Research Article

Novel Spectrophotometric Methods for the Determination of Selegiline Hydrochloride in Bulk and Its Pharmaceutical Preparation

Kumble Divya and Badiadka Narayana

Department of Post-Graduate Studies and Research in Chemistry, Mangalore University, Mangalagangothri 574199, India

Correspondence should be addressed to Badiadka Narayana; nbadiadka@yahoo.co.uk

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A simple and highly selective spectrophotometric method has been developed for the determination of selegiline hydrochloride in bulk and formulations. Method A is based on the oxidation of 3-methyl-2-benzothiazolinone hydrazone in the presence of ceric ammonium sulphate, followed by its coupling reaction with drug to form a colored product having $\lambda_{\max}$ of 629 nm. Method B is based on the coupling reaction of drug with 4-aminoantipyrine to give a new ligand that reacts with copper(II) to give intense bluish red colored chelate which is measured at 539 nm. Beer's law is obeyed in the range of 10.00–85.00 $\mu\text{g mL}^{-1}$ with molar absorptivity of 0.98×10^4 for method A and 20.00–120.00 $\mu\text{g mL}^{-1}$ with molar absorptivity of 0.94×10^4 for method B. The optimum reaction condition and the analytical parameters are evaluated. The results obtained indicate that the methods are free from interference of the ingredients; thus they are successfully applied to pharmaceutical formulations.

1. Introduction

Selegiline hydrochloride (SGE) (Figure 1) (deprenyl), [(R)-(2)-N-methyl-(1-phenyl-2-propyl)-N-propinylamine] hydrochloride [1], is a levomethamphetamine derivative which belongs to a class of drugs called phenethylamines [2]. SEG is a selective, irreversible inhibitor of monoamine oxidase (MAO-A) [3]. It is used for the treatment of early-stage Parkinson's disease, depression, and senile dementia [4]. It is useful adjunct in the treatment of cocaine addiction [5]. SEG (brand name Anipryl) is also used (at extremely high dosages relative to humans) in veterinary medicine to treat the symptoms of Cushing's disease and cognitive dysfunction (canine cognitive dysfunction) in dogs [6]. Recommended dosage of SEG is about 10 mg/day; further increase in the dosage will lead to the nonselective inhibition of MAO [7]. Therefore, it is essential to develop a standard analytical method for monitoring residual drug in bulk and in pharmaceutical formulations. The pharmaceutical importance of drug has prompted us to devise methods for the rapid determination of SEG. A very few methods are reported in the literature for the determination of SEG in

pharmaceutical formulations which include high performance liquid chromatography [8–12], gas chromatography [13, 14], fluorescence polarization immunoassay (FPIA) and gas chromatography-mass spectrometry (GC/MS) [15], spectrofluorometry [16], and stereoselective analyses [17]. The literature survey reveals that so far there is no visible spectrophotometric method for the analysis of SEG.

Unlike gas chromatograph and high performance liquid chromatograph the UV-Visible spectrophotometer is a simple, low-cost instrument and on the other hand, in terms of simplicity and expense, the method could be considered superior in comparison with the previously reported methods. Thus, the present work reports new spectrophotometric methods for the determination of SEG in pure and in pharmaceutical formulations.

2. Materials and Methods

2.1. Apparatus. A UV-Visible spectrophotometer (SHIMADZU, UV 2550, Japan) with 1 cm quartz cells was used for the absorbance measurements.

FIGURE 1: Chemical structure of SEG.

2.2. Chemicals and Reagents. All the reagents were of analytical grade and used without further purification. All the solutions were prepared in distilled water. SEG bulk drug was obtained as gift sample from CAD Pharma Inc., Bangalore, India. Pharmaceutical formulations of SEG were obtained commercially.

2.3. Standard Drug Solution. Stock solution of SEG (1000 $\mu\text{g/mL}$) was prepared by dissolving 100 mg of SEG in distilled water and making the volume 100 mL in a standard volumetric flask. The stock solution was diluted approximately to get working concentration.

3-Methyl-2-benzothiazolinone Hydrazone (MBTH, 0.2% w/v). 0.2 g of MBTH was accurately weighed and dissolved in 100 mL of distilled water.

Ceric Ammonium Sulphate (CAS, 1% w/v). 1 g gm of CAS was dissolved in 20 mL of 0.1N sulphuric acid and the volume became 100 mL.

4-Aminoantipyrine (AAP, 0.1% w/v). 0.1 g of AAP was dissolved in 10 mL of ethanol and made up to be 100 mL with distilled water.

Copper(II) Chloride (1% w/v). 1 g of Cu(II) chloride was accurately weighed and dissolved in 100 mL of distilled water.

2.4. Recommended Procedures

Method A. Aliquots containing 10.00–100.00 $\mu\text{g mL}^{-1}$ of SEG were transferred into a series of 10 mL volumetric flasks. Then 1.0 mL of 0.2% MBTH and 1.0 mL of 1% CAS solutions were added and the flasks were kept aside for 15 min at room temperature. The solutions in each flask were made up to the mark with distilled water and the absorbance was measured at 629 nm against reagent blank.

Method B. Aliquots containing 20.00–120.00 $\mu\text{g mL}^{-1}$ of SEG were transferred into a series of 10 mL volumetric flasks, followed by the addition of 2 mL of 1% AAP and 1 mL of 0.1% Copper chloride. The resulting reddish violet colored mixtures were kept aside for 10 min and make up the solution to be 10 mL with acetone; the absorbance values were measured at 539 nm against the reagent blank solution.

2.5. Preparation of Pharmaceutical Formulation. Twenty tablets of SEG (5 mg) were crushed thoroughly in a mortar,

FIGURE 2: Absorption maximum for method A.

dissolved in 30 mL of ethanol, and diluted to 100 mL by using water. The solution was filtered through Whatman filter paper number 41 and diluted quantitatively with water to obtain a suitable concentration for the analysis. A convenient aliquot was then subjected to the analysis by using proposed method.

3. Results and Discussion

Method A. In this method MBTH undergoes oxidation with Ce(IV), by losing two electrons and one proton to form electrophilic intermediate, which act as the active coupling species [18, 19]. This intermediate undergoes electrophilic substitution reaction with SEG (Scheme 1) to give bluish green colored product that can be measured at 629 nm (Figure 2).

Method B. In this method AAP reacts with SEG to form a new ligand (Scheme 2) having low sensitivity at 329 nm. This sensitivity has been increased by complexation with Cu(II) [20] to give intense bluish red colored chelate which can be measured at 539 nm (Figure 3).

3.1. Determination of Effective Reagents Concentration. Experiments were carried out to optimize the reaction condition for complete color formation. It was found that 1.0 mL of 0.2% MBTH and 1.0 mL of 1% Ce(IV) solutions for method A and 2 mL of 1% 4-AAP and 1 mL of 0.1% Cu(II) solutions for method II were found to be optimum to get the stable and maximum color intensity.

3.2. Quantification. In order to analyze the linearity of the developed methods, absorbance was measured for a series of solutions containing increasing amounts of SEG under optimum condition. Regression analysis of Beer's law plots (Figures 4 and 5) at their respective values revealed good corre-

SCHEME 1: Probable reaction of SEG with MBTH.

SCHEME 2: Probable reaction of SEG with AAP and Cu(II) chloride.

lation. The validity of Beer's law, molar absorptivity, and Sandell's sensitivity values were evaluated and were given in Table 1. The proposed methods showed excellent linearity for the determination of SEG drugs with a good correlation coefficient. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as per the ICH guidelines [21].

3.3. Accuracy and Precision. Accuracy and precision of the proposed methods were tested by carrying out determinations of five replicates of bulk and commercial samples for

both the methods, whose concentrations lie within Beer's law range. The relative standard deviation (RSD) and relative error results indicated that the methods were precise and accurate. The relative error (%) which is a measure of accuracy and RSD (%) which is a measure of precision were summarized in Table 2, revealing the high accuracy and precision of the methods.

3.4. Stoichiometry of the Reaction Product. The stoichiometric ratio of the colored product of method A and method B

TABLE 1: Spectral and statistical data for the determination of SEG.

Parameters	Method A	Method B
$\lambda_{\max}$ (nm)	629	539
Beer's law limits ($\mu\text{g/mL}$)	10.00–85.00	20.00–120.00
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	0.98×10^4	0.94×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	3.33×10^{-2}	1.98×10^{-2}
Limit of detection* ($\mu\text{g mL}^{-1}$)	0.8059	0.4347
Limit of quantification* ($\mu\text{g mL}^{-1}$)	2.4423	1.3173
Regression equation**	$Y = a + bX$	$Y = a + bX$
Slope (b)	0.0124	0.0046
Intercept (a)	0.1428	0.0047
Correlation coefficient (r)	0.9924	0.9987

*Limit of detection calculated according to ICH guidelines.

**Y is the absorbance and X is the concentration in $\mu\text{g mL}^{-1}$.

FIGURE 3: Absorption maximum for method B.

was investigated by applying the continuous variation (Job's) and mole ratio methods [22] using equimolar solutions of the reagents. As shown in Figures 6 and 7, the molar ratio which gave maximum absorbance is found to be 1 : 1 (drug : reagent) for SEG-MBTH system and 1 : 2 for Cu-ligand system. In view of this result, a reaction mechanism is proposed for the developed method.

3.5. Interference Studies. In pharmaceutical analysis, it is important to test the selectivity towards the excipients added to the pharmaceutical preparations. The effects of the excipients associated with formulations of SEG in its pure form and its formulations were investigated using the developed methods. This method does not suffer any interference from commonly associated excipients such as sucrose, lactose, dextrose, starch, and sodium chloride in the preparation of tablets. Data of the interference studies are given in Table 3.

FIGURE 4: Calibration curve for method A.

FIGURE 5: Calibration curve for method B.

3.6. Analytical Application. Commercial formulations (tablets) containing 5 mg of SEG were successfully analyzed by the proposed methods. The results obtained for the Student's t -test at 95% confidence level are less than the theoretical values, which confirm the good accuracy of the methods. Obtained values were listed in Table 4. However, there is no method described in the literature for the assay of SEG in pharmaceutical preparations. Thus the present work reports an elegant method for the determination of SEG in pure and pharmaceutical formulations.

TABLE 2: Evaluation of accuracy and precision.

(a) Method A				
Amount taken ($\mu\text{g mL}^{-1}$)	Amount found* ($\mu\text{g mL}^{-1}$)	RE (%)	SD ($\mu\text{g mL}^{-1}$)	RSD (%)
50.00	49.84	0.32	0.39	0.78
60.00	59.96	0.06	0.11	0.18
70.00	69.83	0.24	0.41	0.58

(b) Method B				
Amount taken ($\mu\text{g mL}^{-1}$)	Amount found* ($\mu\text{g mL}^{-1}$)	RE (%)	SD ($\mu\text{g mL}^{-1}$)	RSD (%)
20.00	20.27	-1.35	0.22	1.08
40.00	39.82	0.45	0.41	1.02
60.00	59.51	0.81	0.81	0.3

* Mean value of five determination processes.
RE: relative error; SD: standard deviation; RSD: relative standard deviation.

FIGURE 6: Application of Job's method to method A.

TABLE 3: Effect of excipients on assay of SEG.

Excipient	Excipient taken (mg)	% recovery* of drug $\pm$ % RSD	
		Method A	Method B
Glucose	50	99.63 $\pm$ 1.43	99.97 $\pm$ 0.22
Lactose	50	99.86 $\pm$ 0.10	99.72 $\pm$ 0.52
Dextrose	35	99.66 $\pm$ 1.27	99.98 $\pm$ 0.27
Sodium chloride	25	99.90 $\pm$ 0.73	99.90 $\pm$ 0.07
Starch	80	99.86 $\pm$ 0.43	99.95 $\pm$ 0.26

* Average for five determination processes.

4. Conclusions

The developed spectrophotometric methods are quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The λ_{max} in both the methods was

TABLE 4: Result of assay of formulation by the proposed method.

Brand name	SEG certified (mg)	Found* $\pm$ SD	
		Method A	Method B
Selegiline	5	5.01 $\pm$ 0.16	5.06 $\pm$ 0.35
		$t = 0.52$	$t = 0.97$

* Mean of five determination processes.
Tabulated t value at 95% confidence level is 2.77.

considerably higher which is a decisive advantage and interferences by common excipients are generally very less. The described procedure is reliable, very simple, and conveniently applicable in most laboratories due to the accessibility of the reagents employed and reasonably low time of analysis. Thus, these methods can be used for routine analysis of SEG in pharmaceutical industries, hospitals, and research laboratories.

FIGURE 7: Application of Job's method to method B.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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