

## DEVELOPMENT AND EVALUATION OF TRANSDERMAL FILM CONTAINING SOLID LIPID NANOPARTICLES OF RIVASTIGMINE TARTRATE

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### ABSTRACT

**Objective:** The objective of present investigation was to develop rivastigmine tartrate transdermal film employing factorial design.

**Methods:** The formulations were designed by Design-Expert software-version10. A series of films were prepared by solvent casting method using polymers, plasticizer, permeation enhancer and other solvents. Transdermal films were evaluated for flatness, drug content, tensile strength, *in vitro* drug release and *ex vivo* skin permeation study.

**Results:** The flatness was found 100% (percentage) for all film formulations. The drug content of transdermal film was found in the range of  $96.51 \pm 0.2$  to  $98.81 \pm 0.3\%$ . The tensile strength of transdermal film was found in the range of  $6.28 \pm 0.06$  to  $11.56 \pm 0.03$  N/mm<sup>2</sup> (newton/millimeter<sup>2</sup>) and *in vitro* drug release at 24<sup>th</sup> h (hour) was found in the range of  $86.24 \pm 0.25$  to  $96.1 \pm 0.48\%$  for various formulations and *ex vivo* skin permeation study results at 24<sup>th</sup> h was found in the range of  $85.83 \pm 0.74$  to  $97.36 \pm 0.93\%$ .

**Conclusion:** These results support the feasibility of developing transdermal film of rivastigmine tartrate for human applications. Thus, transdermal delivery of rivastigmine tartrate film is a safe, painless and cost effective drug delivery system for Alzheimer's patients.

**Keywords:** Alzheimer's disease, Cholinesterase inhibitor, Film, Design-Expert software

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### INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia among older people, accounting for 50-70% of the cases [1, 2]. Dementia is a broader term than AD and refers to any acquired brain syndrome resulting in deteriorating mental functions, severe enough to impair the individual's normal daily life situation [3]. An insidious, progressive, neurodegenerative disease, the pathogenic process in AD starts probably decades before the clinical onset of symptoms.

In the past thirty years, there has been an explosion in the creation and discovery of new medicinal agents. Innovations in drug delivery have not only enabled successful implementation of these pharmaceuticals, but also promoted the developments of new medical treatments with existing drugs. The creation of a transdermal drug delivery system (TDDS) has been one of the most important innovations. Transdermal products for cardiovascular disease, Parkinson's disease, Alzheimer's disease, depression, anxiety, skin cancer, and post-menopausal bone loss are at various stages of formulation and development [4-7].

For decades, a variety of pharmaceutical dosage forms like capsules, tablets, suppositories, liquids, ointments, creams, aerosols, injections, etc. have been used as drug delivery systems to treat chronic and acute diseases. Colloidal drug delivery systems namely micelles, oil-in-water emulsions, micro particles, nanoparticles and liposomes have been employed for controlled drug delivery. Nanoparticles are solid colloidal particles in which the active ingredients are dissolved, entrapped and to which the active principle is attached or adsorbed. A nanoparticle offers so many benefits in drug delivery because of their small particle size and large surface area. Nanoparticles can be used to target the delivery of drugs, to prolong its effect, to enhance bioavailability, to solubilize it for intravascular delivery and to get better its stability against enzymatic degradation [8, 9]. Based on the type of the inactive ingredient used, there are four classes of nanoparticles: Lipid based nanoparticles [10], polymeric nanoparticles [11], metal based nanoparticles [12] and biological nanoparticles [13].

In the present study solid lipid nanoparticles of rivastigmine tartrate were incorporated into transdermal films and evaluated.

### MATERIALS AND METHODS

#### Materials

Rivastigmine Tartrate was obtained as a gift sample from jubilant life sciences Ltd, Nanjangud, Mysuru, India. Hydroxy propyl methyl cellulose was procured from loba chemie, Mumbai, India. eudragit rs 100 and eudragit l 100 was purchased from Degussa India Pvt Ltd, Mumbai, India. Glycerol was obtained from merck specialities pvt ltd, Mumbai, India. All other solvents, reagents and chemicals used were of analytical grade.

#### Experimental methods

##### Preparation of solid lipid nanoparticles (SLN)

SLN of rivastigmine tartrate was prepared by modified solvent emulsification diffusion method. The drug was dissolved in distilled water (internal phase). Required quantity of surfactant (poloxamer 188) and required quantity of lipid (stearic acid) were dissolved in 10 ml (milliliter) of distilled water and heated for 10 min (minutes) and propylene glycol was added to stearic acid solution (external phase). External phase was added to internal phase solution and 10 ml of 70 % aqueous ethanol (co-solvent) was added to above solution and the mixture was homogenized for 15 min at 2000 rpm (rotation per minute), and sonicated for 10 min. By evaporation technique the organic solvents were removed at 40 °C (degree centigrade) under normal pressure, and the nanoparticles were separated by using cooling centrifuge for 15 min at 10000 rpm. Supernatant liquid was removed and nanoparticles were washed with distilled water and freeze dried using mannitol as cryoprotectant [14].

##### Preparation of SLN loaded transdermal film

SLN loaded transdermal films were formulated by solvent casting method [15]. Polymers eudragit rs 100 (E. RS 100) and eudragit l 100 (E. L 100) were dissolved in ethanol and water, respectively to which 12 mg (milligram) rivastigmine tartrate SLN, plasticizer (glycerol 2-4%) and small amount of film forming agent (HPMC) were added. The resultant dispersion was stirred using magnetic stirrer at 1000 rpm for 45 min. The dispersion was then transferred into a petridish placed on the even surface and was allowed to dry for 96 h in desicator with

nitrogen atmosphere. Circular films of 2 cm (centimeter) diameter (3.14 cm<sup>2</sup>) were cut from dried films and placed in desiccators at

reduced pressure. The various formulations obtained by application of factorial design are presented in table 1 [16].

**Table 1: Matrix of 2<sup>3</sup> factorial designs for SLN loaded film**

Formulation code	Rivastigmine SLN (mg)	Polymer (%)		Plasticizer (%)
		(ERS 100)	(EL 100)	
K1	Equivalent to 12 mg of drug	1.5	0.5	2
K2		0.5	1.5	4
K3		1.5	0.5	4
K4		0.5	0.5	4
K5		1.0	1.0	3
K6		0.5	0.5	2
K7		0.5	1.5	2
K8		1.5	1.5	4
K9		1.5	1.5	2

mg: milligram, %: percentage, evaluation of rivastigmine tartrate SLN loaded transdermal films

### Thickness

By using digital vernier calipers, the thickness of the films were measured at three different places, and mean value was calculated [17].

### Weight variation

Weight variation of films was performed by individually weighing 3 randomly selected films and performed for each formulation and mean value was calculated [18].

### Flatness

Three longitudinal strips were cut out from each film: 1 each from the center, left side, and right side. The length of each strip was measured, and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness.

$$\text{Constriction (\%)} = \frac{L1 - L2}{L2} \times 100$$

Where, L1 initial length of each strip, L2, final length.

### Folding endurance

Folding endurance was determined by repeatedly folding the films at the same place until it broke. The number of times the film could be folded at the same place without breaking was the folding endurance value [19].

### Drug content

A prepared film was added to 100 ml saline phosphate buffer (pH 6.8) and stirred vigorously for 24 h followed by ultra-sonication for 15 min. The contents were filtered, and drug was estimated spectrophotometrically at wavelength of 263 nm [20, 21].

### Water vapour transmission rate

Glass vials of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in an oven. About 1 gm (gram) anhydrous calcium chloride was placed in the cells and the respective polymer films were fixed over the brim. The cells were accurately weighed and kept in a closed desiccators containing saturated solution of potassium chloride to maintain a humidity of 84%. The cells were taken out and weighed after 6, 12, 24, 36, 48 and 72 h of storage to note down the weight gain [22].

$$\text{Water vapour transmission rate} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Time} \times \text{Area}}$$

### Swellability study

Completely dried films with a required area were weighed and placed in desiccators for 24 h. the films were removed and exposed to relative humidity (RH) conditions of 75% (containing saturated solution of sodium chloride) in desiccators. Weight was taken on a single pan balance periodically until a constant weight was obtained.

The capacity of the films swelling (in weight %) was calculated in terms of percentage increase in weight of membrane over the initial weight of the specimen. The experiments were conducted in triplicate and the average values were used for the calculation. The percentage degree of swelling (DS) was calculated as

$$\text{DS (\%)} = \frac{W_s - W_d}{W_d} \times 100$$

Where, W<sub>d</sub> and W<sub>s</sub> indicate the weight of the dry and swollen films respectively [23].

### Tensile strength

Mechanical properties of the films were evaluated using universal testing machine (Model 1121, Instron Ltd., Japan) with a 2-kilogram load cell. SLN film of known dimension was positioned between two clamps at a distance of 5 cm. The SLN film was pulled by the clamp at the rate of 50 mm/min. Measurements were run in triplicate for each film.

Tensile strength (N/mm<sup>2</sup>) is the maximum stress applied to a point at which the film breaks and can be computed from the applied force at rupture as a mean of three measurements and the cross-sectional area of the fractured film [24].

$$\text{Tensile strength} = \frac{\text{Force at break (N)}}{\text{Initial cross sectional area of the film (mm}^2\text{)}}$$

### In vitro drug release study

The *in vitro* drug release study of SLN film was performed by using Franz diffusion type cell. The study was performed at 37±0.5 °C, Receptor compartment of diffusion cell contained 20 ml of phosphate buffer (pH-6.8) solution and was constantly stirred by a magnetic stirrer at 100 rpm. Cellophane membrane (molecular weight cut off 10,000-12,000, Hi-Media, India), was employed as release barrier in between receptor and donor compartment which was previously soaked in distilled water. Test samples were withdrawn on definite time intervals from sampling port of the diffusion cell and immediately replaced with an equal volume of fresh buffer. The amount of drug released was quantified using the high performance liquid chromatography (HPLC) method by directly injecting samples to the HPLC system at 263 nm [25, 26].

### Ex vivo permeation study of SLN loaded transdermal films

Animals were purchased from Biogen laboratory animal facility, Reg. No. 971/bc/06, Bill No. 1353. The skin samples of Newzealand white rabbit weighing 3 kg were continuously housed at the institution animal facility. Study was conducted after the approval from institutional animal's ethics committee, JSS College of Pharmacy, Mysuru. (Proposal No. 215/2017).

The skin sample was mounted carefully on franz-type diffusion type cell with the stratum corneum side up with an effective diffusion area of 2.0 cm<sup>2</sup>. The receiver compartments were filled with 20 ml of phosphate buffer (pH-6.8) solution to ensure sink condition. The

diffusion cells were maintained at  $37\pm 0.5^\circ\text{C}$  with stirring at 100 rpm during the experiment.  $2.0\text{ cm}^2$  transdermal film of SLN was mounted onto surface of skin, from the medium 1 ml of the sample was withdrawn at definite time intervals (0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h) and replaced same volume of fresh phosphate buffer. All the test samples were filtered through a whatman filter membrane and by using HPLC the samples were analysed [27-29].

## RESULTS AND DISCUSSION

### Evaluation of SLN loaded transdermal film

The transdermal film containing rivastigmine SLN were prepared and evaluated. The evaluation results were summarized in table 2. The thickness of transdermal films of ranged between ( $0.22\pm 0.06$  to  $0.39\pm 0.02$  mm). The weight variation range of  $132.7\pm 1.79$  to

$141.4\pm 2.45$  mg indicated that different batches of prepared films had similar weights. The % flatness study showed 100% thus indicating a lack of constriction. Folding endurance results ranged between  $66\pm 3.19$  and  $78\pm 4.38$  indicating the capacity of films to maintain their integrity with general skin folding when applied. In a previous study the optimized formulation of film has shown thickness more (0.55 mm) and weight of the film 351.45 mg [30].

### In vitro drug release

The cumulative percentage of drug release from the transdermal film of different formulations is shown in fig. 1 and 2. Rivastigmine tartrate transdermal films showed good in drug release. The percentage release was found to be highest is  $96.1\pm 0.48\%$  at 24<sup>th</sup> h. In a previous study done by Nikhil B *et al.*, the optimized formulation has shown cumulative drug release 95.70% at 24<sup>th</sup> h [31].

Table 2: Evaluation test results of SLN loaded transdermal films

Code	Thickness (mm)*	Wt. variation (mg)*	Flatness (%)*	Folding endurance*	Drug content (%)*	Water vapour transmission ( $\text{g. cm}^2/\text{day}$ ) $\times 10^{-4}$ *	Swellability (%)*
K1	$0.25\pm 0.02$	$135.3\pm 1.18$	100	$68\pm 3.36$	$98.46\pm 0.5$	$4.78\times 10^{-4}$	$31.74\pm 0.82$
K2	$0.35\pm 0.08$	$138.6\pm 1.45$	100	$72\pm 3.68$	$97.62\pm 0.1$	$5.36\times 10^{-4}$	$40.52\pm 0.42$
K3	$0.39\pm 0.02$	$132.7\pm 1.79$	100	$66\pm 4.57$	$98.18\pm 0.7$	$4.64\times 10^{-4}$	$39.76\pm 0.81$
K4	$0.31\pm 0.01$	$134.3\pm 1.52$	100	$74\pm 3.24$	$97.69\pm 0.3$	$4.82\times 10^{-4}$	$38.56\pm 0.77$
K5	$0.29\pm 0.09$	$139.2\pm 1.63$	100	$69\pm 4.71$	$97.78\pm 0.9$	$4.66\times 10^{-4}$	$42.47\pm 0.25$
K6	$0.38\pm 0.06$	$138.9\pm 1.38$	100	$75\pm 3.29$	$98.51\pm 0.2$	$3.93\times 10^{-4}$	$39.85\pm 0.58$
K7	$0.37\pm 0.01$	$136.5\pm 2.02$	100	$72\pm 4.45$	$98.69\pm 0.7$	$5.73\times 10^{-4}$	$38.41\pm 0.75$
K8	$0.22\pm 0.06$	$139.4\pm 1.76$	100	$78\pm 4.38$	$98.97\pm 0.5$	$4.68\times 10^{-4}$	$41.48\pm 0.18$
K9	$0.28\pm 0.08$	$141.4\pm 2.45$	100	$66\pm 3.19$	$98.81\pm 0.3$	$5.17\times 10^{-4}$	$37.34\pm 0.89$

mg: milligram; cm: centimeter; mm: millimeter; %: percentage, \*Average of three readings $\pm$ SD

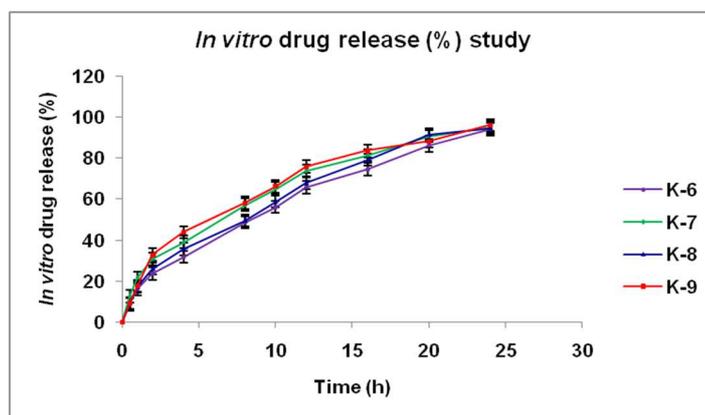


Fig. 1: In vitro drug release profile of formulations (K1-K5) (n=3), n=3 average of three determinations

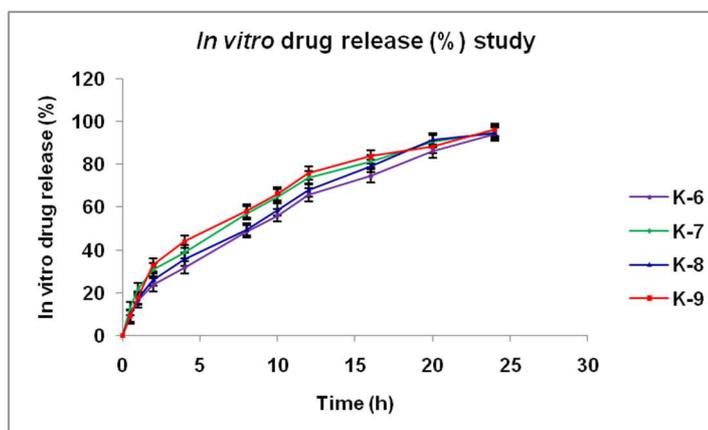


Fig. 2: In vitro drug release profile of formulations (K6-K9) (n=3), n=3Average of three determinations

**Ex Vivo skin permeation study**

The *ex vivo* skin permeation study release from the transdermal film of various formulations is shown in fig. 3 and 4. Rivastigmine

tartrate transdermal films showed good in drug release. The percentage release was found to be highest is  $97.36 \pm 0.93\%$  at 24<sup>th</sup> h. In a previous study done by Nikhil B *et al.*, the permeation study has shown  $96.90 \pm 0.695\%$  drug permeation at 24<sup>th</sup> h [31].

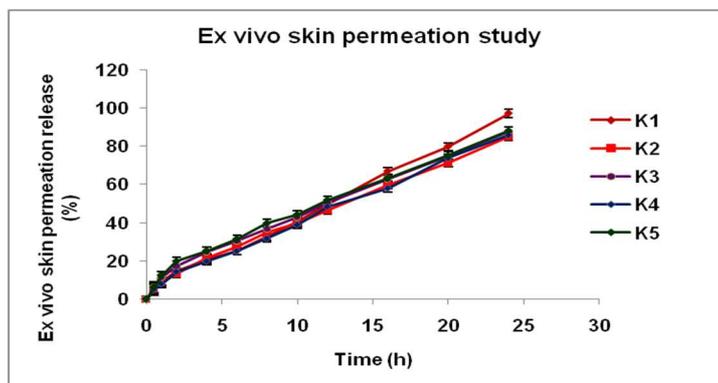


Fig. 3: *Ex vivo* skin permeation profile of formulations (K1-K5) (n=3), n=3 average of three determinations

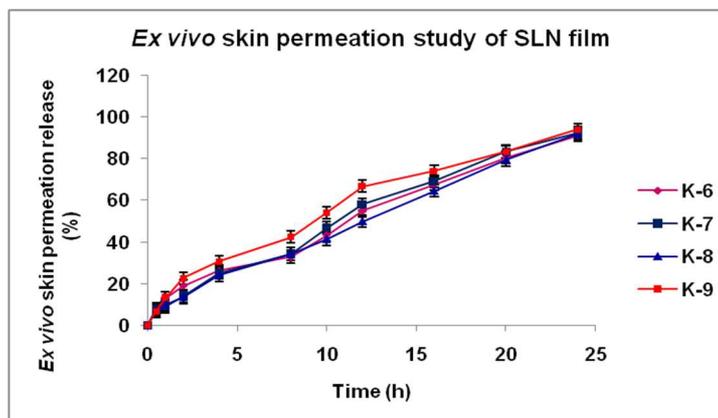


Fig. 4: *Ex vivo* skin permeation profile of formulations (K6-K9) (n=3), n=3 Average of three determinations

By conducting preliminary trials with relative ratio of the selected two components i.e. E. RS 100, E. L100 and plasticizer on the previous related experiences, the low and the higher limits of each variable

were defined (table 3). To evaluate all the possible combination of excipients in the initial formulation system, a full factorial DoE was performed and the results obtained are shown in table 4.

Table 3: Variables in 2<sup>3</sup> factorial designs for SLN loaded transdermal film

Independent variables	Levels	
	Low	High
A: Eudragit RS 100 (mg)	50	150
B: Eudragit L 100 (mg)	50	150
C: Plasticizer (%)	2	4

Dependent variables, R1: Tensile strength (N/mm<sup>2</sup>), R2: Cumulative Drug release (%) 24<sup>th</sup> h

Table 4: Observed response in 2<sup>3</sup> factorial design for SLN loaded transdermal film

Formulation code	Polymer (%)		Plasticizer (%)	Responses	
	E. RS 100	E. L 100		Tensile strength* (N/mm <sup>2</sup> )	Cumulative drug release (%) 24 <sup>th</sup> h*
K1	1.5	0.5	2	11.56±0.03	94.28±0.48
K2	0.5	1.5	4	5.47±0.07	86.24±0.25
K3	1.5	0.5	4	7.1±0.02	89.45±0.57
K4	0.5	0.5	4	6.86±0.09	88.72±0.99
K5	1.0	1.0	3	7.74±0.11	90.57±0.45
K6	0.5	0.5	2	9.28±0.07	92.47±0.86
K7	0.5	1.5	2	8.63±0.08	91.39±0.37
K8	1.5	1.5	4	6.28±0.06	87.43±0.61
K9	1.5	1.5	2	10.41±0.05	93.36±0.44

\*mean±SD, n=3, the results depicts that variables chosen have strong influence on the selected responses, as tensile strength and percentage cumulative drug release values were in the range of 5.47-11.56 N/mm<sup>2</sup> and 86.24-94.28% respectively.

The application of factorial design yielded the following regression equations

**Tensile Strength (N/mm<sup>2</sup>)** = +13.18653+0.012775 \* E RS 100-0.010025 \* E L100-1.77125 \* Plasticizer

**Cumulative drug release(%)** = +95.04694+0.025100 \* E RS 100-6.00000E-004 \* E L100-1.36750 \* Plasticizer+3.10000E-005 \* E RS 100 \* E L100-4.65000E-003 \* E RS 100 \* Plasticizer-6.25000E-003 \* EL100 \* Plasticizer

Where negative values indicate a negative effect of a specific variable on the response factor and positive value indicates positive effect of a specific variable. The polynomial regression results were expressed using Contour graphs, predicted and actual graphs and 3-D graphs.

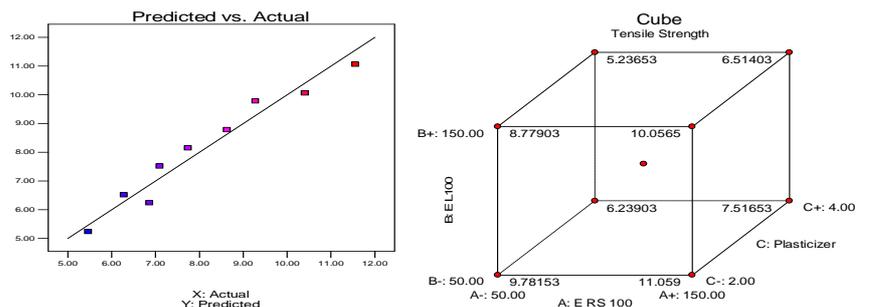


Fig. 5: Predicted V/S actual plot and cube plot depicting the impact of polymer and plasticizer on tensile Strength (N/mm<sup>2</sup>) respectively

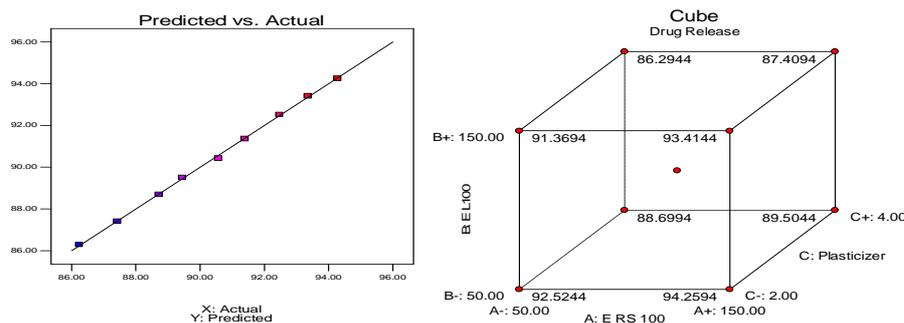


Fig. 6: Predicted V/S actual plot and cube plot depicting the impact of polymer and plasticizer on Cumulative drug release (%) respectively

## CONCLUSION

In the present investigation we, concluded that the *in vitro* drug release as well as skin permeation profiles of rivastigmine tartrate solid lipid nanoparticles from transdermal systems were found to be greatly influenced by the formulation variables such as rivastigmine tartrate SLN loading, polymer and plasticizer concentration and these variables could be suitably altered to achieve the desired controlled release profile of rivastigmine tartrate. The design of experiment with response surface method is an efficient tool to determine and optimize formulation conditions within experimental conditions. Overall, an optimized rivastigmine tartrate SLN loaded transdermal film was successfully developed which could control the release as well as permeation of rivastigmine tartrate up to 24 h.

## CONFLICT OF INTERESTS

Declared none

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