Fabrication of Transdermal Matrix Patch of Lercanidipine Hydrochloride using Natural Polymer and Essential Oil

ABSTRACT

The goal of the present research work was to develop and characterize a transdermal matrix patch of Lercanidipine hydrochloride (LH) for controlled drug delivery using the solvent evaporation method. To achieve controlled drug release, polymers such as psyllium and HPMC K15M were optimized. Moreover, the skin permeation effect of essential oils such as linseed oil, jojoba oil and pumpkin seed oil was investigated on Wistar rat skin. A 3^2 full factorial design was applied to optimize two formulation variables: concentration of essential oil as a permeation enhancer and polymer fixed-weight ratio. To study drug-excipients incompatibility Fourier Transform Infrared Spectroscopy (FTIR) had employed, which showed the absence of chemical interaction. All formulations were evaluated for Physico-chemical parameters, *ex-vivo* drug release study, an *in-vivo* skin-irritation study on Wistar rats and stability study. Developed matrix patch showed optimum Physico-chemical properties with the absence of skin irritation. An *Ex-vivo* drug release study revealed that both formulation variables show an effect on drug release from matrix patches. Effectiveness of the oils as the permeation enhancer was found to be in the following descending order: Pumpkin seed oil > Linseed oil > Jojoba oil. Therefore, pumpkin seed oil was selected as a permeation enhancer in the final formulation that shows the highest flux (164.09±1.49 µg/cm^2/h) and desired drug release for transdermal administration. Stability study shows that the patch was stable up to 6 months at 40±2 ˚C and 75±5 % RH and 30±2 ˚C and 65±5 % RH. The present investigation demonstrates that the prepared matrix patch has a capacity to deliver therapeutically effective controlled release dose of lercanidipine hydrochloride (LH) via transdermal route using pumpkin seed oil as the permeation enhancer.

Keywords: Pumpkin seed oil, Psyllium, hypertension, Lercanidipine hydrochloride, propylene glycol, ex-vivo study, in-vivo skin irritation study.

1. INTRODUCTION

Transdermal devices in a recent time become more popular because it avoids hepatic first-pass metabolism and maintains plasma concentration throughout the treatment, thereby decreasing the dosing frequency and reducing gastrointestinal irritation resulting in improved patient compliance. Easy removal of the patch at any time from the target site will terminate the treatment preventing the chances of overdose and underdose[1-4]. However, the transport of compounds via the skin is a considerable challenge due to the complex structure of the skin. Therefore, a suitable polymer matrix is required through which the drug should be released at a predetermined rate throughout the treatment[5,6]. Psyllium husk obtained from the plant of *Plantago ovata* is rich in polysaccharide and uronic acid contents, which renders it the property of making good thin patches[7-10]. Hence, a polymeric mixture of psyllium husk and HPMC K15M was used as a controlled drug delivery component[11-16]. The success of a transdermal matrix patch depends on the ability of the drug to penetrate into the skin in sufficient quantities to maintain the required therapeutic levels[17,18]. Permeation enhancers are not drugs but they are molecules that reversibly alter the barrier nature of the *Stratum corneum* and allow the drug to penetrate into the skin. The natural permeation enhancers available from the literature review are essential oils, terpenes, terpenoids, fatty acids, glycols and

herbal extracts[19,20]. Essential oils gained more attention from the researchers because they are compatible with a huge range of hydrophilic and lipophilic drugs along with being non-toxic, non-allergic and clinically acceptable[21,22]. Pumpkin seed oil, Linseed oil and Jojoba oil are the well-known essential oils have higher permeability because it contains unsaturated fatty acids which alleviate the lipid stratum corneum by dekeratinization of corneocytes and increasing the permeation of molecules through the skin[23,24]. Propylene glycol, polyethylene glycol 400 and Dibutyl phthalate are the commonly used plasticizers. Therefore, all three here were optimized and PG was selected based on folding endurance study results. Selective drug candidate, Lercanidipine hydrochloride (LH) is a calcium channel blocker used in the treatment of hypertension and several other cardiovascular disorders. It is administered orally with a 10 mg daily dose having 30% bioavailability, so two times required in a day to maintain a therapeutic level. The physicochemical properties such as high lipophilicity (Log P value 6.42 at 20- 25^0 C), low molecular weight (648.19g/mol), high melting point (197-201^0 C), and high pKa (9.36) at 37^0 C indicates its suitability for transdermal matrix patch[22-27]. A solvent evaporation method is used for the preparation of transdermal matrix patch due to its ease of manufacturing and the possibility of achieving a higher release and flux of the lipophilic drug loaded on the matrix as suggested by the literature[28-30]. Hence, in this present research work, hydrophilic polymeric matrix patches formulated using HPMC K15M and psyllium along with essential oils as permeation enhancers.

2. MATERIALS AND METHODS

2.1 Materials

Lercanidipine hydrochloride (LH) got as a gift sample from Glenmark generics limited, Pune, India. Psyllium was purchased from Shiv psyllium Industries, North Gujarat, India. All the investigated oils namely Linseed oil (LO), Jojoba oil (JO) and Pumpkin seed oil (PSO) purchased from Hamdard Laboratories, Ghaziabad, India, Hydroxy Propyl Methyl Cellulose K-15M (HPMC K15M) and propylene glycol (PG) supplied by S.D. Fine Chemicals Ltd., Mumbai, India. All remaining chemicals and solvents were reagent grade. Double distilled water was used throughout the study.

2.2 Animals

Wistar rats (180-200g, 6-8 weeks old) were supplied by Zydus Research center, (Village Moraiya, Near Nova Petrochem, Ahmadabad, Gujarat). The animals received after the study duly approved by the CPSCEA (committee for the purpose of control and supervision on experiments on animals of the government of India) with protocol No. 984/06/2014-2-06. The experiments on animals' *in-vivo* skin-irritation study and *ex-vivo* permeation study using Wistar-rat skin were performed in accordance with the guidelines given by the Animal Ethical Committee, CPCSEA.

2.3 Methods

2.3.1 Dose calculation

The dose of LH for the transdermal patch is calculated on the basis of the value of targeted flux and transdermal flux. Targeted flux of LH is **166.46 µg/cm^2/hr (**calculated by equation: targeted flux Jss= Css×Clt×BW/A, where 3.3 µg/l and 3.37 mL/min/kg are Css and Clt respectively for LH). The oral dose of LH is 10 mg or 20 mg once daily and bioavailability is 40%. Therefore, an orally available dose to maintain plasma concentration is only 4 mg. To surplus the loss of drugs in different layers of skin and for getting the required flux here, a double dose is required. Therefore, in the present study dose of LH for the preparation of the transdermal patch having a 4-cm^2 area is 8mg (73 mg for total 6.8cm Petri plate) and it gives flux 165 µg/hr/cm^2, which is very nearer to the required flux[30,31].

2.3.2 Method for preparation of transdermal matrix patch containing LH

The transdermal matrix patches were prepared using different ratios of psyllium and HPMC K15 M. The polymers concentration was varied with 3%w/v, 4%w/v and 5%w/v by keeping the constant ratio (2:1) of HPMC K15M psyllium and allowed to swell for 2 hrs in water. As per dose calculation, accurately weighed the amount of LH dissolved in ethanol and this drug solution was added into the polymeric solution with continuous stirring using a magnetic stirrer. Then propylene glycol and essential oil are incorporated as plasticizer and penetration enhancers respectively. The inverted funnel was kept over the Petri plate for uniform evaporation, after complete drying biaxial oriented polyethylene, film glued as a backing membrane and a glossy paper having smooth surface used as a release liner. The dried films were removed from the Petri plate and cut into a 4-cm^2 area, wrapped in aluminum foil and stored in desiccators for further studies[30-33].

2.3.3 Preliminary trial study for the optimization of matrix patch formulation

Preliminary trial batches were prepared and evaluated for the optimization of various concentrations of polymers, drugs, plasticizer, and permeation enhancers. Formulations L1 to L3 were prepared with varying concentrations of LH 8mg, 10mg and 12mg to study the effect of LH concentration on drug release and permeation. Batches L4 to L6 were prepared with varying concentrations of polymers with 3%w/v, 4%w/v and 5%w/v, to study the effect of thickness of polymeric matrix on drug release and permeation. Batches PE 1 to PE 7 were prepared with varying concentrations of JO, LO and PSO for the optimization and selection of effective permeation enhancers. Formulations of all preliminary trial batches are displayed in (Table 1).

Table 1. Composition of Preliminary Trial Batches and Results af Dependent Variables (Cumulative Drug Release at 1 Hr(Q1), 16 Hrs (Q16), and Tensile Strength).

Batch code	LCDH loading (mg)	HPMCK15M: Psyllium(2:1) (mg)	EO-Loading (%w/w total wt of polymer dry weight)	CDR at 1 hr(Q1)	CDR at 16hrs(Q16)	Tensile strength
L1	8	300	-	8.22±0.23	68.27±0.19	3.27±0.03
L2	10	300	-	10.19±0.35	73.91±0.27	4.69±0.04
L3	12	300	-	11.11±0.31	79.13±0.17	4.72±0.03
L4	8	300	-	8.22±0.23	68.27±0.19	3.27±0.03
L5	8	400	-	7.18±0.16	57.79±0.21	3.45±0.04
L6	8	500	-	6.54±0.11	50.32±0.19	4.96±0.05
PE1-control	8	300	Without EO	8.22±0.23	61.27±0.19	3.27±0.03
PE2 - LO	8	300	10% w/w	9.19±0.15	64.76±0.06	4.19±0.02

PE3 -LO	8	300	20% w/w	10.41±0.13	66.42±0.19	4.22±0.053
PE4 - JO	8	300	10% w/w	9.12±0.14	68.89±0.11	4.17±0.03
PE5 - JO	8	300	20% w/w	10.08±0.19	69.32±0.17	4.48±0.02
PE6 – PSO	8	300	10% w/w	9.94±0.18	75.11±0.21	4.56±0.03
PE7 – PSO	8	300	20% w/w	11.57±0.13	78.81±0.13	4.67±0.04

2.3.4 Statistical optimization of the formulation variables using Experimental design approach

A preliminary trial study suggested that the concentration of polymers and permeation enhancer mainly affect the release and permeation of LH from the patch. Therefore, further optimization of these two formulation variables performed using experimental designs by the fabrication of transdermal matrix patches having desire drug release and permeation flux. A 3^2 full factorial design, from Design Expert software 9.02 selected (A.M. Abdel Azim, et.al, 2014). This design involved three dependent variables (Y1, Y2 and Y3) and two independent variables (X1 and X2). The release response can be expressed as Y= f (X1, X2). The selected two independent variables for the present investigation were X1, polymer fixed weight ratio; and X2, essential oil concentration in the patches. All other formulation variables were kept constant throughout the study. The dependent variables were Y1, drug release in 1^{st} hr (Q1), Y2, drug release at 16 hrs (Q16) and Y3, the tensile strength of prepared patches. The composition of nine formulations based on this experimental design is displayed in (Table 2). After completion of statistical optimization experiments, polynomial equations and 3-dimensional plots were generated to study the effect of X1 and X2 on Y1, Y2 and Y3, in order to identify the optimized LH, loaded transdermal matrix patch. The final identified batch fabricated and subjected to the validation of statistical optimization design.

Table 2. Composition of LH Loading Factorial Design Batches P1 to P9.

Batch code	P1	P 2	P 3	P 4	P5	P 6	P 7	P 8	P 9
Lercanidipine HCL(mg)	73	73	73	73	73	73	73	73	73
HPMC K15M(mg)	250	250	250	225	225	225	200	200	200
Psyllium(mg)	50	50	50	75	75	75	100	100	100
Water(mL)	12	12	12	12	12	12	12	12	12
Ethanol(mL)	8	8	8	8	8	8	8	8	8
Propylene glycol(%w/w of dry polymer wt)	20	20	20	20	20	20	20	20	20

Pumpkin seed oil(%w/w of dry polymer wt)	10	20	30	10	20	30	10	20	30

2.4 Evaluation study of transdermal matrix patch containing LH

2.4.1 FTIR study

FTIR spectroscopy was used as an analytical tool to find out compatibility between LH, HPMC K15M, psyllium, PSO, LO, JO and PG. FTIR spectra of pure drug and final formulation carried out using the KBR disc method[31,32].

2.4.2 Physicochemical evaluations of LH containing transdermal matrix patch

The experimental design formulations, batches P1 to P9 evaluated for various physicochemical evaluations such as thickness, folding endurance, moisture uptake and loss, tensile strength and drug content according to the method given by A.M. Abdel Azim, et al. 2014 Rajesh Singh Patel and S.S. Poddar, 2009, Pichayakorn W, et al. 2013.

2.4.3 *Ex-vivo* skin permeation study and preparation of rat skin

Wistar rats sacrificed with prolonged ether anesthesia and the abdominal skin of each rat were excised. Hairs on the skin of an animal and subcutaneous tissues were removed with a sharp blade. The skin was washed with phosphate buffer saline, wrapped in aluminum foil and stored in a deep freezer at -20^{o} C until further use. At the time of *ex-vivo* permeation study, the skin was brought to room temperature and hydrated in phosphate buffer solution for half an hour before the study and then placed over the receptor compartment of Franz diffusion cell with a diffusion area of 0.64 cm^{2} and a receptor compartment capacity of 13 mL. The LH loaded transdermal matrix patch was placed over the membrane by keeping the dermal side in contact with the receptor medium. The receptor compartment is filled with 13 mL of pH 6.8 buffer. The temperature of the diffusion medium was maintained at 32 ± 2^{o} C. This whole assembly kept on a magnetic stirrer and solution in the receiver compartment constantly and continuously stirred using a magnetic bead. Samples were withdrawn (2 mL, each time) at different time intervals and replaced with an equal amount of pH 6.8 buffer. The sample was analyzed at 357nm after suitable dilution using a UV spectrophotometer. The Amount of drug permeated per square centimeter at each time interval was calculated and plotted against time[34-40].

The *Ex-vivo* release data were subjected to various kinetic equations to find out the release mechanism and order of drug release. Transdermal flux was calculated using the value of the slope of the cumulative drug release curve that was constructed by the steady-state values of the cumulative amount of drug permeated (mg/cm^{2}) vs time. Permeation coefficients (cm/hr) were calculated by dividing the flux with initial drug loading (mg/cm^{2}). Lag time calculated from back extrapolation. Diffusion coefficient (D/h^{2}) and permeability coefficient (Kp) also calculated from the data of *ex-vivo* studies using given equations, respectively ($D/h^2=1/6\times T_{lag}$, Jss = (dq/dt).1/A, Kp = Jss/Cs). The regression analysis of steady-state data and release rate was calculated. The experiment was performed in triplicate and the mean results were recorded[27,40-43].

2.3.4 Regression analysis of the optimization of formulation

The statistical analysis of factorial design batches performed using Design expert software 9.02. The results of the dependent variables for the factorial design batches are given in (Table 3). To evaluate the contribution of both the factors at three different levels on responses, a two-way analysis of variance (ANOVA) was performed using design expert software 9.02. To demonstrate graphically the influence of each factor on responses the response surface plots such as contour and 3D plots were generated using the software.[44,45] The response surface plots for dependent variables, tensile strength, % drug release in 1 hr(Q1) and % drug release in 16hrs (Q16) are shown in (Fig. 1-3) respectively. The value of $p<0.05$ was considered to be significant.

Table 3. Results of Dependent Variables (Cumulative Drug Release at 1 Hr(Q1), 16 Hrs (Q16), and Tensile Strength.

Batch Code	Coded Value & Actual value		Dependent variables		
	Polymer fixed wt ratio(2:1) (300 mg) X1	PSO (% w/w) X2	% Drug released in 1 hr (%)Y1	% Drug released in 16 hrs (%)Y2	Tensile Strength (Kg/cm^2) Y3
P1	-1	-1	11.42±0.24	73.16±0.52	3.30±0.01
P2	-1	0	12.72±0.45	75.54±0.81	3.49±0.02
P3	-1	1	13.16±0.41	77.28±0.13	3.56±0.03
P4	0	-1	14.41±0.43	80.41±0.16	3.59±0.03
P5	0	0	15.62±0.06	82.78±0.12	3.69±0.02
P6	0	1	16.24±0.31	84.36±0.16	3.84±0.02
P7	1	-1	17.22±0.16	87.64±0.06	3.99±0.03
P8	1	0	18.36±0.21	89.31±0.91	4.45±0.04
P9	1	1	19.18±0.07	87.14±0.14	4.56±0.03

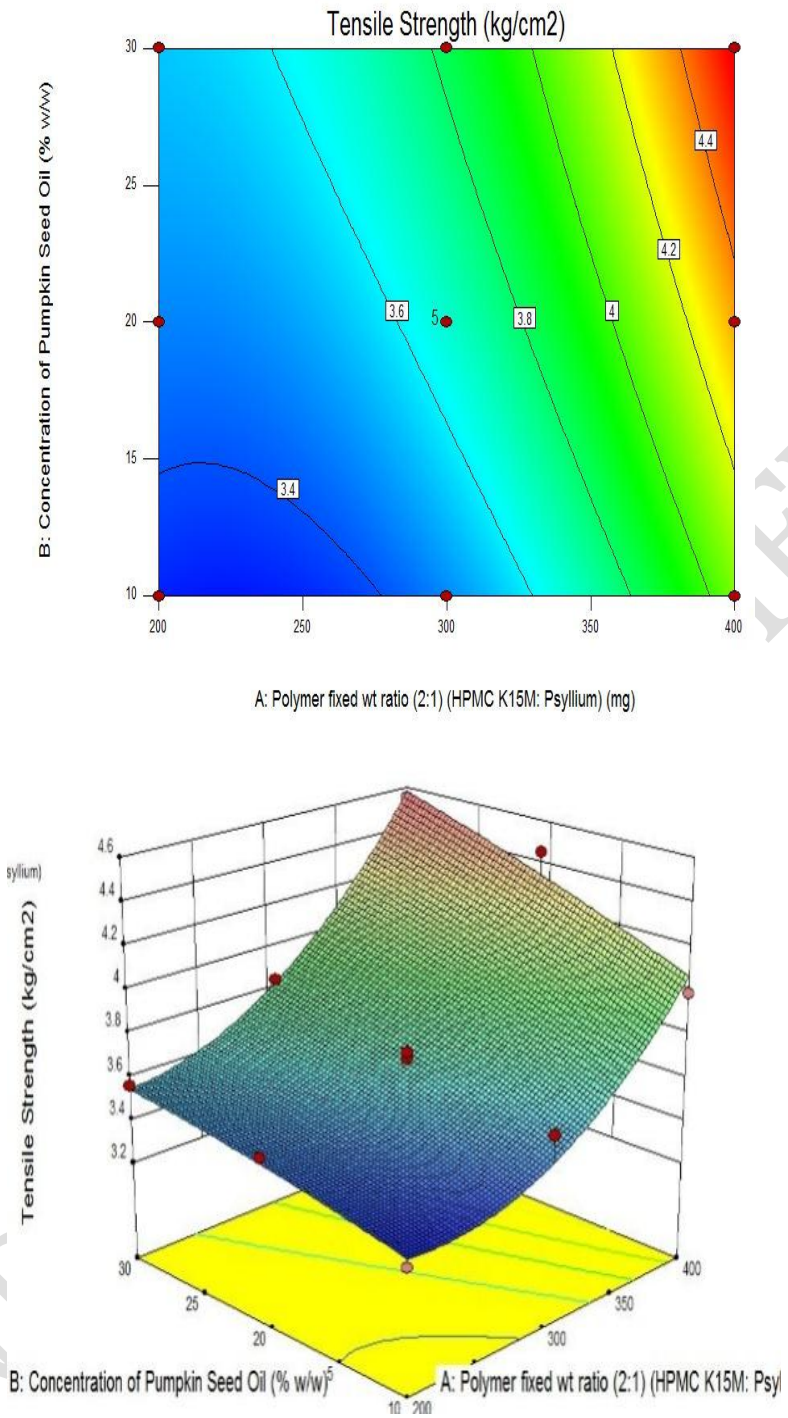

Fig. 1. (A) Counter Plot and (B) Response Surface Plot of the Effect of Psyllium and Pumpkin Seed Oil on Tensile Strength

**Polynomial Equation*

Tensile Strength = +3.67+0.44 × A+0.18× B+0.075 × AB+0.23 × A^2-0.020 × B^2

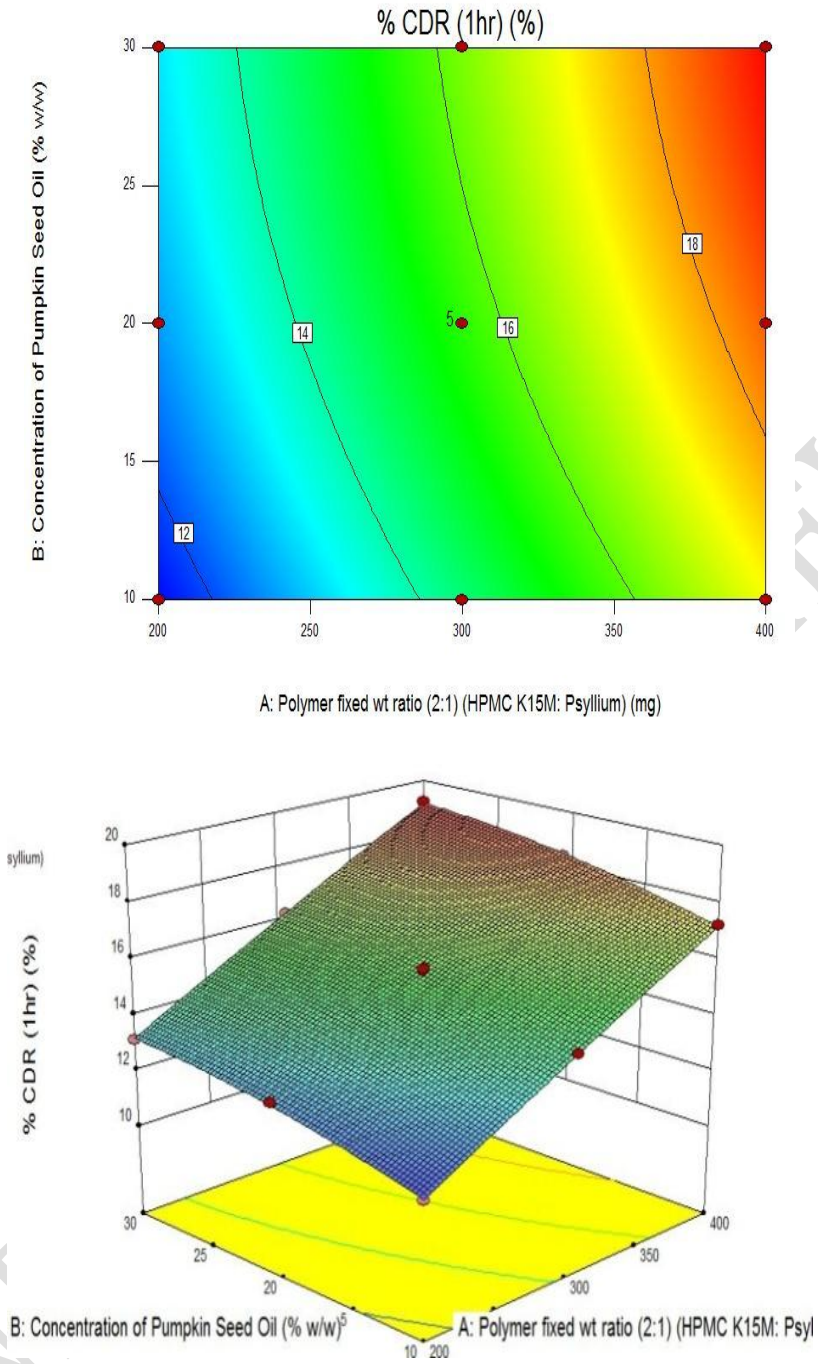

Fig. 2. A) Counter Plot and (B) Response Surface Plot of the Effect of Psyllium and Pumpkin Seed Oil on Percentage Drug Release in 1 Hr

**Polynomial Equation*

% CDR (1Hr) = + 15.62 +2.91 × A+0.92 × B+0.055 × AB-0.078 × A^2-0.29 × B^2

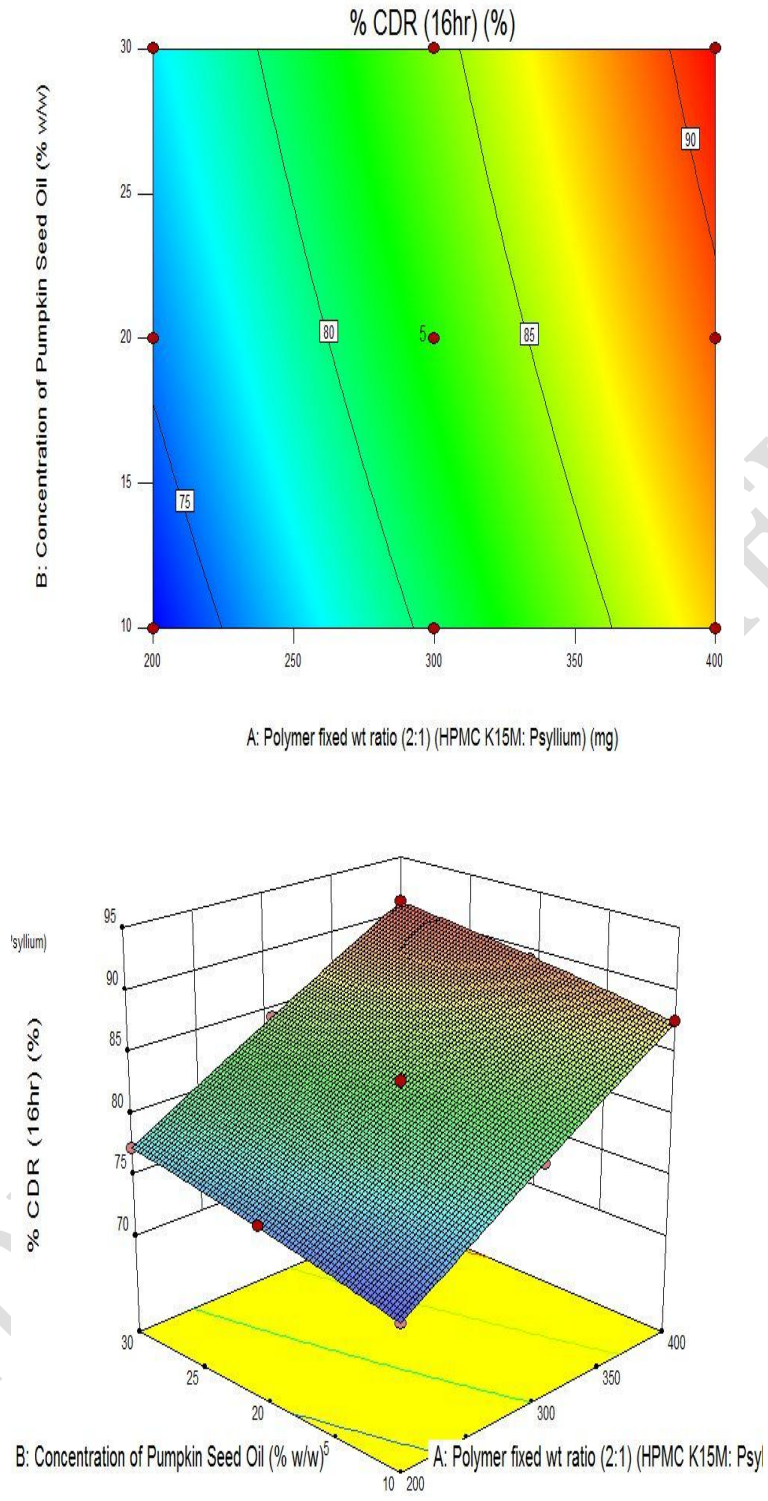

Fig. 3. A) Counter Plot and (B) Response Surface Plot of the Effect of Psyllium and Pumpkin Seed Oil on Percentage Drug Release in 16 Hr

**Polynomial Equation*

% CDR (16Hr) = +82.69+7.02 × A+1.93 × B-0.15 × AB-0.19 × A^2-0.23B^2

2.3.5 *In-vivo* skin irritation study

The study was performed on Wistar rats to determine irritation after a single application of the prepared transdermal matrix patch. Accurately cut 4 cm^2 size patch applied on the clean backside skin of rat and removed after 16 hrs. The exposed skin was evaluated for the formation of edema and erythema and any type of irritation. The rats were divided into 2 groups of 3 rats in each group (n=6), one group as a control and another group as a test (prepared matrix patch). Prior permission takes from the animal ethical committee for this study[46,47].

2.3.6 Stability study

The final optimized batch is subjected to stability study to evaluate any change in appearance and drug release when exposed to accelerated conditions of the environment during storage, handling, transport and use. The study performed according to ICH guideline at 40°C and 75% RH and at 30±2^{o} C and 65±5 % RH in a humidity chamber for a period of six months and it was analyzed for physicochemical parameters at particular time intervals[43,46,47].

3. RESULTS

3.1 FTIR study

Infrared spectra of LH pure drug (A) and LH loaded matrix patch final formulation (B), are shown in (Fig. 4,5). Infrared absorption spectroscopy (IR) of LH shows a sharp band due to stretching vibration bands of OH, N-H and C=O, respectively. From (Fig. 4,5), it was observed that there were no changes in these main peaks in IR spectra of a mixture of drug and polymers, which indicate physical compatibility between LH and all ingredients used in the final formulation of transdermal matrix patch.

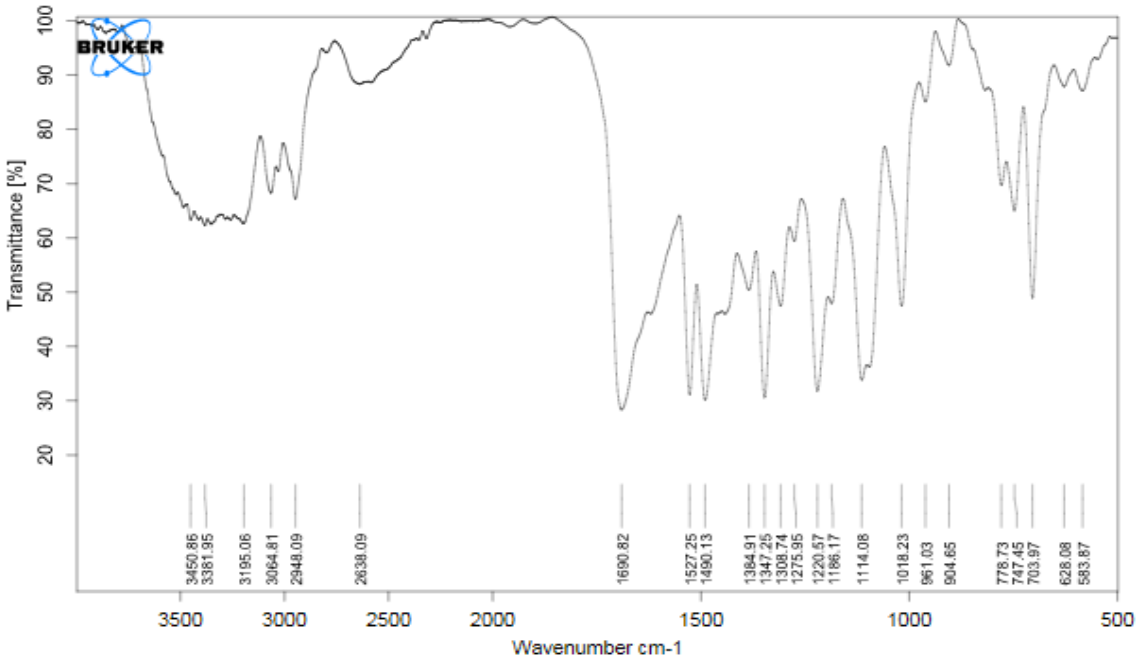

Fig. 4. Infrared Spectra of Lercanidipine Hydrochloride.

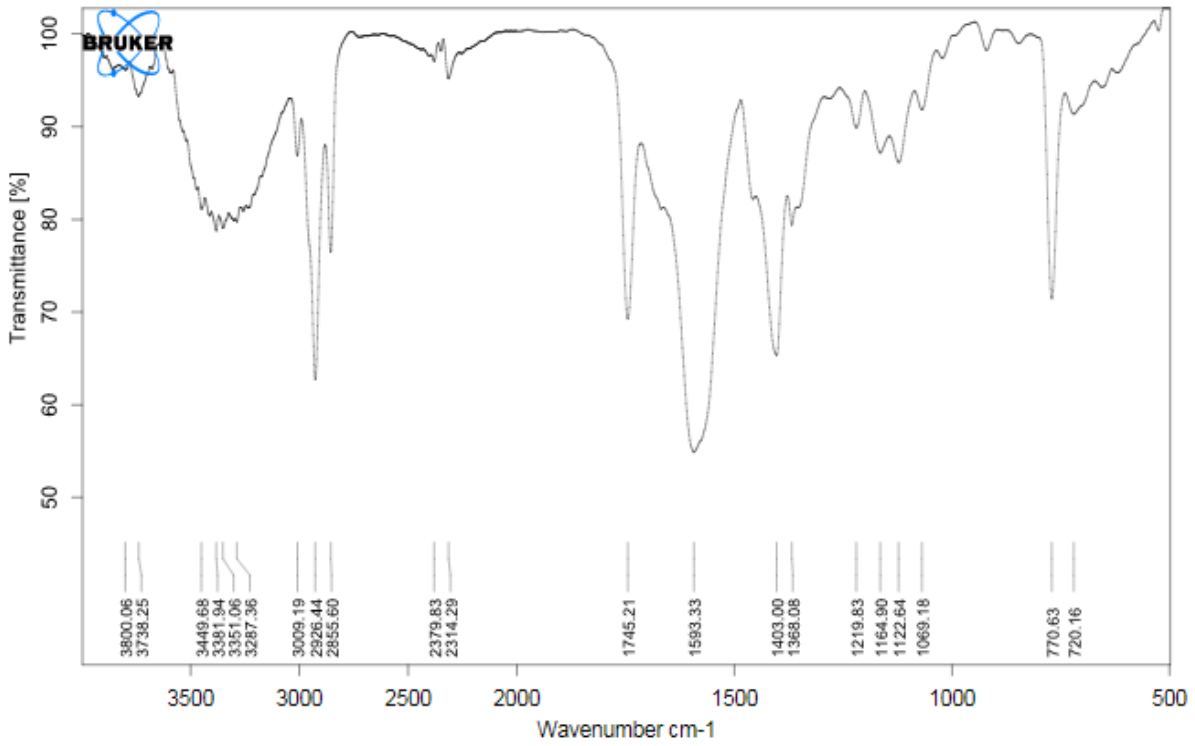

Fig. 5. Infrared Spectra of Final Optimized Formulation.

3.2 Preliminary trial study for the optimization of matrix patch formulation

The preliminary trial batches were prepared and evaluated to investigate the effect of formulation variables such as LH concentration, HPMC K15M and psyllium concentration, essential oils concentration as a permeation enhancer on permeation of LH from the transdermal matrix patch. Obtained results are outlined in above (Table 1), it revealed that as an increase in LH concentration from 8mg, 10mg and 12mg, % cumulative drug release was also increased from 68.27±0.19 % (L1), 73.91±0.27% (L2) and 79.13±0.17% (L3), respectively. This increase in a release is based on Fick's first law of diffusion, in which the drug release is directly proportional to the drug concentration gradient across the membrane, i.e higher amount of drug available for diffusion. The results of batches L4, L5 and L6 suggested that the rate of drug release decreased with an increase in polymer concentration. Batch L4 contains 3%w/v of polymer concentrations shows highest drug release (68.27±0.19%) compare to L5 (57.79±0.21%) and L6 (50.32±0.19%) which contains 4%w/v and 5%w/v, respectively. This appears to be due to an increase in the thickness of the polymer matrix with an increase in polymer concentration. Obtained results of batches PE 1 to PE 7 revealed that permeation increase with an increase in the concentration of essential oils. This is evident from the LH permeation at 16 hrs from formulations PE 2 to PE 7 containing LO, JO and PSO with two different concentrations 10%w/w and 20%w/w. The results in (Fig. 6) also indicate that LO and JO were not sufficient to achieve the desire permeation flux for the controlled release of LH up to 16 hrs. On the other hand, 20% w/w PSO achieves the nearer targeted flux value 157.24 µg/cm^2/hr**,** it was sufficient for controlled release of LH up to 16 hrs and to maintain therapeutic plasma level. Thus, PSO's concentration was selected as one independent variable for further study. Briefly, the effect of the above formulation variables on LH release in the preliminary trial suggested that the amount of LH released from the patch increase with an increase in LH concentration, PSO concentration and decreased with an increase in polymer concentration.

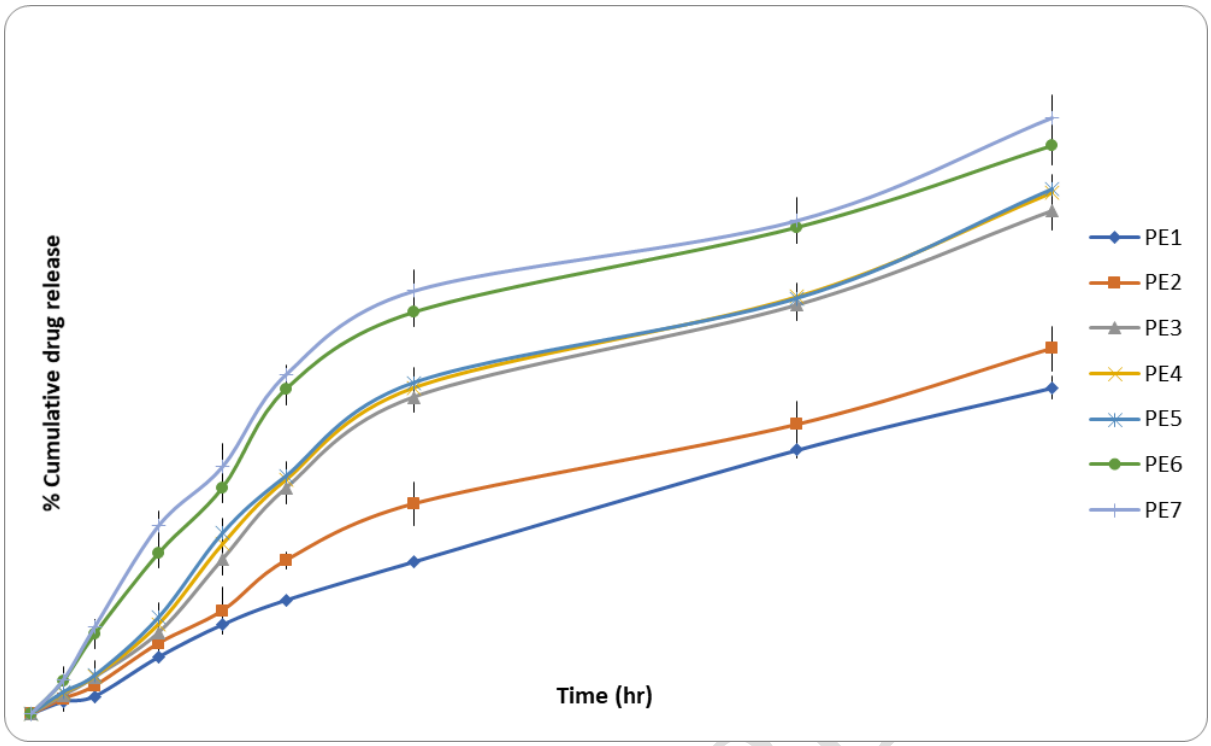

Fig 6. Comparative Drug Release Profile of Batches PE1-PE7

3.3 Statistical optimization of the formulation variables

Based on the results of the preliminary studies further evaluations of formulation variables performed using experimental designs to optimize a suitable combination of independent factors on the fabrication of transdermal matrix patch of LH having a desired rate of drug release as well as permeation flux. A 3^2 full factorial design nine batches are summarized in (Table 2). Drug release at 1hr (Q1), at 16^{th} hr (Q16) and tensile strength selected as dependent variables to find out the final formulation for the LH containing transdermal matrix patch. Prepared nine batches P1 to P9 further evaluated for the physicochemical properties of Matrix patch. Cumulative drug release of LH from Matrix patch and its permeation through the rat skin shown in (Fig. 7), results of dependent variables listed in (Table 3).

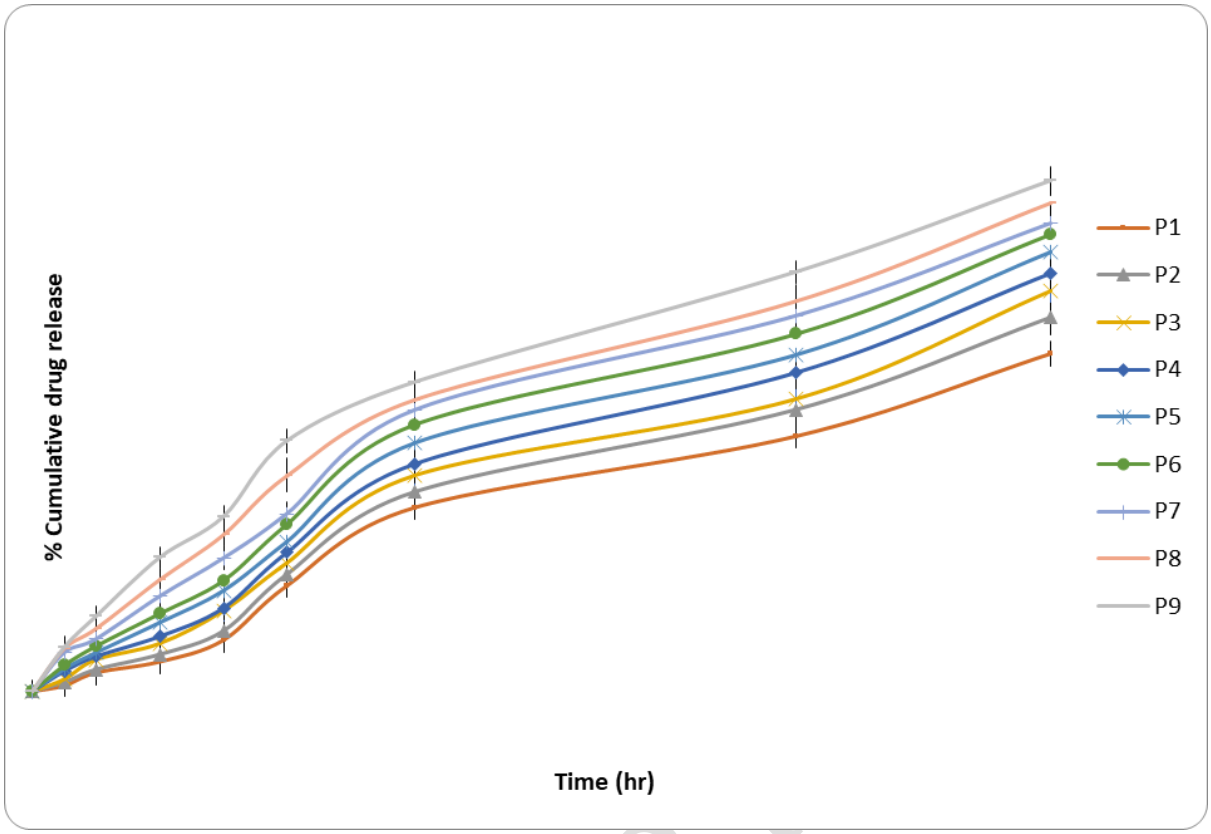

Fig.7. Comparative Drug Release Profile of Batches P1-P9

3.4 Physicochemical evaluations of LH containing transdermal matrix patch

Transparent, flat, flexible and uniform transdermal diffusional matrix patch obtained using a mixture of natural polymer psyllium and synthetic polymer HPMC K15M. The average weight of batches P1 to P9 ranges between 362±1.732 to 524±2.31 mg, which indicates that all the solid excipients uniformly dispersed into the liquid and all batches were relatively in similar weights. The thickness of the patches measured by micrometer screw gauze results found in between 0.81±0.04 to 0.88±0.13 mm. The results revealed that the solution was uniformly cast on a previously lubricated Petri plate and solvent uniformly evaporated from the Petri plate. The drug content of the entire batches lie between 92.85 to 97.60 %, these results revealed that the method select for the preparation of the matrix patch was suitable and reproducible. The results of the flatness study showed that all the batches have the same length before and after cuts. Therefore, nearer to 100% flatness was obtained and it indicates that all patches had a smooth surface. Tensile strength was found between 7.03±0.136 to 8.48±0.127 gm/cm^2, which revealed that the patch had sufficient mechanical strength to withstand during handling, transportation and administration. The same way results of folding endurance study revealed that the patch would not break and maintain their integrity with general skin folding applied. The results are listed in (Table 4).

Table 4: Physicochemical Evaluation of LH Loading Batches P1 to P9. Average of Triplicate Results. Mean ± SD(Standard Deviation).

Batch Code	Weight variation(mg)	Thickness (mm)	Folding endurance	Tensile strength (kg/cm^2)	% Elongation	%moisture Uptake	% moisture Loss
P1	362 ± 1.732	0.84±0.03	335±3.511	7.78±0.15	17.96±0.587	1.88 ± 0.07	2.84 ± 0.09
P2	408 ±2.516	0.86±0.08	348±3.7859	7.03±0.136	18.93±0.450	2.16 ± 0.20	2± 0.06
P3	457 ± 1.527	0.88±0.02	361±3.605	7.21±0.245	18.96±0.585	2.68± 0.06	2.23± 0.03
P4	387 ± 2.087	0.85±0.09	390±4.041	7.32±0.359	19.26±0.351	2.32± 0.08	2.72± 0.05
P5	433 ± 1.127	0.85±0.01	398±6.0277	8.16±0.245	19.50±0.684	2.89± 0.05	2.85± 0.05
P6	486 ± 1.527	0.88±0.07	397±4.00	7.17±0.125	20.46±0.493	2 ± 0.36	1.88± 0.08
P7	425 ± 2.00	0.86±0.11	373±3.605	7.91±0.183	22.50±0.458	1.98 ± 0.11	1.98± 0.09
P8	478 ± 1.527	0.81±0.04	378±2.00	7.76±0.15	20.98±0.602	2.83± 0.05	2.85± 0.06
P9	524 ± 2.51	0.88±0.013	384±3.21	8.48±0.127	21.84±0.335	2.50 ± 0.06	2.5± 0.13

3.5 Ex-vivo skin permeation study of LH

Permeation studies plot of the cumulative amount of drug release versus time was generated and represented in (Fig. 7), from this plot permeation flux, permeability coefficient and enhancement ratio were calculated. The results are listed in (Table 5). The results revealed that batch P9 containing 30%w/w of PSO exhibited the highest flux 164.09 ±0.14 µg/cm^2/hr and 89.93% drug release in 16 hrs. This higher release and permeation occur due to the presence of higher content of fatty acids of PSO. The results of the e*x-vivo* release also suggested that the concentration of PSO and PG both had a major influence on drug release because fatty acids of PSO increases the lipid fluidity and PG water fluidity. Data of e*x-vivo* release fit into different kinetic models to find out release mechanism, the release profiles of the drug seemed to follow zero-order and drug release mechanism was diffusion controlled so, it followed the Higuchi model. The correlation coefficient of R^2 values of batch P9 was r^2 = 0.9976 for zero-order and r^2 = 0.9733 for Higuchi model. A plot of kinetic studies represented in (Fig. 8-9).

Table 5. Results of LH Transdermal Flux and Lag Time, Permeability Coefficient, Diffusion Coefficient, and Enhancement Ratio of Batches P1 to P9.
Average of Triplicate Results. Mean ± SD(Standard Deviation).

Batch Code	Transdermal Flux Jss (µg/cm^2/hr) ±SD	Lag time (hours)	Permeability Coefficient (Kp) (cm/hr) ±SD	Diffusion Coefficient (D) (cm/h×10-8) ±SD	Enhancement Ratio
P1	97.30±0.11	1.25±0.11	1.21×10 $^{-3}$ ±0.21	0.01306±0.11	1.223±0.01
P2	99.17±0.12	1.30±0.12	1.29×10 $^{-3}$ ±0.22	0.01398±0.12	1.113±0.02
P3	119.6±0.12	1.34±0.12	1.48×10 $^{-3}$ ±0.22	0.01416±0.13	1.343±0.03
P4	117.6±0.13	1.35±0.13	1.46×10 $^{-3}$ ±0.23	0.0154±0.14	1.314±0.04
P5	127.4±0.14	1.38±0.14	1.58×10 $^{-3}$± 0.24	0.0156±0.15	1.426±0.05
P6	129.6±0.15	1.29±0.01	1.61×10 $^{-3}$ ±0.25	0.022±0.16	1.449±0.06
P7	137.8±0.16	1.31±0.11	1.71×10 $^{-3}$ ±0.26	0.023±0.17	1.539±0.07
P8	151.2±0.17	1.30±0.12	1.78×10 $^{-3}$ ±0.27	0.026±0.18	1.606±0.08
P9	164.3±0.18	1.25±0.2	1.86×10 $^{-3}$ ±0.28	0.0345±0.19	1.674±0.09

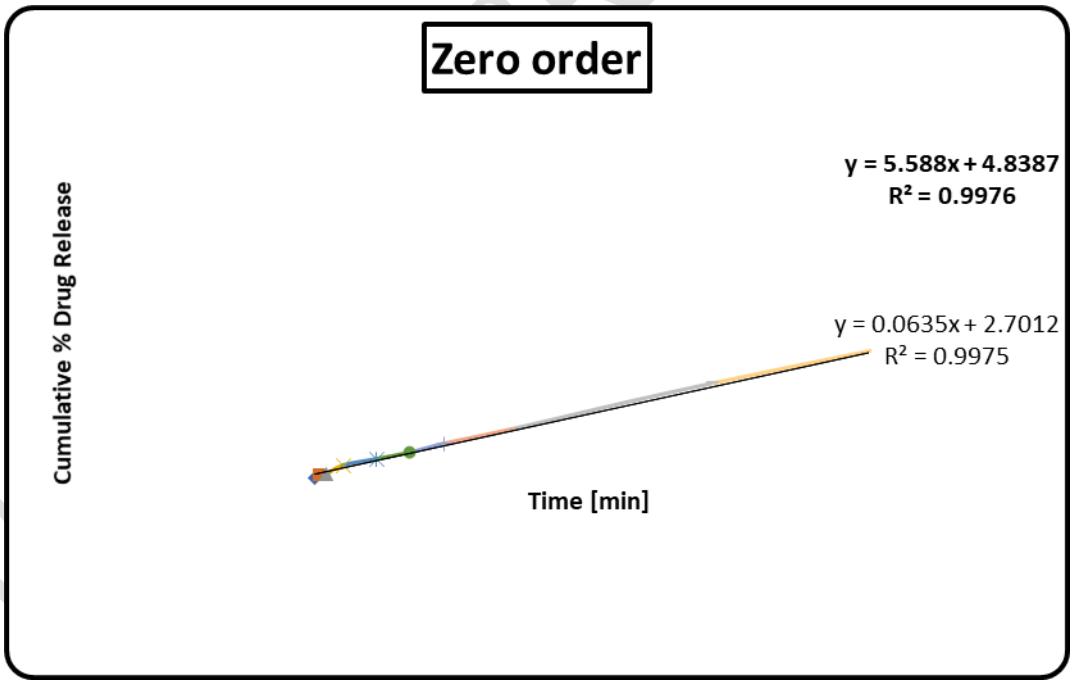

Fig. 8. Zero-Order Plot for Model Release Kinetic for Batch P9

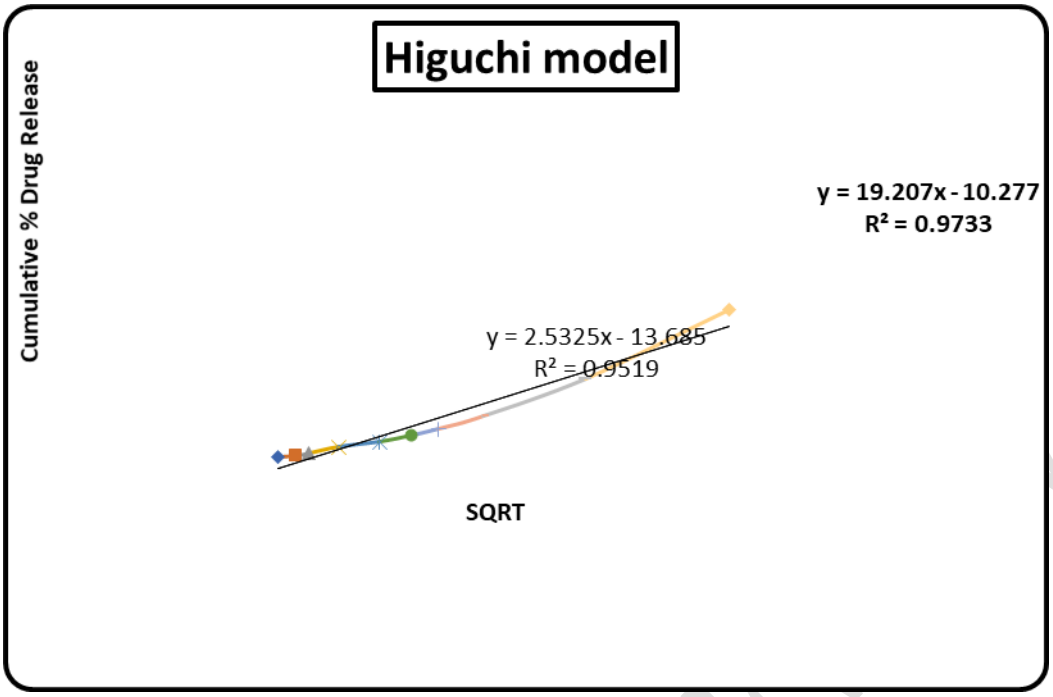

Fig. 9. Higuchi Plot for Model Release Kinetic of Batch P9

3.6 Regression analysis of the optimization of formulation

Based on the values of dependent variables polynomial equations generated and listed with 3D and contour response surface plots, which indicates that both the formulation variables X1 and X2 played an important role in a controlled release of drugs from the transdermal matrix patches. Tensile strength increases with the optimum concentration of PG (20%w/w) and PSO (30%w/w). Obtained results revealed that the selected model was significant and the drug release in a controlled manner for a period of 16 hrs.

3.7 *In-vivo* skin irritation study

The results of the *in-vivo* skin irritation study suggested that optimized batch P9 showed no irritation on rat skin after 16 hrs.

3.8 Stability study

The optimized batch P9 was exposed to stability studies as per ICH guidelines. The results listed in (Table 6) and revealed that prepared patches stable and maintain their physical integrity throughout the study.

Table 6: Stability Studies Results of Optimized Batch P9.

Stability conditions	Sampling time	Folding endurance	Drug content uniformity (%)	Ex-vivo drug release (%)	Visual appearance
Room Storage (30±2˚C and 65±5% RH)	Initial (0 day)	398±1.52	98.39±0.65	93.14±0.85	Clear homogeneous appearance
	After 15 days	398±1.06	98.11±0.21	93.10±0.80	Clear homogeneous appearance
	After 30 days	398±2.64	98.34±0.65	93.12±0.09	Clear homogeneous appearance
	After 90 days	397±2.98	98.77±0.65	93.07±0.89	Clear homogeneous appearance
	After 180 days	397±3.65	98.12±0.12	92.94±0.54	Clear homogeneous appearance
Accelerated condition (40±2˚C and 75±5% RH)	Initial (0 day)	198±1.52	98.45±0.65	90.37±0.88	Clear homogeneous appearance
	After 15 days	218±1.98	98.89±0.35	82.12±0.42	Clear homogeneous appearance
	After 30 days	197±2.98	98.37±0.64	85.07±0.87	Clear homogeneous appearance
	After 90 days	193±3.65	97.19±0.17	86.34±0.54	Clear homogeneous appearance
	After 180 days	230±1.52	96.45±0.65	89.65±0.82	Clear homogeneous appearance

4. DISCUSSION

The aim of the present investigation was to fabricate and evaluate transdermal patch of Lercanidipine hydrochloride (LH) for the treatment of hypertension. In this study, a patch was prepared using psyllium and HPMC K15M by a solvent evaporation method. To overcome the barrier of stratum corneum for drug permeation through the skin, essential oils namely linseed oil, jojoba oil and pumpkin seed oil used as permeation enhancers. Final optimized batch had very good transparency, mechanical strength and compatibility with other ingredients. The controlled release of drug up to 16 hrs, well-observed physicochemical and mechanical properties of matrix patch would be beneficial for the treatment of hypertension with more patient compliance. The use of natural permeation enhancer and polymer makes this research work novel and attractive because they are naturally available with numbers of benefits as well as more economical, capable with any type of physical and chemical modifications.

To study the compatibility between drug and excipients Fourier transforms infrared spectroscopy and differential scanning Calorimetry study performed and obtained results shows the absence of any type of interactions. The transdermal patch of Lercanidipine hydrochloride was prepared with HPMC K15M (200 mg), Psyllium (100 mg), pumpkin seed oil (30 %w/w of dry wt of polymer), propylene glycol (20 % w/w of dry wt of polymer) by a solvent evaporation method. This method prepared matrix diffusion controlled transdermal patch which will improve the therapeutic efficacy and safety of drugs by more precise (i.e. site-specific) placement within the body and releasing the drug at a controlled rate into the systemic circulation without the use of electric current or other electrically heated devices which may distort the stratum-corneum. Prepared drug-loaded patches were evaluated for all physicochemical and mechanical parameters such as folding endurance, tensile strength, flatness, thickness, hardness, weight variation, % moisture uptake and loss, drug content, *ex-vivo* permeation study using Wistar rat, skin irritation study and stability study. Folding endurance and tensile strength

of optimized batch P_9 found to be 384± 3.21 and 4.55 kg /cm2 respectively, these obtained results indicate that prepared patches have sufficient mechanical strength.
To study the drug release from the matrix patch the *ex-vivo* skin permeation study was performed using Franz-diffusion cell, Wistar rat skin was used as membrane and phosphate buffer pH 6.8 uses as a receptor medium at 37°C. Drug permeation of LH optimized batch was found to be 89 %. Skin irritation study confirmed the non-irritant nature of the patch. Stability study showed that transdermal patch which containing psyllium and pumpkin seed oil was stable at accelerated conditions (40°C and 75 % RH) for 180 days. Thus, the patch was successfully prepared for overcoming the drawbacks of oral administration of selected antihypertensive drugs.

5. CONCLUSION

LH is the potent antihypertensive agent and very widely used in the treatment of hypertension but due to the first-pass hepatic metabolism drug bioavailability decreases. Therefore, in the present study the transdermal patch of LH was prepared which showed acceptable physicochemical and satisfactory *ex-vivo* controlled release after 16 hrs could be helpful for the treatment of hypertension with improved patient compliance. Even though, extensive clinical studies are required to prove control release of LH from the transdermal matrix patch.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ABBREVIATIONS USED

LH-Lercanidipine hydrochloride
PSO-Pumpkin seed oil
LO-Linseed oil
JO-Jojoba oil
PG-Propylene Glycol
CPSCEA-Committee for Purpose of Control and Supervision of Experiments on Animals
FTIR-Fourier Transform Infrared Spectroscopy
RH-Relative Humidity
DSC- Differential Scanning Calorimetry
mg-Milligram
mL-Milliliter
hrs-Hours
wt-Weight

7. COMPETING INTERESTS DISCLAIMER:

8.

9. Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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