

Research Article



HPLC ESTIMATION OF RASAGILINE MESYLATE DURING BUCCAL PERMEATION STUDIES

Rama Bukka^{1*} and Kalyani Prakasam²¹ Nargund College of Pharmacy, II Main, Dattatreya Nagar, BSK III stage, Bangalore, India.² The Oxford College of Pharmacy, Bommanahalli, Hosur Road, Bangalore, India.*Corresponding author's E-mail: ramabukka@gmail.com

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ABSTRACT

A simple, isocratic and sensitive high performance liquid chromatographic method was developed and validated for the estimation of Rasagiline Mesylate during buccal permeation studies across porcine buccal mucosa. A reversed- phase (C_{18}) column was used with UV detection at 210 nm. Selected mobile phase contained 10% (v/v) of Isopropanol and 90% (v/v) of triple distilled water containing 0.2% Tri-ethanolamine, pH adjusted to 2.5 using 1% ortho-phosphoric acid. Calibration curve showed good linearity over the concentration range of 1-100 μ g /mL in buccal permeates of distilled water (Blank permeates). The method was validated for linearity, sensitivity, selectivity, accuracy and precision. The applicability of the method was demonstrated by the analysis of buccal permeated samples of Rasagiline Mesylate through porcine buccal mucosa. The steady state permeability Flux (J) and permeability coefficient (k_p) of Rasagiline through buccal mucosa from aqueous solution was found to be 10.63 ± 2.86 (μ g/cm 2 .h $^{-1}$) and 0.010633 ± 0.0025 cm/h respectively.

Keywords: RP-HPLC, Flux, Validation, permeation, Rasagiline Mesylate.

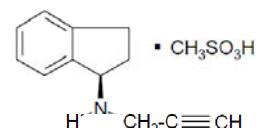
INTRODUCTION

The buccal region of the oral cavity is an attractive target for administration of the drug of choice. Buccal delivery involves the administration of the desired drug through the buccal mucosal membrane lining of the oral cavity. Unlike oral drug delivery, which presents hostile environment for drug, due to acid hydrolysis and hepatic first pass effect, the mucosal lining of the buccal tissue provides a much milder environment for drug absorption.

The mucosal lining of the oral cavity offers some distinct advantages. It is richly vascularised and more accessible for the administration and removal of the dosage form. Additionally buccal drug delivery has a high patient acceptability compared to other non-oral routes of drug administration.¹ Permeability rates of solutes will depend on their molecular characteristics ex: size, lipophilicity and extent of ionization. There are two principal routes of penetration: Transcellular and paracellular. A compound may access both these routes, although paracellular route is principal route for hydrophilic compounds where as for lipophilic molecules transcellular route predominates.²

At present, the study is planned to develop a simple, isocratic and sensitive HPLC method for the analysis of Rasagiline Mesylate during buccal permeation studies. Rasagiline mesylate is an irreversible inhibitor of monoamine oxidase (MAO) used for the treatment of the signs and symptoms of idiopathic Parkinson's disease as initial mono therapy and as adjunct therapy to levodopa.³ It is selective for MAO type B over type A by a factor of fourteen. It undergoes extensive hepatic biotransformation.⁴ The absolute bioavailability of Rasagiline is about 36%. Steady-state half-life is 3 hours.³

Literature survey reveals few analytical methods for the estimation of the drug using high performance liquid chromatography⁵⁻⁶ Stability indicating RP-LC method⁷ and a validated LC-MS/MS method for the determination of Rasagiline in plasma is also reported.⁸

**Figure 1:** Chemical structure of Rasagiline Mesylate**MATERIALS AND METHODS**

Rasagiline Mesylate was kindly donated by Apotex Research Pvt Ltd. HPLC grade solvents were purchased from Ranbaxy fine chemicals Ltd. Porcine buccal mucosa obtained from local slaughter house.

Chromatographic conditions

The apparatus used for the HPLC analysis was Shimadzu LC equipped with LC 20 AT prominence liquid chromatograph pump, and a Shimadzu SPD-20A UV prominence UV/Vis detector. An Inertsil ODS-3V (4.6 mm \times 250mm; 5 μ m particle size) column was employed during the analysis. An isocratic method was used with a mobile phase containing a mixture of Iso-propanol and triple distilled water (TD) containing 0.2% Triethanolamine (10:90, v/v). The pH of the triple distilled water containing 0.2% Triethanolamine (TEA) was adjusted to pH 2.5 with 1% ortho-phosphoric acid solution followed by the addition of Iso-propanol. Flow rate of the mobile phase was 1.0 mL per minute. The mobile phase was vacuum-filtered through 0.22 μ m Millipore membrane and degassed by ultra-sonication.



The injection volume was 20 μ L. After equilibration with the solvent to obtain a stable baseline, aliquots of samples were injected.

The total run time was 25 minutes. The absorbance of the eluent was monitored at 210nm with a detection sensitivity of 0.250 aufs. All the analyses were performed at room temperature.

Preparation of calibration standards

Standard stock solution of Rasagiline Mesylate (1560 μ g/mL) was prepared in triple distilled water such that each mL will have Rasagiline base equivalent to 1000 μ g. The solution was stored under refrigeration (4.0 \pm 0.5 °C). A secondary stock solution (100 μ g/mL) was prepared by dilution of the primary stock solution with triple distilled water. Aliquots of the secondary stock were further diluted with blank permeates to obtain eight calibration standards (1, 5, 10, 20, 30, 40, 50 and 100 μ g/mL). The tissue permeates of the TD water (blank permeates) was prepared by placing the porcine buccal tissue in Franz diffusion cell and TD water in the donor (3 ml) as well as receptor compartment (35 ml) of the diffusion cells. The entire set up was placed over magnetic stirrer and temperature was maintained at 37°C by placing the diffusion cell in a water bath for 10 h at 37°C. The permeates collected after 10 hours used as diluent for the preparation of calibration standards. The prepared calibration standards were analysed after filtering through 0.22 μ m Millipore membrane.

As some of the tissue components will also diffuse into the receptor compartment during the study and which are found to be eluted in a time range of 1–4 minutes, the mobile phase was selected to give a retention time of greater than 4 minutes for the drug.

The mobile phase and calibration standards were freshly prepared on the day of usage.

Method validation

Method validation was carried out under the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures.⁹ The method was validated with respect to linearity, precision, accuracy, selectivity, sensitivity of the calibration standard solutions.

Linearity

The linearity of the Calibration curves was obtained from eight different drug concentrations (1, 5, 10, 20, 30, 40, 50 and 100 μ g /mL) of Rasagiline Mesylate. The solutions were made in triplicate and each solution was injected three times. The curves were generated by plotting the peak area against Rasagiline Mesylate concentration. Linearity was evaluated by Microsoft Excel.

The precision of the method was determined by instrumental precision, repeatability (intra-day) and intermediate precision (inter-day) and was expressed as percentage relative standard deviation.

Instrumental precision was determined by performing six replicate injections of a sample of 10 μ g /mL of Rasagiline Mesylate diluted with blank permeates.

Repeatability was evaluated by analysis of three concentration levels (5, 10 and 20 μ g /mL Rasagiline Mesylate) on the same day. Each solution were made in triplicate and injected three times the same day under the same experimental conditions.

The intermediate precision (inter-day precision) was determined by analyzing three independent samples in triplicate during three different days.

Accuracy/ Recovery from buccal samples

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. In this study, accuracy was determined based on the recovery (percentage) of known amounts of Rasagiline Mesylate. This was performed by analyzing Rasagiline Mesylate at three concentration levels (5, 10 and 20 μ g /mL). Samples were prepared in triplicate. The accuracy was determined by comparing the found concentration with the added concentration.

Selectivity

Method selectivity was evaluated through possible interference from the tissue components. For this, the blank permeates was prepared placing the triple distilled water with buccal tissues in Franz diffusion cells for 10 h at 37°C and then analyzed by the HPLC procedure with a run time of 25 minutes.

Sensitivity

Sensitivity of the method was determined by means of the detection limit (LOD) and quantification limit (LOQ). The LOD and LOQ were measured based on the method described by the International Conference on Harmonization.⁹ Calculations for LOD were based on the standard deviation of the intercept of the calibration curve (σ) and the slope of curve (S), using the equation $LOD = 3.3 \sigma / S$. LOQ was calculated by the equation $LOQ = 10 \sigma / S$.

Ex vivo permeation study of Rasagiline Mesylate through Porcine Buccal mucosa:

Tissue preparation

Porcine buccal tissue was obtained from a local slaughter house immediately after the pigs were slaughtered. Buccal tissue of freshly slaughtered pigs was immediately placed in cold kerb's buffer and transferred to our laboratory.

The buccal mucosa was carefully separated from fat and muscles using micro-dissecting forceps and scissors. The permeation studies were initiated within 2 h of isolating buccal epithelium.



Permeation study

The drug permeation from the solution was studied using the Franz diffusion cell. The buccal epithelium was carefully mounted in between the two compartments of a Franz diffusion cell with internal diameter of 2.1 cm (3.46 cm² area) with a receptor compartment volume of 35 ml. Distilled water was placed in the receptor compartment. The buccal epithelium was allowed to equilibrate with the receptor for one hour to enhance the permeation. 3 mg of Rasagiline in 3 ml of distilled water was taken in the donor compartment. The entire set up was placed over magnetic stirrer and temperature was maintained at 37°C by placing the diffusion cell in a water bath. 0.5 ml sample was collected at predetermined time intervals from receptor compartment and replaced with an equal volume of the distilled water. The samples were analyzed by above mentioned method after filtering through 0.22 µm Millipore membrane.

The Rasagiline Mesylate concentration in the buccal mucosa permeates was corrected for sampling effects according to following equation.¹³

$$C^1n = Cn (VT/VT-VS) (C^1n-1/Cn-1)$$

Where C^1n is the corrected concentration of the n^{th} sample, Cn is the measured concentration of Rasagiline Mesylate in the n^{th} sample, C^1n-1 is the corrected concentration of Rasagiline Mesylate in the $(n-1)^{\text{th}}$ sample, $Cn-1$ is the measured concentration of Rasagiline Mesylate in the $(n-1)^{\text{th}}$ sample, VT is the total volume of the receptor fluid, VS is the volume of the sample drawn.

The flux ($\mu\text{g}/\text{cm}^2 \cdot \text{h}$) of Rasagiline Mesylate was calculated from the slope of the plot of the cumulative amount of Rasagiline Mesylate permeated per cm^2 of buccal epithelium at steady state against the time using linear regression analysis. The steady state permeability coefficient (kp) of the drug through porcine, buccal mucosa was calculated by using the following equation $kp = J/C$, where ' J ' is the drug flux and ' C ' is the initial concentration of Rasagiline Mesylate in donor cell.¹³

The results of pure drug permeation studies were expressed as mean \pm S.D. (n=5)

RESULTS AND DISCUSSION

Chromatography

Under the chromatographic conditions used, Rasagiline had retention time of 7.05 minutes. Representative chromatograms of blank buccal permeate and a permeation of drug is shown in Fig. 2. The method was selective for Rasagiline mesylate as there was no interference from the endogenous compounds of the buccal mucosa. The peak Asymmetry factor observed in this study was (T_f) 1.5, which was less than the permissible limits (recommended $T_f \leq 2.0$). The Asymmetry factor observed to be closer to 1, if the sample of Rasagiline mesylate was diluted in mobile phase, indicating that polarity of the calibration samples was more than the mobile phase as the samples of the

diffusion study was injected as such in TD water without further dilution/modification.

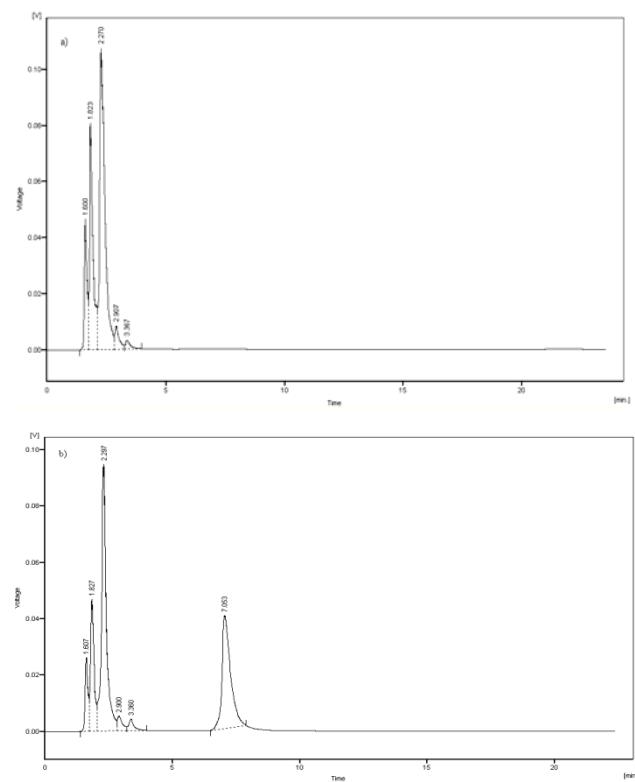


Figure 2: Chromatograms of (a) blank buccal permeate and (b) permeation sample withdrawn during diffusion study

Method validation

Linearity and sensitivity

Excellent linearity was obtained in the concentration range of 1-100 µg /mL. The correlation coefficients were greater than 0.999 for all the point standard curves. The equation for the calibration lines were as follows: $y=83.8x-62.93$, where x is the drug concentration ($\mu\text{g} / \text{mL}$) and y is the peak area. The LOQ and LOD were 0.51 and 0.170 µg /mL respectively. The results indicate that the proposed method is sensitive to detect and quantify 0.17 µg mL⁻¹ and 0.5 µg mL⁻¹ respectively.

Accuracy and precision

Percentage Recovery of Known amounts of Rasagiline Mesylate from blank permeate was performed by analyzing at three concentration levels (5, 10 and 20 µg /mL). The results of accuracy are summarized in table 1. The mean percentage recovery of Rasagiline was found to be $101.6\% \pm 1.3$ to 99.6 ± 0.7 indicating complete recovery of Rasagiline. The calibration standards (5, 10 and 20 µg /mL) were analyzed on three consecutive days to assess precision (intra-and inter-day; %R.S.D.). The precision values are less than 2% RSD. The results of precision are summarized in table 2. Instrumental precision was determined by performing six replicate injections of a sample of 10.0 µg /mL of Rasagiline Mesylate diluted with blank permeates and mean of 10.05 µg/mL and % RSD



found to be 0.85. Therefore, the values obtained in this study are within permissible limits.

Table 1: Percentage Recovery of Rasagiline from buccal sample (accuracy)

Drug concentration		Mean % recovered ^a	% RSD
added $\mu\text{g/mL}$	Found $\mu\text{g/mL}$		
5	5.08	101.6	1.30
10	9.96	99.6	0.9
20.	19.96	99.8	0.7

^a mean of 3 determinations, RSD = Relative standard deviation

Table 2: Precision of the proposed method

Concentration of Rasagiline $\mu\text{g/mL}$	Measured concentration of Rasagiline			
	Intra-day		Inter-day	
	Mean ^a	% RSD	Mean ^a	% RSD
5	5.05	1.1	5.48	1.90
10	10.05	0.96	10.27	1.82
20	20.1	0.88	20.08	1.31

^a mean of 3 determinations, RSD = Relative standard deviation

Application of the chromatographic method

The applicability of the above method was demonstrated by permeation study of Rasagiline Mesylate from aqueous solution across the buccal mucosa.

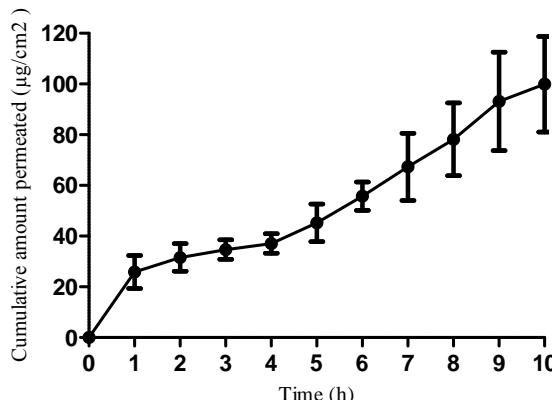


Figure 3: Buccal permeation of Rasagiline through porcine buccal mucosa

Rasagiline was used as a mesylate, having a $\log P$ of 2.2¹⁰. Manuel A. Navia and Pravin R. Chaturvedi discussed the role of partition coefficient ($\log P$) in the prediction of absorptive potential of drugs. It can be argued that $\log P$ values between 0 and 3 constitute an optimal window for drug absorption. Values that are too high (>6) or too low (<-3) can be associated with poor transport characteristics. The low absorption observed for compounds with a high $\log P$ value can be attributed to the poor aqueous solubility of these compounds and their preferential residence in lipophilic regions. In turn, the suboptimal lipophilicity of the very polar compounds precludes their ability to penetrate membrane barriers. Although a higher $\log P$ value tends to favor absorption across the gastro intestinal tract wall, it also tends to render compounds more susceptible to metabolism and/or biliary clearance.¹¹ However, it should be noted that in addition to permeant lipophilicity, the Permeability of ionizable molecules depends on the pH

conditions and drug pKa . The pKa of the drug molecule is found to be 7.16 at 37°C.¹²

Flux of Rasagiline through buccal mucosa is found to be $10.63 \pm 2.86 (\mu\text{g}/\text{cm}^2 \cdot \text{h}^{-1})$ and steady state permeability coefficient (k_p) is found to be $0.010633 \pm 0.0025 \text{cm}/\text{h}$. Fig 3 gives cumulative amount of the drug permeated per square cm of buccal epithelium against time in hour from solution through porcine buccal mucosa (graph pad prism version 5.03). The HPLC method developed and validated in this study can be implemented in the investigation of buccal permeation enhancement mechanisms of existing and novel enhancers.

CONCLUSION

A simple, isocratic and sensitive HPLC method was developed and validated for the estimation of Rasagiline during buccal permeation studies.

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