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**Research Article**


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## STABILITY INDICATING RP-HPLC METHOD FOR SELEGILINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATION

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### ABSTRACT

Stability indicating RP-HPLC method has been developed for the determination of Selegiline hydrochloride in pharmaceutical formulation. Chromatographic separation of Selegiline hydrochloride and its known impurities was achieved by using BDS Hypersil C18 (250 x 4.6 mm, 5  $\mu$ m) column at temperature of 40°C. Mobile phase composed of Acetonitrile:Potassium di-hydrogen phosphate buffer adjusted at pH 4.0 $\pm$ 0.5 (30:70v/v). Flow rate was 1.00 ml per min and the absorbance was measured at 205nm. The retention time of Selegiline hydrochloride was 5.7min, at runtime 10min. System suitability parameters like, tailing factor 1.1 and theoretical plates 6010 were calculated. The linearity of the method was evaluated from 25 $\mu$ g per ml to 100 $\mu$ g per ml for Selegiline hydrochloride by injecting 50 $\mu$ l of standard solution giving correlation coefficient 0.999. The developed method can be used or applied for the quantification of Selegiline hydrochloride in pharmaceutical products.

**Keywords:** Selegiline Hydrochloride, RP-HPLC, Validation and Degradation.

### INTRODUCTION

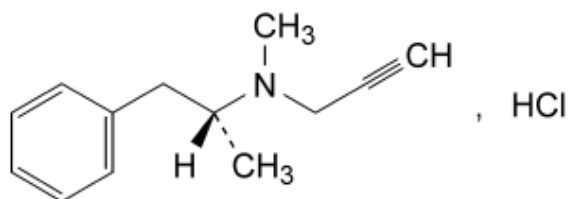
Selegiline hydrochloride is an selective, irreversible inhibitor of MAO-type B; MAO-B metabolizes dopamine and phenylethylamine. Selegiline hydrochloride exhibits little therapeutic benefit when used independently, but it enhances and prolongs the anti-Parkinson effects of levodopa.

Selegiline hydrochloride is widely used in Parkinson's disease and also may be given with Levodopa upon onset of disability, in the treatment of mild to moderate Alzheimer disease and at the higher doses for the treatment of depression.

Chemically, Selegiline hydrochloride is (R)-(-)-N-2-propynylphenethylamine hydrochloride [1-3]. Selegiline hydrochloride is absorbed from GIT, bioavailability 4-5% (oral, fasted), 20% (oral, after food) and half life is about 1.5(oral, single dose). Metabolism occurs in liver and excretion through urine. Selegiline hydrochloride is soluble in water and methanol. Route of administration of Selegiline hydrochloride is oral and transdermal dosage form. Based on literature survey, some HPLC methods are reported for determination of Selegiline hydrochloride [4-6]. However, stability methods are not reported on Selegiline hydrochloride. So, the present study is to develop a stability indicating RP-HPLC method for estimation of Selegiline hydrochloride in pharmaceutical formulation. The developed HPLC method is precise, robust, specific, sensitive and accurate and has been validated as per ICH guidelines [7]. Chemical structure shown in Figure 1.

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**Fig. 1:** Chemical structure of Selegilinehydrochloride[2]

## MATERIALS AND METHODS:

### *Instrumentation:*

High Performance Liquid Chromatography (Shimadzu HPLC, LC-2010 CHT), UV detector and BDS Hypersil C18 column (250 x 4.6 mm, 5  $\mu$ m).

### *Reagents and Chemicals:*

AR grade of potassium dihydrogen phosphate and HPLC Grade of methanol, acetonitrile were used for analysis. They were purchased from Merck Chemicals, Mumbai and LOBA Chemical Pvt. Ltd., Mumbai. HPLC grade water was obtained from Elga Lab water purifier.

All purified standard materials (not less than 98.5%) were used. Marketed formulation which is available in 5 mg strength of tablets (SELGIN – Intaspharma) were analysed with this method.

### *Chromatographic Conditions:*

Mobile phase composed of Acetonitrile: Potassium dihydrogen phosphate buffer (dissolved 2.04 gram in 1000 ml of HPLC grade water and adjusted the pH to  $4.00 \pm 0.05$  with ortho phosphoric acid), Acetonitrile and Buffer was in the ratio of 30:70(v/v) respectively. Injection volume was 50 $\mu$ l with flow rate 1.00ml per minute. Detection of the Selegiline hydrochloride was achieved by using a UV detector and absorbance measured at 205nm. BDS Hypersil C18 column (250mm x 4.6mm, 5 $\mu$ m) and column oven temperature maintained at 40°C.

### *Standard preparation:*

Standard stock solution (1000 $\mu$ g/ml) was prepared in water which is used as diluent. Weighed accurately 50mg of Selegiline hydrochloride standard and transferred into 50ml volumetric flask and dissolved in diluent with intermediate sonication, diluted to volume(50ml) with diluent and mixed. Further diluted 0.5 ml of this solution transferred to 10 ml volumetric flask and q.s. to 10ml with diluent and mixed well to achieve a concentration of 50 $\mu$ g per ml.

### *Sample preparation for 5mg strength:*

Weighed and transferred 5 tablets into a 25 ml volumetric flask and make up volume with water to 25ml volumetric flask and sonicated for 20 minutes with intermediate shaking and filtered the solution using Whatman's filter paper, and the filtrate used as sample.

## RESULTS AND DISCUSSION:

### *Selection of analytical wavelength ( $\lambda_{max}$ ) by ultraviolet spectroscopy (UV):*

UV spectra of 10  $\mu$ g/ml solution of Selegiline hydrochloride standard in HPLC grade water was scanned between 200-400 nm with medium scan speed against HPLC grade water blank and recorded the absorbance obtained at 205 nm, where maximum absorption occurs, showed in Figure 2.

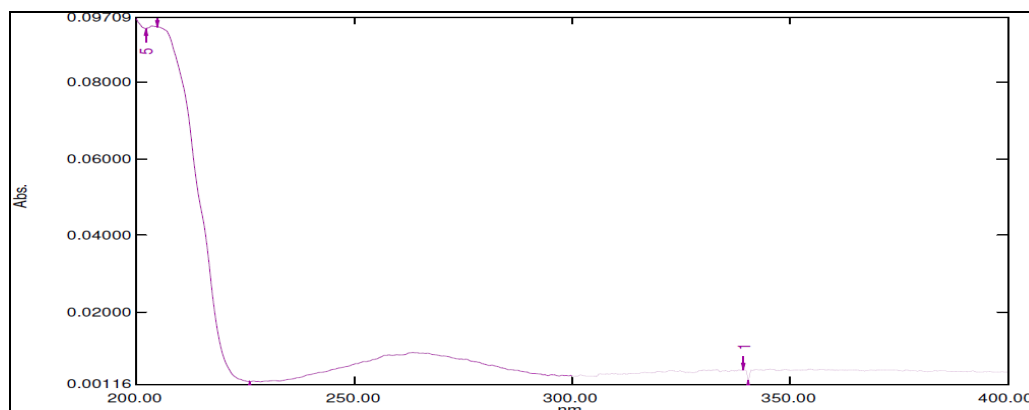


Fig. 2: UV chromatogram of Selegiline hydrochloride

#### Method development:

Method development trials were performed with Potassium phosphate, monobasic ammonium phosphate and acetate buffers and different organic modifiers such as methanol, acetonitrile and water but the peak shape of Selegiline hydrochloride was poor. Finally after 5 trails, the resolution and sharp peak shapes were achieved with, Hypersil C18

(250mm x 4.6mm, 5 $\mu$ m) column and the separation was achieved by Potassium dihydrogen phosphate buffer and acetonitrile. Based on Selegiline hydrochloride absorbance spectra, samples absorbance was measured at 205nm. See Selegiline hydrochloride HPLC chromatogram was represented in Figure 3. The retention time of Selegiline hydrochloride was 5.78min, at runtime 10.0min.

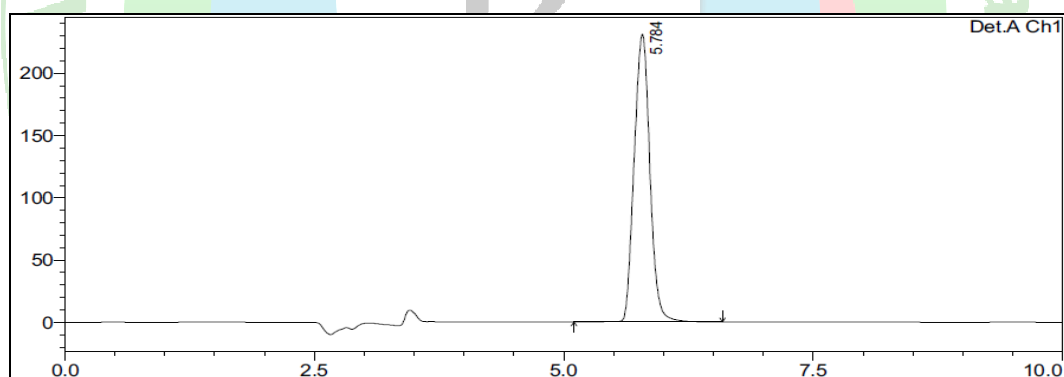


Fig. 3: Standard chromatogram of Selegiline hydrochloride

#### Method Validation [7]:

Validation was performed as per ICH Q2 (R1) guidelines for parameters like specificity, precision, accuracy, linearity and range, robustness, limit of detection and limit of quantification.

#### Linearity and Range

Dilutions of Selegiline hydrochloride shows good correlation coefficient in concentration

range of 25-100 $\mu$ g/ml ( $r^2=0.999$ ). Linearity was evaluated for a set of six standard working solutions containing 25-100 $\mu$ g/ml for Selegiline hydrochloride. The linear regression data is presented in Table I. The linearity of calibration graphs and adherence of the system to Beer's law was validated by determining correlation coefficient and S.D. values which were found to be well within the accepted limit as per ICH guidelines.

Table I : Linear regression data for calibration curves

Parameters	SelegilineHCl
Linearity range $\mu\text{g/ml}$	25-100 $\mu\text{g/ml}$
$r^2 \pm \text{SD}^*$	0.999
Slope $\pm \text{SD}^*$	52862 + 179
Intercept $\pm \text{SD}^*$	17052 + 987

\*n=6

**Precision studies**

Repeatability was studied by injecting six replicates of the working standard (50  $\mu\text{g/ml}$ ).

Results of precision were satisfactory as per ICH guidelines.

The results for repeatability studies of HPLC are shown in Table II.

Table II: Repeatability studies

Precision	Amount ( $\mu\text{g/ml}$ )	Area	Mean Area $\pm \text{SD}^*$	%RSD*
Repeatability (*n=6)	50	2413224	2414198+2596.783	0.107
	50	2417586		
	50	2415937		
	50	2416216		
	50	2410139		
	50	2412086		

**Intraday and Inter-day**

Intraday precision studies were performed by 3 repeated injections of standard drug solutions at three concentrations (40, 50 and 60  $\mu\text{g/ml}$ ). Inter day precision studies were performed using same concentrations on three

different days over a period of 1 week. Precision was expressed as % relative standard deviation values. The results for intra-day and inter day precision studies of HPLC were satisfactory as per ICH guidelines are shown in Table III and Table IV.

Table III: Intraday precision studies

Precision	Amount ( $\mu\text{g/ml}$ )	Area	Mean Area $\pm \text{SD}$	%RSD*
Intra-day (*n=3)	40	2291231	2293013 $\pm$ 2060.30	0.089
	40	2292539		
	40	2295269		
	50	2317331	2320055 $\pm$ 3194.53	0.137
		2318295		
		2324538		
	60	2323910	2346053 $\pm$ 3258.85	0.138
		2322120		
		2392129		

Table IV: Inter-day precision studies

Precision	Amount ( $\mu\text{g/ml}$ )	Area	Mean Area $\pm$ SD	%RSD*
Intra-day (*n=3)	40	2345580	2342139 $\pm$ 15174.98	0.647
	40	2325539		
	40	2355298		
	50	2413851	2424318 $\pm$ 17465.01	0.720
	50	2410177		
	50	2448926		
	60	2455098	2432409 $\pm$ 21462.11	0.882
	60	2403605		
	60	2438525		

**Accuracy (Recovery studies)**

Drug sample when at three given concentration levels (80%, 100%, 120%) showed satisfactory recovery of Selegiline

hydrochloride. The mean recovery was found to be 99.75%. The limit for mean recovery is 90-107%. Thus the method was found to be accurate as per ICH guidelines. The data is represented in Table V.

Table V: Results for accuracy studies

Spike level	Amount added ( $\mu\text{g/ml}$ )	Area	Amount recovered ( $\mu\text{g/ml}$ )	% Recovery	Mean recovery $\pm$ S.D.	% R.S.D.*
80%	40	1896756	40.43	101.09	100.67 + 0.42	0.4172
		1881081	40.10	100.25		
100%	50	2399666	49.91	99.83	99.75+0.0775	0.0776
		2384499	49.84	99.68		
120%	60	2833550	58.87	98.11	98.87 $\pm$ 0.755	0.763
		2811169	59.78	99.63		

\*n=2

Acceptance criteria: % Recovery 98-102 % & % RSD  $\leq$  2**Robustness of the Method**

Each factor described under section 1.2.8(IV) of ICH guidelines Q2 (R1), was changed at three levels (-1, 0, 1). One factor was changed at one time to estimate the effect. Robustness of the method was evaluated at a concentration level 50  $\mu\text{g/ml}$  for Selegiline hydrochloride. Results are presented in Table VI. Insignificant differences in peak areas and less

variability in retention time were observed, indicating that robustness of Selegiline hydrochloride were satisfactory as per ICH guidelines.

**LOD & LOQ**

The LOD and LOQ values for Selegiline hydrochloride are shown in Table VII.

**Table VI: Robustness data in terms of retention time for Selegiline hydrochloride  
(change in flow rate, temperature and wavelength)**

Factor	Level	SelegilineHCl ( $t_R$ in min)	Peak area
<b>A: Change in Flow Rate (1 ml/min)</b>			
0.8	-2	7.1	3120125
1	0	5.7	2416077
1.2	+2	4.7	2135177
	<b>% RSD*</b>	<b>0.162</b>	
<b>B: Change in temperature (40<sup>0</sup>c)</b>			
38 <sup>0</sup> c	-2	5.9	2187393
40 <sup>0</sup> c	0	5.7	2162031
42 <sup>0</sup> c	+2	6.01	2167679
	<b>% RSD*</b>	<b>0.62</b>	
<b>C: Change in Wavelength</b>			
202	-3	5.6	2529539
205	0	5.7	2412969
208	+3	5.8	2490103
	<b>% RSD*</b>	<b>0.239</b>	

\*n=3

**Table VII: LOD and LOQ**

Parameters	LOD ( $\mu\text{g/ml}$ )	LOQ ( $\mu\text{g/ml}$ )
SelegilineHCl	0.061	0.186

#### System Suitability Parameters

System suitability tests (resolution, tailing factor, theoretical plates) were performed to verify the resolution and reproducibility of the chromatographic system. System suitability parameters for the optimized method were found to be within the acceptable limit as per ICH guidelines.

#### Forced degradation studies [8] :

Stress studies were carried out on the drug sample according to ICH guideline Q1A (R2). The Selegiline HCl was subjected to different stress conditions like acid induced, base induced, neutral, dry heat and hydrogen peroxide. The stress conditions were optimized in such way that the drug will degrade at least 20-30%.

#### Acid Induced Degradation

SelegilineHCl was treated with 0.1M HCl solution at 80<sup>0</sup>C for 6 hrs and showed no degradation. The chromatogram is shown in Figure 5.

#### Base induced degradation

Selegiline HCl was treated with 0.1M NaOH solution at 80<sup>0</sup>C for 6 hrs and showed no degradation. The chromatogram is shown in Figure 6.

#### Hydrogen Peroxide Degradation

Selegiline HCl was treated with hydrogen peroxide (3%) solution at room temperature for 15 mins. The degradation peaks were obtained at 4.76 mins and 5.36 mins along with peak of Selegiline hydrochloride at 5.82. The degradation was found to 59.59 %. The chromatogram is shown in Figure 7.

**Dry heat degradation**

Accurately weighed 50mg of Selegiline Hydrochloride evenly spread in separate

petridish and kept in oven at 105°C for 24 hours. It showed no degradation. The chromatogram is shown in Figure 8.

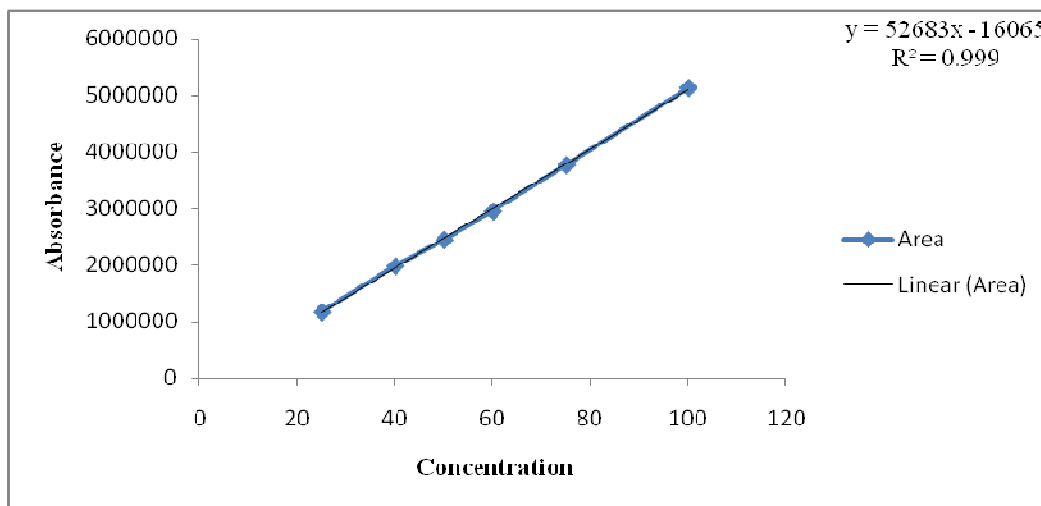


Fig. 4: Calibration curve for linearity of Selegiline hydrochloride dilutions.

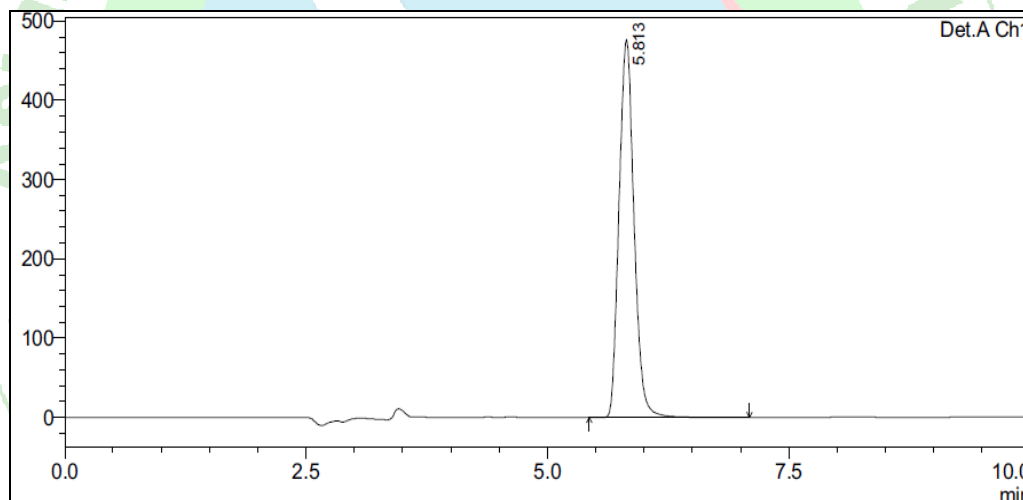


Figure 5: Chromatogram of acid degradation (0.1M HCl, 6hrs at 80°C),  $t_R$ -5.81

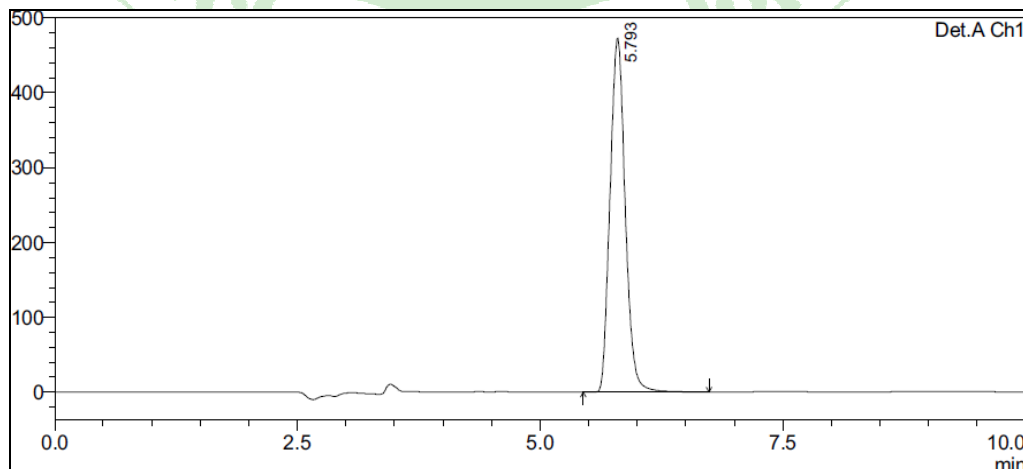
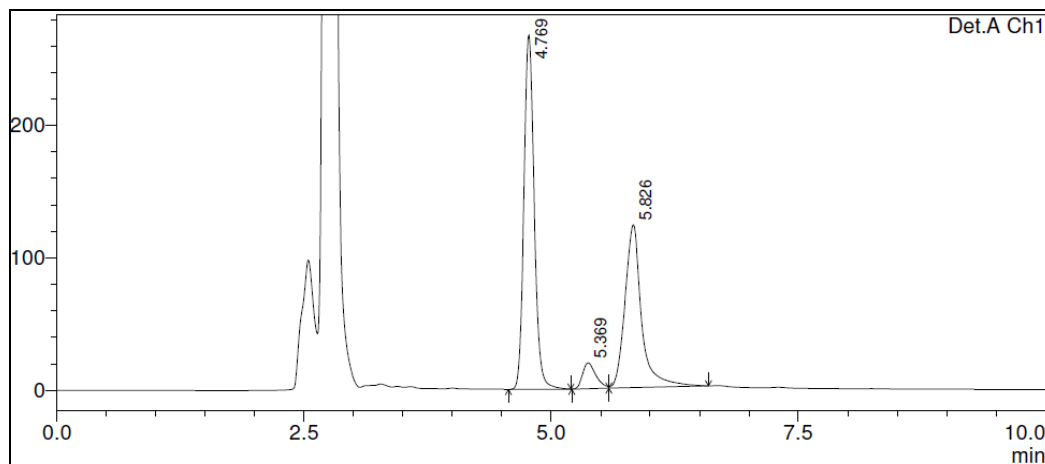
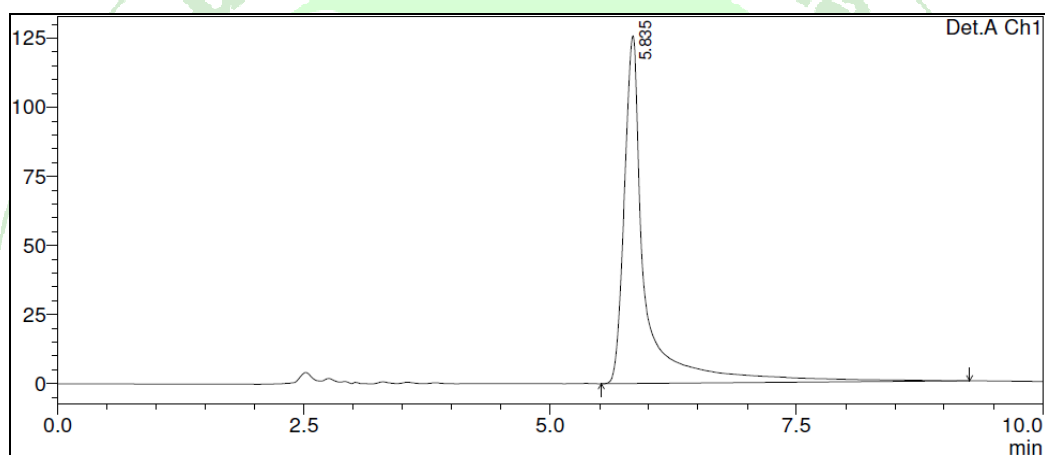


Figure 6: Chromatogram of base degradation (0.1M NaOH, 6hrs at 80°C),  $t_R$ 5.79

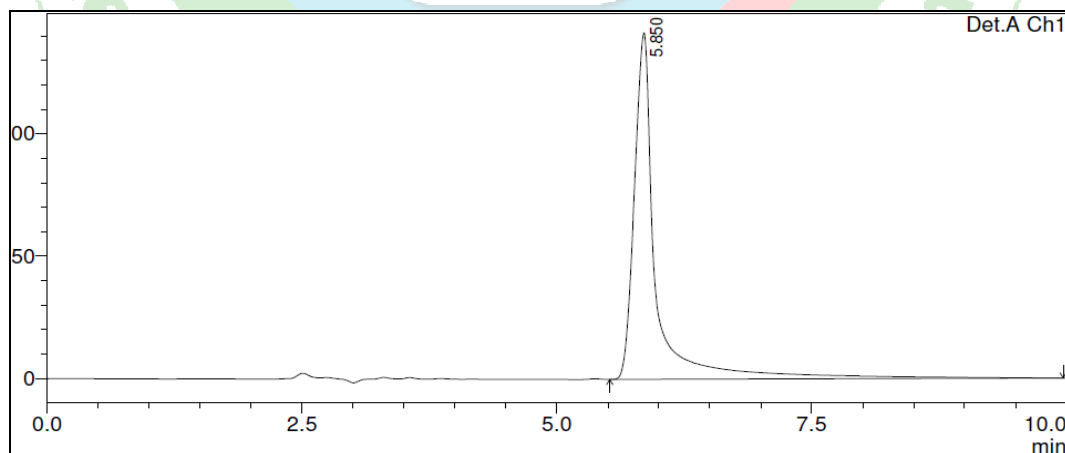




**Figure 7:** Chromatogram of peroxide (3% H<sub>2</sub>O<sub>2</sub>, 15 mins.),  $t_R$ - 5.82 (Selegiline hydrochloride), 4.76 and 5.36 (Degradation products of Selegiline hydrochloride)



**Figure 8:** Chromatogram of Dry heat degradation (24hrs at 105°C) , $t_R$ - 5.83 Selegiline hydrochloride



**Figure 9:** Chromatogram of Neutral degradation (HPLC grade water, 6hrs at 80°C) , $t_R$ - 5.85 Selegiline hydrochloride.

#### **Neutral degradation**

SelegilineHCl was treated with HPLC grade water at 80°C for 6 hrs and showed no degradation. The chromatogram is shown in Figure 9. Results of the forced degradation studies are enlisted in Table VIII.

Table VIII: Results of forced degradation studies of Selegiline hydrochloride

Stress condition	Amount of SelegilineHCl degraded (%)	Amount of SelegilineHCl recovered (%)	$t_R$ of degraded products (min)	Relative retention
0.5M HCl (6hrs at 80°C)	-	100	-----	-----
0.5M NaOH (6hrs at 80°C)	-	100	-----	-----
H <sub>2</sub> O <sub>2</sub> (15min. at RT)	59.59	40.41	4.7,5.3	5.8
Dry heat (24hrs at 105°C)	-	100	-----	-----
Neutral (6hrs at 80°C)	-	100	-----	-----

**CONCLUSION:**

A new precise, accurate, robust, stability indicating RP-HPLC method has been developed for the estimation of Selegiline hydrochloride (API) and in pharmaceutical formulation. The method was validated per ICH guidelines the results for which were within specified limits. The developed method was applied for the stability studies of Selegiline hydrochloride in pharmaceutical dosage form. The results of forced degradation studies reveal that the method is stability indicating. The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies. The method can be employed for the routine analysis of Selegiline hydrochloride.

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