Int. J. Electrochem. Sci., 11 **(2016)** 4802 – 4811, doi: 10.20964/2016.06.64

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Square Wave Voltammetric Determination of Rasagiline Mesylate on Hanging Mercury Drop Electrode and its application in Dosage Form and Biological Fluids

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Received: 16 February 2016 / *Accepted:* 26 March 2016 / *Published:* 4 May 2016

A new square wave voltammetric (SWV) method has been developed and validated for the determination of Rasagiline mesylate in bulk, dosage form and human plasma due to a cathodic behaviour of its acetylene group at Hanging Mercury Drop Electrode (HMDE). Many facts such as different supporting electrolytes, scan rate, pulse amplitude, accumulation time and accumulation potential were studied. A good linearity was obtained over a concentration range of (2.8 x10^{-8} to1.4 x10^{-7}mol L^{-1}) with 99.78% as mean recovery and 1.09%, as the relative standard deviation and limits of detection and quantification were found to be (2.7 x10^{-9} and 9.24 x1 0^{-9}mol L^{-1}), respectively. The accuracy and precision of the method were within acceptable limits. The method was applied for determining the active ingredients in its tablets with % recoveries ± relative standard deviation of 95.46 ± 3.7 6 and in spiked human plasma with 94.3 ± 2.8 9%, respectively.

Keywords: Rasagiline mesylate; Square Wave Voltammetry; Hanging Mercury Drop Electrode; acetylene group.

1. INTRODUCTION

Rasagiline mesylate (RAS) [(R) -N-(prop-2-ynyl) -2,3-dihydro-1H-inden-1-amine methane sulfonate] of molecular weight 267.3 g mol^{-1} is an enzyme blocker of monoamine oxidase type B (MAO inhibitor) which works by slowing the breakdown of neurotransmitters such as dopamine, norepinephrine and serotonin in the brain. This medication is used alone or with other medications like levodopa or carbidopa) to treat movement disorders caused by Parkinson's disease. It does not cure Parkinson's disease, but it improves tremor and muscle stiffness ("on-off" problems). It is rapidly absorbed from the gastrointestinal tract and its peak plasma concentration occurs in about 30 minutes to 1 hour. Its bioavailability is reported to be about 36%. It is contraindicated in patients with moderate to severe hepatic impairment [1].

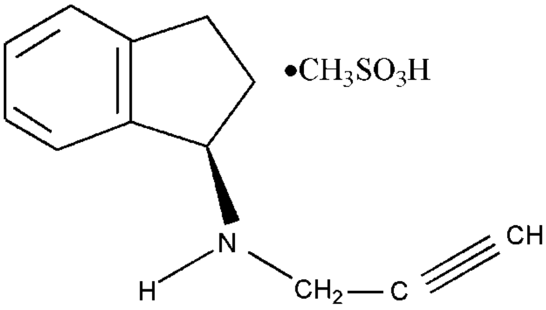

Figure 1. Rasagiline mesylate structure.

Literature Survey reveals that different analytical techniques have been applied for determination of RAS including, High performance Liquid Chromatography (HPLC) methods with ultra violet (UV) or diode-array detection [2-7], HPLC methods for drug impurity profiling and stability-indicating assays [8-12], High performance Liquid Chromatography-mass spectrometry detection (HPLC-MS) [13-15], spectrophotometric methods [16-19] and electrochemical method [20].

The objective of this investigation is to study the effect of the Rasagiline mesylate on Hanging Mercury Drop Electrode (HMDE) owing to its acetylenic (alkynes) electro active group in an attempt to develop a sensitive electrochemical method for quantitative analysis of it in bulk, tablets and spiked plasma through several factors which will be studied and discussed later.

2. EXPERIMENTAL

2.1 Materials and reagents

2.1.1 Pure materials

Pure Rasagiline mesylate of purity 99.0% supplied by (Al-Rowad Pharmaceutical Industries; Cairo-Egypt).

2.1.2 Pharmaceutical dosage form

RASANOPARK, Rasagiline tablets *B.N* 2223 provided by (Al-Rowad Pharmaceutical Industries, Cairo-Egypt) contain 1.56 mg of Rasagiline mesylate equivalent to1 mg Rasagiline.

2.1.3 Reagents

Phosphoric acid, boric acid and acetic acid are all of analytical grade for three different buffer solutions of 0.04 mol L^{-1} which was used to prepare Britton-Robinson (BR) buffer solution at pH11. Sodium hydroxide, potassium hydroxide, potassium chloride, lithium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate are also of analytical grade.

Acetonitrile, methanol, n-hexane, dichloromethane and isopropanol are of HPLC grade. Double distilled water was used in all work. Fresh human plasma was obtained from the blood bank (VACSERA, Cairo, Egypt).

2.1.4 Standard solutions

A stock solution of the standard RAS was first prepared by dissolving 25 mg RAS in double distilled water and adjust the volume in 25 mL volumetric flask with the same solvent. A second stock was prepared by transferring 0.05 µL of stock 1 into 50 mL volumetric flask and complete to the total volume with double distilled water to obtain a final concentration of 1µg mL^{-1} and stored in the refrigerator at 4 ^{0}C for 72 hours.

2.2 Apparatus

The Metrohm model of the 693 VA processor and of 694 VA stand equipped with three electrodes is employed. The electrodes are a reference electrode of Ag/AgCl in 3 mol L^{-1} KCl and a platinum counter electrode. The Hanging Mercury Drop Electrode (HMDE) is used as a working electrode for electrochemical measurements with drop size 4. The Metrohm model 654 pH meter is also used.

2.3. Procedures

2.3.1 Construction of calibration curve of RAS

After purging the electrolyte blank solution for 150 Sec with nitrogen gas, a fresh drop of HMDE is produced in 10 ml of 0.1 mol L^{-1} NaOH and 0.5 mol L^{-1} KCl solution as a blank solution and electrically activated by Square Wave Voltammetry (SWV) sweep with three replicates from (-100 to -700 mV) with scan rate 60 mVs^{-1}, pulse amplitude 50 mV, and accumulation time 90 s with stirring on accumulation potential-100 mV at600 rpm. Aliquots of RAS solutions from stock two, covering the concentration range (2.8 x 10^{-8} to 1.4 x 10^{-7}mol L^{-1}) in the final dilution were added to the blank solution and then analysed under the same conditions on a renewable mercury drop.

2.3.2 Application to pharmaceutical formulation

Five tablets were thoroughly grounded into a fine powder and mixed well. An average weight of one tablet was accurately weighed and transferred to a 100 mL measuring flask to prepare a stock solution containing 15.6 µg mL^{-1} RAS, sonicated for 45 min in 50 mL of double distilled water then completed to the final volume with the same solvent and mixed well. 1 mL was taken from the clear supernatant and diluted to 10 ml with double distilled water preparing stock 2. An aliquot was then analysed according to the proposed voltammetric procedure based on the standard addition method.

2.3.3 Application to spiked human plasma

0.5 ml of drug-free serum samples were transferred into10 ml centrifuge tube, fortified with (0.5 and 5 µg) RAS solution. The solution mixture was treated with 0.05 mL methanol-water mix (50:50 v/v) and0.1 mL phosphate buffer pH7 and then vortexed with a 4ml (n-hexane–dichloromethan –isopropanol) mix (20:10:1 v/v) for10 min then centrifuged for 5 min at 2000 rpm. Required volume of the organic layer was transferred to another centrifuge tube and evaporated at40 ºC under nitrogen [13]. The residue was reconstituted in volume of 0.01 mol L^{-1} sulphuric acid equal to that taken from the extract. All was done as the same on blank except 0.5 mL water instead of plasma. This blank was used for standard addition. An aliquot was transferred to the voltammetric vessel and diluted to10 ml with the supporting electrolyte and then analysed according to the proposed voltammetric procedure based on the standard addition method.

3. RESULT AND DISCUSSION

The voltammetric behaviour of the RAS was studied on HMDE in 0.1 mol L^{-1} NaOH and 0.5 mol L^{-1} KCl as supporting electrolyte. RAS manifests a prominent cathodic peak at potential - 468. This peak is irreversible and diffusion controlled as will be discussed later.

Previously it had been reported that RAS produce anodic peak on glassy carbon electrode [20] by oxidation of RAS over a concentration range of (0.85 -94.1 µg mL^{-1}) with a correlation coefficient of 0.9939 but in the present work, HMDE was used and had been applied successfully for determining RAS by square wave voltammetry through reduction of (−C≡CH) group to (−C=CH) [21,22] in concentration range of ng mL^{-1} as described below .Several factors have been studied for optimization.

3.1 Optimization of experimental conditions

3.1 Effect of supporting electrolyte

The electrochemical behavior of Rasagiline mesylate on the HMDE was carefully studied in different types of supporting electrolytes like acidic (0.1 mol L^{-1} H_2SO_4), basic (0.1 mol L^{-1} of borate buffer, pH11), (universal buffer, pH11), (0.1-0.01 mol L^{-1} NaOH) and (0.1 mol L^{-1} KOH) it gave a highest peak current in 0.1 mol L^{-1} NaOH which was chosen ,also by studying the effect of presence of salt like (KCl, NaCl and $LiCl_3$) on the peak potential in combination with 0.1 mol L^{-1} NaOH, 0.5 mol L^{-1} KCl had the highest current as compared to the other electrolytes and also showed more stability in peak potential of RAS.

3.1.2 Effect of scan rate

By scanning the effect of different scan rates from 20 to120 mVs^{-1} on the peak current of 25 ng mL^{-1} RAS at cathodic mode, it was shown that the 60 mVs^{-1} scan rate was the favourable because of its lowest standard deviation.

By plotting the peak potential (E_p/mV) against scan rate $(\upsilon/\text{mVs}^{-1})$ it was shifted to more negative potential by increasing the scan rate (Figure 2a) confirming the irreversibility of the reduction electrode reaction of RAS at HMDE.

From the graphical representations of (E_p) versus (υ) for RAS (Figure 2), the $E°$ value at HMDE can be deduced from the intercept which is equal to be E°= - 468.16 mV and αn value can then be calculated from the equation [23].

$$E_{p_a} - E_{p_{\frac{a}{2}}} = \frac{1.857RT}{\alpha nF}$$

And at 25 ºC, then

$$E_{p_a} - E_{p_{\frac{a}{2}}} = \frac{47.7}{\alpha n}$$

αn=1.315 and by $\alpha=0.65$ (range from 0.30 to 0.70), thus the number of electrons transfer n for reduction step is equal 2.02 nearly≈2 which indicates that two electrons were involved in the reduction of the acetylene group [21,22] of RAS on the HMDE.

Scan rate studies were carried out to assess whether the process at the HMDE electrode was under diffusion or adsorption controlled process as shown in (Figure 2b).

By plotting the log current (log I_p) versus log scan rate (log υ) and extrapolating the line to υ=0 it was found that the logarithm of reduction peak current (log I_p) is linear to the logarithm of scan rate (log υ) with the linear regression equation of log $I_p=0.708 +0.22$ log (υ) (r=0.981). From the previous equation, the slope was found (0.22) which is less than 0.5. This approved that the electrochemical reduction process of RAS at HMDE is a diffusion controlled process.

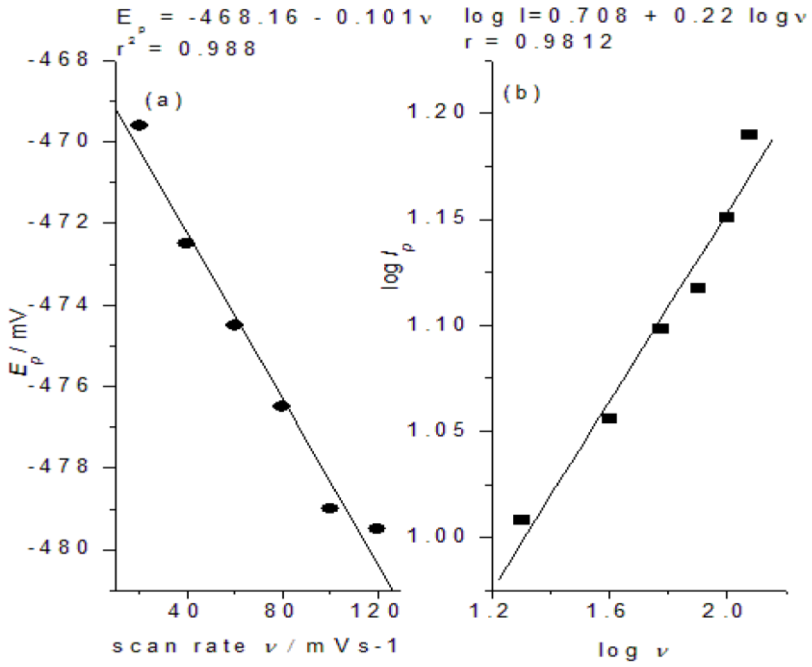

Figure 2. Plot of (a) potential (E_p/mv) against scan rate (υ/Vs^{-1}), (b) log current (log I_p) against log scan rate (log υ) on 25 ng mL^{-1} Rasagiline mesylate in 0.1 mol L^{-1} NaOH and 0.5 mol L^{-1} KCl as supporting electrolyte, accumulation time 90 s with stirring and stop stirring for 10 son HMDE.

3.1.3 Effect of accumulation potential and accumulation time

When we scan the *effect of accumulation potential* starting from -200, -100 and-50 mV to-700 mV on RAS it was found that they are nearly the same in the produced current so we choose-100 mV as the accumulation potential. For the *effect of accumulation time* we made scan on low concentration (10 ng mL^{-1}) and high concentration (50 ng mL^{-1}) on accumulation potential -100 mV, it was found that 90 s has nearly the lowest standard deviation and giving high current.

3.1.4 Effect of the rotation speed of stir

By scanning the different rotation speed of stirring the solution, it was found that rotation speed of 600 rpm/min gave a current with the lowest standard deviation.

3.2 Linearity and validation parameters

3.2.1 Linearity and range

Calibration curve (Figure 3) was constructed as a function of the concentrations of standard RAS in ng mL^{-1} (X) versus their peak currents in nA (Y) on (SWV) mode over a potential range from -100 to -700 mV in the presence of 0.1 mol L^{-1} NaOH and 0.5 mol L^{-1} KOH. The responses were linear over different concentration ranges in ng mL^{-1} according to the SWV sweep mode with equation $Ip = 0.586\,X + 2.049$(r^2=0. 9998). The calibration data were presented in (Table 1).

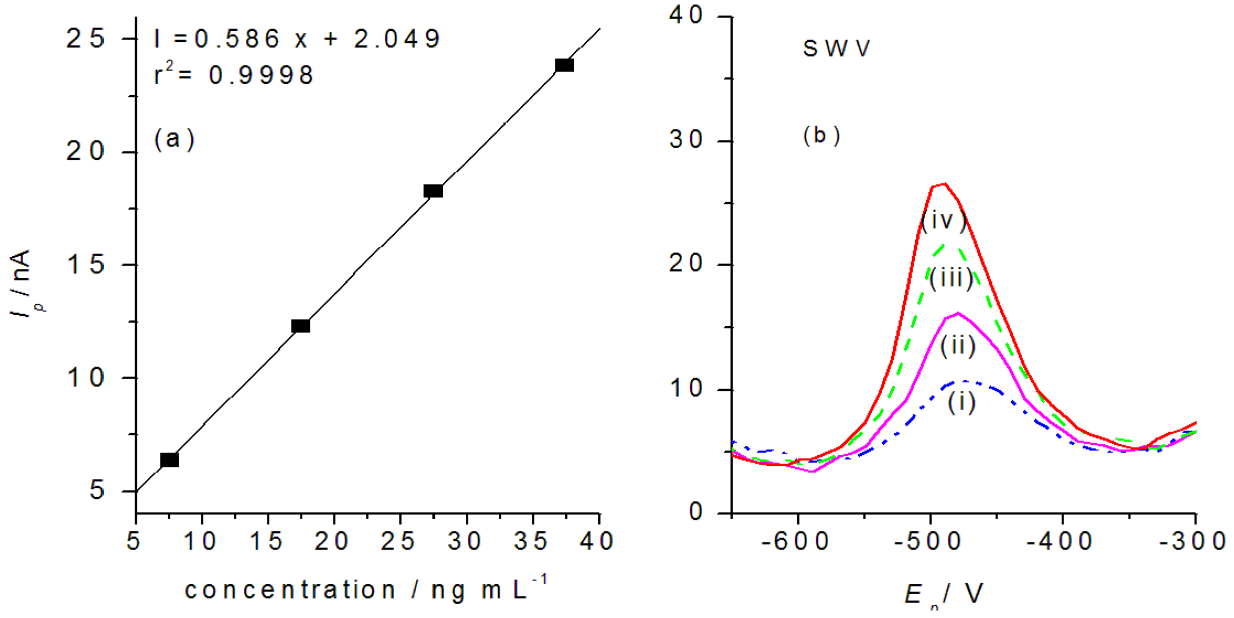

Figure 3. Showing (a) the calibration curve of RAS on SWV mode and (b) the voltammogram of SWV mode on different concentrations of RAS (i) 7.5 ng mL^{-1} (ii) 17.48 ng mL^{-1} (iii) 27.45 ng mL^{-1} (iv) 37.41 ng mL^{-1}, scan rate 60 mVs^{-1}, accumulation time 90 s with stirring, an accumulation potential-100, pulse amplitude 50 mV, electrolyte 0.1 mol L^{-1} NaOH and 0.5 mol L^{-1} KOH on HMDE.

Table 1. Linearity data of RAS in pure form over SWV Voltammetry.

Mode	**SWV**
Concentration range (ng mL^{-1})	(7.49-37.4)
Slope	0.59
Intercept	2.05
Correlation (r)	0.9999
SDV Y/X	0.14
Average	99.78
%RSD	1.09
% Err	0.22
LOD (ng mL^{-1})	0.74
LOQ (ng mL^{-1})	2.47

*standard deviation y/x

3.2.2 Detection and quantification limits

It showed a lower detection limit (LOD) of 0.74 ng mL^{-1} and a lower quantification limit (LOQ) of 2.47 ng mL^{-1} which indicates the sensitivity of the method in determining of RAS (Table 1).

3.2.3 Accuracy and precision

The accuracy and precision data have been presented in (Table 2). The intraday and interday data which were evaluated through replicate analysis of three different concentrations (17.4, 27.4 and 37.4 ng mL^{-1}) of authentic drug three times a day for three constitutive days with the same standard.

Table 2. Precession data of the proposed SWV method for determination of RAS in pure form.

	interday (between days)			**intraday (within day)**		
Concentration (ng mL^{-1})	**17.48**	**27.45**	**37.41**	**17.48**	**27.45**	**37.41**
	100.26	100.96	99.49	99.89	102.66	98.71
	98.15	102.75	98.92	98.41	103.74	98.42
	100.71	102.30	98.74	100.98	103.29	98.24
Average, %	99.71	102.01	99.05	99.76	103.23	98.46
RSD, %	1.37	0.91	0.39	1.29	0.52	0.24
ER, %	0.504	2.24	1.04	0.11	2.66	1.29

3.3 Applications

3.3.1 Determination of RAS in Tablets

To assess the applicability of the proposed method, RAS was analysed in commercial film-coated tablets (RASANOPARK). On the basis of above results, new developed SWV method was applied to the direct determination of RAS in its pharmaceutical product of current therapeutic use and compared with HPLC method as reference method [4]. Well-defined SWV peaks were obtained and it is clear from statistical data that no significance difference between the proposed method and reference HPLC method (Table 3).

Table 3. Analysis of Rasagiline mesylate in film-coated tablets (RASANOPARK) by the proposed SWV method and reference HPLC method.

Parameter	SWV mode	HPLC [4]
	90.23	98.60
	94.85	97.97
	96.52	95.77
	95.48	
	100.24	
Average, %	95.46	97.45
RSD, %	3.76	1.52
F test	5.59 (19.24)	
Student t-test	1.09 (2.44)	

Tabulated F and t values at 95% confidence level

3.3.2 Application on spiked plasma

The recovery studies of drug in serum samples were performed using the standard addition and calculated by intercept method.

Table 4. Analysis of Rasagiline mesylate in spiked plasma by the proposed SWV method.

	Spiked concentrations	
Plasma spiked	**5 μg**	**0.5 μg**
1	95.19	95.80
2	91.24	88.90
3	96.49	
Average, %	94.3	92.34
RSD, %	2.89	5.28

*numbers of replicates are 3 for each determination.

The mean recoveries for RAS spiked in (5 and 0.5 µg on serum, plasma) were achieved after liquid-liquid extraction method, then evaporated under nitrogen at 40 ºC followed by reconstitution in 0.01 mol L^{-1} H_2SO_4 and analysed as prescribed before, were of % recoveries of (94.3 and 92.34, respectively) and %RSD of (2.89 and 5.28, respectively) (Table 4). The procedure had been carried out on free drug serum in order to check the effect of interfering compounds. The experiment revealed a negative effect of interfering substances which indicate the selectivity of the procedure.

4. CONCLUSION

The reduction of RAS is through an irreversible process in 0.1 male L^{-1} NaOH and 0.5 Mol L^{-1} KOH, which involves a transfer of two electrons in the acetylene group on HMDE. This voltammetric response is used for electroanalytical measurements of the drug molecule in nano concentration range through new valid SWV method for the quantification of RAS on HMDE in film-coated tablets and human plasma with high precision and accuracy.

ACKNOWLEDGEMENTS
The authors would like to express their gratitude to Department of Analytical Chemistry at Faculty of Pharmacy (Helwan University) and Applied Organic Chemistry Department, National Research Centre, (Dokki, Egypt) for providing instruments and the means necessary to accomplish this work. Thanks are due to Volkswagen Stiftung, Kastanienallee 35, 30519 Hannover, Germany, for the financial support of purchasing the Metrohm model 693 VA processor and 694 VA stand.

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