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# HPLC BASED *IN VIVO* PHARMACOKINETIC STUDIES OF SELEGILINE HYDROCHLORIDE MICROSPHERES FOR PARKINSON'S DISEASE

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**ABSTRACT**

Selegiline hydrochloride functions as an irreversible mono amino oxidase inhibitor and is typically prescribed to treat the symptoms of idiopathic Parkinson's disease. The microspheres were created for prolonged drug retention in the gastrointestinal tract, leading to improved oral bioavailability and superior absorption. Mucoadhessive microspheres were developed using the ionotropic gelation technique using different concentrations of sodium alginate, calcium chloride, carbopol 940p, and karaya gum with different levels of stirring speed. The optimization process was carried out using Design Export 13 software. Followed by investigating and comparing the pharmacokinetic profiles of selegiline hydrochloride pure drug and its microsphere optimized formulation in rat plasma by RP-HPLC using tetradeuteroselegiline as internal standard with a single oral administration of 0.129 mg. When compared with pharmacokinetic parameters of selegiline hydrochloride, the $AUC_{0\text{-}t}$, $AUC_{0\text{–}\infty}$, $T_{max}$ and $t_{1/2}$ of selegiline hydrochloride microspheres were increased, while the $C_{max}$ was decreased. These results suggested that formulation modification of selegiline hydrochloride into microspheres enhanced bioavailability.

**Keywords: Selegiline hydrochloride, Parkinson's disease, Mucoadhesive microspheres, Pharmacokinetics, HPLC**

## INTRODUCTION

A microsphere can have a diameter of between 1 and 1000 µm. The sphere-shaped, free-flowing particles are made of proteins or polymers. In addition to the first two, they are made with waxes, disintegrating synthetic polymers, and natural polymers. A second-generation monoamine oxidase type B inhibitor called selegiline hydrochloride permanently and specifically blocks dopamine in the central nervous system. Parkinson's disease-related motor problems have been treated with selegiline hydrochloride. On the other hand, selegiline hydrochloride undergoes first-pass metabolism, having a low bioavailability (10%) and a short half-life between 1.5 to 3.5 h. Due to the drug's short half-life, poor bioavailability, and need to maintain therapeutic levels, a gastro retentive formulation must be created **[1]**. For initial monotherapy with selegiline hydrochloride, a dosage of 5 mg once daily is advised and further cases as adjunctive therapy with levodopa. Literature review on analytical methods of selegiline hydrochloride reveals that there are methods such as reverse phase high-performance liquid chromatography (RP-HPLC) **[2]**.

## MATERIALS AND METHODS

### Materials

Methanol of the HPLC grade was purchased from E. Merck Ltd. in Mumbai. Sun Pharma, Mumbai, India, provided the reference standard Selegiline hydrochloride. tetradeuteroselegiline (internal standard) received from Beryl Drugs Limited. In Mumbai, India and S.D. Fine sold potassium dihydrogen orthophosphate and ammonia solution. Methocel purchased from Sakshi private limited, Nagpur. The reagents were all HPLC grade. Wherever necessary, solutions were prepared using milli-Q grade (Millipore, France) water that had been filtered via a 0.45 µm membrane filter before to use. Waters HPLC system and a C-18 cosmosil packed column used for analysis **[2]**.

### Preparation and evaluation of selegiline hydrochloride mucoadhessive microspheres

Using the ionic gelation method, mucoadhesive microspheres containing selegiline hydrochloride have been created. In the purified water, sodium alginate was mixed with mucoadhesive polymers such carbopol 940P and karaya gum. Selegiline hydrochloride added to polymer dispersion on a magnetic stirrer. The gelation medium, which facilitates in the creation of stable microspheres, was produced by dissolving 10% calcium chloride in a 2% solution of glacial acetic acid. The homogenous alginate solution extruded into the gelation medium while stirring with a 21G syringe needle. Particle size, cumulative

percent drug release, compatibility studies (FTIR and DSC), and scanning electron microscopy were all evaluated **[3, 4]**.

***In vivo* studies of selegiline hydrochloride**

**Experimental animals**

Healthy Wistar rats (weighing an average of 250 g) were chosen as the study's subjects. The animals were all healthy and remained so throughout the trial. The animals were housed in areas with a complete exchange of fresh air, a constant supply of power and water, and controlled environmental conditions (25°C and 45% Relative humidity, and a 12 h of light and dark cycle). Rats were given access to unlimited amounts of water and a regular meal **[5-7]**. The protocol for the study received approval from the institution's animal ethics committee (IAEC NO:1447/PO/Re/S/11/CPCSEA-40/A).

**Calibration curve**

Different concentrations of Selegiline hydrochloride were examined for the linearity study: 0, 1, 2, 4, 6, 8, 10 and 12 ng/ml. 50 ml of an internal standard (100 ng/ml) were added to each of these samples. By using least-squares linear regression analysis to plot the area ratios of selegiline to internal standard versus the concentration of selegiline, the calibration curve was plotted **[8]**.

**Study design**

Randomly selected rats were split into Group A and B. Each group contains six rats. The following treatments were done to the rats. They went without food for 24 h before to the experiments. Four hours following the dose, food was available. Group A received selegiline hydrochloride pure drug suspension of 0.5% methocel, whereas second group B received oral administration of prepared microspheres diluted in 0.5% methocel of 0.129 mg - animal dose **[8]**.

**Blood sampling**

At periods 0, 0.50, 1, 1.50, 2, 4, 6, 12 and 24 h after the post dose, 0.3 ml of blood samples were routinely drawn from the tail vein using cannula and put into eppendorf tubes with heparin to avoid coagulation of blood and centrifuged at 4000 rpm for 5 to 10 min to extract the plasma. Frozen stored at -20°C until analysis. Before injecting in to HPLC, mobile phase was added, followed by vortexed for 30 secs; and centrifuged at 9000 rpm for 10 min, and 20 µl supernatant aliquot was injected into the HPLC column **[9, 10]**.

**Instruments**

HPLC (Waters 2487) equipped with isocratic pump and UV-detector with Empower 2 software. Balance (AUX 220, Shimadzu Corporation, Kyoto, Japan), magnetic stirrer (Remi Instruments Pvt. Ltd., Mumbai, India), pH meter (Hicon Scientific Instruments, Delhi, India), centrifuge (Remi Instruments Pvt. Ltd., India).

**HPLC system:** Waters HPLC system with Empower 2 software (C-18 cosmosil column) with 1 ml/min flow rate

**Injector:** Manual syringe of volume- 20 µl.

**Detector wavelength** – 206 nm.

**Mobile phase preparation**

Methanol and phosphate buffer solution with a pH of 7.0 ± 0.05 were combined 95:5 % v/v. After filtering through a 0.45 µm nylon membrane filter, the mixture was degassed for 10 min. The mobile phase was the diluents. A 0.45 µm membrane filter and a power sonicator were used to filter and sonicate the mobile phase **[10]**.

**PHARMACOKINETIC ANALYSIS**

**Peak plasma concentration ($C_{max}$)**

The maximum concentration of medication in plasma ($C_{max}$) that can be attained following oral drug administration. $C_{max}$ is the maximum concentration that can be determined by visual examination of the concentration-time curve.

**Peak plasma concentration time ($T_{max}$)**

$T_{max}$ is a measure of how long it takes for a drug to reach its peak concentration after being taken.

**Elimination half-life ($t_{½}$)**

A certain amount of time is needed to reduce the drug's plasma concentration by half, which is $t_{½}$. The corresponding $t_{½}$ was calculated by-

$$t_{½} = \frac{0.693}{K_{el}}$$

**Area under curve (AUC)**

The following equation determines the area under the drug plasma concentration versus time curve, $AUC_{0-t}$

$$AUC_{0-t} = \int_0^t C_{(t)} dt$$

where, $C_t$ represents plasma drug concentration at t h.

$(AUC_{t-\infty}) = C_t/K_{el}$, where $C_t$ - at time t final measurable concentration and $K_{el}$ - terminal elimination rate constant.

$$AUC_{0-\infty} = AUC_{0-t} + AUC_{t-\infty}$$

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_t}{K_{el}}$$

Using Win Nonlin 3.3® pharmacokinetic software, the non-compartmental analysis of the pharmacokinetic parameters was carried out. All values stated as mean ± SEM **[11,12]**.

**STABILITY STUDIES**

Optimized Selegiline hydrochloride microspheres formulation was tested for stability at two different temperatures as per ICH guidelines, long term studies (30 ± 2°C with 65 ± 5% RH) and accelerated stability studies (40 ± 2°C with 75 ± 5% RH). Tested different evaluation parameters like particle size, percent entrapment efficiency, cumulative percent drug release at initial stage, three months and six months **[13]**.

**RESULTS**

**Calibration curve**

The calibration curve requirement was set at a correlation coefficient ($r^2$) of 0.9993.

Results were given in **Table 1** and shown in **Figure 1**.

**Pharmacokinetic study**

Standard, non-compartmental methods were used to analyze the pharmacokinetic parameters. The maximum plasma drug concentration ($C_{max}$) as well as the time needed to reach this concentration ($T_{max}$) were calculated using the curve of plasma concentration-time. **Figure 2** and **Figure 3**, respectively provides standard selegiline hydrochloride and internal standard tetradeuteroselegiline HPLC chromatograms in rat plasma. Selegiline hydrochloride standard with tetradeuteroselegiline, formulation chromatogram of selegiline hydrochloride with tetradeuteroselegiline in rat plasma given in **Figure 4 and Figure 5**, respectively.

**Pharmacokinetic data of selegiline hydrochloride**

**Table 2** provides the plasma concentrations of selegiline after oral administration of the pure medication and optimized selegiline microspheres. **Figure 6** displays the corresponding plasma concentration-time curves. According to the equations previously described, the pharmacokinetic parameters were computed, and the results are displayed in **Table 3**.

Following an oral administration of selegiline hydrochloride microspheres formulation as compared to selegiline hydrochloride pure drug. **Figure 6** gives wistar rat's plasma concentration-time curve.

$C_{max}$ of the microspheres and pure drug was found to be 4.61±0.13 ng/ml and 5.45±0.26 ng/ml. $T_{max}$ of both microspheres and drug was 2.0 ± 0.09 h and 1.02±0.02 h.

**Stability study**

Stability studies data given in **Table 4**.

**Table 1: Standard calibration curve of Selegiline hydrochloride**

| S. No | Concentration(ng/ml) | Peak area |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 2 | 121851 |
| 3 | 4 | 225824 |
| 4 | 6 | 326475 |
| 5 | 8 | 430115 |
| 6 | 10 | 530527 |
| 7 | 12 | 638156 |

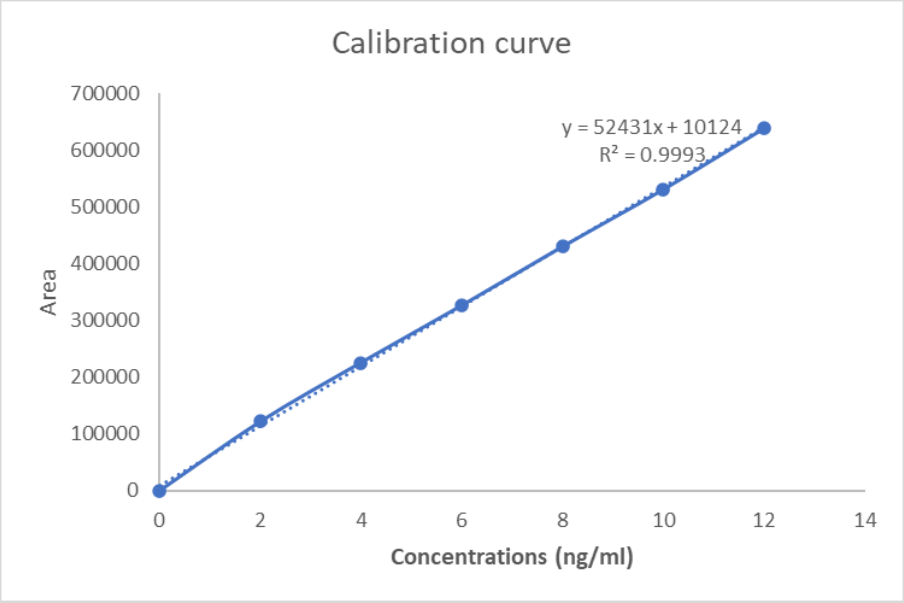


**Figure 1: Standard calibration curve of selegiline hydrochloride in rat plasma**

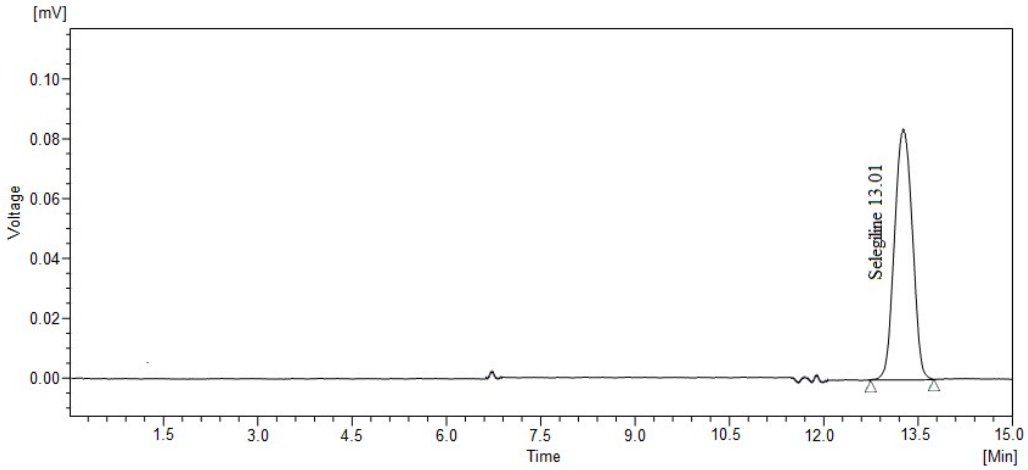


**Figure 2: Standard HPLC chromatogram of selegiline hydrochloride in rat plasma**

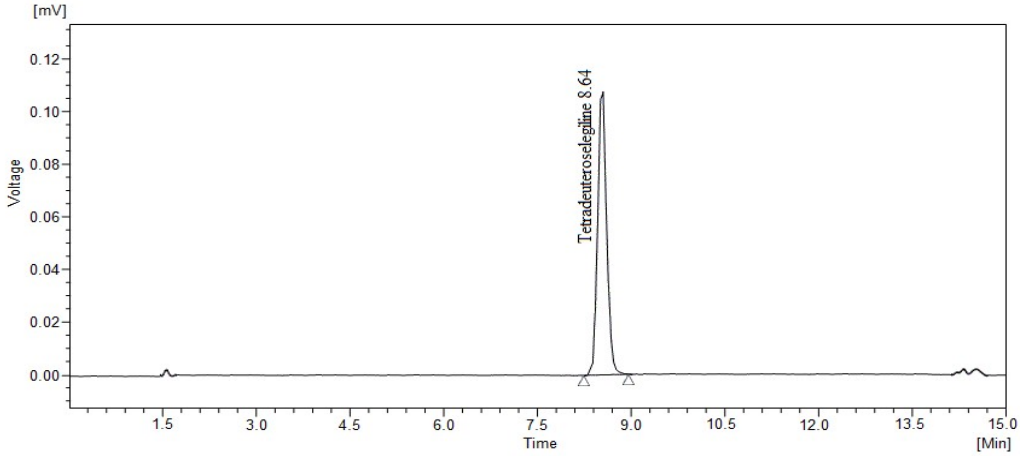


**Figure 3: Standard HPLC chromatogram of internal standard tetradeuteroselegiline in rat plasma**

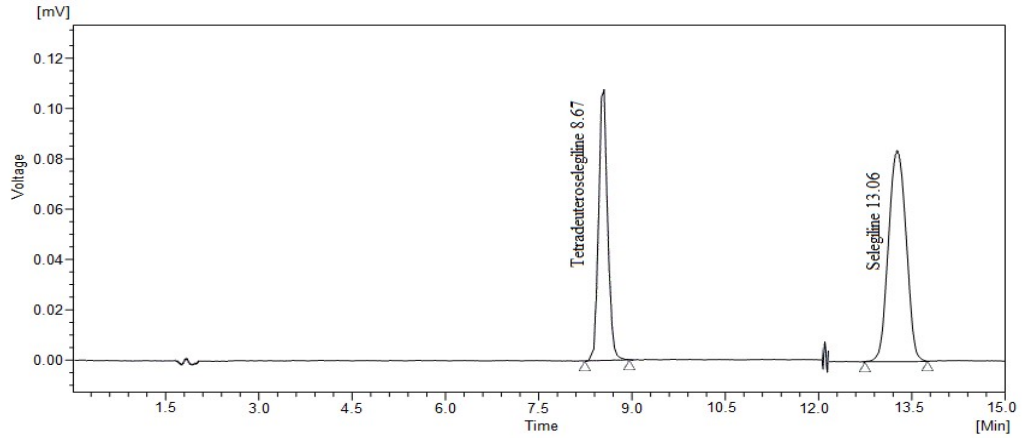


**Figure 4: Standard HPLC chromatogram of Selegiline hydrochloride and tetradeuteroselegiline (IS) in rat Plasma**

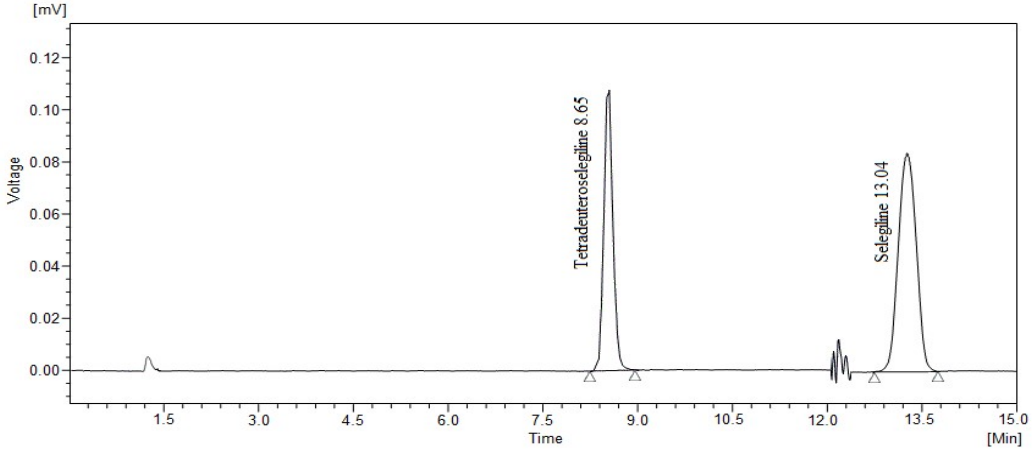


**Figure 5: Formulation chromatograms of selegiline hydrochloride and tetradeuteroselegiline (IS) in rat plasma**

**Table 2: Plasma concentration profiles of selegiline hydrochloride pure drug and selegiline optimised microspheres formulation**

| Time (h) | Selegiline hydrochloride pure drug (ng/ml) | Selegiline hydrochloride microspheres optimized formulation (ng/ml) |
|---|---|---|
| 0 | 0 | 0 |
| 0.5 | 3.02±0.16 | 1.24±0.10 |
| 1 | 5.45±0.12 | 2.81±0.16 |
| 1.5 | 3.21±0.09 | 3.29±0.12 |
| 2 | 2.32±0.12 | 4.61±0.13 |
| 4 | 0.24±0.03 | 2.72±0.15 |
| 6 | 0 | 1.39±0.10 |
| 12 | 0 | 0.67±0.08 |
| 24 | 0 | 0 |

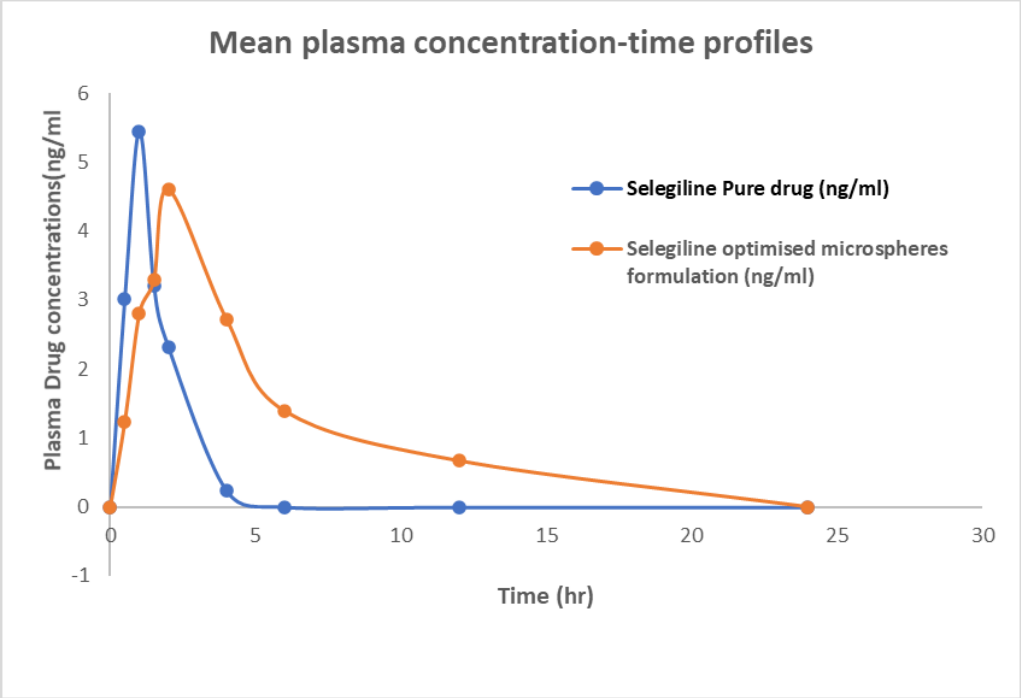


**Figure 6: Mean plasma concentration-time profiles for selegiline hydrochloride pure drug and selegiline hydrochloride optimized microspheres formulation in rats (n=6).**

**Table 3: Mean pharmacokinetic parameters of selegiline hydrochloride pure drug and selegiline hydrochloride optimised microspheres formulation**

| Pharmacokinetic parameters | Selegiline Pure drug | Selegiline–microspheres Optimized Formulation |
|---|---|---|
| $C_{max}$ (ng/ml) | 5.45±0.26 | 4.61±0.13 |
| $AUC_{0-t}$ (ng. h/ml) | 28.5±0.19 | 34.4±0.36 |
| $AUC_{0-inf}$ (ng. h/ml) | 35.1±0.23 | 42.1±0.29 |
| $T_{max}$ (h) | 1.02±0.02 | 2.00±0.09 |
| $t_{1/2}$ (h) | 3.56±0.08 | 6.15±0.09 |

**Table 4: Stability studies for optimized formulation**

| Time in months | 0 | 3 | 6 |
|---|---|---|---|
| Condition | | 30 ± 2°C / 65 ± 5% RH | |
| Particle size (µm) | 451.95 ± 1.53 | 451.43 ± 1.87 | 451.04 ± 0.94 |
| Drug entrapment efficiency (%) | 84.83 ± 1.16 | 89.26 ± 0.72 | 89.18 ± 1.05 |
| *In vitro* drug release (%) | 96.87 ± 0.95 | 96.63 ± 0.87 | 96.08 ± 1.62 |
| Condition | | 40 ± 2°C / 75 ± 5% RH | |
| Particle size (µm) | 451.25 ± 1.28 | 450.83 ± 1.62 | 450.27 ± 1.18 |
| Drug entrapment efficiency (%) | 84.83 ± 1.54 | 84.51 ± 1.12 | 84.16± 1.93 |
| *In vitro* drug release (%) | 96.87 ± 1.15 | 96.34 ± 0.81 | 95.82 ± 1.39 |

## DISCUSSION

AUC, $T_{max}$, $t_{½}$ values were more in optimized formulation than pure drug which indicates more bioavailability and sustained release, even though $C_{max}$ was more for Pure drug. AUC is a critical indicator of the bioavailability of a medicine from a dosage form, showing the total area under curve, which is the total amount of drug that reaches the systemic circulation after oral administration. $AUC_{0-\infty}$ infinity for microspheres formulation was

higher 34.4±0.36 ng.h/ml than the pure drug 28.5±0.19 ng.h/ml. $AUC_{0-t}$ of the microsphere's formulation, 34.4±0.36 ng.h/ml was significantly higher ($p<0.05$) as compared to pure drug. On the other hand, $C_{max}$ is more for pure drug than optimized formula, it indicates only highest concentration achieved, which might not fully capture the drug's overall exposure. There were no marked changes in formulation after stability Studies. The findings indicated that there were no significant changes in particle size, drug entrapment efficiency, *in vitro* percent drug release. Formulation was stable after performing stability studies.

## CONCLUSION

Numerous clinical studies have demonstrated that a drug's ability to be absorbed, distributed, metabolised, excreted, and harmful when consumed is strongly influenced by these processes. This study illustrates that, by transforming poorly absorbable Selegiline hydrochloride to improved permeability. The selegiline hydrochloride pure drug and mucoadhessive microspheres pharmacokinetic results revealed notable differences in the pharmacokinetic parameters, suggesting that the formulation modification of selegiline hydrochloride can successfully increase membrane permeability increases gastrointestinal absorption and relative bioavailability. The obtained pharmacokinetic information can be used to better understand the kinetic profile of selegiline hydrochloride mucoadhessive microspheres and to set the groundwork for future drug development in *in vivo* investigations for the treatment of Parkinson's disease.

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**Conflict of interest**

The authors declare no conflicts of interest relevant to this article.

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