Introduction

Alzheimer’s disease (AD) is a progressive, irreversible brain disorder with no known cause or cure. It attacks and slowly steals the disease including memory loss, confusion, impaired judgment, personality changes, disorientation, and loss of language skills. Always fatal, AD is the most common form of irreversible dementia.

AD is the commonest cause of dementia affecting older people. It is becoming tragically common. It is estimated that there are currently 18 million people worldwide with AD. This figure is projected to nearly double by 2025 to 34 million people.

AD is a neurodegenerative disorder characterized by progressive decline of cognitive functions. The significant deficits in presynaptic cholinergic markers observed in brains of AD patients have led to the formulation of the cholinergic hypothesis of AD. Current approaches to the treatment of cognitive and behavioral symptoms of AD emphasize the use of cholinesterase inhibitors. Rivastigmine (Exelon®), a carbamate type pseudo-irreversible acetylcholinesterase (AChE) inhibitor, may be advantageous in treating AD, as it plays specificity for central AChE over peripheral AChE or butrylcholinesterase1,2.
Exelon® is brand name for the drug rivastigmine. Exelon® is not claimed to be a cure for AD. It treats the symptoms only and there is no evidence that it could halt or reversible AChE inhibitor. It is selective for the cholinesterase and is used for the symptomatic treatment of mild moderately severe dementia in AD³.

It is given as the hydrogen tartrate but doses are expressed in terms of the equivalent of base.

Exelon® (rivastigmine tartrate) is a reversible cholinesterase inhibitor and is known chemically as (S)-N-Ethyl-N-methyl-3-[1-(dimethylamino)ethyl]-phenyl carbamate hydrogen-(2R,3R)-tartrate. Rivastigmine tartrate is commonly referred to in the pharmacological literature as SDZ ENA 713 or ENA 713.

It has an empirical formula of C₁₄H₂₂N₂O₂·C₄H₆O₆ (hydrogen tartrate salt)⁴.

![Chemical structure of Rivastigmine hydrogen tartrate.](image)

Pharmacopoeia does not give any official determination method for RVT. Few chromatographic methods for the determination of rivastigmine (RVT) have been described⁵-⁸. LC-MS-MS method have been used to quantitate analysis of RVT and its major metabolite (NAP 226-90) in biological fluids⁹.

In the previous study¹⁰ three spectrophotometric methods, direct absorbance measurement, first and second derivative methods were applied to quantitative analysis of RVT in capsules. When the previous literatures cited, no electrochemical studies on the analysis of RVT in bulk drugs and pharmaceutical formulations were reported. Electrochemical methods have already proved to be very useful in the field of drug analysis due to their simplicity, low cost and relatively short analysis time when compared with other techniques.
In this study, an investigation on the electrochemical behaviors of RVT was done. In the case of the electrochemical analysis, phosphate buffer (pH=6) as a supporting electrolyte and scan rate of 20 mV s\(^{-1}\) were found to be optimal condition in the application of DPV technique to the determination of RVT in pharmaceutical dosage forms. The developed electrochemical technique was compared with capillary zone electrophoresis\(^{11}\) and a good agreement between results was observed.

2. Experimental

2.1. Apparatus

Voltammetric measurements were made using a BAS 100 W/B model electrochemical analyser and a HP 1100 laserjet printer. The three-electrode system comprised a BAS MF 2012 glassy carbon disc electrode, a BAS MF 1063 type Ag/AgCl reference electrode and a BAS MV 1032 Pt wire auxiliary electrode.

2.2. Reagents

RVT was kindly obtained from Novartis Ltd. without prior purification. Exelon\(^{\circledast}\) capsules containing a 1.5 mg dose were obtained from local drugstores.

Analytical grade phosphoric acid was purchased from Merck&Co. All other chemicals were of analytical-reagent grade and were used as received.

2.3. Solution preparation

Stock solution of 10\(^{-3}\) M RVT was prepared in phosphate buffer. Diluted working standard solutions were then prepared daily from fresh stock solution and contained phosphate buffer. Phosphate buffers were prepared according to the USP pharmacopoeial procedure.

2.4. Sample Preparation

The determination of RVT was performed in capsules that are commercially available in Turkey. The content of 10 capsules was finely powdered and thoroughly mixed. An accurately weighed amount of powder equivalent to 1.5 mg of RVT was dissolved in the supporting electrolyte
solution (pH=6.0). Filtration of solution through Whatman No: 42 filter paper was performed to remove any remaining insoluble matter.

In this case, working solutions of the pharmaceutical formulations were prepared exactly as the standard solutions. All these solutions were prepared daily.

2.5. Electrochemical condition

2.5.1. Pretreatment of the working electrode

To provide a reproducible active surface and improve the sensitivity and resolution of the voltammetric peaks, the working electrode was polished with 0.5 mm alumina powder on a polishing cloth prior to each electrochemical measurement. The electrode cleaning procedures require only 2 min. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper.

2.5.2. Voltammetric Method

$10^{-3}$ M stock standard solution of RVT was prepared in phosphate buffer. The standard series of RVT in the concentration range of $(7 \times 10^{-5} - 8 \times 10^{-4})$ M was prepared by using the above stock solution. In all the analysis procedure, phosphate buffer (pH = 6.0, 0.2 M) were used as supporting electrolyte. After the voltammogram of supporting electrolyte was recorded known aliquots of standards were added and voltammograms were recorded to obtain the calibration graph. The same procedure was repeated for sample analysis.

3. Results And Discussion

3.1. Influence of pH of supporting electrolyte

In the application of DPV technique, some supporting electrolytes namely Britton-Robinson buffer and phosphate buffer were tested. Phosphate buffer system of them was found to be suitable for the electrochemical analysis.

Differential pulse voltammetric behaviour of RVT at a glassy carbon electrode was examined varying pH over a wide range of values from acidic to alkaline media (between 2.0 and 10.0) as indicated in Figure 2. Figure 3 shows that the highest peak current was obtained in from phosphate
VOLTAMMETRIC BEHAVIOUR OF RIVASTIGMINE HYDROGEN TARTRATE AND ITS DETERMINATION IN CAPSULE DOSAGE FORM

Figure 2

Effect of pH on peak current for 1.0x10^-4 M RVT solutions in different phosphate buffer by means of differential pulse voltammetry at a glassy carbon electrode.

Figure 3

Curve obtained from relationships between pH values and their corresponding Ip values for 1.0x10^-4 M RVT solutions in phosphate buffer.
buffer at pH 6.0. According to the experimental results, phosphate buffer at pH 6.0 (0.2 M) was selected as an appropriate supporting electrolyte. This study indicated that RVT is electroactive and that the mechanism of oxidation is dependent of pH. When pH is lower than 6 no noticeable oxidation peak is observed and the maximum peak current was observed at 1030.0 mV vs Ag/AgCl electrode. The shift in the differential pulse peak potential as a function of pH was studied and a linear decrease in potential was observed in the range of pH=6 and pH=10 (Figure 4). Figure 5 shows the relationship between peak current and RVT concentration in phosphate buffer (pH: 6.0).

![Figure 4](image)

**Figure 4**

Effect of pH on peak potentials for 1.0x10^-4 M RVT solutions in phosphate buffer at pH: 6.0 by means of differential pulse voltammetry at a glassy carbon electrode (vs Ag/AgCl)

### 3.2. Development of the differential pulse voltammetry

To improve the sensitivity and selectivity of the determination of RVT, differential pulse voltammetric parameters such as pulse amplitude was investigated. It was found that the peak currents increased significantly with increasing pulse amplitude from 10 to 50 mV. The pulse amplitude chosen for practical determination was 50 mV.

In these experiments faster scan rates resulted in higher peak currents but background currents also increased. Taking peak current and background current into consideration the scan rate used in this study was 20 mVs\(^{-1}\).
The effect of RVT concentration on peak current at 1030 mV was investigated. Optimum experimental and instrumental conditions for the differential pulse voltammetric determination of RVT were found as pH: 6.0 phosphate buffer, 20 mVs\(^{-1}\) scan rate, 50 mV pulse amplitude, 17 ms sample width, 50 ms pulse width, 200 ms pulse period.

The effect of potential scan rate on the peak current and the peak potential for cyclic voltammetric experiments of RVT was evaluated. Figure 6 shows the influence of the square root of the scan rate on the peak current. A linear relationship was observed between 10 and 100 mVs\(^{-1}\) which is typical for diffusion-controlled currents. The logarithm of peak current - logarithm of scan rate (log \(i_p\) - log \(v\)) relationship was also linear (log \(i_p\) = 0.57 log \(v\) + 0.59). The slope means that the reaction is greatly diffusion controlled, but adsorption is also effective the reaction rate. From the curves obtained in phosphate buffer of pH = 6.0 with different concentrations of RVT the currents at 1030.0 mV were recorded. Log \(i\) - log \(C\) relationship was also obtained as log \(i\) = 0.36 log \(C\) +3.185 (\(r^2 = 0.995\)). It can be seen from the slope that the reaction order was found to be 0.36.
Tafel plot was obtained with a scan rate of 5 mVs\(^{-1}\) beginning from a steady-state potential in phosphate buffer pH 6.0 and from the slope of the linear part \(\alpha\) was found to be 0.33. Regarding this value and the position and heights of anodic and cathodic peaks, the reaction seems not reversible.

![Figure 6](image)

**Figure 6**

Variation of peak current, \(I_p\) (\(\mu\)A), with scan rate, \(\sqrt{V}\) (mVs\(^{-1}\))

On the basis of the electrochemical behavior of RVT at a glassy carbon electrode, a method was developed, namely, differential pulse voltammetry for the determination of the drug. The results are given in Table I.

**TABLE I**

Linear regression analysis and its statistical results obtained by DPV technique for RVT

<table>
<thead>
<tr>
<th>Parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (M)</td>
<td>(7 \times 10^{-5} - 8 \times 10^{-4})</td>
</tr>
<tr>
<td>Regression equation ((Y))</td>
<td>(Y = 9.4 \times 10^{-3} \times C + 4.0 \times 10^{-6})</td>
</tr>
<tr>
<td>Slope ((b))</td>
<td>(9.4 \times 10^{-3})</td>
</tr>
<tr>
<td>Std. error of slope ((S_b))</td>
<td>(8.8 \times 10^{-4})</td>
</tr>
<tr>
<td>Intercept ((a))</td>
<td>(4.0 \times 10^{-6})</td>
</tr>
<tr>
<td>Std. error of intercept ((S_a))</td>
<td>(4.0 \times 10^{-7})</td>
</tr>
<tr>
<td>Correlation coefficient ((r))</td>
<td>(0.9954)</td>
</tr>
<tr>
<td>Std. error of correlation coefficient ((S_r))</td>
<td>(5.9 \times 10^{-7})</td>
</tr>
</tbody>
</table>

\(Y = a + b \cdot C\) where \(C\) is concentration in M and \(Y\) in peak current units for differential pulse voltammetric method, respectively.
3.3. Validation

Validation of the procedures for the quantitative assay of the drug were examined by using the limit of detection (LOD), limit of quantitation (LOQ), recovery, repeatability, specificity. The values of LOD and LOQ were 1.69x10\(^{-5}\) M and 5.65x10\(^{-5}\) M, respectively. The linearity of the method was observed between 7x10\(^{-5}\) - 8x10\(^{-4}\) M. The limit of quantification (LOQ) and the limit of detection (LOD) were calculated according to USP 25 guidelines\(^{12,13}\). These values were calculated for seven replicates. Precision and accuracy of the results obtained by applying the method to the synthetic samples (see Table II) were demonstrated by calculating the percent mean recovery and relative standard deviation. No systematical error and the effect of excipient on the analysis of capsules were observed, according to the experimental results obtained from recovery study and capsules analysis.

In the present study, the performance of the applied electrochemical method was tested in the linear concentration range of 7.0x10\(^{-5}\) – 8.0x10\(^{-4}\) M RVT and satisfactory results for accuracy and precision were obtained as presented in Table II. The application of the method to the quantitative evaluation of capsules was explained in the following sub-section.

### TABLE II

Recovery data obtained in synthetic samples

<table>
<thead>
<tr>
<th>Added (M)</th>
<th>Found (M)</th>
<th>SE</th>
<th>Recovery (%)</th>
<th>RSD % n=5</th>
<th>% Bias’</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0x10(^{-5})</td>
<td>6.8x10(^{-5})</td>
<td>6.0x10(^{-7})</td>
<td>97.1</td>
<td>1.98</td>
<td>2.86</td>
</tr>
<tr>
<td>8.0x10(^{-5})</td>
<td>7.5x10(^{-5})</td>
<td>6.9x10(^{-7})</td>
<td>94.1</td>
<td>2.05</td>
<td>5.94</td>
</tr>
<tr>
<td>2.0x10(^{-4})</td>
<td>2.0x10(^{-4})</td>
<td>1.7x10(^{-6})</td>
<td>100.5</td>
<td>1.89</td>
<td>-0.50</td>
</tr>
<tr>
<td>4.0x10(^{-4})</td>
<td>4.0x10(^{-4})</td>
<td>4.8x10(^{-6})</td>
<td>100.6</td>
<td>2.67</td>
<td>-0.56</td>
</tr>
<tr>
<td>5.0x10(^{-4})</td>
<td>5.0x10(^{-4})</td>
<td>4.2x10(^{-6})</td>
<td>100.1</td>
<td>1.89</td>
<td>-0.10</td>
</tr>
<tr>
<td>6.0x10(^{-4})</td>
<td>6.0x10(^{-4})</td>
<td>5.9x10(^{-6})</td>
<td>100.3</td>
<td>2.2</td>
<td>-0.25</td>
</tr>
<tr>
<td>8.0x10(^{-4})</td>
<td>7.6x10(^{-4})</td>
<td>6.8x10(^{-6})</td>
<td>95.3</td>
<td>1.99</td>
<td>4.72</td>
</tr>
</tbody>
</table>

**Mean** 98.3

**RSD %** 2.81

\[ \text{% Bias} = \frac{\text{Added} - \text{Found}}{\text{Added}} \times 100 \]
3.4. Application to the Pharmaceutical Preparations

The experimental results obtained by applying the DPV technique to the commercial pharmaceutical capsule preparation were presented in Table III. When a comparison was made between the applied DPV technique and the reported method\textsuperscript{11}, a good coincidence was observed. In the cases of two methods, t-test values at 95% confidence level were given in the Table III. No significant difference was found statistically.

Based on the above results, the DPV technique may be recommended for routine and quality control analysis of the investigated drug in pharmaceutical dosage forms.

**TABLE III**

Assay results obtained from commercial sample

<table>
<thead>
<tr>
<th>Commercial dosage (form\textsuperscript{a})</th>
<th>Analysis techniques</th>
<th>DPV</th>
<th>CZE(\textsuperscript{*})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.508</td>
<td>1.498</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.498</td>
<td>1.500</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.500</td>
<td>1.528</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.528</td>
<td>1.570</td>
</tr>
<tr>
<td>Mean\textsuperscript{b}</td>
<td></td>
<td>1.52</td>
<td>1.48</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.030</td>
<td>0.024</td>
</tr>
<tr>
<td>RSD (%)</td>
<td></td>
<td>1.961</td>
<td>1.600</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>0.173</td>
<td>0.001</td>
</tr>
<tr>
<td>Confidential limit (p=0.05)</td>
<td></td>
<td>0.026</td>
<td>-</td>
</tr>
<tr>
<td>Calculated t value</td>
<td></td>
<td>2.120</td>
<td>-</td>
</tr>
<tr>
<td>Theoretical t value (p=0.05)</td>
<td></td>
<td>2.260</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a.} (Exelon \textcopyright) contains 1.5 mg RVT per capsule

\textsuperscript{b.} Mean value is the average of five experiments

SE = Standard error

(\textsuperscript{*}) literature method (11).
Summary

The electrochemical behaviour of rivastigmine hydrogen tartrate (RVT) was studied by differential pulse voltammetry (DPV) with glassy carbon electrode and an analytical method was developed for the determination of RVT in phosphate buffer at pH: 6.0 as supporting electrolyte. The developed method was applied to the quantitative analysis of RVT. By using Phosphate and Britton-Robinson buffers, the influence of pH and scan rate was investigated on electrochemical behaviour of RVT. Applying the proposed method, the calibration graph was obtained from 7.0x10^{-5} to 8.0x10^{-4} M. In this study, the values of LOD and LOQ were calculated as 1.69x10^{-5} M and 5.65x10^{-5} M, respectively. Synthetic samples were analysed and mean recoveries and relative standard deviations were found as 98.3 % and 2.81 %. A good agreement was observed for the obtained recovery results. The electrochemical results in this study were compared with those obtained by capillary zone electrophoresis method in literature and a comparable result was reported.

Keywords: Rivastigmine; Differential pulse voltammetry; Pharmaceutical dosage form

Özet

Rivastigmin Hidrojen Tartarat'ın Voltametrik Davranışı ve Kapsüllerdeki Tayini

Rivastigmin hidrojen tartarat’ın (RVT) elektrokimyasal davranışı camı karbon elektrot kullanılarak diferansiyel puls voltametri (DPV) ile çalışılmıştır. RVT tayini için, destek elektrolit olarak pH:6 fosfat tamponunun kullanıldığı analitik bir metod geliştirilmiştir. Geliştirilen metod RVT’nin kantitatif analizine uygulanmıştır. Fosfat ve Britton-Robinson tamponları kullanılarak RVT’nin elektrokimyasal davranışı üzerinde pH etkisi ve tarama hızı incelenmiştir. Kalibrasyon grafiği, önerilen metod uygulanarak 7x10^{-5} - 8x10^{-4} M arasında elde edilmiştir. Bu çalışmada LOD ve LOQ değerleri sırasıyla 1.69x10^{-5} M ve 5.65x10^{-5} M olarak hesaplanmıştır. Sentetik örnekler analiz edilmiştir. Ortalama değer ve relatif standart sapma %98.3 ve % 2.81 olarak bulunmuştur. Elde edilmiş geri kazanım sonuçları uygunluck göstermektedir. Bu çalışmadaki elektrokimyasal sonuçlar literatürdeki kapiler zon elektroforesis metodu ile kıyaslanabilir sonuçlar vermiştir.

Anahtar kelimeler: Rivastigmin, Diferansiyel puls voltametri, Farmasöütik dozaj şekli
REFERENCES


