Available online on 15.01.2025 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Formulation and Evaluation of Betulinic Acid Loaded Transdermal Patches

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Article Info:

Article History:

Received 18 Oct 2024
Reviewed 01 Dec 2024
Accepted 27 Dec 2024
Published 15 Jan 2025

Cite this article as:

Singh H, Bose P, Singh AP, Singh AP, Formulation and Evaluation of Betulinic Acid Loaded Transdermal Patches, Journal of Drug Delivery and Therapeutics. 2025; 15(1):52-58
DOI: http://dx.doi.org/10.22270/jddt.v15i1.6951

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Abstract

Many disorders, including cardiovascular diseases, Parkinson's disease, Alzheimer's disease, fungal diseases, depression, anxiety, and Attention Deficit Hyperactivity Disorder (ADHD), skin cancer, female sexual dysfunction, post-menopausal bone loss, and urine incontinence, can now be treated with transdermal delivery systems. A naturally occurring pentacyclic triterpenoid, betulinic acid possesses antiretroviral, antimalarial, and anti-inflammatory qualities. More recently, it has been shown to have anticancer potential through topoisomerase inhibition. The goal of the current study was to create transdermal patches with a controlled release action that included several polymers and an anti-inflammatory medication, such as betulinic acid..The solvent evaporation approach was successfully used to create the betulinic acid transdermal patch. Betulinic acid transdermal patches for transdermal medication administration were assessed. A calibration curve was acquired, a transdermal film was made, and the drug's bioavailability constraints were addressed.

Keywords: Controlled DDS, Transdermal DDS, Betulinic acid, Transdermal Patch, solvent evaporation method.

INTRODUCTION

Natural products have long been utilized to treat human diseases due to their broad range of biological activities, which can be harnessed for medicinal purposes. These naturally occurring compounds are playing an increasingly important role in drug discovery and development. Notably, a significant proportion of anticancer and antimicrobial agents are derived from natural sources. The anticancer effects of these natural substances are often attributed to their ability to activate cell death pathways, such as apoptosis, in cancer cells. Apoptosis, or programmed cell death, is a fundamental process that helps maintain tissue balance and is conserved across species. Disruptions in apoptosis can lead to various diseases, including cancer. One of the hallmarks of cancer is its ability to evade apoptosis, with many cancer cells disabling the mitochondrial (intrinsic) apoptosis pathway. Mitochondria, which are essential for cellular energy production, also regulate the point of no return in apoptosis. Betulinic acid, a natural compound, demonstrates potent antitumor activity by triggering the mitochondrial pathway to apoptosis. Agents that target mitochondria, like Betulinic acid, could offer new ways to overcome drug resistance in cancer therapy[1].

Conventional oral drug formulations—such as tablets, capsules, and liquid forms—often face challenges related to gastrointestinal (GI) absorption, local irritation, dilution of the drug's strength, liver metabolism, enzyme degradation, and protein binding at the absorption site. These issues result in reduced bioavailability and a shorter duration of action, necessitating frequent doses, which can be a barrier to patient adherence and increase treatment costs. The parenteral route is typically preferred for patients with moderate to severe conditions, though it suffers from low patient compliance due to its invasive nature, requiring regular needle injections. Most conventional dosage forms, except intravenous infusions, follow second-order kinetics, where the drug is initially released at a fast rate, causing a rapid rise in blood concentration, followed by an exponential decline. This leads to fluctuating drug levels, often above or below the therapeutic range. The inability to maintain a steady drug concentration in the body, along with issues like unpredictable absorption and

repeated dosing, has led to the development of more advanced drug delivery systems[2].

Betulinic acid is a pentacyclic triterpenoid compound known for its antiretroviral, antimalarial, anti-inflammatory, and anticancer properties, the latter through the inhibition of topoisomerase. It is found in various plants, including the white birch (Betula pubescens), the ber tree (Ziziphus mauritiana), selfheal (Prunella vulgaris), and others. In 1995, Betulinic acid was recognized for its selective inhibitory effects on human melanoma and was later shown to induce apoptosis in human neuroblastoma, both in vitro and in animal models. It was considered for drug development with support from the National Cancer Institute's Rapid Access to Intervention Development program. Betulinic acid has also demonstrated activity against several other types of cancer, including neuroectodermal cancers, human leukemia, and malignant head and neck cancers. However, epithelial cancers such as breast, colon, lung, and renal cell carcinomas, along with T-cell leukemia, showed no response to Betulinic acid treatment. Triterpenes like Betulinic acid are promising candidates for developing new multi-targeted therapies. Despite its potential, Betulinic acid's limited water solubility restricts its bioavailability, posing a challenge for its effective use. This underscores the need for novel drug delivery systems to improve its therapeutic potential. The present study aims to develop transdermal patches containing Betulinic acid, combined with various polymers, for controlled drug release and enhanced bioavailability[3-4].

MATERIALS AND METHODS

Materials

Betulinic acid is obtained from Sigma Aldrich (Merck), Hydroxypropyl & methylcellulose E5 is from loba chemie and Dimethyl sulphoxide by Hi media and Dibutylphthalate, Potassium chloride, Fused calcium chloride from CDH Chemicals.All other solvents and reagents were used of analytical grade.

Determination of melting point

By placing a tiny quantity of the medication in a capillary tube that was closed at one end, the drug's melting point was ascertained. The temperature at which the drug melted was measured by placing the capillary tube in a melting point apparatus. This was done three times, and the average result was recorded.

Determination of drug-excipients compatibility

FT-IR [5]: Because the drug and polymer are in close proximity to one another during the film formulation preparation process, they may interact, potentially causing the drug to become unstable. preformulation studies pertaining to the drug-polymer interaction are therefore crucial in choosing the right polymers. To determine whether betulinic acid and the chosen polymers were compatible, FT-IR spectroscopy was utilized. The drug with excipients and the pure drug were scanned independently.

Procedure: Drugs and polymers were combined with potassium bromide, and spectra were obtained. The FT-IR spectra of betulinic acid and betulinic acid with polymer were compared. Peaks of betulinic acid that vanished or shifted in any of the spectra were examined.

Calibration curve of Betulinic acid

Standard solution of drug

Ten milligrams of betulinic acid were weighed and put into a 100 ml volumetric flask to create a stock solution. To achieve a solution of 100 µg/ml, the volume was adjusted using the mobile phase. To get a secondary stock solution of 10 µg/ml, it was further diluted. Final concentrations of 0.5, 2.0, 3.0, 4.0, and 10.0 µg/ml of the model drug were obtained by diluting appropriate aliquots of secondary stock solution (10 µg/ml) in 10 ml volumetric flasks to the appropriate level with mobile phase. Plotting peak areas against concentrations allowed for the construction of a calibration curve, and the regression equation for the model drug was calculated.

UV Spectrophotometer method

Preparation of standard solution

To create the stock solution, 10 mg of precisely weighed betulinic acid were dissolved in 10 ml of volumetric flask of ethanolic phosphate buffer (7.4), and the volume was adjusted with the ethanolic phosphate buffer (7.4) to produce a clear solution with a concentration of 1000 µg/ml. To get a secondary stock solution of 100 µg/ml, it was further diluted. To create a solution of 5, 10, 15, 20, 25, and 50 µg/ml, a series of drug concentrations were prepared from the secondary stock solution. Specifically, 0.5, 1.0, 1.5, 2.0, 2.5, and 5.0 ml solutions were pipetted out of the secondary stock solution and transferred into 10 ml volumetric flasks. The solution was then made up to 10 ml with ethanolic phosphate buffer (7.4).

Analysis

Using a UV double beam spectrophotometer, the absorbance of each of these solutions was measured at a λmax of 215 nm against a blank, which is ethanolic phosphate buffer (7.4). The standard calibration curve of the drug is obtained by plotting the absorbance against the drug's concentration. The in-vitro drug release of betulinic acid in formulation was ascertained using this curve.

Preparation of Transdermal Films

Preparation of Transdermal patches

The solvent evaporation method was used to create transdermal patches with betulinic acid in cylindrical glass molds that were open on both sides. After pouring a 2% (m/V) polyvinyl alcohol (PVA) solution and drying it for six hours at 60 °C, the backing membrane was cast. D-PC was dissolved in a 1:1 mixture of chloroform and methanol to create the drug reservoir. As a plasticizer, 15% (w/w of the dry polymer composition) dibutylphthalate was utilized. A magnetic stirrer was used to slowly stir the 50 mg of drug (in a 5 ml solvent mixture of chloroform: methanol) into the homogenous

dispersion. After casting the homogeneous mixture onto a PVA backing membrane, it was allowed to dry at room temperature. The films were kept in a desiccator between wax paper sheets.

Evaluation of Transdermal patches[6-9]

Physical appearance

All the prepared patches were visually inspected for color, clarity, flexibility and smoothness.

Thickness uniformity

The aim of the present study was to check the uniformity of thickness of the formulated films. The thickness of the film was measured at 3 different points using a digital caliper and average thickness of three reading was calculated.

Weight uniformity

For each formulation, three randomly selected patches were used. For weight variation test, 3 films from each batch were weighed individually and the average weight was calculated.

Folding endurance

The folding endurance was measured manually for the prepared films. A strip of film (5 x 5 cm) was cut and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance.

Percentage moisture absorption

The films were weighed accurately and placed in the desiccators containing 100 ml of saturated solution of potassium chloride, which maintains 80-90% RH. After 3 days, the films were taken out and weighed. The study was performed at room temperature. The percentage moisture absorption was calculated using the formula:

Percentage moisture absorption = Final Weight – Initial Weight/ Initial Weight X 100

Percentage moisture loss

The films were weighed accurately and kept in a desiccators containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed. The moisture loss was calculated using the formula:

Percentage moisture loss = Final Weight –Initial Weight/ Initial Weight X 100

Water vapors transmission rate

Five milliliter glass vials were cleaned and dried in an oven to a consistent weight. An adhesive tape was used to secure the 1.44 cm2 polymer films over the brim of the vials containing approximately 1 gm of fused calcium chloride. After that, the vials were weighed and kept for a full day in a humidity chamber with an RH of 80–90%. To record the weight gain, the vials were taken out and weighed every 24 hours for three days in a row.

Water vapour transmission rate = Final Weight –Initial Weight/ Time X Area X 100

Tensile strength

The Universal Strength Testing Machine (Hounsfield, Slinfold, Horsham, U.K.) was used to measure the film's tensile strength. The machine had a sensitivity of one gram. There were two load cell grips on it. The upper one was movable, while the lower one was fixed. Between these cell grips, a test film measuring 4 × 1 cm2 was fixed, and force was applied gradually until the film broke. The dial reading in kilograms was used to determine the film's tensile strength. The following is an expression for tensile strength:

Tensile strength = Tensile load at Break/ Cross Sectional Area

Drug content uniformity of films

The 1 cm^2 patches were cut and put into a beaker with 100 ml of pH 7.4 phosphate buffered saline. A magnetic bead was used to stir the medium. Whatmann filter paper was used to filter the contents, and the filtrate's drug content was measured spectrophotometrically at 215 nm using a reference solution made up of drug-free placebo films. To confirm the outcome, the experiment was conducted again.

In vitro drug release studies

In vitro skin permeation studies were performed by using a modified Franz diffusion cell with a receptor compartment capacity of 20 ml. The synthetic cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The formulated patches were cut into size of 1cm^2 and placed over the drug release membrane and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37 ± 0.50C. The samples of 1ml were withdrawn at time interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h, analyzed for drug content spectrophotometrically at 215 nm against blank. The receptor phase was replenished with an equal volume of phosphate buffer at each time of sample withdrawal. The cumulative amounts of drug permeated per square centimeter of patches were plotted against time.

The diffusion kinetics of the drug Betulinic acid were analyzed by graphical method .

- Zero order graphs were made by plotting Cumulative % drug release against Time in hours.
- First order graphs were made by using Log cumulative % drug remaining against Time in hours.
- The diffusion pattern release of the formulation was studied by plotting Higuchi's graph using Cumulative % drug released against Square root of time.
- The Peppas exponential equation was explained by plotting a graph of Log of cumulative % drug release against Log time.

RESULTS

Table 1: Pre-formulation Studies

S.No.	Drug	Melting Point	Solubility	Partition Coefficient(P)
1.	Betulinic acid	317.32 °C	5mg/ml	4.7

Drug excipients compatibility studies

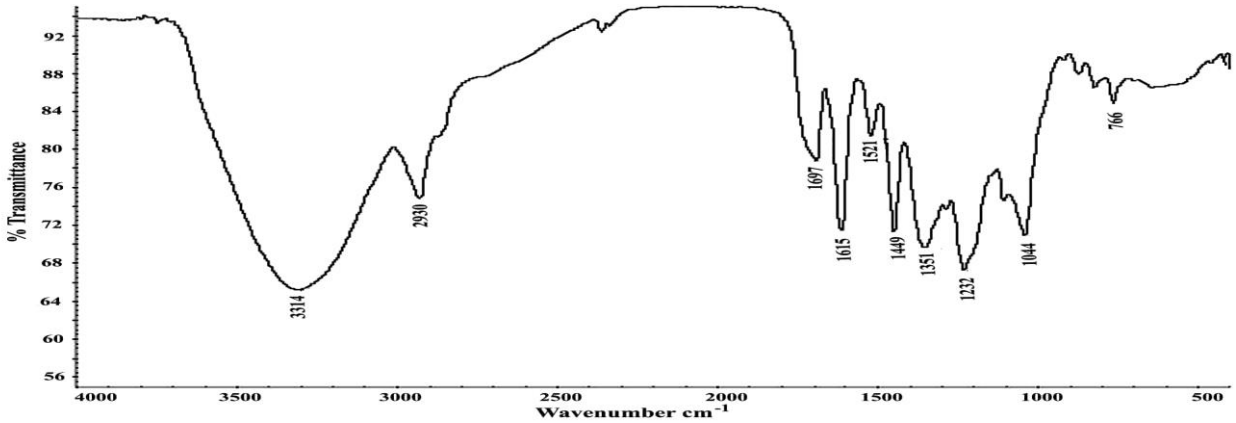

Figure 1: IR Spectrum of Pure Betulinic acid

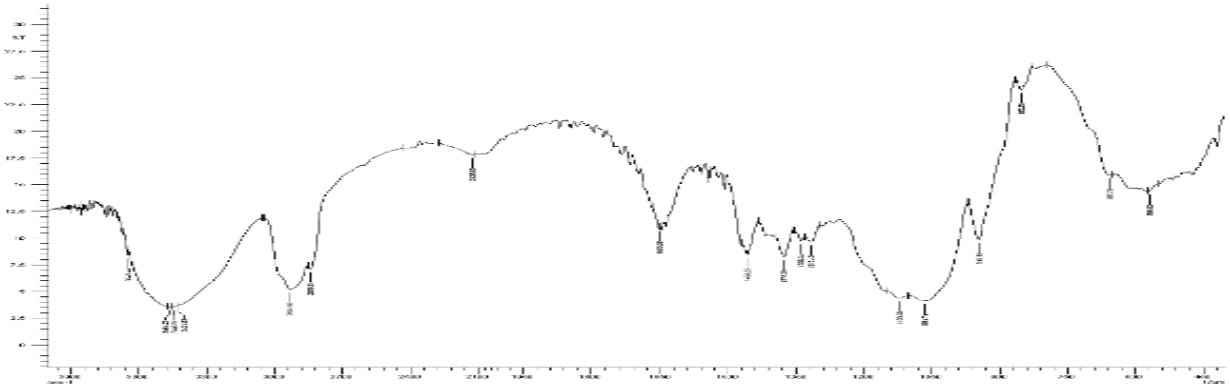

Figure 2: IR Spectrum of Pure HPMC E5

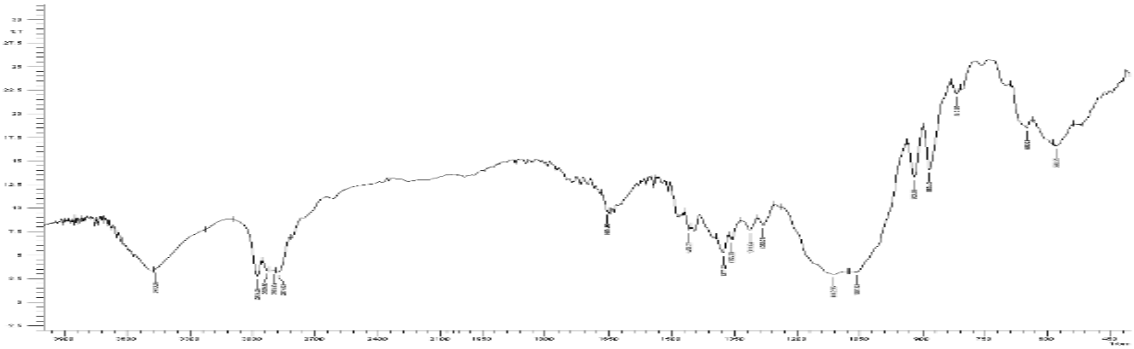

Figure 3: IR Spectrum of Pure EC

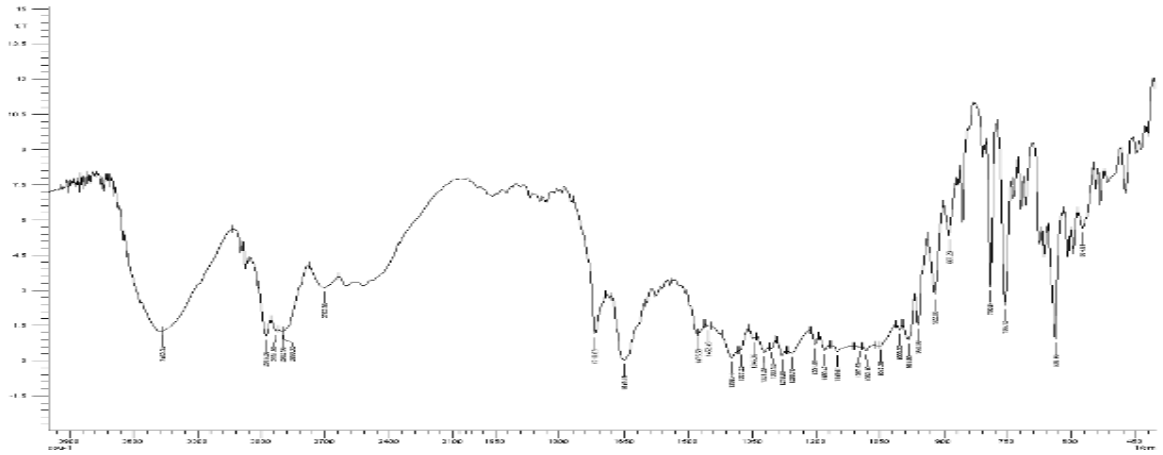

Figure 4: IR Spectrum of Betulinic acid +HPMC E5+EC mixture

Formulation of Transdermal patches

Table 1: Compositions of different formulations containing Betulinic acid

Formulations	F1	F2	F3	F4	F5	F6	F7
Betulinic acid, mg	30	30	30	30	30	30	30
Ethylcellulose,mg	300	*	30	60	90	120	150
HPMC E(5cps),mg	*	300	270	240	210	180	150
Dibutylphthalate (2drop),ml	0.12	0.12	0.12	0.12	0.12	0.12	0.12
DMSO,ml	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Chaloroform:Ethanol (1:1),ml	5	5	5	5	5	5	5

*No ingredient used, HPMC=Hydroxypropyl Methylcellulose, DMSO=Dimethyl sulfoxide

Evaluation of Transdermal Patches

Table 2: Thickness uniformity of F1 to F7 patch formulation (SD, n=3)

S.No.	Formulation code	Average Thickness (mm)			
		Trial 1	Trial 2	Trial 3	Mean±S.D
1.	F1	0.20	0.18	0.22	0.202±0.022
2.	F2	0.19	0.21	0.21	0.204±0.012
3.	F3	0.18	0.21	0.21	0.208±0.018
4.	F4	0.20	0.17	0.22	0.198±0.026
5.	F5	0.15	0.14	0.17	0.156±0.016
6.	F6	0.20	0.22	0.20	0.204±0.014
7.	F7	0.17	0.18	0.20	0.192±0.028

Table 3: Weight uniformity of F1 to F7 patch formulation (SD, n=3)

S.No.	Formulation code	Average Weight			
		Trial 1	Trial 2	Trial 3	Mean±S.D
1.	F1	0.40	0.43	0.42	0.418±0.016
2.	F2	0.38	0.36	0.36	0.368±0.014
3.	F3	0.40	0.38	0.37	0.384±0.016
4.	F4	0.41	0.39	0.38	0.394±0.016
5.	F5	0.35	0.41	0.38	0.382±0.032
6.	F6	0.38	0.34	0.36	0.362±0.022
7.	F7	0.43	0.40	0.41	0.414±0.016

Table 4: Folding endurance of F1 to F7 patch formulation (SD, n=3)

S.No.	Formulation code	Folding Endurance			
		Trial 1	Trial 2	Trial 3	Mean±S.D
1.	F1	116	110	107	112.10± 4.580
2.	F2	53	63	50	55.66±6.806
3.	F3	60	67	73	66.64±6.506
4.	F4	74	84	88	82.22±7.214
5.	F5	85	79	94	86.00±7.546
6.	F6	78	91	85	84.66±6.506
7.	F7	93	104	90	95.66±7.376

Table 5: Data of percentage Moisture Absorption (SD, n=3)

S.No.	Formulation code	Percentage moisture absorption			
		Trial 1	Trial 2	Trial 3	Mean±S.D
1.	F1	4.65	6.97	9.3	6.974±2.322
2.	F2	0	2.63	2.63	1.754±1.516
3.	F3	0	2.94	2.94	1.962±1.6978
4.	F4	2.70	2.70	5.50	3.632±1.618
5.	F5	2.43	2.43	4.87	3.243±1.406
6.	F6	2.70	5.40	5.40	4.506±1.5586
7.	F7	4.761	7.142	7.142	6.348±1.376

Table 6: Data of percentage Moisture Loss (SD, n=3)

S.No.	Formulation code	Percentage moisture loss			
		Trial 1	Trial 2	Trial 3	Mean±S.D
1.	F1	10.0	12.5	15.0	12.66±2.58
2.	F2	7.89	10.52	10.52	9.64±1.52
3.	F3	7.50	10.06	10.0	9.16±1.46
4.	F4	2.5	5.06	7.5	5.00±2.58
5.	F5	2.85	2.85	5.71	3.82±1.68
6.	F6	0	5.26	7.89	4.38±4.02
7.	F7	6.97	9.30	11.62	9.28±2.36

Table 7:- Data of percentage Water vapors transition rate (SD, n=3)

S.No.	Formulation code	Water vapour transition rate			
		Trial 1	Trial 2	Trial 3	Mean±S.D
1.	F1	.0043	.0046	.0046	45±.0006
2.	F2	.0020	.0031	.028	26±.0008
3.	F3	.0026	.0032	.0034	30±.0006
4.	F4	.0028	.0023	.0034	28±.0006
5.	F5	.0031	.0031	.0028	30±.0002
6.	F6	.0037	.0034	.0046	37±.0006
7.	F7	.0049	.0043	.0037	42±.0008

Table 8: Data of percentage Tensile strength (SD, n=3)

S.No.	Formulation code	Tensile strength Kg/mm²			
		Trial 1	Trial 2	Trial 3	Mean ± S.D
1.	F1	3.85	3.96	3.71	3.84±.012
2.	F2	2.85	2.96	3.07	2.96±.112
3.	F3	3.05	3.14	3.13	3.13±.082
4.	F4	3.18	3.29	3.21	3.22±.058
5.	F5	3.22	3.31	3.28	3.27±.048
6.	F6	3.27	3.39	3.36	3.34±.064
7.	F7	3.32	3.47	3.44	3.41±.078

Table 9: Percentage of drug content of F1 to F7 formulation (SD, n=3)

S.No.	Formulation Studies	Concentration Mean ± SD*(mg/cm²)	Percentage drug content
1.	F1	1.176±0.072	92.66
2.	F2	1.056±0.072	87.68
3.	F3	1.083±0.048	90.26
4.	F4	1.083±0.056	90.26
5.	F5	1.114±0.076	92.86
6.	F6	1.114±0.038	92.86
7.	F7	1.114±0.035	95.44

CONCLUSION

The present study was designed to investigate the possibility of preparing Transdermal patches of a known herbal bio-active compound Betulinic acid to combat poor solubility and poor bioavailability profile.The Transdermal patch of Betulinic acid was prepared successfully by solvent evaporation method.The prepared patches exhibited satisfactory physical characteristics such as weight uniformity, thickness uniformity and folding endurance. This may be due to the HPMC E5 which is more hydrophilic than, EC, which is less permeable to water vapour. Among all the developed formulations, batch F7 containing HPMC E5 and EC (5:5) showed maximum rate of drug release of 95.44 ± 0.262 within 24 h.This research work has established the foundation for future study on the potential of Betulinic acid loaded patches for a Transdermal delivery system. One of the additional advantages of the formulation is better stability profile. The formulated Transdermal film in the study is simple in preparation without using any special or costly excipients thus making it cost effective also.

Disclosure Statement: There are no conflicts of interest.

Acknowledgment: It's our privilege to express the profound sense of gratitude and cordial thanks to our respected chairman Mr. Anil Chopra and Vice Chairperson Ms. Sangeeta Chopra, St. Soldier Educational Society, Jalandhar for providing the necessary facilities to complete this research work.

Funding: Nil

Authors Contributions: All the authors have contributed equally.

Ethics approval: N/A

Informed Consent Statement: N/A

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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