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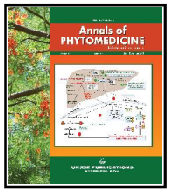

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HPLC based *in vivo* pharmacokinetic studies of rasagiline mesylate microspheres for Parkinson's disease

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Abstract

Rasagiline mesylate 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 934, and xanthan gum. The optimization process was carried out using Design Export 13 software. Followed by investigating and comparing the pharmacokinetic profiles of rasagiline mesylate pure drug and its microsphere optimized formulation in rat plasma by RP-HPLC using pseudoephedrine as internal standard with a single oral administration of 0.0258 mg. When compared with pharmacokinetic parameters of rasagiline mesylate, the AUC0-t, AUC0–8, Tmax and $t_{1/2}$ of rasagiline mesylate microspheres were increased, while the C_{max} was decreased. These results suggested that formulation modification of rasagiline mesylate into microspheres enhanced bioavailability.

1. 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 rasagiline mesylate permanently and specifically blocks dopamine in the central nervous system. Parkinson's disease-related motor problems have been treated with rasagiline mesylate. On the other hand, rasagiline mesylate undergoes first-pass metabolism, having a low bioavailability (36%) 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 (Sharma *et al.,* 2015; Ali *et al.,* 2020). For initial monotherapy with rasagiline mesylate, a dosage of 1 mg once daily is advised, whereas an initial dose of 0.5 mg per day is advised for adjunctive therapy with levodopa. Literature review on analytical methods of rasagiline mesylate reveals that there are methods such as reverse phase high-performance liquid chromatography (RP-HPLC) (Sundaramurthy *et al.,* 2011), high performance thin layer chromatography and UV-visible spectrophotometry for estimation of pharmacokinetic parameters (Singaram *et al.*, 2012).

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2. Materials and Methods

2.1 Materials

Methanol of the HPLC grade was purchased from E. Merck Ltd. in Mumbai. Sun Pharma, Mumbai, India, provided the reference standard rasagiline mesylate. Pseudoephedrine (internal standard) received from Beryl Drugs Limited. In Mumbai, India, Merck sold HPLC acetonitrile, 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 (Omar *et al.,* 2020; Parasuraman *et al.,* 2010).

2.2 Preparation and evaluation of rasagiline mesylate mucoadhessive microspheres

Using the ionic gelation method, mucoadhesive microspheres containing rasagiline mesylate have been created. In the purified water, sodium alginate was mixed with mucoadhesive polymers such carbopol 934P and xanthan gum. Rasagiline mesylate 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 (Prasanthi *et al.,* 2023; Ravi Kumar *et al.,* 2018; Anusree *et al.,* 2018; Dewi Melani *et al.,* 2020; Veerendra *et al.,* 2021).

2.3 *In vivo* studies of rasagiline mesylate

2.3.1 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. The protocol for the study received approval from the Institution's Animal Ethics Committee (IAEC NO:1447/PO/Re/S/11/CPCSEA-40/A).

2.3.2 Calibration curve

Six different concentrations of rasagiline mesylate were examined for the linearity study: 0, 1, 2, 4, 6, 8 and 10 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 rasagiline to internal standard versus the concentration of rasagiline, the calibration curve was plotted.

2.3.3 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 rasagiline mesylate pure drug suspension of 0.5% methocel, whereas second Group B received oral administration of prepared microspheres diluted in 0.5% methocel of 0.0258 mg - animal dose (Rohith *et al.,* 2019; Ying *et al.,* 2018).

2.3.4 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 (Hitesh Kumar *et al.,* 2021; Pramod *et al.,* 2014).

2.3.5 Instruments

HPLC (Waters 2487) equipped with isocratic type 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 1.5 ml/min flow rate

Injector: Manual syringe of volume-20 µl.

Detector wavelength-210 nm.

2.3.6 Mobile phase preparation

Acetonitrile and phosphate buffer solution with a pH of 7.0 ± 0.05 were combined 60:40% 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 (Omar *et al.,* 2020; Parasuraman *et al.,* 2010).

2.4 Pharmacokinetic analysis

2.4.1 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.

2.4.2 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.

2.4.3 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:

$$t_{1/2} = \frac{0.693}{K_{el}}$$

2.4.4 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.

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

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

Using Win Nonlin 3.3® pharmacokinetic software, the non-compartmental analysis of the pharmacokinetic parameters was carried out. All values stated as mean ± SEM (Yuan *et al.,* 2017; Sutar *et al.,* 2021; Raseshkumar *et al.,* 2021, Nitin *et al.,* 2021).

2.5 Stability studies

Optimized rasagiline mesylate microspheres formulation were 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 (Ameena Yasmeen *et al.,* 2019; Bhuvaneswari *et al.,* 2021).

3. Results

3.1 Calibration curve

The calibration curve requirement was set at a correlation coefficient (r^2) of 0.9989. Results were given in Table 1 and shown in Figure 1.

Table 1: Standard calibration curve of rasagiline mesylate

S. No.	Concentration (ng/ml)	Peak Area
1	0	0
2	1	3081
3	2	5500
4	4	10204
5	6	15389
6	8	20132
7	10	25132

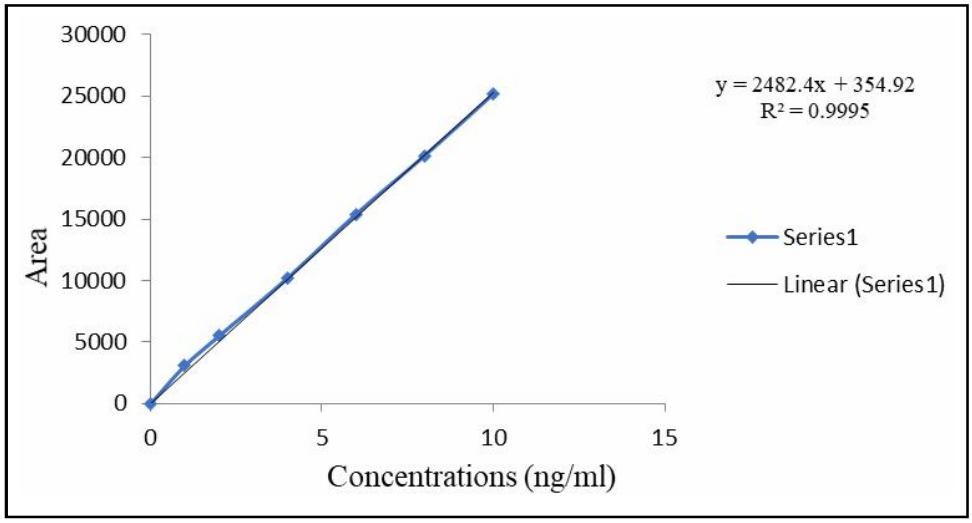

Figure 1: Standard calibration curve of rasagiline mesylate in rat plasma.

3.2 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 rasagiline mesylate and internal standard pseudoephedrine HPLC chromatograms in rat plasma. Rasagiline mesylate standard with pseudoephedrine, formulation chromatogram of rasagiline mesylate with pseudoephedrine in rat plasma given in Figure 4 and Figure 5, respectively.

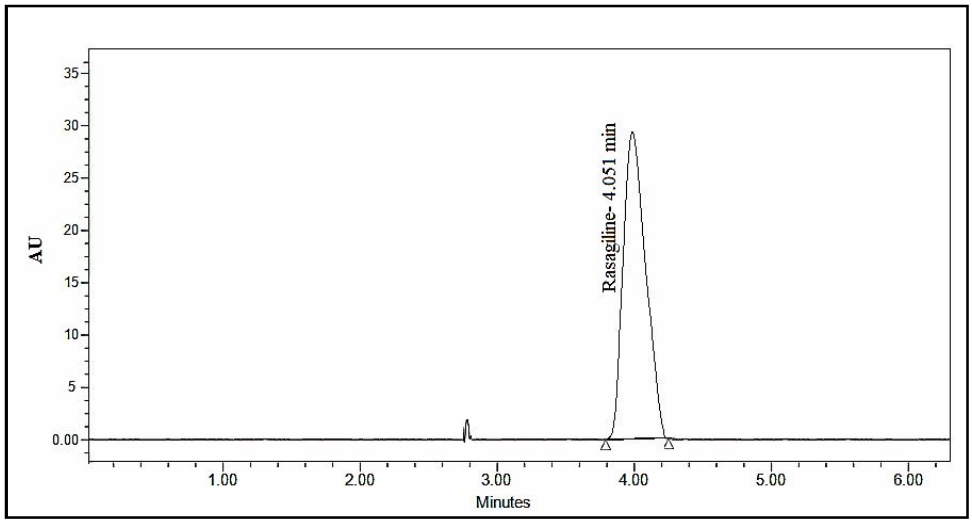

Figure 2: Standard HPLC chromatogram of rasagiline mesylate in rat plasma.

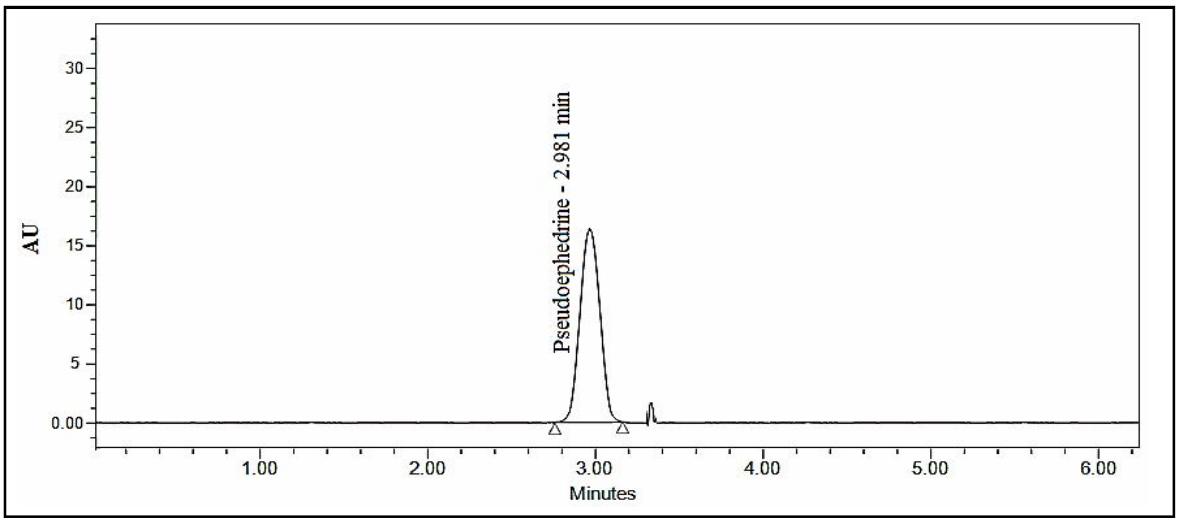

Figure 3: Standard HPLC chromatogram of internal standard pseudoephedrine in rat plasma.

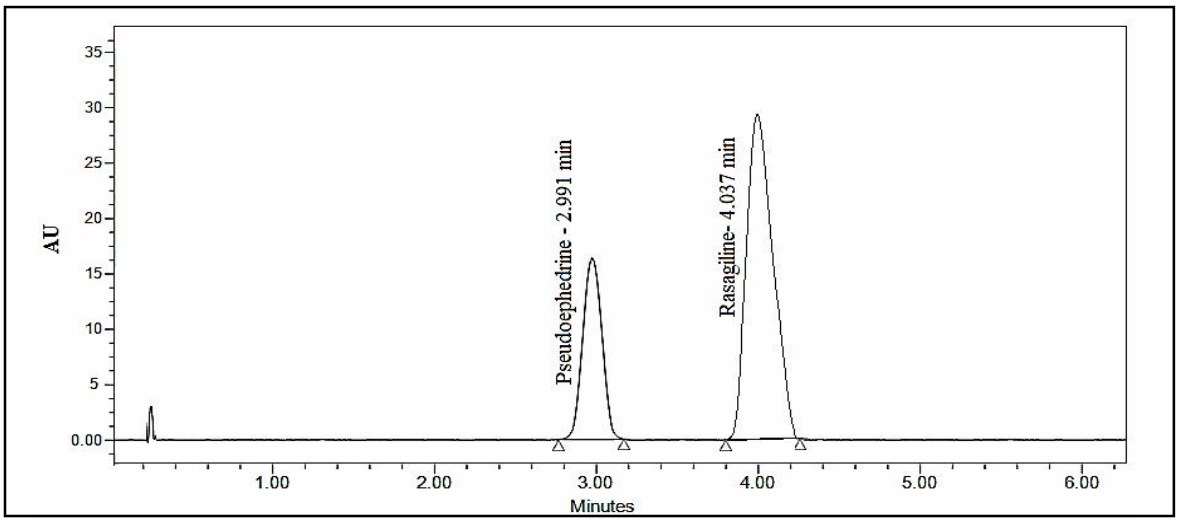

Figure 4: Standard HPLC chromatogram of rasagiline mesylate and pseudoephedrine (IS) in rat plasma.

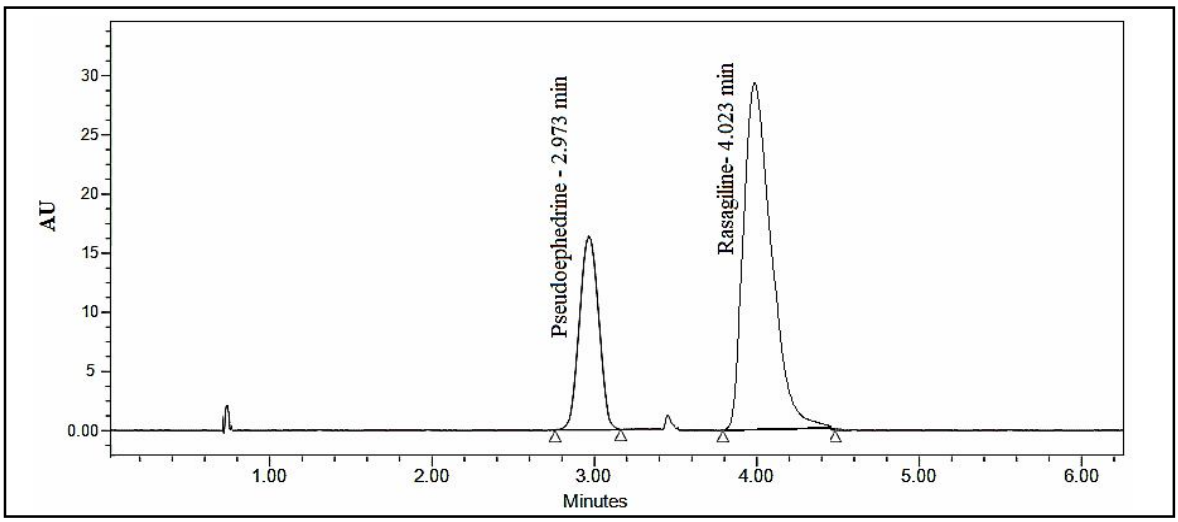

Figure 5: Formulation chromatograms of rasagiline mesylate and pseudoephedrine (IS) in rat plasma.

3.3 Pharmacokinetic data of rasagiline mesylate

Table 2 provides the plasma concentrations of rasagiline after oral administration of the pure medication and optimised rasagiline 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.

Table 2: Plasma concentration profiles of rasagiline mesylate pure drug and rasagiline optimised microspheres formulation

Time (h)	Rasagiline mesylate pure drug (ng/ml)	Rasagiline mesylate microspheres optimized formulation (ng/ml)
0	0	0
0.5	2.55 ± 0.26	1.23 ± 0.13
1	4.32 ± 0.25	1.61 ± 0.71
1.5	3.12 ± 0.23	2.89 ± 0.57
2	2.98 ± 0.26	3.54 ± 0.67
4	1.37 ± 0.22	2.11 ± 0.39
6	0.75 ± 0.09	1.65 ± 0.10
12	0.11 ± 0.03	1.15 ± 0.03
24	0	0

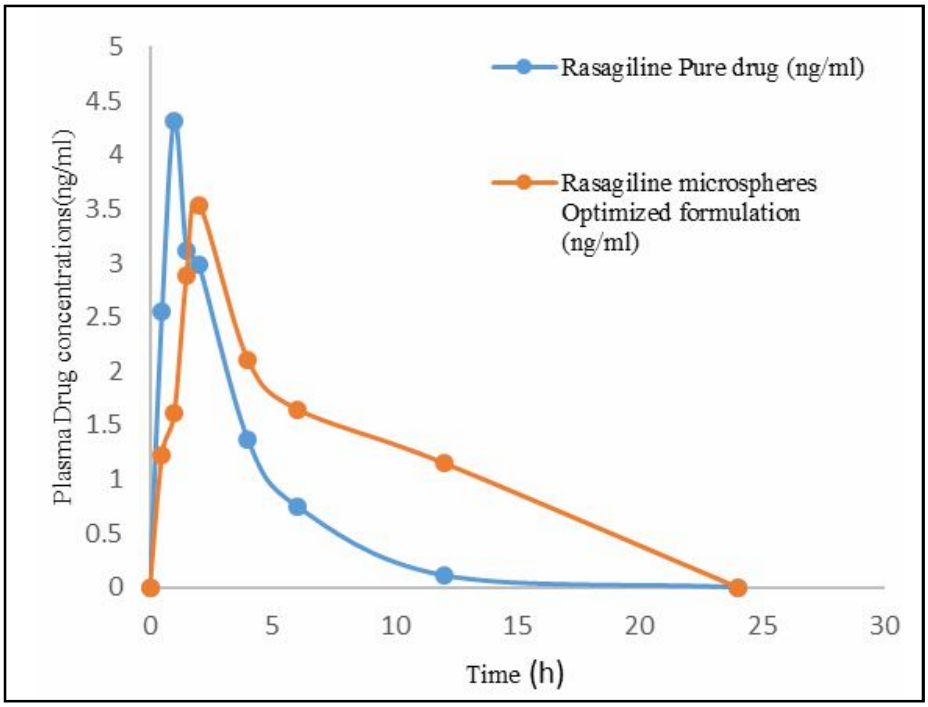

Figure 6: Mean plasma concentration-time profiles for rasagiline mesylate pure drug and rasagiline mesylate optimized microspheres formulation in rats (n=6).

Table 3: Mean pharmacokinetic parameters of rasagiline mesylate pure drug and rasagiline mesylate optimised microspheres formulation

Pharmacokinetic parameters	Rasagiline mesylate pure drug	Rasagiline mesylate optimised microspheres
C_{max} (ng/ml)	4.32 ± 0.25	3.54 ± 0.67
AUC_{0-t} (ng. h/ml)	22.63 ± 2.65	34.22 ± 2.56
AUC_{0-inf} (ng. h/ml)	32.1 ± 1.58	43.8 ± 2.48
T_{max} (h)	1.0 ± 0.05	2.0 ± 0.06
$t_{1/2}$ (h)	3.2 ± 0.83	6.32 ± 0.53

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

C_{max} of the microspheres and pure drug was found to be 3.54 ± 0.67 ng/ml and 4.32 ± 0.25 ng/ml. T_{max} of both microspheres and drug was 2.0 ± 0.06 h and 1.0 ± 0.05 h.

3.4 Stability study

Stability studies data given in Table 4.

Table 4: Stability studies for optimized formulation

Time in months	0	3	6
Condition	30 ± 2°C / 65 ± 5% RH		
Particle size (µm)	472.35 ± 1.24	471.85 ± 2.17	471.59 ± 1.38
Drug entrapment efficiency (%)	89.42 ± 1.55	89.26 ± 0.72	89.18 ± 1.05
In vitro drug release (%)	97.14 ± 1.79	97.07 ± 1.42	97.02 ± 1.35
Condition	40 ± 2°C / 75 ± 5% RH		
Particle size (µm)	472.61 ± 2.15	471.49 ± 1.39	471.04 ± 1.71
Drug entrapment efficiency (%)	89.67 ± 1.26	89.23 ± 1.78	89.02 ± 2.36
In vitro drug release (%)	97.14 ± 1.79	97.07 ± 1.42	97.02 ± 1.35

4. 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 43.8 ± 2.48 ng.h/ml than the pure drug 32.1 ± 1.58 ng.h/ml. AUC_{0-t} of the microsphere's formulation, 34.22 ± 2.56 ng.h/ml was significantly higher (p<0.05) as compared to pure drug, 22.63 ± 2.65 ng.h/ml. 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* per cent drug release. Formulation was stable after performing stability studies.

5. 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 rasagiline mesylate to improved permeability. The rasagiline mesylate pure drug and mucoadhessive microspheres pharmacokinetic results revealed notable differences in the pharmacokinetic parameters, suggesting that the formulation modification of rasagiline mesylate 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 rasagiline mesylate 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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