DEVELOPMENT AND CHARACTERIZATION OF SILDENAFIL CITRATE LOADED TRANSDERMAL PATCHES FOR TREATMENT OF PEDIATRICS PULMONARY ARTERIAL HYPERTENSION USING QBD APPROACH

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Abstract
The goal of this research work was to develop sildenafil citrate loaded transdermal patch to prolong the drug's release, enhance bioavailability, and improve patient compliance. A variety of formulas were created using design of experiment method by Solvent casting process with Duratak 2516 polymer, TEC plasticizer, and a skin Permeation enhancer containing 20% IPM. The thickness, tensile strength, folding endurance, percent elongation, percent moisture content, percent moisture absorption, percent drug content, *in vitro* drug release, *in vitro* permeation, and drug excipient compatibility of the optimised formulations were all examined. The effect of varying the concentrations of Duratak 2516 polymer (X1) and Triethyl citrate (percent) concentration (X2) on the responses, Thickness (Y1), percent Drug content (Y2), and percent Moisture uptake (Y3), percentage drug released in 48 hours, was investigated using a three level two full factorial design method. To determine the kinetics of drug release, *in vitro* release data was fitted to multiple models. For dependent variables, regression analysis and analysis of variance were used. To choose the best batch, the statistics between factorial design batches and the theoretical profile were employed. Batch F9 was deemed the best batch since it contains Duratak 2516 and TEC (20%) and had a release rate of 99 percent for up to 48 hours.
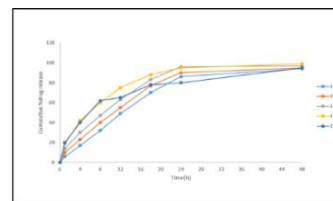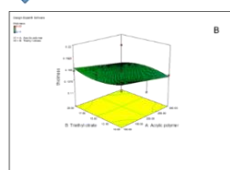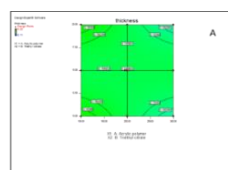
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Graphical Abstract
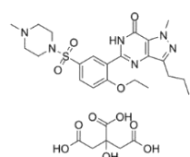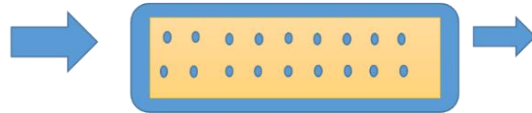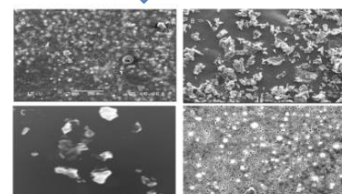

Sildenafil citrate → **Transdermal Patch** → *In Vitro* **Dissolution profile**

QbD Approach Optimised Batch **SEM Study of Patch**

Keywords: Transdermal patch; Skin permeation enhancer; *In vitro*; Sildenafil; Transdermal delivery, Pediatric pulmonary arterial hypertension (PAH).

Abbreviation:
Sildenafil (SNF)
Transdermal drug delivery system (TDDS)
Cyclic guanosine monophosphate (cGMP)
Triethyl citrate (TEC),
Acetyl tributyl citrate (ATBC)
Duratak 2516 (DUT)
Psyllium (PSY)
Eudragit RL 100 (ER)
Ethyl cellulose (Ethocel) (EC)

1. Introduction

Pulmonary hypertension (PH) is a disease characterized by elevated pulmonary artery pressure, which can result in right ventricular failure. In children, PH is most commonly associated with underlying cardiac or lung disease (eg, bronchopulmonary dysplasia [BPD]). Pediatric pulmonary arterial hypertension (PAH) shares common features of adult disease, Pediatric Pulmonary arterial hypertension is a rare blood vessel disorder of the lung in which the pressure in the pulmonary artery (the blood vessel that leads from the heart to the lungs) rises above normal levels. An increase of the number of smooth muscle cells in the walls of small lung arteries (a phenomenon called proliferation) that are remodeling the vessels, may lead to obstructions in the microcirculation, which will then lead to an increase in the blood pressure. Chronic thromboembolic pulmonary hypertension is a complication representing less than 1% of all cases of acute pulmonary embolism (the sudden blocking of a lung artery by a clot or foreign material which has been brought to its site by the blood current), which directly leads to pulmonary hypertension. Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension are chronically debilitating and life-threatening.

Sildenafil (SNF) protects cyclic guanosine monophosphate (cGMP) from degradation by cGMP-specific phosphodiesterase type 5 (PDE5) in the corpus cavernosum. Nitric oxide (NO) in the corpus cavernosum of the penis binds to guanyl ATB Cyclase receptors, which results in increased levels of

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cGMP, leading to smooth muscle relaxation (vasodilation) of the intimal cushions of the helicine arteries (Kukreja RC et al., 2012).

Transdermal drug delivery system (TDDS) is the topically administered medications in self-contained, discrete dosage forms of patches which when applied to the skin deliver the drug, through the skin portal to systemic circulation at a predetermined and controlled rate over a prolonged period of time in order to increase the therapeutic efficacy and reduced side effect of drug (Ali S et al., 2015). TDDS maintains drug concentration within the therapeutic window for prolong period of time ensuring that drug levels neither fall below the minimum effective concentration nor exceed the maximum effective concentration. It means the delivery of drugs across the skin and into the systemic circulation taking advantage of the relative accessibility of the skin, is altogether different from topical drug delivery which can only target the local affected areas (Kriplani P et al., 2021). The thorough understanding of morphological, biophysical and physicochemical structure and properties of the skin is immensely important in order to deliver therapeutic agents through the human skin for systemic and desired effects (Thakur G et al., 2016).

Transdermal delivery provides an important edge over any other forms of injectable and oral routes by increasing patient compliance and avoiding first pass metabolism (FPM) respectively. At one side it provides controlled and sustained administration of drug and at the other side it allows continuous input of drugs with short biological half-life thereby avoiding pulsed entry into systemic circulation, which often causes undesirable side effects (Indermun S et al., 2014). It will be convenient, especially notable in patches which require only once weekly application. With such a simple dosing regimen, the patient compliance and adherence to drug therapy gets aided. Overall, with all its beneficiary properties TDDS can be considered as a potential alternative to oral as well as other routes of drug administration (Bénès L et al., 1997).

2. Materials and methods

Table 1: Materials used

Name of Ingredients	Name of Manufacturer
Sidenafil citrate (SNF)	MSN Organic Ltd, Hyderabad.
Acrylate polymer (Durotak 2516)	Henkel, USA
Isopropyl myristate	BASF, USA
Triethyl citrate (TEC)	Merck, USA
Acetyl tributyl citrate (ATBC)	KLJ Group, Gujrat, India
Ethyl cellulose (EC)	SD Fine Chem Ltd., Mumbai, India
Mineral Oil (Drakeol 7 LT)	Henkel, USA
Silicon Oil	Supreme Silicons, Pune, India
Psyllium	SD Fine Chem Ltd., Mumbai, India
Eudragit RL 100 (ER)	Evonic Degussa, Mumbai, India
Supor® Membrane filter	PALL Life Sciences, Mumbai, India

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2.1. Methods

2.1.1. Optimization of concentration of Excipients for Formulation of sildenafil citrate Loaded Transdermal Matrix Patch

2.1.1.1. Screening Trial Batches for Selection of Polymers & Plasticizer
Preliminary trial batches prepared for selection of patch forming polymers with suitable plasticizer and their concentrations. For response surface analysis of variance was generated by Design Expert 12.0 software. Trial batches for screening were prepared with following polymers levels and plasticizer levels-

Table 2: Selected Polymers & Plasticizer for study

Polymers		Plasticizer	
Name	Range (mg)	Name	Range (% w/w of polymer)
Acrylate polymer (Duratak 2516) (DUT)	100 – 500	Triethyl citrate (TEC)	10 – 30
Ethyl cellulose (Ethocel) (EC)	100 – 500	Acetyl tributyl citrate (ATBC)	10 – 30
Eudragit RL 100 (ER)	100 – 500	-	
Psyllium (PSY)	100 – 500		

Compositions of different formulations represented in **Table 3**. Prepared patches evaluated for physicochemical parameters.

Table 3: 2^2 full factorial design for screening trial of Polymers & Plasticizers

Trials	Level			
	Factor	-1	0	+1
Set 1	DUT	100	250	500
	ATBC	10	20	30
Set 2	DUT	100	250	500
	TEC	10	20	30
Set 3	EC	100	250	500
	ATBC	10	20	30

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Set 4	EC	100	250	500
	TEC	10	20	30
Set 5	EUR	100	250	500
	ATBC	10	20	30
Set 6	EUR	100	250	500
	TEC	10	20	30
Set 7	PSY	100	250	500
	ATBC	10	20	30
Set 8	PSY	100	250	500
	TEC	10	20	30

2.2.2. Conclusion of Screening Trial Batches for Selection of Polymers & Plasticizer

Table 4: Summary of data of screening batches

No. of trials	Factor 1 Polymer (100 – 500 mg)	Factor 2 Plasticizer (10 – 30 % of polymer)	Statistical evaluation		Physical evaluation	
			Response 1 Thickness (mm)	Response 2 Drug content %	Response 3 % Flatness	Response 4 Folding endurance (Nos.)
7	Acrylate polymer (Duratak 2516) (DUT)	Acetyl tributyl citrate (ATBC)	0.09 – 0.18	88 - 97	90 – 98	335 – 365
7	Acrylate polymer (Duratak 2516) (DUT)	Triethyl citrate (TEC)	0.10 – 0.16	90 – 103	93 – 99	330 -360
7	Ethyl cellulose (Ethocel) (EC)	Acetyl tributyl citrate (ATBC)	0.12 – 0.18	92 – 100	91 – 97	330 - 378

No. of trials	Factor 1 Polymer (100 – 500 mg)	Factor 2 Plasticizer (10 – 30 % of polymer)	Statistical evaluation		Physical evaluation	
			Response 1 Thickness (mm)	Response 2 Drug content %	Response 3 % Flatness	Response 4 Folding endurance (Nos.)
7	Ethyl cellulose (Ethocel) (EC)	Triethyl citrate (TEC)	0.14 – 0.20	92 – 98	93 – 98	290 – 366
7	Eudragit RL 100 (ER)	Acetyl tributyl citrate (ATBC)	0.11 – 0.17	91 – 97	90 - 98	300 - 362
7	Eudragit RL 100 (ER)	Triethyl citrate (TEC)	0.13 – 0.19	93 – 99	96 – 100	360 - 410
7	Psyllium (PSY)	Acetyl tributyl citrate (ATBC)	0.10 – 0.17	89 – 95	90 – 95	250 – 364
7	Psyllium (PSY)	Triethyl citrate (TEC)	0.13 – 019	90 – 96	92 – 97	255 - 369

From above data, it can be concluded that combination of Acrylate polymer (Duratak 2516) (DUT) and Triethyl citrate (TEC) shows optimum results for all four response parameters based on statistical & physical evaluation.

2.2.3. Optimization of Polymers concentration on the basis of Evaluation parameters
Preliminary trial batches DUT1 to PS5 were prepared for the selection of patch forming polymer and its concentration.

Table 5: Preliminary trial batches for Optimization of Polymer concentration

Ingredient(s) (mg)	DUT1	DUT2	DUT3	DUT4	DUT5
SNF	75	75	75	75	75
Acrylic polymer (Duratak 2516)	100	200	300	400	500

Ingredient(s) (mg)	EC1	EC2	EC3	EC4	EC5
SNF	75	75	75	75	75
EC	100	200	300	400	500

Ingredient(s) (mg)	ER1	ER2	ER3	ER4	ER5
SNF	75	75	75	75	75
ER	100	200	300	400	500

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Ingredient(s) (mg)	PS1	PS2	PS3	PS4	PS5
SNF	75	75	75	75	75
Psyllium	100	200	300	400	500

Table 6: Physicochemical Evaluation Parameters of Batches DUT1 to PS5

Batch code	Thickness (mm)	Drug content (%)	Flatness (%)	Folding Endurance (Nos)
DUT1	0.11±0.002	96.3 ± 0.3	95 ± 1	358 ± 4
DUT2	0.12±0.003	98.5 ± 0.4	96 ± 1	355 ± 5
DUT3	0.13±0.002	97.2 ± 0.1	94 ± 2	352 ± 2
DUT4	0.14±0.001	95.6 ± 0.1	93 ± 1	352 ± 5
DUT5	0.14±0.003	96.7 ± 0.4	94 ± 1	355 ± 7
EC1	0.10±0.002	95.4 ± 0.2	98 ± 2	365 ± 4
EC2	0.11±0.001	96.9 ± 0.1	95 ± 1	345 ± 5
EC3	0.12±0.001	98.3 ± 0.2	94 ± 1	356 ± 6
EC4	0.14±0.002	96.1 ± 0.2	93 ± 1	352 ± 6
EC5	0.15±0.003	97.6 ± 0.3	94 ± 2	354 ± 3
ER1	0.09±0.001	96.9 ± 0.1	96 ± 2	358 ± 4
ER2	0.11±0.002	98.1 ± 0.2	97 ± 1	348 ± 4
ER3	0.12±0.002	96.3 ± 0.1	91 ± 1	342 ± 5
ER4	0.14±0.003	95.4 ± 0.2	93 ± 1	342 ± 6
ER5	0.14±0.001	95.1 ± 0.2	92 ± 1	346 ± 4
PS1	0.11±0.001	97.9 ± 0.1	93 ± 2	338 ± 6
PS2	0.13±0.002	96.5 ± 0.2	94 ± 1	364 ± 4
PS3	0.13±0.003	95.3 ± 0.2	95 ± 1	250 ± 5
PS4	0.15±0.002	96.3 ± 0.2	96 ± 1	252 ± 5
PS5	0.16±0.001	98.1 ± 0.1	94 ± 2	253 ± 6

Discussion:
Results of physicochemical parameters of batches DUT1 to PS5 represent in table 6. The results suggested that there was no much difference in thickness of all the batches. Which revealed that thickness were increases as concentration of polymer increases. Drug content results also found uniform in all batches in a range of 95 % to 98 %, it suggested that the drug and polymers uniformly distributed in matrix dispersion. It also suggested that method of preparation was suitable for the formulation of transdermal matrix patch of SNF. An obtained result of flatness study suggested that the length of patch strip, before and after cuts were remain same. Results found in a range of 97 % to 98 %. Overall, the results of preliminary trial batches suggested that batches prepared with DUT shows good mechanical properties compare to other polymers but poor adhesive properties. On the other side batches prepared with psyllium shows very good adhesive and mechanical strength, but psyllium alone was not sufficient to prepare flexible, uniform and transparent patches. Therefore, to improve physicochemical properties of prepared patches attempt was made to use Acrylic polymer (DUT) and polymer fixed weight ratio as one independent factor X1 for the optimization of final formulation.

2.2.4. Preliminary Trial Batches for the Optimization of Plasticizers
Preliminary trial batches were prepared for the selections of plasticizer concentrations. Here, two plasticizers namely Triethyl citrate (TEC1 to TEC5), Acetyl tributyl citrate (ATBC1 to ATBC5) were tried at 5 different concentrations, 10 % w/w, 15 % w/w, and 20 % w/w, 25 % w/w, 30 % w/w respectively. All the batches were prepared with 300 mg Acrylic polymer (Duratak 2516), 75 mg sildenafil citrate, 2 ml ethanol and 8 ml water.
Compositions of different formulations represented in **Table 7**. Prepared patches evaluated for physicochemical parameters.

Table 7: Preliminary Trial Batches for Plasticizers Ingredient(s) (%w/w)

Ingredient(s) (mg)	TEC1	TEC2	TEC3	TEC4	TEC5
SNF	75	75	75	75	75
TEC	10	15	20	25	30

Ingredient(s) (mg)	ATBC 1	ATBC 2	ATBC 3	ATBC 4	ATBC 5
SNF	75	75	75	75	75
ATBC	10	15	20	25	30

2.2.4.1. Optimization of Plasticizers concentration
Preliminary trial batches TEC1 to TEC5, ATBC 1 to ATBC 5 were prepared for the selection of suitable plasticizer and its concentration for the preparation of transdermal matrix patch of sildenafil citrate Results of physicochemical parameters represented in table 8.

Table 8: Physicochemical Evaluation Parameters of Patches from TEC1 to ATBC5

Batch code	Thickness (mm)	Drug content (%)	Flatness (%)	Folding Endurance (Nos)
TEC1	0.15 ± 0.002	96.2 ± 0.2	98 ± 2	308 ± 4

TEC2	0.13 ± 0.001	98.5 ± 0.1	94 ± 2	304 ± 3
TEC3	0.14 ± 0.002	97.7 ± 0.1	98 ± 1	304 ± 2
TEC4	0.15 ± 0.002	98.8 ± 0.2	95 ± 1	302 ± 3
TEC5	0.14 ± 0.001	97.5 ± 0.1	97 ± 2	304 ± 4
ATBC1	0.14 ± 0.003	95.6 ± 0.1	95 ± 1	224 ± 6
ATBC2	0.19 ± 0.002	95.7 ± 0.1	97 ± 1	247 ± 7
ATBC3	0.2 ± 0.002	95.1 ± 0.2	94 ± 2	252 ± 9
ATBC4	0.22 ± 0.001	95.8 ± 0.1	93 ± 2	269 ± 6
ATBC5	0.21 ± 0.002	95.4 ± 0.2	95 ± 2	282 ± 7

Discussion:

Results of batches TEC1 to TEC5 revealed that all the batches possess good physicochemical parameters. But the results of batches ATBC1 to ATBC5 suggested that obtained patches were having poor flexibility and mechanical strength compared to TEC formulations. Results of batches TEC1 to TEC5 showed that patches were possess good mechanical strength and all physicochemical parameters were in uniform range as shown in table. Therefore, for further study, among all two plasticizers, TEC with 10 % w/w weight of total dry weight of polymer was selected. Moreover, additional trials carried out with 5% and 7.5% concentration of TEC to select optimum concentration of TEC.

2.2.5. Selection of Permeation enhancers

The skin flux of SNF obtained without permeation enhancer was 77.082 µg / cm2 / hr and it was not sufficient to acquire targeted flux, to maintain the therapeutic concentration of drug up to predetermined period. Therefore, in this present research work suggested from literature review three permeation enhancers namely Isopropyl myristate, mineral oil (Drakeol 7LT), silicon oil were selected in the concentration of 10 % and optimized for the improvement in skin flux. Formulations evaluated with varying concentrations for the selection of effective permeation enhancer.

2.2.5.1. *In-Vitro* Release Testing (IVRT) or *In-Vitro* permeation study method by HPLC:

Table 9: Preliminary Trial Batches for permeation enhancer (s) (%w/w)

Ingredient(s) (mg)	IM	MI	SO
SNF (mg)	75	75	75
Acrylic polymer (mg)	300	300	300
TEC (%)	10	10	10
Isopropyl myristate (%)	10	--	--
Mineral oil (%)	--	10	--
Silicon oil (%)	--	--	10

IM – Patches prepared with Isopropyl Myristate as penetration enhancer
MI – Patches prepared with Mineral Oil as penetration enhancer
IM – Patches prepared with Silicon Oil as penetration enhancer

Table 10: Diffusion Cell Apparatus parameters

Instrument	:	Diffusion cell apparatus (Robotic diffusion station) Make: Teledyne Hanson Research, Model: Pheonix RDS.
Type	:	Vertical Diffusion Cell Apparatus
Sampling mode	:	Auto sampler
Membrane	:	25 mm, 0.45 µm Supor® Membrane filter, (Make-PALL Life Sciences P/N.- 60172)
Cell Volume	:	10 mL
Surface Area	:	1.0 sq. cm
Medium	:	0.09% NaCl aqueous solution: 99.9% Ethanol (7:3)
Stirrer speed	:	600 RPM
Bath temperature	:	32.5 + 0.5°C
Replacement volume	:	0.4 mL
Sampling/Collect volume	:	0.4 mL
Test length	:	48 Hours
Sampling time points	:	1, 4, 8, 12, 18, 24 & 48 hours

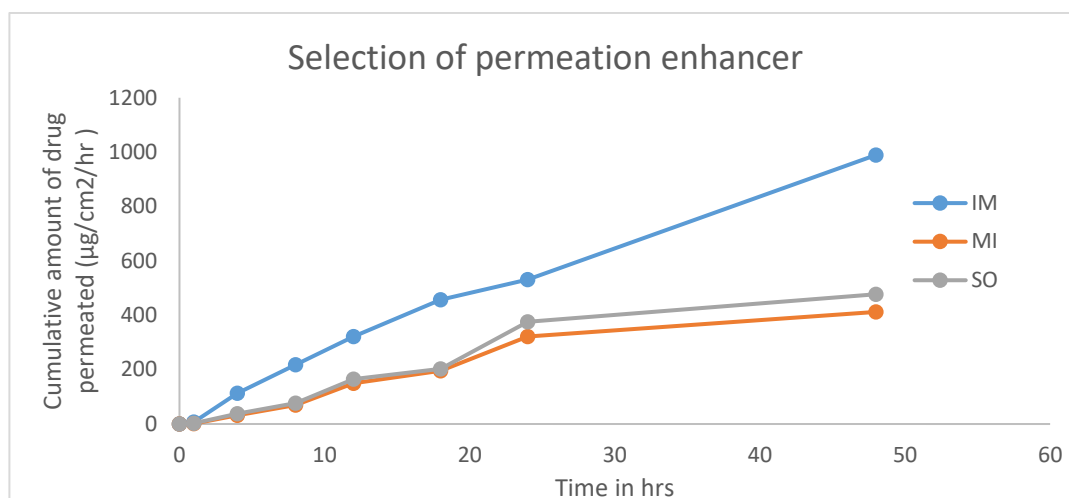| TIME (h)	Cumulative amount of drug permeated (µg/cm2/hr)		
	IM	MI	SO
1	7.52 ± 3.48	1.08 ± 0.23	2.81 ± 0.13
4	113.32 ± 9.87	32.16 ± 3.52	38.52 ± 1.45
8	218.23 ± 37.26	69.60 ± 8.30	76.54 ± 7.43
12	321.43 ± 23.36	149.34 ± 6.10	164.84 ± 7.56
18	457.50 ± 52.32	196.15 ± 12.35	203.15 ± 12.65
24	531.92 ± 45.28	322.34 ± 24.23	375.38 ± 12.03
48	989.42 ± 31.23	412.29 ± 12.23	477.23 ± 13.89

Figure 1: Amount of drug permeation at different time interval with different permeation enhancer

Discussion:
Results of batches IM, MI and SO revealed that all the batches possess different level of permeation through the cell membrane filter. Among the all three permeation enhancer Isopropyl myristate (IM) was showing significantly high drug permeation, whereas Mineral Oil and Silicon Oil shows almost similar and low permeation till the 48 hrs. Therefore, for further study, was planned with isopropyl mystriate with different concentration i.e 10%, 15% & 20% w/w weight of total dry weight of polymer to optimize the quantity of isopropyl mystriate in the patch.

2.2.6. Statistical Optimization of the Formulation Variables Using 3^2 full factorial Experimental Design
Preliminary trial batches were prepared and evaluated for the selection of various concentrations of polymers, plasticizers and permeation enhancers. Results of preliminary trial batches suggested that batches prepared with Acrylic polymer shows good mechanical properties. On the other side batches prepared with psyllium shows very good adhesive and mechanical properties, but psyllium alone was not sufficient to prepare flexible, uniform and transparent patches. Therefore, to improve physicochemical property of prepared patch attempt was try to use Acrylic polymer fixed weight ratio select as one independent factor X1 for the optimization of final formulation. The results of preliminary trial batches for the selection of permeation enhancers also suggested that among three selected, permeation of pure drug improve with isopropyl myristate and it was sufficient to achieve targeted flux to maintained therapeutic concentration and controlled release of drug for a predetermined period. Therefore, concentration of Triethyl citrate (TEC) as a plasticizer selected as another independent factor X2.
3^2 full factorial design were selected from Design Expert software 7.0 for the optimization of final formulation. This design involved three dependent variables (Y1, Y2, and Y3) and above mentioned two independent variables (X1 and X2). The dependent variables Y1 was *folding endurance* of prepared patches, Y2 was *drug content* and Y3 was *thickness*. The composition of 13 formulations based on this experimental design, after completion of statistical optimization experiments, polynomial equations and contour plots generated to study the effect of selected independent variables on dependent variables in order to identify the optimized drug loaded transdermal patch. The final identified batch prepared and subjected to validation of statistical optimization design.

2.2.6.1. Method of Preparation of Transdermal Matrix Patch of Batches F1-F8 Using 3^2 Full Factorial Designs

The transdermal matrix patches containing SNF were prepared using different concentration of Acrylic polymer. The polymers concentration was varied from 100 mg, 200 mg, to 300 mg than allowed to swell for two hrs in water. As per dose calculation and result of drug permeability study, accurately weighed amount of SNF 75 mg dissolved in ethanol and this drug solution added into the polymeric solution with continuous stirring using magnetic stirrer. Then Triethyl citrate (TEC) as per the table 9 and 10% Isopropyl myristate incorporated as plasticizer and penetration enhancer respectively. Inverted funnel was kept over the petri plate for uniform evaporation at room temperature for 24 hrs in dark condition, after complete drying biaxial oriented polyethylene film used as a backing membrane and a smooth glossy paper used as a release liner. Finally the prepared Single-layer drug in adhesive patches removed from the petri plate and cut into 4 cm^2 areas backing layer and release liner was attached and cover it with an aluminum foil. At last cover, patches put into zipper bag tightly close it and stored into desiccators for further evaluation studies.

Table 11: Formulation of SNF Loaded transdermal patch 3^2 Factorial Design Batches F1 to F8

Batch code	X1 Acrylic polymer (mg)	X2 Triethyl citrate (%)	Y1 Thickness (mm)	Y2 Drug content (%)	Y3 Moisture uptake (%)
F1	200	15	0.11 ± 0.002	98.1 ± 0.4	2.10 ± 0.13
F2	200	20	0.16 ± 0.001	97.8 ± 0.7	1.86 ± 0.05
F3	100	15	0.15 ± 0.003	98.6 ± 0.3	1.85 ± 0.12
F4	300	10	0.14 ± 0.002	99.1 ± 0.8	1.82 ± 0.21
F5	300	20	0.16 ± 0.001	96.3 ± 0.7	1.78 ± 0.25
F6	100	10	0.17 ± 0.001	95.6 ± 0.4	1.76 ± 0.21
F7	200	10	0.16 ± 0.002	92.3 ± 0.8	1.75 ± 0.12
F8	200	15	0.13 ± 0.003	96.8 ± 0.8	1.75 ± 0.11

Physicochemical Evaluations of Single-layer drug in adhesive patch of Batches F1 to F8

Factorially designed batches F1 to F8 were evaluated for following physicochemical parameters.

Table 12: Physicochemical Evaluation of SNF Loading Batches F1 to F8

Batch code	Thickness (mm)	Drug content (%)	Folding Endurance (%)	% moisture loss
F1	0.11 ± 0.002	98.1 ± 0.4	356 ± 3	2.2 ± 0.2
F2	0.16 ± 0.001	97.8 ± 0.7	345 ± 5	2.1 ± 0.2
F3	0.15 ± 0.003	98.2 ± 0.7	386 ± 2	1.9 ± 0.1

F4	0.14 ± 0.002	99.1 ± 0.8	354 ± 6	1.8 ± 0.2
F5	0.16 ± 0.001	96.3 ± 0.7	346 ± 7	1.9 ± 0.3
F6	0.17 ± 0.001	95.6 ± 0.4	389 ± 8	1.8 ± 0.2
F7	0.16 ± 0.002	95.6 ± 0.8	372 ± 8	1.7 ± 0.1
F8	0.13 ± 0.003	96.8 ± 0.8	351 ± 4	1.7 ± 0.3

Discussion

Transdermal matrix patch for sildenafil citrate was successfully prepared using Acrylate polymer (Duratak 2516) (DUT) as a patch forming polymers by solvent evaporation method and final drug loaded patch was found out by design of experiments from the software 7.0 of design expert. Prepared batches F1 to F8 were evaluated for different physiochemical parameters. Results of physicochemical parameters of batches F1 to F8 represented in table 12. Drug loaded films (4 m^2) were weighed using Digital electronic balance, Shimadzu, Japan. The weight of 4 cm^2 patches ranged from 351 ± 0.642 mg to 377 ± 0.345 mg. In all the cases, the calculated standard deviation values were very low which indicates that the prepared patches were uniform in weight, and therefore all the batches passed the weight variation as per limits given in USP. Obtained results suggested that the drug was uniformly dispersed in to polymeric dispersion. With the help of micrometer gauge, the thickness of patches were measured at six positions and the average thickness was note down. The result of batches F1 to F8 revealed that there were minor differences between the thicknesses of all the formulations, it obtained in between 0.11 ± 0.01 mm to 0.22 ± 0.04 mm. Batch F6 shows highest thickness and F1 shows lowest thickness, this happen due to the different in polymer concentration and distribution difference over the Petri plate. Drug content of the transdermal Single-layer drug in adhesive patches were performed to find out whether the loading of drug is uniform in the formulation. The results of drug content study was found in a range of 92.26 ± 0.21 to 98.56 ± 0.324 mg. Folding endurance of prepared patches was in range of 258 to 399 folds. Depending upon the concentration of TEC and ATBC, results of folding endurance were differed. Batches F8 shows highest folding endurance with 399 ± 1.6 indicates that the patches had sufficient mechanical strength and it would be remain as such during the treatment on the application site. The smoothness was measure manually for the prepared transdermal patch. An obtained result of flatness study suggested that the length of patch strip, before and after cuts was remain same and it shows 2 to 3% constriction in all the nine batches with 97.7 % to 98.9 % flatness. Prepared batches F1 to F8 evaluated for percentage moisture uptake and loss as well. The results shows that batch F8 exhibit lowest moisture uptake value of 1.75 ± 0.02 % which decrease chances of microbial contamination. Percentage moisture loss was in range of $1.96 \pm 1.2\%$, this low moisture loss prevents patches to become brittle.

2.3. Drug release Study

The formulation F5 was showed increase in the concentration of polymer, the rate of drug release decreased. This may be attributed to the previous finding that higher concentration of polymer may decrease the crystalline drug in patch and thus deceased drug releases. The highest cumulative percentage of drug release i.e. 97% was observed from formulation F3 in 48 hrs.

Table 13: *In-vitro* drug release of sildenafil citrate from transdermal patches

Time	Cumulative % drug release				
(h)	F1	F2	F3	F4	F5

1	15 ± 0.21	12 ± 0.50	20 ± 0.46	14 ± 0.15	12 ± 0.21
4	35 ± 0.46	30 ± 0.69	35 ± 0.35	31 ± 1.13	30 ± 0.40
8	42 ± 0.23	38 ± 1.73	50 ± 1.02	35 ± 0.63	32 ± 1.16
12	50 ± 1.28	45± 1.58	63 ± 3.05	45± 1.65	42 ± 2.81
18	62± 2.13	69± 2.08	83 ± 4.46	65± 3.11	63 ± 0.64
24	74 ± 1.77	85± 4.19	96 ± 1.78	85 ± 1.57	80± 1.13
48	87 ± 2.86	95 ± 3.53	97 ± 1.77	93 ± 2.28	89 ± 2.39
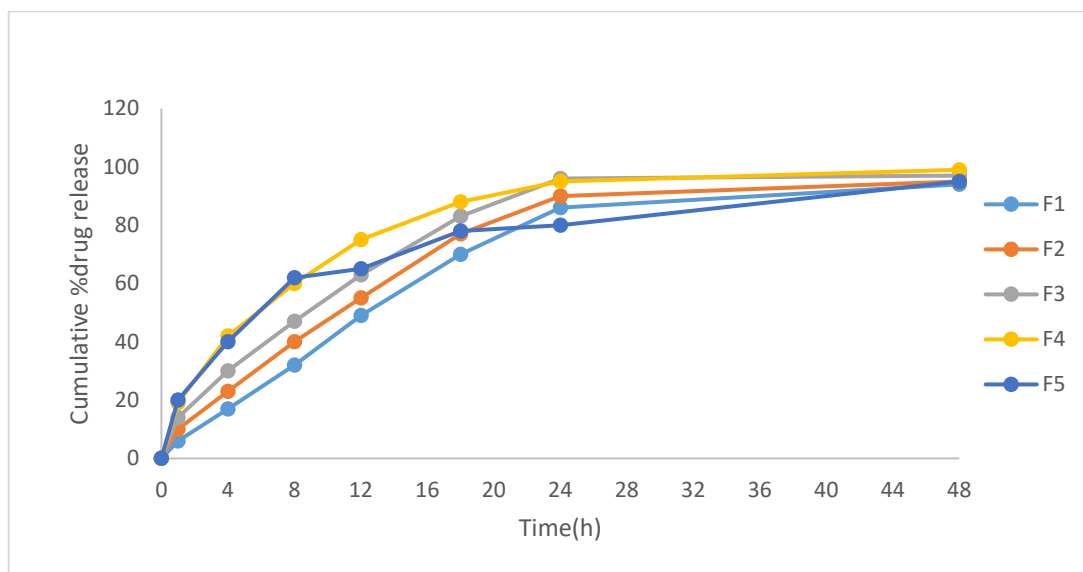
Figure 2: *In-vitro* drug release profile of sildenafil citrate from transdermal patches

Observation:
Formulation F3 was showing faster release among all formulation, formulation F2 and F4 were also showing drug release more than 90%, Hence these 3 formulation were used for *In-vitro* permeation study evaluation.

Table 14: *In-vitro* permeation study of sildenafil citrate from transdermal patches

Time	Cumulative amount of drug permeated (μg/cm2/hr)		
(h)	F2	F3	F4
1	3.24 ± 0.56	2.75 ± 0.42	3.48 ± 0.76
4	36.66 ± 2.12	30.34 ± 1.78	42.56 ± 3.32
8	79.28 ± 3.56	69.52 ± 2.98	89.61 ± 4.19
12	158.46 ± 7.19	143.19 ± 6.98	189.84 ± 9.67

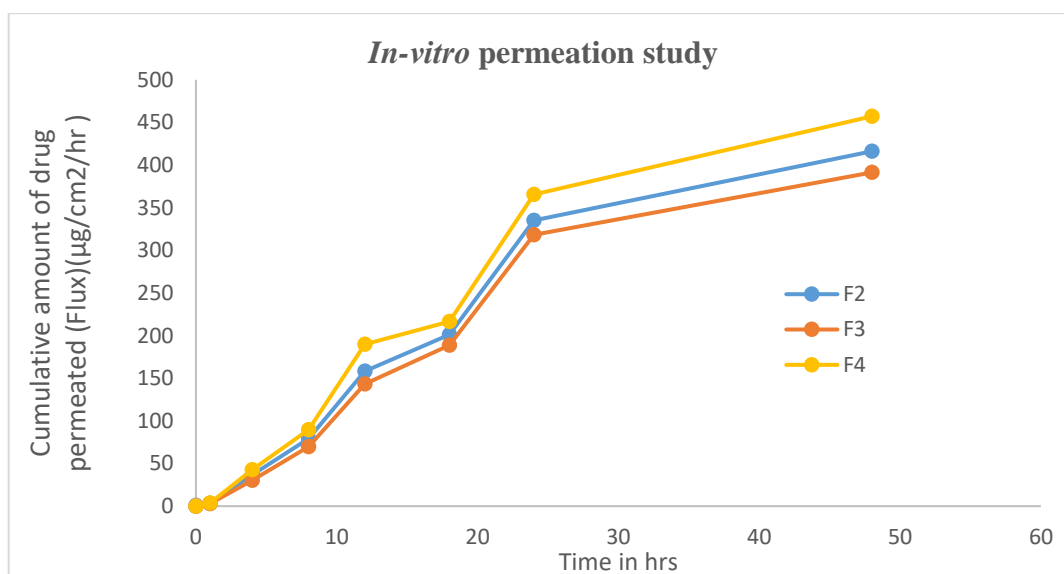|			
18	200.98 ± 10.98	188.57 ± 8.23	216.51 ± 11.45
24	335.23 ± 12.12	318.12 ± 10.26	365.48 ± 13.77
48	416.15 ± 15.53	391.39 ± 14.23	457.19 ± 16.11

Figure 3: *In-vitro* permeation study of sildenafil citrate from transdermal patches

Observation:
Formulation F4 was showing significantly more *in-vitro* permeation than the F2 and F3.

Conclusion:
Since, the formulation F4 was showing better *in-vitro* permeation among all other formulations, the formulation F4 was stored in the stability to evaluate further.

3.0. Effect of acrylic polymer:
3.1. Effect of acrylic polymer with respect to *In-Vitro* dissolution profile:
It was observed that the formulations containing acrylic polymer, release rate was found to be the patch which was having higher concentration of acrylic polymer was having more extend of drug release than the patch which was having lower concentration of acrylic polymer (98%, 93% and 90% drug release with 133%, 117% and 100% of acrylic polymer concentration respectively in 48 hrs.) in the transdermal patches. This was due to the nature of the acrylic polymer which tent to increase the solubility of drug substance.
The result of *in-vitro* drug release study is presented in table 15 and figure 4.

Table 15: *In-vitro* drug release of sildenafil citrate from transdermal patches containing polymer at different concentration of 133% (FA1), 117% (FA2) and 100% (FA3)

TIME (h)	Cumulative % drug release		
	FA1	**FA2**	**FA3**
1	10± 1.73	12± 1.5	12± 1.14

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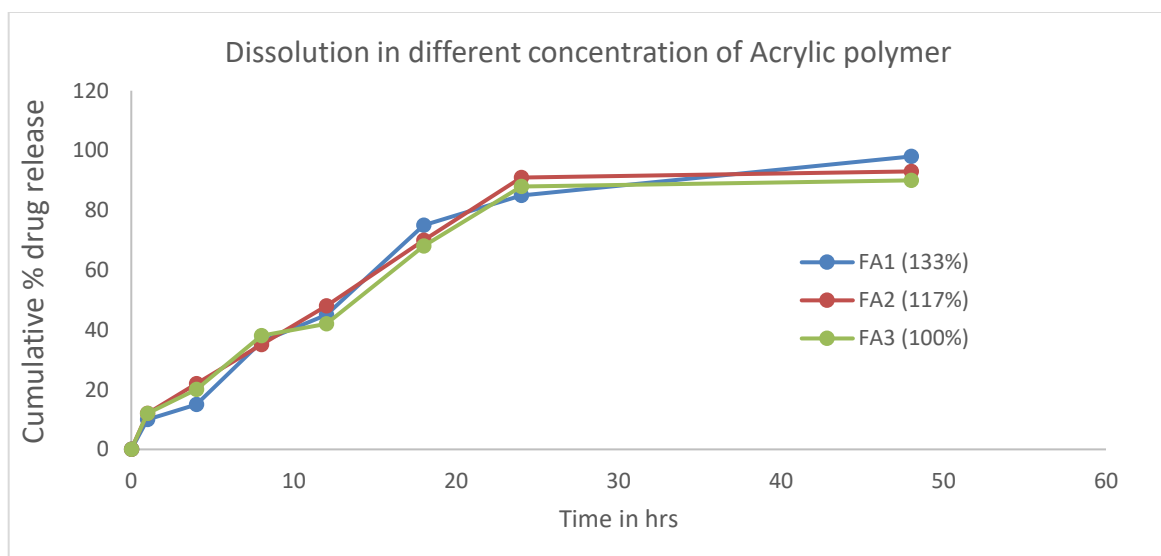| 4 | 15± 1.72 | 22± 1.5 | 20± 1.36 |
8	36± 1.51	35± 1.5	38± 1.42
12	45± 1.21	48± 1.23	42± 1.12
18	75± 1.52	70± 1.12	68± 1.52
24	85± 1.50	91± 1.53	88± 1.53
48	98± 1.21	93± 1.23	90± 1.23

Figure 4: *In-vitro* drug release profile of sildenafil citrate from transdermal patches containing polymer at different concentration of 133% (FA1), 117% (FA2) and 100% (FA3)

Observation:
Above *In-vitro* dissolution profile evident that the acrylic polymer enhance the solubility of the sildenafil citrate.

3.2. Effect of acrylic polymer with respect to *In-Vitro* permeation study:
It was observed that the formulations containing acrylic polymer, permeation rate was found to be higher with the high concentration of acrylic polymer as like *In-Vitro* drug release profile. The result of *in-vitro* permeation study result is presented in table 16 and figure 5.

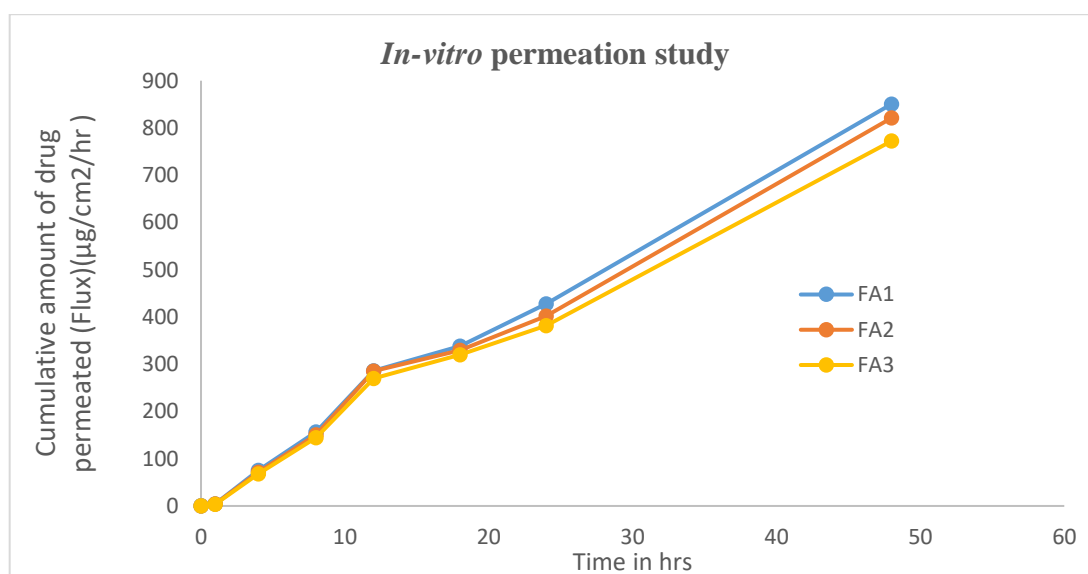Table 16: *In-vitro* permeation study of sildenafil citrate from transdermal patches containing polymer at different concentration of 133% (FA1), 117% (FA2) and 100% (FA3)

TIME (h)	Cumulative amount of drug permeated ($\mu g/cm2/hr$)		
	FA1	**FA2**	**FA3**
1	4.42 ± 0.42	4.00 ± 0.31	3.68 ± 1.26
4	75.53 ±	71.17 ±	68.35 ±

	6.98	6.45	5.11
8	157.06 ± 7.45	150.75 ± 6.22	144.80 ± 8.45
12	285.92 ± 13.22	285.37 ± 7.84	269.71 ± 7.76
18	338.55 ± 18.42	329.68 ± 12.54	320.29 ± 11.54
24	427.78 ± 13.76	401.88 ± 11.33	381.66 ± 12.65
48	850.68 ± 20.56	821.29 ± 16.54	772.38 ± 15.69

Figure 5: *In-vitro* permeation study profile of sildenafil citrate from transdermal patches containing polymer at different concentration of 133% (FA1), 117% (FA2) and 100% (FA3)

Observation:
Formulation FA1 was showing more *in-vitro* permeation than the FA2 and FA3.

Conclusion:
Since, the formulation FA1 was showing better *in-vitro* permeation among all other formulations, the formulation FA1 was stored in the stability to evaluate further.

4.0. Effect of plasticizer:
4.1. Effect of plasticizer with respect to *In-Vitro* drug release profile:
It was observed that the formulations containing triethyl citrate (TEC) as plasticizer, release rate was found to be almost similar or without significant different with the change in concentration of the TEC (98, 95 and 93% for 20, 15 and 17.5% respectively in 48 hrs.) in the transdermal patches. The result of *in-vitro* drug release study is presented in table 17 and figure 6.
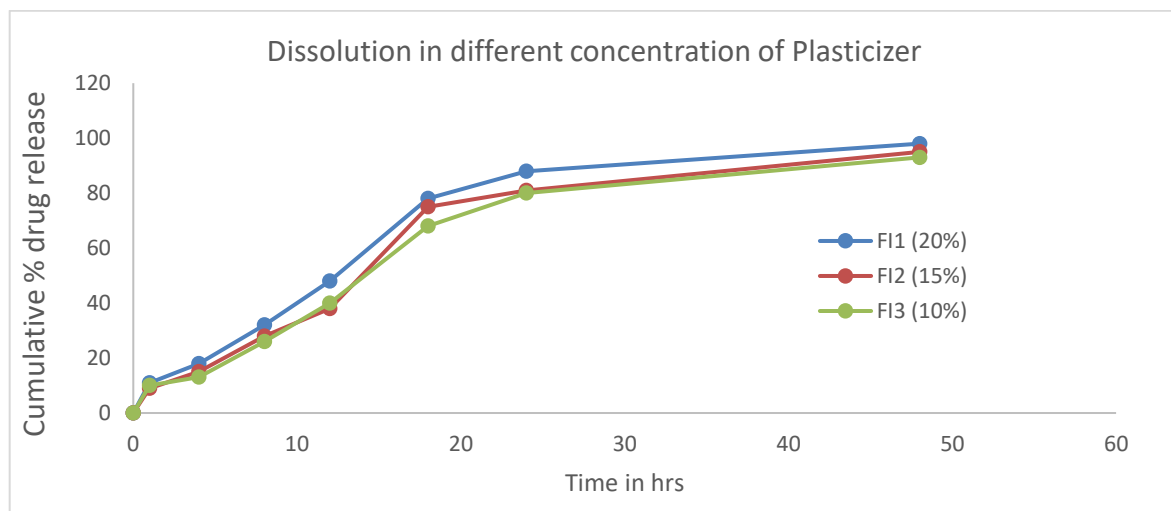
Table 17: *In-vitro* drug release profile of sildenafil citrate from transdermal patches containing plasticizer at different concentration of 20% (FI1), 15% (FI2) and 17.5% (FI3)

TIME (h)	Cumulative % drug release

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	FI1	FI2	FI3
1	11± 1.2	9± 1.23	10± 1.21
4	18± 1.1	15± 1.24	13± 1.20
8	32± 1.2	28± 1.23	26± 1.25
12	48± 1.2	38± 1.21	40± 1.23
18	78± 1.1	75± 1.20	68± 1.18
24	88± 1.2	81± 1.32	80± 1.23
48	98± 1.3	95± 1.12	93± 1.21

Figure 6: *In-vitro* drug release profile of sildenafil citrate from transdermal patches containing plasticizer at different concentration of 20% (FI1), 15% (FI2) and 10% (FI3)

Observation:
Above *In-vitro* drug release profile evident that the change in the concentration of Triethyl Citrate had not significantly impacted the *in-vitro* drug release profile of the formulation.

4.2 Effect of plasticizer with respect to *In-Vitro* permeation study:
It was observed that the formulations containing triethyl citrate (TEC) as plasticizer, drug permeation rate was found to be similar in the initial time points with respect to 20%, 15% and 17.5%. However, drug permeations were differed in the last time points. i.e Formulation was manufactured with 17.5% TEC was showing significantly faster permeation at 24 hrs and 48 hrs. The result of *in-vitro* drug permeation study data is presented in table 18 and figure 7.

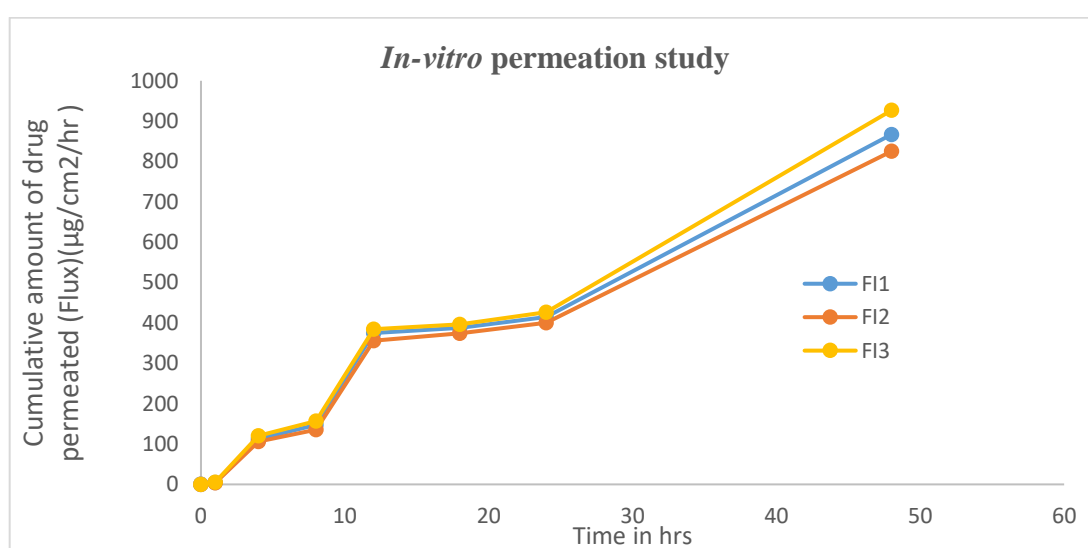Table 18: *In-vitro* permeation study of sildenafil citrate from transdermal patches containing plasticizer at different concentration of 20% (FI1), 15% (FI2) and 17.5% (FI3)

TIME (h)	Cumulative amount of drug permeated (µg/cm2/hr)		
	FI1	FI2	FI3

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1	4.73 ± 2.11	4.03 ± 3.54	5.21 ± 1.76
4	112.53 ± 3.01	106.24 ± 4.11	120.32 ± 3.41
8	147.79 ± 5.12	135.50 ± 6.34	157.31 ± 5.76
12	375.09 ± 7.98	355.76 ± 9.03	384.24 ± 8.14
18	387.90 ± 6.34	374.24 ± 10.64	396.23 ± 3.23
24	414.99 ± 7.22	400.49 ± 12.21	426.86 ± 7.28
48	867.10 ± 10.78	825.25 ± 13.56	926.86 ± 5.56

Figure 7: *In-vitro* drug permeation profile of sildenafil citrate from transdermal patches containing plasticizer at different concentration of 20% (FI1), 15% (FI2) and 17.5% (FI3)

Observation:
Formulation FI3 was showing more *in-vitro* permeation than the formulations FI1 and FI2.

Conclusion:
Since, the formulation FI3 was showing better drug release profile and *in-vitro* permeation among all other formulations due to the optimized quantity of plasticizer (triethyl citrate), the formulation FI3 was stored in the stability to evaluate further.

5.0. Effect of isopropyl myristate:
5.1. Effect of isopropyl myristate with respect to *In-Vitro* dissolution profile:
The transdermal patches containing 20% (FT1), 15% (FT2) and 10% (FT3) of isopropyl myristate showed the release rate almost similar or without significant difference with the change in concentration of the permeation enhancer. The result of *in-vitro* drug release study is presented in table 19 and figure 8.

Table 19: *In-vitro* drug release profile of sildenafil citrate from transdermal patches containing permeation enhancer at different concentration of 20% (FT1), 15% (FT2) and 10% (FT3)

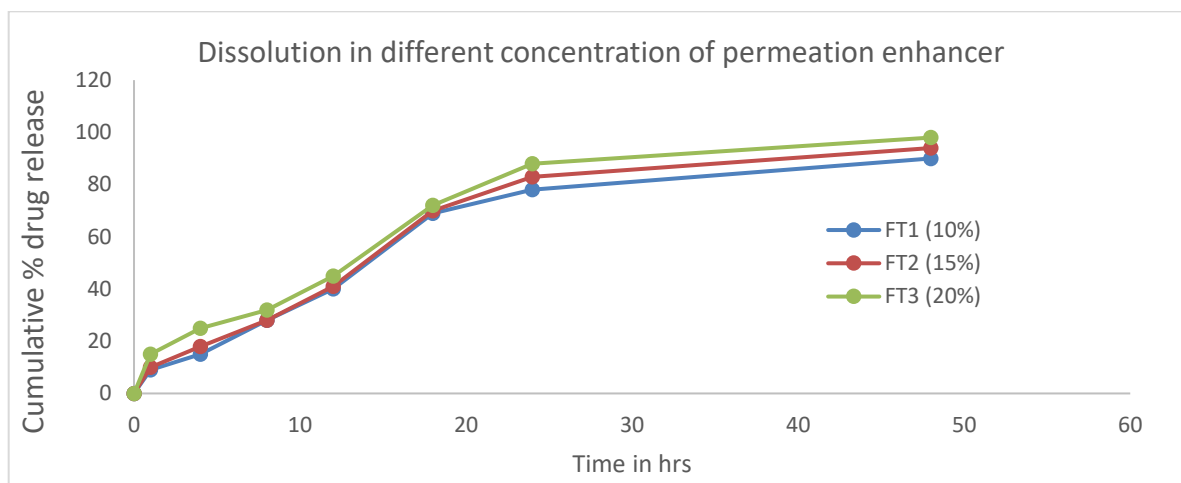| TIME (h)	Cumulative % drug release		
	FT1	FT2	FT3
1	9± 0.75	10± 0.75	15± 0.45
4	15± 0.55	18± 0.53	25± 0.56
8	28± 0.45	28± 0.36	32± 0.36
12	40± 0.36	41± 0.34	45± 0.54
18	69± 0.65	70± 0.52	72± 0.45
24	78± 0.70	83± 0.45	88± 0.36
48	90± 0.32	94± 0.63	98± 0.42

Figure 8: *In-vitro* drug release profile of sildenafil citrate from transdermal patches containing permeation enhancer at different concentration of 10% (FI1), 15% (FI2) and 20% (FI3)

Observation:
Above *In-vitro* drug release profile evident that the change in the concentration of Triethyl Citrate had not significantly impacted the *in-vitro* release profile of the formulation.

5.2. Effect of isopropyl myristate with respect to *In-Vitro* permeation study:
The transdermal patches containing 10% (FT1), 15% (FT2) and 20% (FT3) of isopropyl myristate showed 90, 94 and 98% drug release in 48 h respectively. It was observed that concentration of isopropyl myristate increased, the cumulative amount of drug release was also increased as shown in Table 20 and Figure 9.

Table 20: *In-vitro* drug permeation profile of sildenafil Citrate from transdermal patches containing permeation enhancer at different concentration of 10% (FT1), 15% (FT2) and 20% (FT3)

TIME (h)	Cumulative amount of drug permeated (µg/cm2/hr)

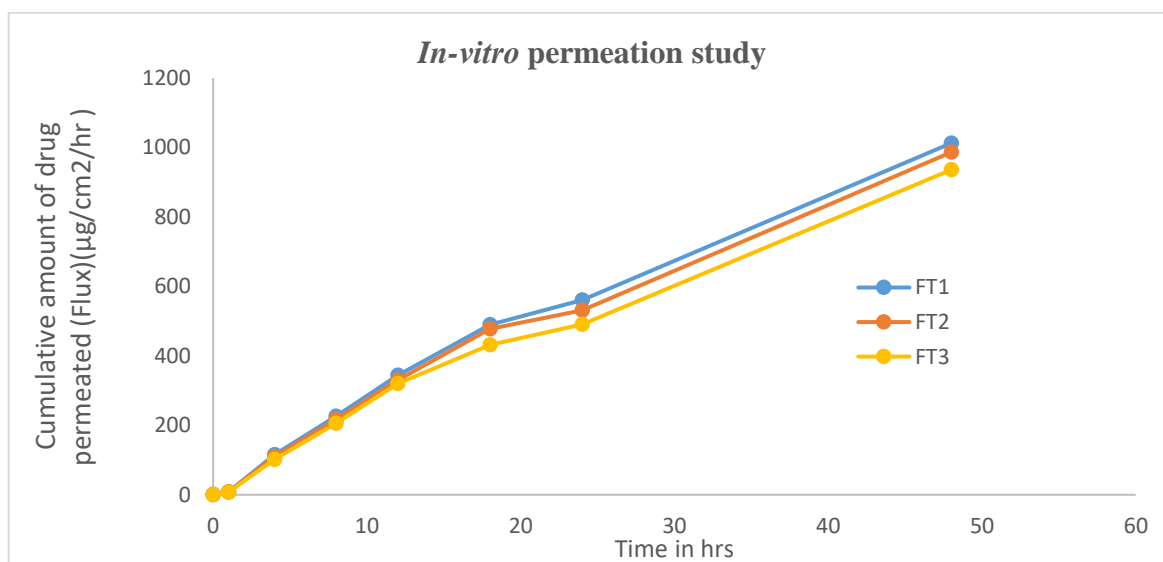|	FT1	FT2	FT3
1	8.59 ± 0.89	8.01 ± 1.12	7.17 ± 3.17
4	115.21 ± 2.23	109.50 ± 4.21	101.80 ± 3.56
8	226.39 ± 4.15	216.41 ± 5.78	205.51 ± 4.32
12	343.80 ± 3.67	329.76 ± 3.54	319.69 ± 6.23
18	489.75 ± 7.12	477.47 ± 7.23	431.23 ± 7.43
24	560.29 ± 3.76	530.85 ± 6.27	490.46 ± 5.23
48	1012.29 ± 6.11	986.14 ± 8.33	935.59 ± 6.32

Figure 9: *In-vitro* drug permeation profile of sildenafil citrate from transdermal patches containing permeation enhancer at different concentration of 20% (FT1), 15% (FT2) and 10% (FT3)

Observation:
Formulation FT1 was showing more *in-vitro* permeation than the FT2 and FT3.

Conclusion:
Since, the formulation FT1 was showing better *in-vitro* permeation among all other formulations due to the optimized quantity of penetration enhancer (isopropyl myristate), the formulation FT1 was stored in the stability to evaluate further.

6.0. Selection formulation of Sildenafil citrate patches for Stability study:
On the basis of *in-vitro* dissolution studies and *in-vitro* permeation study, the best formulations F4, FA1, FT1, and FI3 selected for further stability studies.

Table 21: *In-vitro* permeation study data of sildenafil citrate from optimized transdermal patches containing batch (F4), acrylic polymer 133 % (FA1), TEC 17.5% (FI3), and Isopropyl myristate 20% (FT1).

TIME (hrs)	Cumulative amount of drug permeated (µg/cm2/hr)			
	F4	FA1	FT1	FI3
1	3.48 ± 0.38	4.42 ± 1.67	8.59 ± 2.58	5.21 ± 2.13
4	42.56 ± 2.35	75.53 ± 9.10	115.21 ± 8.77	120.32 ± 23.61
8	89.61 ± 7.90	157.06 ± 22.57	226.39 ± 32.22	157.31 ± 36.20
12	189.84 ± 8.10	285.92 ± 29.01	343.80 ± 29.69	384.24 ± 58.34
18	216.51 ± 17.56	338.55 ± 51.60	489.75 ± 59.61	396.23 ± 52.39
24	365.48 ± 14.33	427.78 ± 31.61	560.29 ± 40.88	426.86 ± 60.25
48	457.19 ± 14.33	850.68 ± 56.61	1012.29 ± 40.88	926.86 ± 60.25

6.1. Scanning Electron Microscopic Study of Sildenafil citrate Patch

The surface morphology of the transdermal patches before and after *in-vitro* permeation study was scanned using a scanning electron microscope (SEM) **(**Zeiss Evo, Germany**).** The result shown in Figure 10, indicated that the drug is uniformly distributed in prepared transdermal patch and after permeation study; it was observed that the drug is released from the patch in to the skin, which can be then permeated through skin into the systemic circulation.
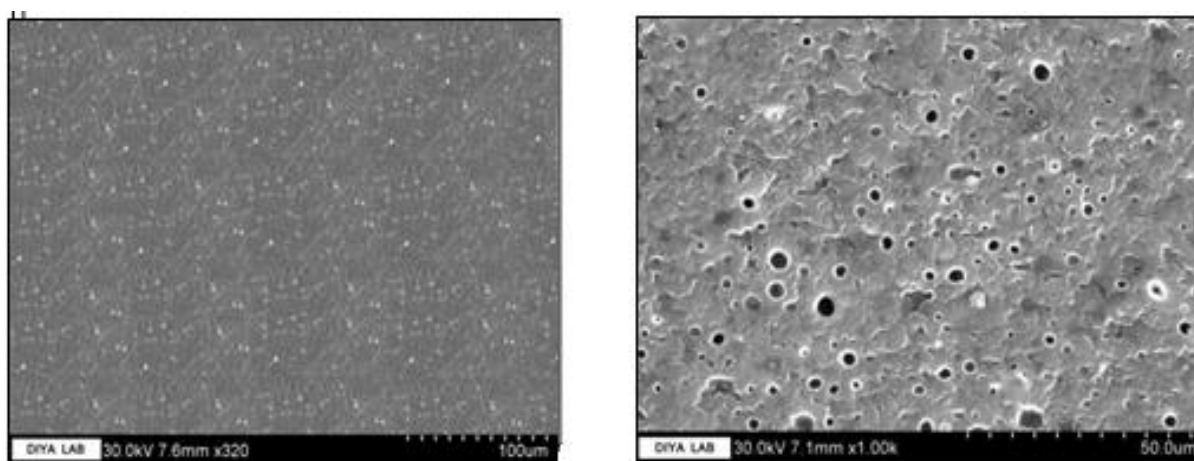

Figure 10: SEM photographs of sildenafil citrate patch before permeation study, (A); after permeation study, (B)

7.0. Stability Study

The stability study of optimized formulations (F4, FA1, FT1, FI3) were conducted according to the ICH guidelines; the formulations were stored at 40 ± 2°C /75± 5% RH, 5°C ± 3°C and 25 ± 2°C /60± 5% RH for 3 months.

The result indicated that no change in physical appearance was observed after 90 days. The drug content of the patches at initial and stability were tabulated below. These data's indicated that no significant (p

>0.05) change after 3 months in the formulation. The results of in-vitro permeation studies of fresh batch and 3 month old batch is shown in Figure 11, 12, 13 & 14.

Table 22: Drug Content in stability

| Batch Code | Initial | Refrigerator temperature | | | Room temperature | | | Accelerated temperature | | |
| | | 5°C ± 3°C | | | 25°C± 2°C/60%RH±5%RH | | | 40°C± 2°C/75%RH±5%RH | | |
		30 day	60 Day	90 Day	30 day	60 Day	90 Day	30 day	60 Day	90 Day
F4	98.45 ± 1.20	98.54 ± 2.15	98.87 ± 1.58	99.81 ± 2.72	98.43 ± 1.90	98.43 ± 1.45	98.41 ± 1.52	98.21 ± 2.31	98.12 ± 1.76	98.1 ± 0.95
FA1	97.89 ± 2.11	99.76 ± 0.65	98.67 ± 1.43	99.13 ± 1.82	98.18 ± 0.34	98.01 ± 1.87	98.03 ± 1.02	97.98 ± 0.78	97.92 ± 1.51	98.12 ± 0.75
FT1	99.32 ± 1.23	100.56 ±0.25	99.32 ± 1.15	98.12 ± 2.13	99.12 ±0.55	99.16 ± 1.65	98.78 ± 1.11	98.99 ± 0.15	99.05 ± 1.42	99.12 ± 1.87
FI3	96.87 ± 1.25	96.22 ±1.12	97.99 ± 1.94	98.51± 1.23	97.12 ±2.01	97.18 ± 1.52	97.56 ± 1.72	96.90 ± 2.80	97.13 ± 1.45	97.20 ± 2.56

7.1. Cumulative amount of drug permeated from the stability samples:

Table 23: Optimized Batch: F4

| Time (hr) | Batch No : F4 | | | |
| | Cumulative amount of drug permeated (ug/cm2/hrs) | | | |
	Initial	3 Month 5°C ± 3°C	3 Month 25°C/60%RH	3 Month 40°C/75%RH
0	0	0	0	0
1	3.48 ± 0.76	4.12 ± 2.12	3.96 ± 1.34	3.84 ± 2.65
4	42.56 ± 3.32	48.76 ± 3.67	46.51 ± 5.37	51.26 ± 3.27
8	89.61 ± 4.19	102.57 ± 3.43	101.25 ± 8.76	101.26 ± 5.63
12	189.84 ± 9.67	210.78 ± 4.54	207.56 ± 4.12	213.56 ± 7.18
18	216.51 ± 11.45	252.42 ± 6.54	249.84 ± 9.62	287.61 ± 6.28
24	365.48 ± 13.77	417.67 ± 6.87	406.84 ± 8.87	389.54 ± 7.12
48	457.19 ± 16.11	771.43 ± 8.65	751.46 ± 10.13	715.64 ± 9.67
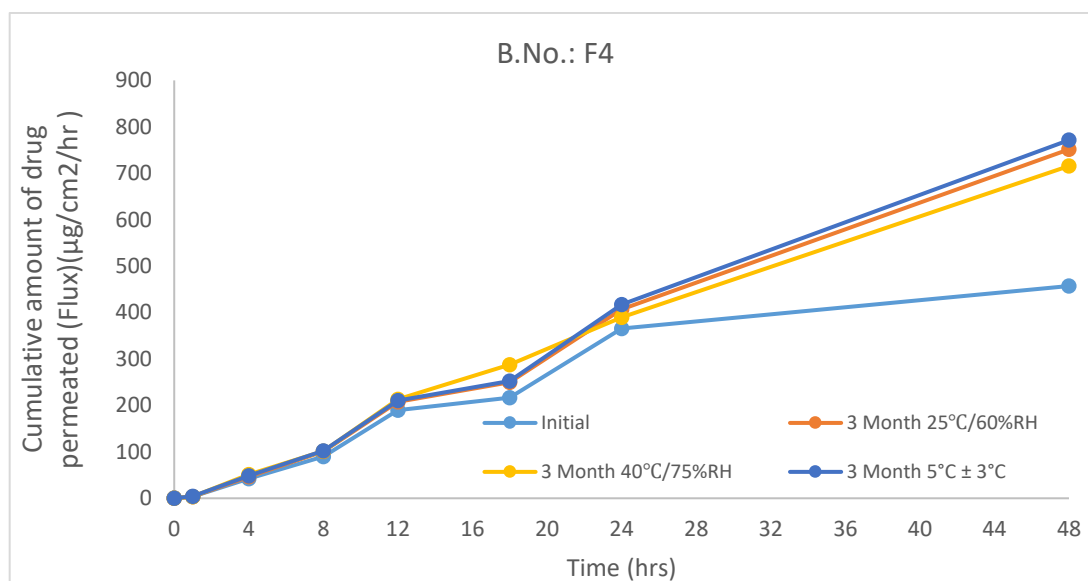
Figure 11: *In-vitro* drug permeation profile of fresh and 3 month old transdermal patch of optimized formulation (F4)

Observation:
The stability data reveals that no change observed in the drug content in stability of the formulation. However, drug permeation was significantly increased from Initial to 3 Month in refrigerator, long term and accelerated condition. Since all stability conditions are behaving in same manner in permeation profile, it can be consider these changes due to ageing effect.

Table 24: Optimized Batch: FA1

| Time (hr)	Batch No: FA1			
	Cumulative amount of drug permeated (ug/cm2/hrs)			
	Initial	3 Month 5°C ± 3°C	3 Month 25°C/60%RH	3 Month 40°C/75%RH
0	0	0	0	0
1	4.42 ± 0.42	4.45 ± 0.98	3.65 ± 1.57	3.88 ± 2.03
4	75.53 ± 6.98	82.88 ± 2.54	72.33 ± 3.23	80.44 ± 3.76
8	157.06 ± 7.45	158.23 ± 5.10	154.67 ± 5.67	168.34 ± 4.76
12	285.92 ± 13.22	284.83 ± 6.65	278.51 ± 7.65	304.76 ± 8.54
18	338.55 ± 18.42	327.09 ± 7.18	321.45 ± 6.87	352.56 ± 6.26
24	427.78 ± 13.76	421.40 ± 6.87	416.01 ± 7.62	442.47 ± 7.62
48	850.68 ± 20.56	856.19 ± 8.65	837.86 ± 9.78	875.42 ± 9.67
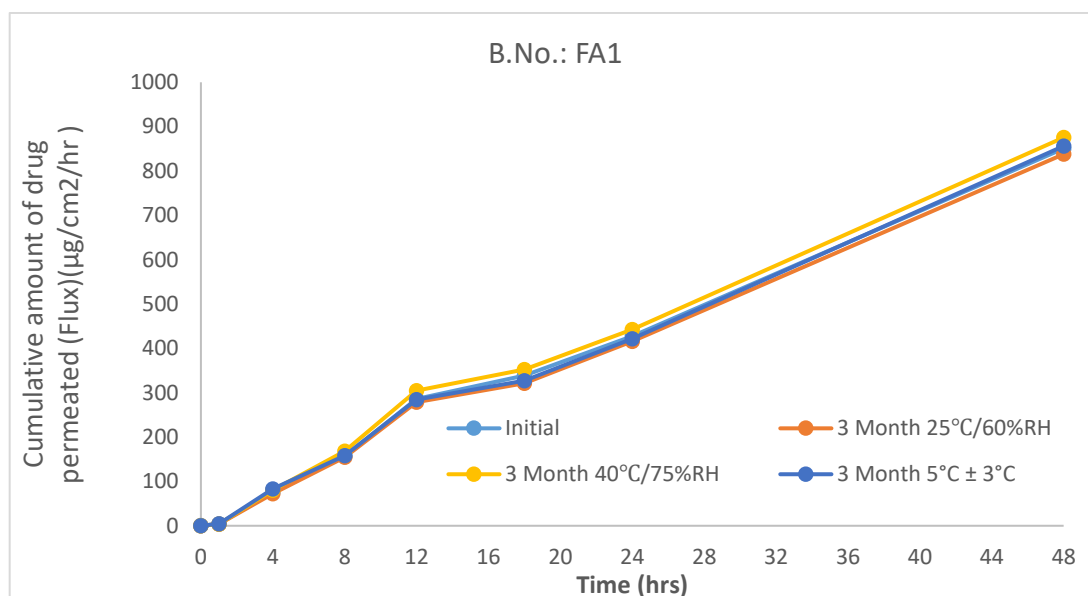
Figure 12: *In-vitro* drug permeation profile of fresh and 3 month old transdermal patch of optimized formulation (FA1)

Observation:

The above stability data reveals that no changes observed in the drug content and *In-Vitro* drug permeation profile between initial and stability of the formulation.

Table 25: Optimized Batch: FT1

| Time (hr)	Batch No: FT1			
	Cumulative amount of drug permeated (ug/cm2/hrs)			
	Initial	3 Month 5°C ± 3°C	3 Month 25°C/60%RH	3 Month 40°C/75%RH
0	0	0	0	0
1	8.59 ± 0.89	10.98 ± 1.24	9.12 ± 1.98	11.26 ± 0.98
4	115.21 ± 2.23	128.28 ± 2.31	124.80 ± 4.12	134.53 ± 1.67
8	226.39 ± 4.15	246.87 ± 2.98	236.54 ± 3.89	265.59 ± 4.23
12	343.80 ± 3.67	381.45 ± 3.43	362.88 ± 5.21	389.77 ± 5.34
18	489.75 ± 7.12	517.98 ± 4.12	510.23 ± 6.81	528.84 ± 6.14
24	560.29 ± 3.76	597.12 ± 5.65	587.45 ± 7.62	603.43 ± 7.13
48	1012.29 ± 6.11	1284.19 ± 7.23	1134.76 ± 8.12	1267.45 ± 8.42
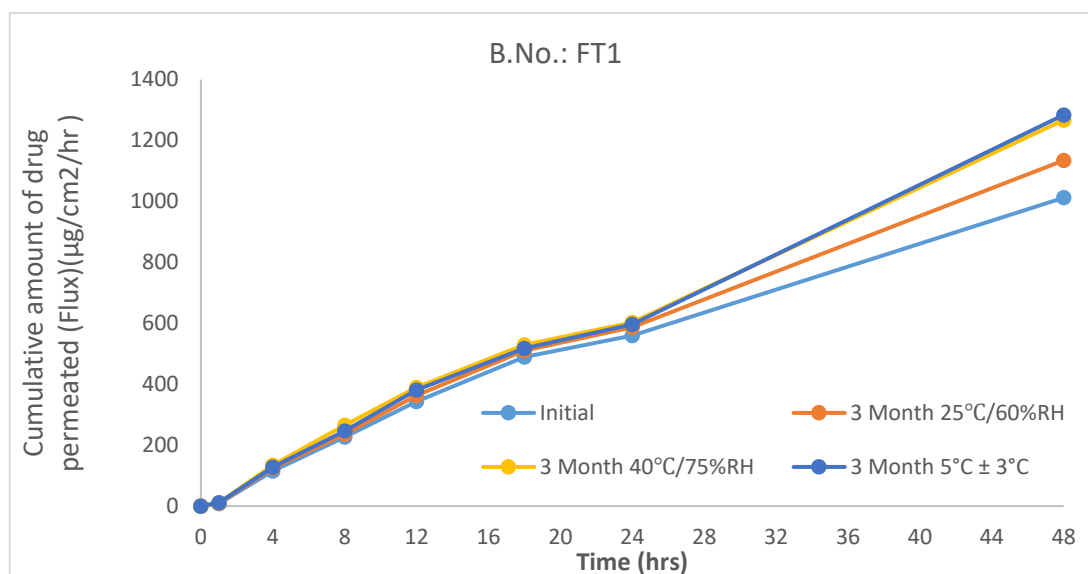
Figure 13: *In-vitro* drug permeation profile of fresh and 3 month old transdermal patch of optimized formulation (FT1)

Observation:

The above stability data reveals that no changes observed in the drug content and there was slight change in drug permeation profile observed, but these change cannot be considered as significant.

Table 26: Optimized Batch: FI3

| Time (hr)	Batch No: FI3			
	Cumulative amount of drug permeated (ug/cm2/hrs)			
	Initial	3 Month 5°C ± 3°C	3 Month 25°C/60%RH	3 Month 40°C/75%RH
0	0	0	0	0
1	5.21 ± 1.76	4.98 ± 1.25	4.67 ± 1.87	2.55 ± 1.03
4	120.32 ± 3.41	110.28 ± 2.32	105.34 ± 2.56	76.45 ± 2.43
8	157.31 ± 5.76	143.72 ± 5.54	132.67 ± 3.58	105.64 ± 3.12
12	384.24 ± 8.14	366.98 ± 6.81	351.78 ± 4.17	307.65 ± 3.89
18	396.23 ± 3.23	375.25 ± 6.12	358.12 ± 6.54	315.98 ± 6.03
24	426.86 ± 7.28	401.26 ± 7.87	378.56 ± 7.10	337.88 ± 8.63
48	926.86 ± 5.56	887.29 ± 6.23	876.80 ± 6.87	717.53 ± 9.21
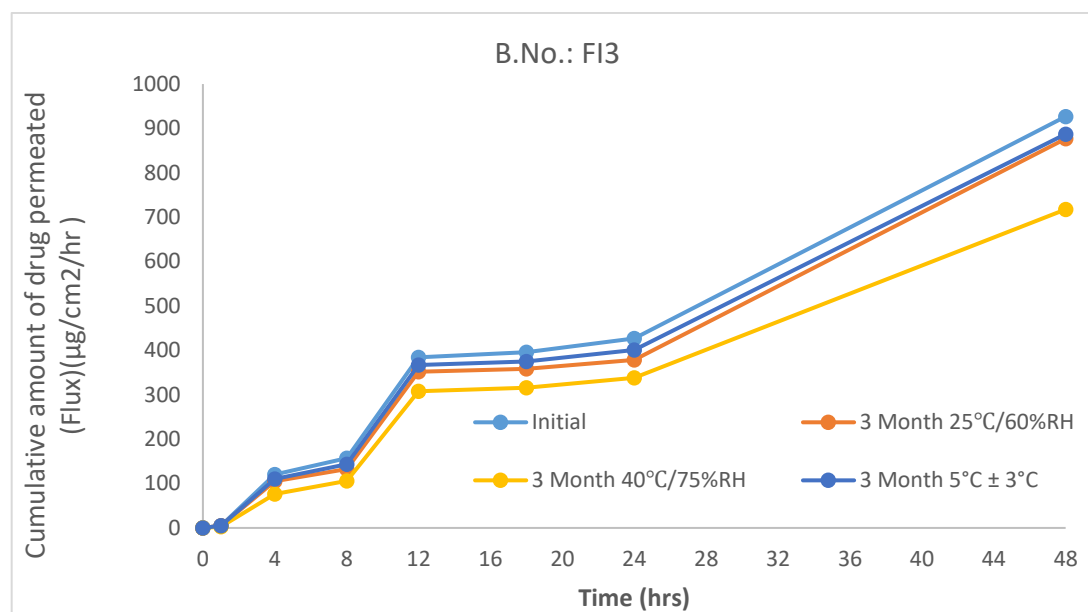
Figure 14: *In-vitro* drug permeation profile of fresh and 3 month old transdermal patch of optimized formulation (FI3)

Observation:

The stability data reveals that no change observed in the drug content in stability of the formulation. However, drug permeation was significantly decreased from Initial to 3 Month in refrigerator, long term and accelerated condition. Also it was observed that refrigerator and long term condition was slightly better than accelerated condition.

Conclusion:

The formulation F4 was showing better *in-vitro* permeation among all other formulations, the formulation F4 was stored in the stability study, since all stability conditions are behaving in same manner in permeation profile, it can be consider these changes due to ageing effect.

The formulation FA1 was showing better *in-vitro* permeation among all other formulations, the formulation FA1 was stored in the stability study, in stability no changes observed in the drug content and there was slight change in drug permeation profile observed, but these changes cannot be considered as significant.

The formulation FT1 was showing better *in-vitro* permeation among all other formulations due to the optimized quantity of penetration enhancer (isopropyl myristate), the formulation FT1 was stored in the stability study, in stability no changes observed in the drug content and there was slight change in drug permeation profile observed, but these changes cannot be considered as significant.

The formulation FI3 was showing better drug release profile and *in-vitro* permeation among all other formulations due to the optimized quantity of plasticizer (triethyl citrate), the formulation FI3 was stored in the stability study, in stability no change observed in the drug content in stability of the formulation. However, drug permeation was significantly decreased from Initial to 3 Month in refrigerator, long term and accelerated condition. Also it was observed that refrigerator and long term condition was slightly better than accelerated condition.

Based on the above formulation stability data, it can be concluded that B.No.: FA1 and FT1 were quite stable in drug content and *in-vitro* drug permeation profile. Hence these two formulations were selected for the candidate for *In-vivo* pharmacokinetic study.

Conflict of interest
The authors declare no conflicts of interest.

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