Reports in Electrochemistry

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ORIGINAL RESEARCH

Electrochemical behavior and assay of anti-Parkinson drug selegiline using cathodic adsorptive stripping square wave voltammetry in bulk form

This article was published in the following Dove Press journal:
Reports in Electrochemistry
23 October 2015
Number of times this article has been viewed

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Abstract: The electrochemical reduction behavior of selegiline was investigated by cyclic voltammetry using glassy carbon electrode and validated by square wave cathodic adsorptive stripping voltammetry. Selegiline gave one well-defined reduction peak in the potential range −0.4 to −0.5 V versus Ag/AgCl electrode. The reduction process is irreversible and partial diffusion controlled. Various chemical and instrumental parameters affecting the electroanalytical response for the determination of selegiline were investigated and optimized. Under optimized conditions, the adsorptive stripping peak current is found to be linear over the concentration range of 3.0×10^{-7}–2.5×10^{-5} M with a detection limit of 3.04×10^{-8} M and a lower limit of quantification of 1.01×10^{-7} M.

Keywords: selegiline, anti-Parkinson drug, glassy carbon electrode, square wave cathodic adsorptive stripping voltammetry, diffusion controlled

Introduction

Selegiline (L-deprenyl) is a drug that is used for the treatment of early-stage Parkinson's disease, depression, and dementia. Selegiline is an irreversible and relatively selective inhibitor of monoamine oxidase (MAO-B). MAO-Bs are flavoenzymes sited in the outer mitochondrial membranes of brain, which catalyze the oxidation of a large variety of amine neurotransmitters into the corresponding imines.[1] Selegiline has negligible oral bioavailability due to extensive first-pass metabolism.[2]

It takes a protective action against DNA damage, oxidative stress, and excitotoxic damage from glutamate by trapping hydroxyl and peroxyl radicals. It also stimulates the release of superoxide dismutase (SOD).[3,4] SOD is a key enzyme that helps to quench the production of damaging free radicals. Selegiline may prevent or reverse iron-induced memory impairment. The deposition of excess iron in the brain is implicated in several neurodegenerative diseases.[5]

Selegiline has a structure of *N*-methyl-*N*-(2-propynyl)-2-methyl-1-phenyl ethyl-2-amine and is synthesized by the alkylation of (−)-methamphetamine using propargyl bromide. The chemical structure of selegiline is shown in Figure 1.

Quantitative analysis of selegiline in human plasma by high-performance liquid chromatography (HPLC) along with some other metabolites is reported; further, its determination in urine has also been reported by capillary electrophoresis.[6–8]

There has been no electrochemical study published on quantitative determination of selegiline in bulk form as well as pharmaceutical formulations. The widespread use of

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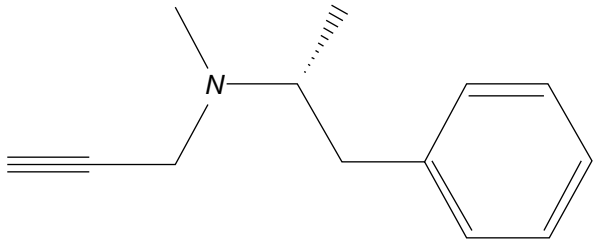

Figure 1 Structure of selegiline.

this compound and the need for clinical and pharmacological studies require fast and sensitive analytical techniques to assay the drug in pharmaceutical dosage forms. This paper deals with the voltammetric determination of drug in bulk form with a low detection limit, hence making it more sensitive.

The low value of limit of detection (LOD) validates the analytical procedure and provides a fast and reliable technique for the assay of the sample without consuming excess time.

Materials and methods

Selegiline was obtained in pharmaceutical dosage form from Intas Pharmaceuticals Pvt Ltd (Ahmedabad, Gujarat, India), and was used after purification. A stock solution of selegiline (1.5×10^{-3} mol/L) was prepared in distilled ethanol. Double distilled water, obtained from laboratory distillation assembly, was used throughout the studies. The solutions for recording voltammograms were prepared by mixing appropriate volume of stock solution and buffers. All chemicals used were of analytical grade and obtained from Sigma-Aldrich (St Louis, MO, USA).

Instrumentation

Electrochemical measurements were performed using model 1230A [SR 400] electrochemical analyzer (CHI Instrument, Bee Cave, TX, USA), purchased from Sinsil International, Mumbai, India. Electrodes used were: glassy carbon electrode (GCE) as working electrode, Ag/AgCl (3.0 mol/L KCl) as reference electrode, and a platinum electrode as auxiliary electrode. All the solutions examined by electrochemical techniques were purged for 10–15 minutes with purified nitrogen gas in which a continuous stream of nitrogen was passed over the solutions before each of the measurements. Nitrogen gas was deoxygenated by passing it through acidic sodium (meta) vanadate solution. All pH-metric measurements were made on a CHINO (Chino Scientific Instruments Mfg, Ajmer, Rajasthan, India) digital pH meter fit with a glass electrode standardized with buffers of known pH.

Analytical procedure

A portion of the finely grounded material equivalent to 10 mg of selegiline was accurately weighed and dissolved in 20 mL of ethanol; then the solution was diluted with 15 mL of water and subjected to sonication for 15 minutes in order to get a homogenous solution. The contents of the beaker were transferred into a centrifuging tube and centrifuged at 3,000 rpm for 30 minutes. An aliquot of 1.5×10^{-3} mol/L of the solution was then analyzed according to the proposed voltammetric procedure.

Pretreatment of GCE

The working GCE was polished with 0.05 µm alumina slurry and further subjected to sonication for a short duration prior to each measurement in order to remove all impurities that remained on the surface of the electrode, and then it was dried at 40°C in an oven.

Results and discussion

The electrochemical behavior of selegiline at GCE was studied using cyclic voltammetry (CV) and square wave cathodic adsorptive stripping voltammetry (SWCAdSV). In all electrochemical methods, selegiline gave one well-defined cathodic peak in Britton Robinson (BR) buffer of pH 11.0 at GCE.

Cyclic voltammetric behavior

Typical cyclic voltammograms for selegiline were recorded within the potential range of 0.0 to −0.8 V versus Ag/AgCl reference electrode at different scan rates and concentrations. Selegiline (1.5×10^{-3} mol/L) in BR buffer gave a well-defined cathodic reduction peak in the chosen potential range, while no peaks were obtained in the anodic direction, thus indicating the irreversible nature of reduction.[9]

Effect of scan rate

Cyclic voltammograms of selegiline, recorded at different scan rates, clearly showed that as the scan rate is increased from 20 to 160 mV/s at a certain concentration of selegiline, the peak potential shifted toward more negative value, supporting the irreversible nature of the reduction process (Figure 2A).[10]

Furthermore, peak current was found to be linearly dependent on square root of scan rate consistent with the Randles–Sevcik equation, which can be expressed as:

$$I_p = \left(2.99\times10^{-5}\right)n\left[\alpha n'\right]^{\frac{1}{2}} A C_o D_o^{\frac{1}{2}} \nu^{\frac{1}{2}} \qquad (1)$$

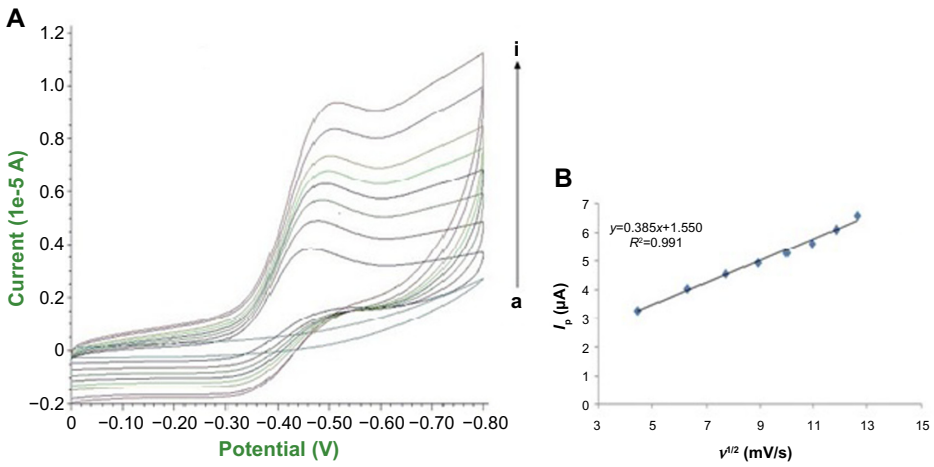

Figure 2 Peak current as a response to the potential in triangular wave form with varying scan rate.
Notes: (**A**) Cyclic voltammogram of selegiline at different scan rates (a) blank, (b) 20 mV/s, (c) 40 mV/s, (d) 60 mV/s, (e) 80 mV/s, (f) 100 mV/s, (g) 120 mV/s, (h) 140 mV/s, and (i) 160 mV/s at pH 11.0 in BR buffer (concentration 1.5×10^{-3} M). (**B**) Plot of I_p versus $v^{1/2}$ from voltammogram in (**A**) for selegiline in 1.5×10^{-3} M concentration in BR buffer of pH 11.0.
Abbreviation: BR, Britton Robinson.

where n is the number of electrons exchanged in reduction, n' is the number of electrons involved in the rate determining step, α is the charge transfer coefficient, A (cm^2) is the apparent surface area of the electrode, C_o (mol/L) is the concentration of the electroactive species, I_p (μA) is the cathodic peak current, D_o (cm^2/s) is the diffusion coefficient of the electroactive species, and v (mV/s) is the scan rate.

For diffusion controlled process, I_p is directly proportional to $U^{½}$ ($I_p \alpha v^{½}$).[11,12] Hence, a graph (Figure 2B) between peak current versus square root of scan rate shows linear behavior, providing indications about the diffusion process as the rate-determining step of the reduction mechanism, which is expressed by the following linear regression equation:

$$I_p\,(\mu A) = 0.385\, v^{\frac{1}{2}}\left(\frac{mV}{s}\right) + 1.550\,(\mu A) \qquad r^2 = 0.991 \quad (2)$$

A slope of 0.38 confirms the diffusive nature of reduction of selegiline. Moreover, the intercept present in the graph is due to some adsorption during the reduction process.

Square wave cathodic adsorptive stripping voltammetry

On the basis of the electrochemical reduction of selegiline at GCE, SWCAdSV method was optimized for trace determination of selegiline by its square wave potential waveforms. Voltammograms of bulk selegiline in the BR buffer recorded by

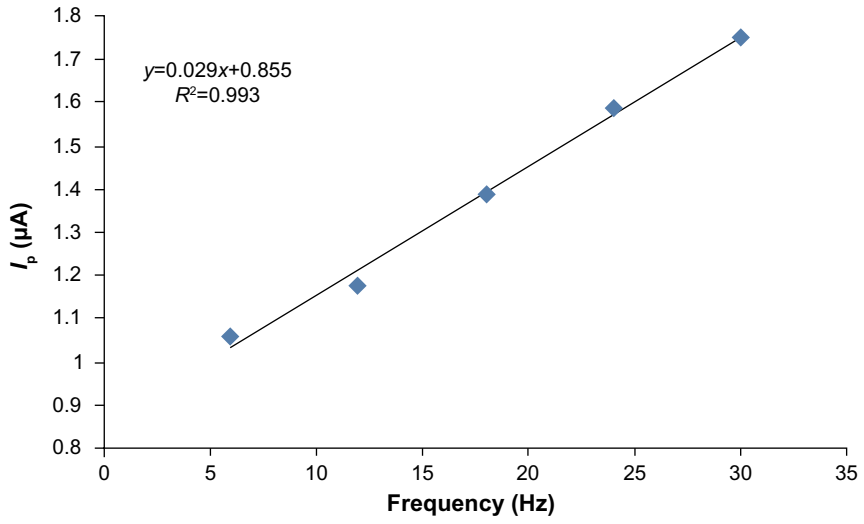

Figure 3 Plot of I_p versus frequency (f) for selegiline in 1.5×10^{-3} M concentration in BR buffer at pH 11.0.
Abbreviation: BR, Britton Robinson.

Table 1 Optimized operational parameters for SWCAdSV

Operational parameters	Results
Frequency (Hz)	30
Square period (s)	0.03
Scan increment (mV)	04
Pulse amplitude (mV)	25
Peak-to-peak amplitude (mV)	50

Abbreviation: SWCAdSV, square wave cathodic adsorptive stripping voltammetry.

square wave voltammetry following its preconcentration onto the GCE by adsorptive accumulation for 15 seconds exhibited a well-defined single irreversible cathodic peak at pH 11.0.

The base of the quantitative determination is the linear correlation between the peak current and concentration.[13] The best single peak shape, peak current sensitivity, and reproducibility of these techniques were obtained in alcoholic solution.

Optimization of operational parameters

The dependence of peak current on instrumental conditions such as frequency (f), scan increment (ΔS), and pulse amplitude (E_{sw}) were examined, and optimum operational conditions were obtained.

Effect of frequency

The peak current of the reduction of selegiline using SWCAdSV was found to be linearly dependent on the operational frequency in the range of 10–50 Hz, while a well-defined peak was obtained at 30 Hz. At higher frequencies, the peak shape broadened, and hence 30 Hz frequency was chosen for the further determination.[14]

Figure 3 shows a linear graph between I_p versus frequency (f) with a regression equation of:

$$I_p\,(\mu A) = 0.029 f + 0.855 \qquad r^2 = 0.993 \qquad (3)$$

Effect of scan increment and pulse amplitude

Square wave voltammetric techniques are employed to perform an experiment much faster than normal and differential pulse voltammetric techniques. An increase in scan increment (ΔS) in the range of 2–10 mV increased the peak current, but the preferred scan increment was taken as 4 mV suitable with respect to peak shape and size, as beyond this scan increment, the peak shape was broadened. The best peak definition was obtained at 25 mV of pulse amplitude (E_{sw}).

The staircase step period (square period) =0.03 seconds according to the fact:

$$\tau\,(s) = \frac{1}{f} \qquad (4)$$

where τ is square period in seconds and f is the square wave frequency in Hz.

Furthermore, the peak-to-peak amplitude was calculated as:[15]

$$\text{Peak-to-peak amplitude} = 2E_{SW} \qquad (5)$$

All operational parameters are listed in Table 1.

Effect of concentration

In the optimized conditions, a linear variation of the peak current (I_p) with concentration of bulk selegiline was examined within the concentration range of 4.2×10^{-10}–1.5×10^{-4} M.[16] The square wave cathodic adsorptive stripping voltammograms of selegiline at different concentrations are shown in Figure 4A.

The SWCAdSV peak current linearly increased with increasing concentrations (Figure 4B), and corresponding

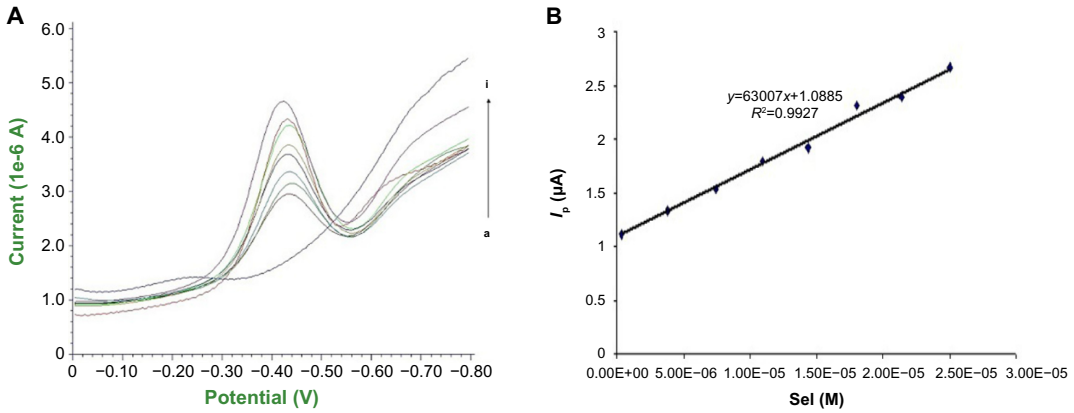

Figure 4 Peak current as a response to the potential in square wave form with varying concentration.
Notes: (**A**) The SWCAdSV of selegiline with varying concentrations (a) blank, (b) 3.0×10^{-7} M, (c) 3.8×10^{-6} M, (d) 7.4×10^{-6} M, (e) 1.09×10^{-5} M, (f) 1.44×10^{-5} M, (g) 1.80×10^{-5} M, (h) 2.14×10^{-5} M, and (i) 2.5×10^{-5} M at pulse amplitude 25 mV in BR buffer at pH 11.0. (**B**) Plot of I_p (µA) versus concentration (µM) from voltammogram in (**A**) of selegilne with varying concentrations in BR buffer of pH 11.0.
Abbreviations: BR, Britton Robinson; SWCAdSV, square wave cathodic adsorptive stripping voltammetry; Sel, concentration of selegiline.

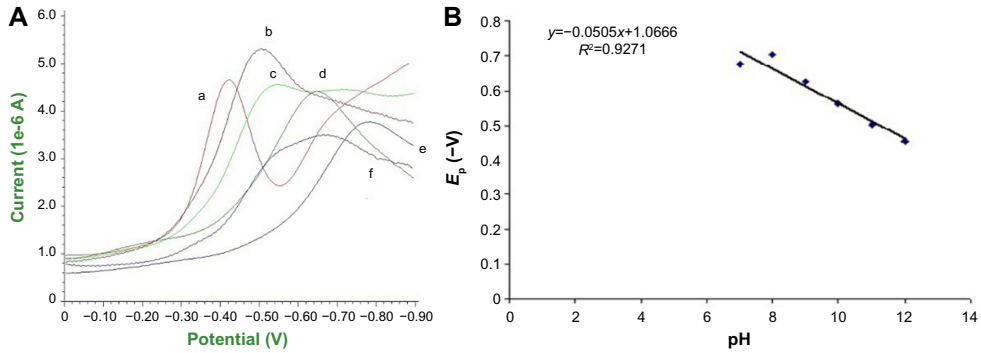

Figure 5 Effect of pH on peak current and peak potential.
Notes: (**A**) The SWCAdSV of Selegiline with varying pH (a) 12.0, (b) 11.0, (c) 10.0, (d) 9.0, (e) 8.0, and (f) 7.0 at pulse amplitude 25 mV in BR buffer. (**B**) Plot of E_p (–V) versus pH of 1.5×10^{-3} M selegiline solution.
Abbreviations: BR, Britton Robinson; SWCAdSV, square wave cathodic adsorptive stripping voltammetry.

regression equation for the graph between I_p versus concentrations is as follows:

$$I_p\,(\mu A) = 6.3E+04\ \mathrm{Sel}\left(\frac{\mu A}{M}\right) + 1.0885\,(\mu A) \qquad r^2 = 0.9927 \tag{6}$$

Effect of pH

The effect of pH on the monitored electroanalytical signal was investigated by varying the pH in the range 7.0–12.0 in BR buffer at a target concentration of 1.5×10^{-3} mol/L selegiline solution. Linear pH dependence of the peak potential for reduction wave clearly shows that the proton participates directly in the reduction process carried out at GCE (Figure 5A and B).[17–19]

The relation between E_p of the reduction wave and pH over the range 7.0–12.0 may be expressed by the following regression equations:

$$\text{SWCAdSV: } E_p\,(V) = -0.0505\ \text{pH} + 1.0666 \qquad r^2 = 0.9271 \tag{7}$$

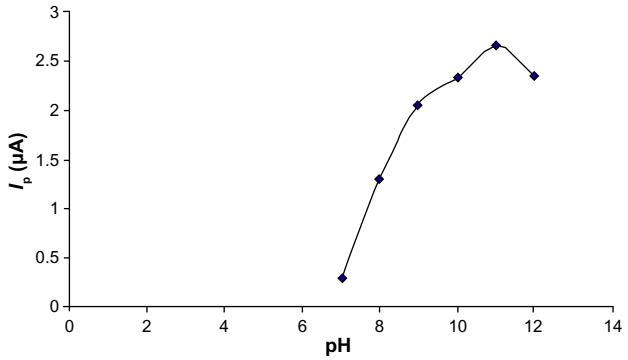

Figure 6 Influence of pH on SWCAdCV peak current of 1.5×10^{-3} M selegiline in BR buffer (pH 7.0–12.0).
Abbreviations: BR, Britton Robinson; SWCAdSV, square wave cathodic adsorptive stripping voltammetry.

As depicted in Figure 6, the peak height attains maxima at pH 11.0 and thereafter decreases. Therefore, pH 11.0 was selected as the optimum pH for the determination of selegiline.

Validation of the analytical procedure

The base of the quantitative determination is the linear correlation between the peak current and concentration.[20,21] The validation of proposed method for quantification in bulk form of selegiline was carried out via estimation of linearity range of concentration, LOD, limit of quantification (LOQ), and % recovery.

LOD and LOQ

The smallest concentration of the sample that can be detected with appreciable certainty was calculated using the equation:

$$\mathrm{LOD} = \frac{3s}{m}, \tag{8}$$

where s is the standard deviation of intercept and m is the slope of the calibration curve (Ip versus concentration). LOD for the

Table 2 Analytical parameters for voltammetric determination of selegiline in bulk form using SWCAdSV

Parameters	Results
SWCAdSV	
Measure potential (V)	–0.436
Linearity range (M)	3×10^{-7}–2.5×10^{-5}
Slope (µA/mol/L)	6.3×10^{4}
Intercept (µA)	1.0885
Correlation coefficient	0.9927
LOD (mol/L)	3.04×10^{-8}
LOQ (mol/L)	1.01×10^{-7}
SD	0.000639
% RSD	0.0588

Abbreviations: LOD, limit of detection; LOQ, limit of quantification; SWCAdSV, square wave cathodic adsorptive stripping voltammetry; RSD, relative standard deviation; SD, standard deviation.

Table 3 Result of accuracy for assay of selegiline in bulk form using SWCAdSV

Serial number	Conc added (mg/L)	Mean conc found[a] (mg/L)	Mean accuracy/ error[a]	Mean % recovery[a]	SD	% RSD
SWCAdSV						
1	1.0	0.982	0.018	98.2	0.008367	0.85
2	2.0	1.984	0.016	99.2	0.005477	0.28
3	3.0	2.974	0.026	99.1	0.011402	0.38

Notes: [a]Mean values were calculated by five independent repeated measurements. Mean ± SD (% recovery) =98.83+0.025246. Mean ± SD (% recovery) from RP-HPLC =97.97±3.308277.

Abbreviations: conc, concentration; RP-HPLC, reverse phase high-performance liquid chromatography; SWCAdSV, square wave cathodic adsorptive stripping voltammetry; RSD, relative standard deviation; SD, standard deviation.

standard solution of the sample using the technique SWCAdSV was found to be 3.04×10^{-8} M.

The lower LOQ for precise measurements was determined using the equation:

$$\text{LOQ} = \frac{10s}{m} \quad (9)$$

The LOQ for the proposed method was found to be 1.01×10^{-7} M. The low values of LOD and LOQ proved the good sensitivity of the method.

Similarly, low value of % relative standard deviation (RSD) indicates less spread of sets of data, which shows a good precision in the method. All data are tabulated in Table 2.

Percentage recovery

A certain amount of the sample was added and the method was applied to experimentally investigate the concentration present in the solution. The amount found was expressed in terms of mean percentage recovery. The data were collected based on the five separate determinations. All the results obtained were compared with the results obtained from the determination of selegiline by reverse phase high-performance liquid chromatography (RP-HPLC) and were found to be more accurate.[22]

Table 4 Result for repeatability

Serial number	Conc (mg/L)	Peak current (μA)	Mean ± SD	% RSD	% RSD from RP-HPLC
1	1	1.475	1.43±0.042101	0.02944	0.162331
2	1	1.464	1.43±0.042101	0.02944	0.162331
3	1	1.437	1.43±0.042101	0.02944	0.162331
4	1	1.396	1.43±0.042101	0.02944	0.162331
5	1	1.378	1.43±0.042101	0.02944	0.162331

Abbreviations: conc, concentration; RP-HPLC, reverse phase high-performance liquid chromatography; RSD, relative standard deviation; SD, standard deviation.

Accuracy

Since accuracy is the nearness of a calculation to the true value, the accuracy of the procedure was investigated by calculating the error between measured mean concentrations found and the concentration that was actually added.

The mean recovery was found to be 98.83%±0.025246% for selegiline, and the result for accuracy was further compared with the result from RP-HPLC. The result was found to be more accurate than the result obtained from RP-HPLC (Table 3).

Precision

Repeatability

The repeatability study, conducted using the test selegiline solution of concentration 5 mg/L in five replicate readings (n=5), shows an RSD of 0.02944%. It was concluded that the analytical technique has good repeatability. Data are listed in Table 4.

Intermediate precision (intraday and interday study)

The intraday studies were conducted on the test selegiline solution of concentrations 1, 2, and 3 mg/L in four replicate

Table 5 Result for intraday study

Serial number	1	2	3
Conc (mg/L)	1	2	3
Peak current (μA)			
0 h	1.485	1.79	2.179
2 h	1.492	1.792	2.335
4 h	1.476	1.76	2.301
6 h	1.488	1.78	2.298
Conc found (mg/L)			
0 h	1.023	2.008	2.993
2 h	1.024	2.002	3.019
4 h	0.999	2.001	2.991
6 h	0.990	2.03	3.007
Mean ± SD	1.009±0.017	2.010±0.013	3.002±0.013
% RSD[a]	1.68	0.65	0.43

Note: [a]Mean % RSD is 0.92.

Abbreviations: conc, concentration; h, hour(s); RSD, relative standard deviation; SD, standard deviation.

Table 6 Result for interday study (day1)

Serial number	1	2	3
Conc (mg/L)	1	2	3
Peak current (μA)			
A	1.479	1.791	2.112
B	1.477	1.798	2.130
C	1.473	1.764	2.149
Conc found (mg/L)			
A	1.004	1.995	2.990
B	1.003	1.997	2.995
C	0.989	1.991	2.997
Mean ± SD	0.998±0.008	1.994±0.003	2.994±0.004
% RSD[a]	0.801	0.150	0.133

Notes: [a]Mean % RSD is 0.361. A, B, and C indicate triplicate readings for each concentration (ie, 1, 2, and 3mg/L) and were taken on the same day at a time interval of 15 minutes (0 min, 15 min, and 30 min).
Abbreviations: conc, concentration; min, minutes; RSD, relative standard deviation; SD, standard deviation.

Table 7 Result for interday study (day 2)

Serial number	1	2	3
Conc (mg/L)	1	2	3
Peak current (μA)			
A	1.476	1.782	2.298
B	1.468	1.778	2.274
C	1.481	1.768	2.299
Conc found (mg/L)			
A	1.001	2.002	3.001
B	1.003	1.999	2.991
C	1.005	1.996	3.002
Mean ± SD	1.003±0.002	1.999±0.003	2.998±0.006
% RSD[a]	0.19	0.15	0.20

Notes: [a]Mean % RSD is 0.18. A, B, and C indicate triplicate readings for each concentration (ie, 1, 2, and 3mg/L) and were taken on the same day at a time interval of 15 minutes (0 min, 15 min, and 30 min).
Abbreviations: conc, concentration; min, minutes; RSD, relative standard deviation; SD, standard deviation.

Table 8 Result for interday study (day 3)

Serial number	1	2	3
Conc (mg/L)	1	2	3
Peak current (μA)			
A	1.449	1.73	2.202
B	1.477	1.775	2.211
C	1.481	1.745	2.221
Conc found (mg/L)			
A	0.999	1.998	2.998
B	1.001	2.001	2.999
C	1.001	1.999	2.999
Mean ± SD	1.0003±0.001	1.999±0.0015	2.998±0.0005
% RSD[a]	0.099	0.075	0.019

Notes: [a]Mean % RSD is 0.064. A, B, and C indicate triplicate readings for each concentration (ie, 1, 2, and 3mg/L) and were taken on the same day at a time interval of 15 minutes (0 min, 15 min, and 30 min).
Abbreviations: conc, concentration; min, minutes; RSD, relative standard deviation; SD, standard deviation.

Table 9 Overall % RSD

Serial number	Conc (mg/L)	Conc found (mg/L)			Mean	SD	RSD
		Day 1	Day 2	Day 3			
1	1	0.998	1.003	1.0003	1.0004	0.0025	0.25
2	2	1.994	1.999	1.999	1.997	0.0028	0.14
3	3	2.994	2.998	2.998	2.996	0.0023	0.076

Notes: Overall RSD =0.15. Overall RSD from RP-HPLC =0.00086.
Abbreviations: conc, concentration; RP-HPLC, reverse phase high-performance liquid chromatography; RSD, relative standard deviation; SD, standard deviation.

readings (n=4) at an equal time interval of 0, 2, 4, and 6 hours, while interday analysis was carried out on the aforementioned same three test solutions in triplicate readings (n=3) at a time interval of 24 hours continuously for 3 days. The value of RSD for intraday and interday analysis was found to be 0.92% and 0.15% (overall), respectively, thus demonstrating the precision of the method. Data were further compared with the result obtained from RP-HPLC. All data for intraday analysis are listed in Table 5, and data for interday analysis are listed in Tables 6 (day 1), 7 (day 2), 8 (day 3), and 9 (overall RSD).

Conclusion

The investigated voltammetric behavior supports the elucidation of the possible cathodic process and gives an idea of diffusion-controlled rate-determining step of reduction mechanism, while the proposed SWCAdSV procedure can be successfully applied for the determination of selegiline in pharmaceutical formulations. The method allows for direct dissolution and estimation of the drug with good accuracy and precision, and hence is a comparatively quick, reliable, and relatively cheap method to perform in quality control laboratories.

Acknowledgments

This work was financially supported by the Council of Scientific and Industrial Research, New Delhi, India, by grant File Number 09/149(0654)/2014/EMR-I. The authors would also like to thank Professor Suresh K Bhargava, Deputy provice chancellor (International), Royal Melbourne Institute of Technology, Australia, for suggestions and providing instrumental accessories.

Disclosure

The authors report no conflicts of interest in this work.

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