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RASAGILINE MESYLATE, A BCS CLASS III DRUG; *EX-VIVO* PERMEATION ENHANCEMENT STUDY THROUGH EXCISED RAT ABDOMINAL SKIN

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ABSTRACT: Rasagiline Mesylate (RM), an antiparkinson drug, belongs to BCS class III drug having high solubility with low permeability. It undergoes extensive first-pass metabolism with oral bioavailability of only 35%. In the present research work, efforts were made to improve its permeation as requisite for the transdermal drug delivery system. RM was authenticated by determination of melting point. Further confirmed by ATR-FTIR, DSC and XRD. The solubility and pH dependant solubility profile was established. The purity of the received drug and estimation of the drug during the study was performed by an in-house developed novel RP-HPLC method. To determine the drug flux, permeation experiments were performed in modified Franz diffusion cells using dialysis membrane and excised rat abdominal skin as a barrier and the flux found to be 45.4 and 29.9 µg/cm^2 / h, respectively. To increase flux and permeation, permeation enhancers with different mechanisms like Isopropyl myristate (IPM), Hyaluronidase, Ethanol, Oleic acid, Dimethyl sulfoxide (DMSO), Polyethylene glycol (PEG 400) and Propylene glycol (PG) were employed in the concentration of 5%. The flux, permeability coefficient, and enhancement ratio of solutions were determined. IPM improved the drug permeation among all the permeation enhancers by 3.3 folds higher than the pure drug alone. The order of enhancing the permeation by all the permeation enhancers used was in the order PG <PEG 400<DMSO<Oleic acid <Ethanol<Hyaluronidase<IPM. This research study concluded that the IPM was selected from all the permeation enhancers used for the transdermal drug delivery system of RM.

INTRODUCTION: Rasagiline mesylate (RM), a selective irreversible dose-dependent monoamine oxidase B (MAO-B) inhibitor that also exhibits neuroprotective effects, is prescribed for Parkinson's disease (PD), the most prevalent neurodegenerative disease; characterized by symptoms of tremor, rigidity and bradykinesia.

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In advanced conditions, PD patients generally have a mean intake of 9.9 tablets per day which is estimated to be approximately 3600 tablets per year. There is a need for improved treatment with a lesser frequency of dose intervals.

The drug RM has a molecular weight of 267.34 Da (>500), lesser half-life of only about 3 h with oral bioavailability of 35% and an oral dose being 0.5 to 1 mg daily [1]. These drug properties are highly suitable for formulating it as transdermal drug delivery systems (TDDS). The increasing market for transdermal products is evidence of the very high acceptability of TDDS by patients of various age groups.

The transdermal product market worldwide today is estimated to be nearly 10 billion dollars. Majority of analgesics during cancer therapy occupied by the TDDS at the present market (opioid analgesics) [2]. As TDDS has numerous advantages over conventional dosage forms such as avoiding frequent drug administration, providing safe and convenient drug delivery, surpassing first-pass metabolism and gastrointestinal tract with poor bioavailable drugs, and many others, it is desirable with high patient compliance. With the latest programmable systems, multiple dosing on-demand at variable rate drug delivery were also made possible [3]. However, to succeed with TDDS, the lower permeability of the skin need to be addressed and is a challenging aspect in the formulation development [4].

The presence of the stratum corneum, which acts as the main barrier, limits the drug delivery through skin. Being a BCS class III drug RM has high solubility but low permeability. The passive permeation of RM through the skin was not enough to reach an effective therapeutic concentration. To enhance the drug permeation through the skin, different permeation enhancers that alter skin barrier properties are employed. Several mechanisms were proposed for their increased penetration action, such as lipids disruption in stratum corneum, keratin protein modification, pore opening, improving the drug partition or solvents into the layers [5].

The safe and suitable penetration enhancers to promote percutaneous absorption of numerous drugs, their classification and mechanisms of action were discussed in detail by Sinha V R *et al.*, to help in the suitable penetration enhancer selection for improved permeation of poorly absorbed drugs and low permeable drugs [6]. In a review by Yang C *et. al.*, they presented an overview of the investigations in this field [7]. Hui *et al.*, studied the influence of varied contents of azone and tween 80 on the transdermal permeation of levimazone [8]. In another study, Jantharappa *et. al.*, demonstrated the effects of different combinations of co-solvents and permeation enhancers on meloxicam permeation [9]. Ahmed *et al.* in their study, discussed the feasibility of formulating fenoterol in TDDS by utilizing various penetration enhancers to achieve increased drug diffusion [10]. Rabinarayan used a combination of acrylic adhesives and penetration enhancers like isopropyl myristate (IPM), cineol and limonene for successful transdermal delivery of simvastatin across pork ear skin [11]. In this study, various permeation enhancers were evaluated to investigate their suitability to include in TDDS for Rasagiline Mesylate (RM).

MATERIALS AND METHODS:

Materials: Rasagiline mesylate (RM) reference standard and bulk were gift samples from Apotex Pvt. Ltd, Bangalore, India. Dialysis membrane (Sigma Aldrich 12 kDa), Isopropyl myristate (IPM), Ethanol, Oleic acid, Dimethyl sulfoxide (DMSO), Polyethylene glycol (PEG 400) and Propylene glycol (PG), Hyaluronidase (1500IU) was purchased locally and all other chemicals and ingredients used were of pharmaceutical and analytical grade.

Preformulation Studies:

Melting Point: The melting point was determined by taking approximately 5 mg of the sample in a capillary glass tube sealed at one end. The sample containing capillary was placed in melting point apparatus (Lab India, Visual Melting Range Apparatus, MR-VIS') and the temperature was increased gradually. At the temperature in which sample starts melting, *i.e.*, melting point of sample in degree Celsius was given as auto-generated report by the instrument in auto mode [12-17].

pH-Dependent Solubility Profile: The drug RM was passed through #200 to minimize the time required to attain equilibrium. Then, an accurately weighed excess quantity of the drug was placed in a vial along with the different solvents (2 ml). The tightly closed vial was agitated at room temperature for 24 h; the mixture was centrifuged and filtered. The drug concentration in the supernatant was determined (n=3) by developed and validated RP-HPLC method [12]. The observations are presented in **Table 1**.

Partition Coefficient: The partition coefficient between 1-octanol and water at room temperature was determined by following method. An equal volume of (10 ml) of 1-octanol and water were added to a separating funnel to which added 10 mg of accurately weighed RM. The mixture was shaken for 24 h. The solute (drug) concentration in

the aqueous and 1-octanol phase was determined by a newly developed and validated in-house RP-HPLC method.

The partition coefficient between 1-Octanol: Phosphate Buffer Saline (PBS) pH 7.4 was also determined by the same procedure.

Fourier Transform Infra-Red Spectroscopy: ATR-FTIR studies were carried out in Shimadzu IR Affinity-1S instrument. The samples were placed on the germanium prism and ATR-FTIR spectra were recorded in the scanning measurement range of 600 to 4000 cm^{-1}.

Differential Scanning Calorimetry: DSC studies were carried out in Perkin Elmer DSC-8000 (Germany) instrument. About 1-3 mg of samples were placed in aluminium pans and the pans were closed by crimping the lids. The filled aluminium pans and empty sealed pan were placed in a sample holder where the empty pan acts as a reference. The thermal behaviour of samples was investigated under nitrogen purging at a scanning rate of 10 °C/min, covering the temperature range of 30-350 °C. Heat runs for each sample, using nitrogen as purging gas and the samples were analyzed.

X-Ray Diffraction Study: The sample for the XRD studies was mounted on the sample cell and scanned between 2 θ of 0-60° with a counting time of 0.1 s step size. The X-ray patterns were obtained with XPERT-PRO equipment (PAN analytical, The Netherland) having an XRD commander programme. Measurement conditions consisted of a target Cu-Ka radiation anode, voltage 30 kV, and current 15 mA. Diffraction patterns were obtained using a step size of 0.03° 2 θ between 2° and 60° 2 θ at a rate of 2° /min at ambient temperature.

Skin Partition: A piece of whole excised rat skin was weighed accurately and was placed in a stoppered test tube containing 10 ml of PBS pH 7.4. Added to it accurately weighed quantity of the drug RM (10 mg). The mixture was equilibrated for 36 h. The solution mixture was centrifuged, filtered and analyzed for drug concentration by developed and validated RP-HPLC method [12]. The amount of drug partitioned in the skin (n) was calculated using the equation (n)=(Cb-Ca), where Ca and Cb are the drug concentration in the solution after and before equilibrium, respectively. The solubility of the drug in whole skin (Cs) was obtained by Cs=n/(w/o), where w and o are the weight and density of the whole skin, respectively.

Novel RP-HPLC Method for RM Estimation: The drug estimations were performed on Shimadzu Prominence Liquid Chromatography (Shimadzu, Japan) equipped with LC solution software using a Phenomenex 100 C18 (250 × 4.6 mm), 5 µm column at room temperature using a flow rate of 0.8 ml/min with run time 6.0 min and UV detection wavelength at 268 nm. The injection volume was set as 50 µl.

Acetonitrile and water in the ratio of 50:50% v/v, adjusted to pH to 3.0±0.05 using orthophosphoric acid, was used as a mobile phase which was filtered (0.2 µm finer porosity nylon membrane filter) and degassed by sonication. The mobile phase is used as the diluent.

A standard stock solution was prepared by dissolving RM standard equivalent to 25 mg in 25 ml of diluent. The above stock solution was diluted to get a final standard concentration of 100 µg/ml. 50 µl of standard and sample solutions were injected to the system and chromatographs were recorded. The amount of RM was calculated.

***In-vitro* Permeation Study of Pure Drug Solution Through Dialysis Membrane (Sigma Aldrich 12kDa):** The *in-vitro* drug permeability was determined using a modified Franz-diffusion cell. This cell consisted of donor and receptor compartments. A dialysis membrane (Sigma Aldrich 12 kDa) (DM) was placed in between the two compartments to act as a barrier. A 10 ml of aqueous drug solution (10 mg/ml) was placed in donor compartment. The receptor compartment was double jacketed of 18 ml capacity and is fitted with sampling port, was filled with phosphate buffer saline pH 7.4.

The content was stirred continuously at 100 rpm on a magnetic stirrer. The temperature of the receptor compartment was maintained at 37 ± 0.05 °C by continuously circulating hot water of 45 °C into the jacket of the receptor compartment. Two ml aliquots were withdrawn and replaced by the same volume of PBS pH 7.4 periodically for 8 h. The drug content in each aliquot was determined spectrophotometrically at 265 nm using UV-1800

Shimadzu spectrophotometer. The cumulative percentage of drug permeated was calculated and reported [10, 11].

***Ex-vivo* Permeation through Excised Rat Abdominal Skin of Drug Alone and Along With Permeation Enhancers:** *Ex-vivo* permeation experiments for permeation enhancers evaluation of RM in albino rats were approved by the IAEC of Government College of Pharmacy, Bangalore with certificate No. DCD/GCP/20/E.C/ADM/2017-2018 Dated 03.06. 2017. The animals used for *in-vivo* experiments were adult albino rats (4-6 weeks old) of either sex, weighing 180-220 g. Freshly excised rat abdominal skin sample was prepared as follows; Rat abdominal skin was carefully clipped and shaved to remove the hair with electrical clipper, full-thickness skin was then excised from sacrificed rats, cleaned with normal saline. The subcutaneous fat was carefully removed using surgical scissors, and the skin was sectioned to pieces of the required size [10-15].

The prepared rat abdominal skin was placed between donor and receptor compartments of the modified Franz diffusion cell. The *ex-vivo* drug permeation of pure drug solution was determined by placing 10 ml aqueous solution (10 mg/ ml) of RM into the donor compartment. The screening of permeation enhancers like Isopropyl myristate (IPM), Hyaluronidase, Ethanol, Oleic acid, DMSO, PEG 400 and PG belonging to esters, enzymes, alcohols, fatty acids, nonproteic sulfoxides and glycols categories respectively, was performed by adding 5% w/v of each permeation enhancer to the drug solutions containing 1% w/v of drug (RM). Two ml aliquots were withdrawn from the receptor compartment through sampling port periodically for 8 h.

The drug content of each aliquot was determined spectrophotometrically at 265 nm using UV-1800 Shimadzu spectrophotometer. The drug content of aliquots of samples from IPM as a permeation enhancer was determined by in-house developed novel RP-HPLC method since IPM showed the highest permeation enhancing activity among all the permeation enhancers used. The cumulative percentage of drug diffused was calculated and reported in **Table 2**. The accumulative amounts of RM penetrated through excised rat abdominal skin, Q ($\mu g/cm^2$), were plotted versus time. The steady-state flux, Jss ($\mu g/cm^2/h$), was determined from the slope of straight line of the plot. All data were presented as mean ± standard deviation (n=3).

RESULTS AND DISCUSSION:

Preformulation Studies: The received drug RM was authenticated by determining melting point which was found to be 156.8 ºC and DSC analysis with a very sharp peak at 157.65 **Fig. 1** were concurrent with the available literature value. The purity of received RM was determined by in-house developed novel RPHPLC method and assay value was found to be 99.02% (within the limits of 90 to 110%). The purity of compound further confirmed by obtaining sharp endothermic peak in DSC thermograph. Further ensured by ATR-FTIR spectral analysis and XRD studies. The principle peaks obtained by ATR-FTIR as illustrated in **Fig. 2** (3364 cm^{-1}, Secondary Amine group (3300-3400 cm^{-1}) 2992 cm^{-1}, Aromatic CH (2850-3000 cm^{-1}) 2764 cm^{-1}, Aliphatic CH (2700-2800 cm^{-1}) 1201 cm^{-1}, Aliphatic CN (1000-1350 cm^{-1})) and these values were concurrent with available literature. 2θ values of 9.08, 13.56, 13.69, 16.37, 18.23, 22.86, 27.49 and 36.92° obtained for RM by XRD analysis suggested that RM was crystalline in nature **Fig. 3**.

In-house novel RP-HPLC method with a retention time of 2.53 min makes the developed method economical. It requires lesser time for analysis, lesser volume of the mobile phase, and a high turnout of samples **Fig. 4**. The results of pH-dependent solubility profile study suggested that RM was highly soluble in various solvents and different pH as it belongs to BCS class III drug **Table 1**. Partition coefficient values were found for both octanol: water (2.57) and octanol: PBS pH7.4 (2.02). The results of partition study attributed that 0.086 mg/ml of drug retained in within the skin.

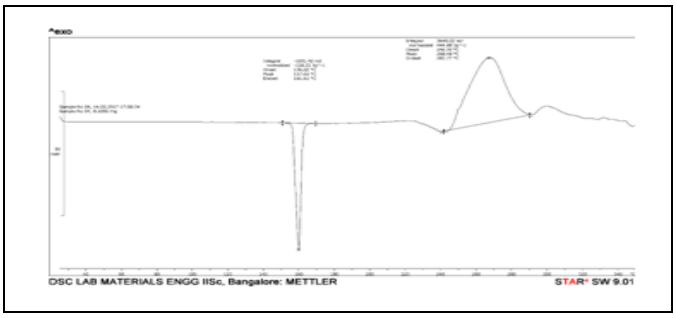


FIG. 1: DSC THERMOGRAM FOR RM

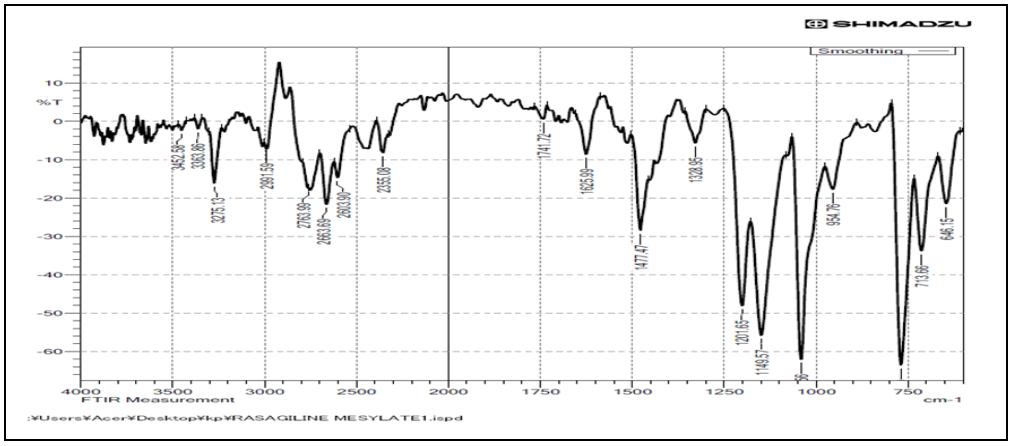


FIG. 2: FTIR SPECTRUM FOR RM

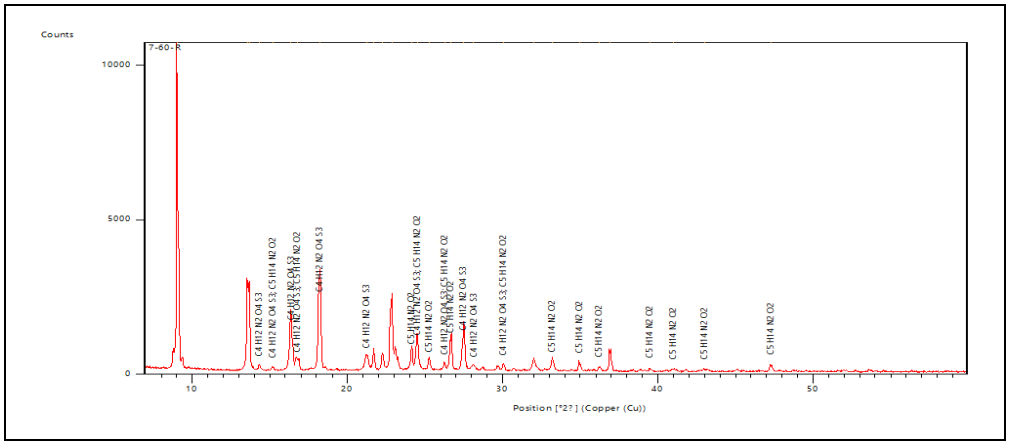
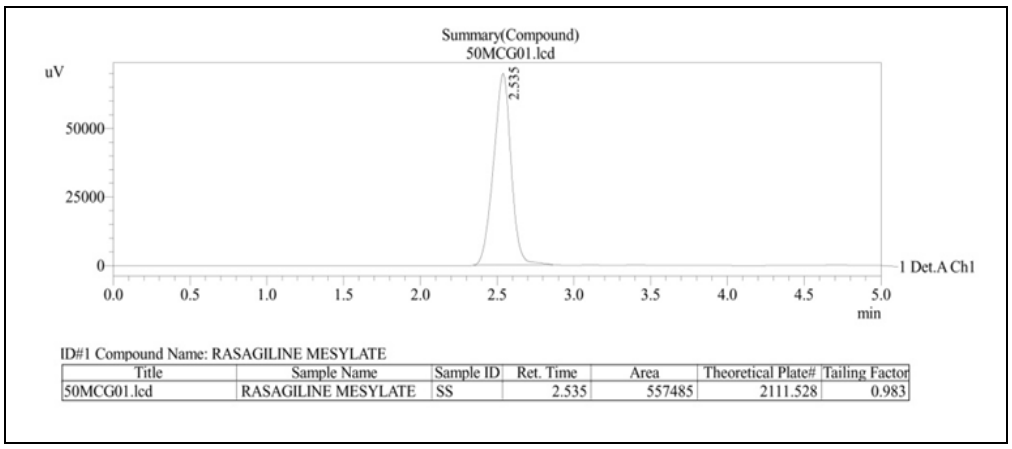


FIG. 3: XRD PATTERN FOR RM



Title	Sample Name	Sample ID	Ret. Time	Area	Theoretical Plate#	Tailing Factor
50MCG01.lcd	RASAGILINE MESYLATE	SS	2.535	557485	2111.528	0.983

FIG. 4: A TYPICAL HPLC CHROMATOGRAPH FOR RM

TABLE 1: SOLUBILITY OF RM IN DIFFERENT SOLVENTS

S. no.	Solvents	Solubility (mg/ml)
1	Water	55.96±0.28
2	pH 7.4 PBS	63.32±0.19
3	pH 6.8	95.82±0.32
4	pH 4.5	122.13±0.29
5	pH 1.2	131.52±0.24
6	Octanol	0.07±0.01
7	Methanol	319.89±0.42
8	Ethanol	59.66±0.37
9	Acetone	3.70±0.12
10	IPA	23.35±0.54

***In-vitro* Permeation Study:** In the *in-vitro* experiments using dialysis membrane, the flux was found to be 45.4 µg/cm^2/h.

The cumulative drug permeation and the flux for *in-vitro* permeation study suggested that the barriers used in the study dialysis membrane (Sigma Aldrich 12 kda) have less resistance for the permeation of drug compared to excised rat abdominal skin.

TABLE 2: PERMEATION ENHANCERS EVALUATION

Permeation Enhancer	Steady State Flux(µg/cm^2/h)	Enhancement Ratio
Pure drug (RM)	29.9±0.52	1
RM + IPM	99.1±0.38	3.3143
RM+DMSO	54.3±0.32	1.8161
RM+Ethanol	84.1±0.24	2.8127
RM+Oleic acid	66.5±0.61	2.2241
RM+Hyaluronidase	92.6±0.26	3.0970
RM+PG	31.9±0.23	1.0668
RM+PEG400	35.4±0.21	1.1839

***Ex-vivo* Permeation through Excised Rat Abdominal Skin of Drug Alone and Along with Permeation Enhancers:** The drug RM belongs to BCS class III drug, having high solubility and low permeability in spite of having good partitioning between aqueous and lipoidal solvents it has low permeability.

The flux for pure drug solution alone was found to be 29.9 µg/cm^2/h, and the permeation coefficient Kp of 2.99 × 10^{-3} suggested that RM has low permeation. To improve its permeation, various permeation enhancers with different mode of mechanisms were tried. All the permeation enhancers were enhancing the permeation of RM through excised rat abdominal skin. IPM has the highest flux and enhancement ratio (3.3143) as given in **Table 2**. This could be due to the permeation enhancing ability of the IPM, to act on subcutaneous lipid matrices by incorporating into them, thereby disrupting lipid packing arrangement and lamellar lipids bilayer perturbation and disordering. This mechanism was predominant over the permeation enhancing mechanism of other permeation enhancing agents which were used for the study. The order of permeation enhancing ability of various permeation enhancers was found to be in the order of; PG <PEG 400 < DMSO < Oleic acid < Ethanol < Hyaluronidase < IPM.

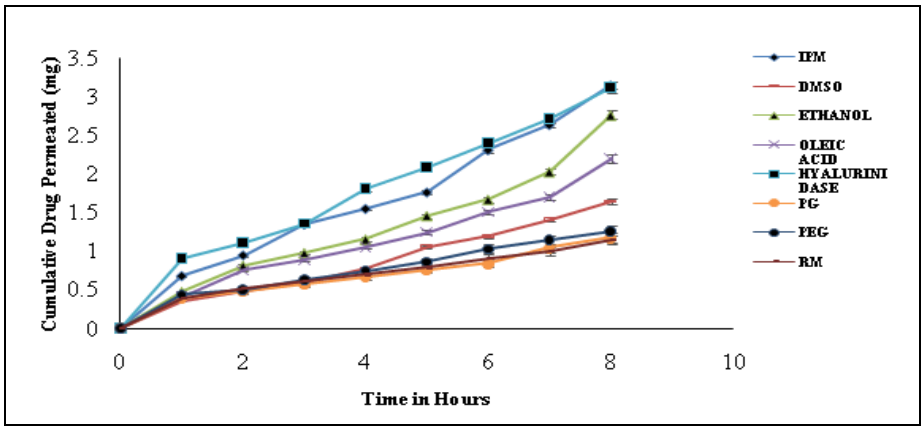


FIG. 5: SCREENING OF PERMEATION ENHANCERS

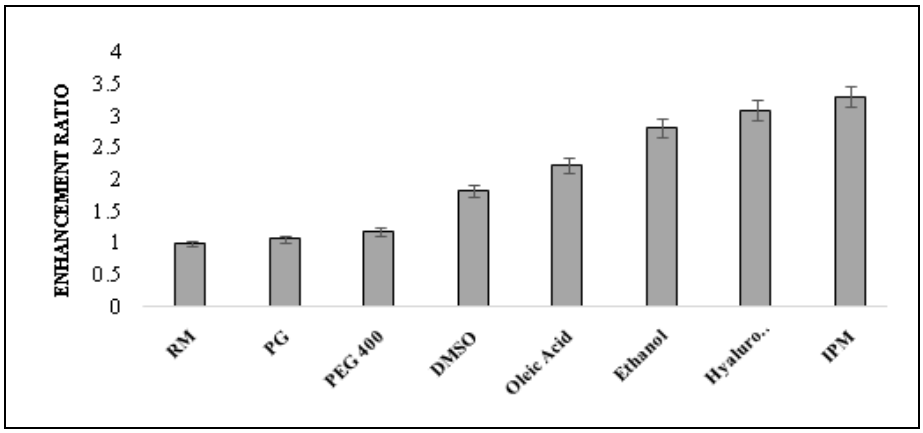


FIG. 6: ENHANCEMENT RATIOS OF DIFFERENT PERMEATION ENHANCERS

CONCLUSION: The RM, antiparkinson drug belongs to BCS class III, having low permeability and low oral bioavailability. The primary aim of the present work was to enhance its permeation by several permeation enhancers. During the study, the flux, permeation coefficient and enhancement ratios were determined for pure drug alone and along with the various permeation enhancers.

The present study concluded that IPM enhances the permeation by 3.3 folds among all the permeation enhancers and could be the ideal choice as a permeation enhancer for RM in a transdermal drug delivery system.

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CONFLICTS OF INTEREST: Authors declare no conflict of interest.

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