7.0 on MWCNT-GCE at different scan rates: 10 (1), 20 (2), 30 (3), 40 (4), 60 (5), 80 (6) and 100 (7) mV/s.
Figure 5. Differential pulse voltammograms of IPH at different concentrations: 1.25 (1), 2.5 (2), 5 (3), 10 (4), 18.8 (5), 25 (6), 35 (7), 45 (8) and 55 (9) μM.

Figure 6. Plot showing the relation between $I_{pa}$ and concentration of IPH.
Table 1. Characteristics of calibration plot of IPH.

<table>
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<tr>
<td>Linearity range (µM)</td>
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<tr>
<td>LOD (µM)</td>
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<tr>
<td>LOQ (µM)</td>
<td>0.949</td>
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<td>Inter-day assay RSD (%)</td>
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<tr>
<td>Intra-day assay RSD (%)</td>
<td>1.36</td>
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Table 2. Effects of interferents on differential pulse voltammetric response of 2.5 µM IPH at MWCNT- GCE.

<table>
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<th>Interferent</th>
<th>Concentration (µM)</th>
<th>Current (µA)</th>
<th>Signal change (µA)</th>
<th>RSD* (%)</th>
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<tbody>
<tr>
<td>IPH (no interferent)</td>
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<td>3.510</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ascorbic acid</td>
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<td>0.140</td>
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<td>Glucose</td>
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<td>Sucrose</td>
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<td>Talc</td>
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<td>3.525</td>
<td>0.015</td>
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<td>Acacia powder</td>
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<td>0.076</td>
<td>1.87</td>
</tr>
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<td>Starch</td>
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<td>3.621</td>
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<td>2.05</td>
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</table>

* Average of 4 determinations
### Table 3. Results of analysis of IPH in spiked urine and serum samples.

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<th>IPH added (μM)</th>
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<th>Amount found (μM)</th>
<th>Average recovery (%)</th>
<th>RSD (%)</th>
</tr>
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</tr>
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<td>4</td>
<td>1.99</td>
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</tr>
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