



Response Surface Methodology for Optimization of Ultrasound-Assisted Transdermal Delivery and Skin Retention of Asenapine Maleate

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Abstract

Purpose Asenapine maleate (ASP) is an antipsychotic agent used in the treatment of schizophrenia and bipolar disorder. It has extremely low oral bioavailability of < 2%, necessitating the utilization of alternate route of administration. The objective of this work is to study and optimize the sonophoretic transdermal delivery and skin retention of ASP statistically, in Sprague-Dawley rat skin, using response surface methodology in Design of Experiments (DoE).

Method I-optimal design was employed, using the ultrasound (US) parameters viz., duration of US application, amplitude of US, and mode of US application (simultaneous application or pretreatment) as the input variables. Steady-state flux (J_{ss}) of ASP and amount of drug retained in skin after 24 h was taken as the output responses.

Results The model and dependent variables were found to be significant and representative of the data and response surface. While passive diffusion yielded J_{ss} of 2.575 $\mu\text{g}/\text{cm}^2/\text{h}$, the same values with US application ranged from 8.18 to 127.68 $\mu\text{g}/\text{cm}^2/\text{h}$. Passive diffusion of drug showed 46.22 \pm 5.2 $\mu\text{g}/\text{cm}^2$ of ASP retained in 24 h, while US application resulted in 99.07 to 1495.6 $\mu\text{g}/\text{cm}^2$ of drug retained in skin in 24 h. Based on the findings from optimization studies, 30 min of US application, with amplitude of 27–28, and simultaneous application mode was found to achieve the optimal transdermal drug flux, with slightly lower retention values in skin.

Conclusion The study found that the sonophoretic transdermal permeation and retention of ASP in vitro could be optimized using response surface methodology.

Keywords Asenapine maleate · Transdermal · Ultrasound · Sonophoresis · Design of Experiments · Response surface methodology

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Introduction

Asenapine maleate (ASP) is an atypical antipsychotic agent, used in treating schizophrenia and acute manic or mixed episodes of bipolar I disorder [1]. Upon oral administration, it undergoes extensive hepato-gastrointestinal first-pass metabolism via N-oxidation and/or 11-hydroxylation occurring in the liver and gut, resulting in bioavailability of < 2%. As a result, ASP is administered as a sublingual tablet [2]. However, due to the drawbacks with this route, researchers have investigated alternate approaches, including transdermal, intranasal, and injectable routes for delivery of this agent [3–6].

Transdermal drug delivery is the non-invasive route of delivering drugs across skin for systemic effect. It is ideal for molecules that undergo hepato-gastrointestinal first-pass metabolism. It also prevents GI sensitization, permits prolonged

drug release, improves patient compliance, and is self-administrable [7]. This route is also highly advantageous in administering drugs to patients who are unable to swallow orally. However, the skin permeation of drugs is highly limited by the stratum corneum (SC), necessitating the use of various permeation enhancement techniques. These techniques could be classified into physical strategies, chemical strategies, and a combination of both. The physical strategies consist of ultrasound, iontophoresis, magnetophoresis, microneedles, laser ablation, and heat [8–10]. Chemical strategies include the use of chemical permeation enhancers like azone, terpenes, polyalcohols, alkanols, sulfoxides, and surfactants, among others [11, 12].

One of the important physical strategies for enhancing transdermal drug delivery is sonophoresis. Sonophoresis or phonophoresis refers to the application of low-frequency ultrasound (US) to skin, to cause improved drug permeability [13]. On the basis of frequency, US can be classified into two main types, viz., low-frequency US (20–100 kHz) and high-frequency US (≥ 0.7 MHz), with the former showing greater transdermal permeability [14, 15]. Apart from frequency, other sonophoretic parameters like duration of US application, intensity of US, duty cycle, and others have been shown to modulate the drug permeation through the skin [10, 16]. While the goal of transdermal delivery is to achieve drug release into systemic circulation, the drug may also get entrapped in the skin layers, forming a drug depot from where the drug may diffuse slowly, even after removal of the applied formulation [17]. Therefore, for transdermal applications, a balance between permeation and skin retention would be sought. In this research paper, we seek to study and optimize the sonophoretic transdermal delivery of ASP statistically, using response surface methodology in design of experiments (DoE). The parameters evaluated were drug permeation, and drug retention *in vitro*, in male Sprague-Dawley rat skin.

DoE is a tool widely used in pharmaceutical formulation development, which helps to model the effects of different input variables on select output responses, predict responses in the experimental design space, identify factor-interactions, and analyze synergism or antagonism among various factors [18, 19]. The use of DoE for transdermal drug delivery is limited [20], and this is the first report on the use of this methodology for analyzing both sonophoretic transdermal drug permeation, along with drug retention in skin.

Materials and Methods

Materials

ASP was received as a kind gift from Orbicular Pharmaceutical Technologies Pvt. Ltd., Hyderabad, India. Acetonitrile and methanol (HPLC grade) and triethylamine

were procured from Merck Specialties Pvt. Ltd. (Mumbai, India). All other chemicals used were of analytical grade.

Analytical Method for Estimation of ASP

Asenapine maleate was analyzed by RP-HPLC by modification of an earlier method [5, 21]. The HPLC system (LC-2010, Shimadzu, Kyoto, Japan) comprised a pump, degasser (Shimadzu), auto-injector, UV/Vis detector, system controller (Shimadzu), and LC Solution software (version 1.24 SP1). The column used was Hyperclone BDS C18 (5 μm ; 250 \times 4.6 mm), maintained at temperature of 25 °C. The mobile phase was a mixture (40:60 *v/v*) of acetonitrile and phosphate buffer pH 3.0 (20 mM, containing 0.1% *v/v* triethylamine), with flow rate of 1.0 mL/min. The wavelength of detection was 230 nm and injection volume was 20 μL . The developed method was linear ($R^2 > 0.9995$) in the range of 0.1–20 $\mu\text{g/mL}$. Accuracy was within 98.0–100.1% and precision values (inter- and intraday) were within RSD < 2%. Recovery of drug from skin extracts was found to be more than 96.5%.

In Vitro Skin Permeation and Retention Studies

Animals

Abdominal skin from male Sprague-Dawley rats was used for the drug permeation and retention experiments. The animals, 6–8 weeks old, weighing 180–220 g were housed at 24–26 °C, exposed to an equal light/dark cycle, with unrestricted access to water and standard chow. The experiments were performed with approval of the experimental protocol by the Institutional Animal Ethical Committee, Manipal Academy of Higher Education, Manipal (Approval No. IAEC/KMC/25/2015). The fur on the abdominal portion of the rats was removed 24 h prior to the experiment, with the help of an electric clipper. The rats were sacrificed and the skin was excised. The underlying subcutaneous tissue and fat were removed, and the skin pieces were washed thoroughly under running water. The skin was then secured between the donor and receptor compartments of a vertical diffusion cell (with diffusion area of 1 cm^2), with the stratum corneum towards the donor compartment. Hydration of the skin was undertaken for 1 h, using phosphate buffer (pH 7.4), with the setup maintained on a magnetic stirrer. After this, the solutions from both the donor and the receptor compartments were discarded, and the experiments were performed using this hydrated skin.

Passive Diffusion Studies

Dispersion of ASP in pH 7.4 phosphate buffer was taken in the donor compartment of the diffusion cell. Plain pH 7.4 phosphate buffer was taken in the receptor compartment, and the setup was maintained on a magnetic stirrer.

Sampling (volume 0.5 mL) was carried out from the receptor compartment at different intervals up to 24 h. After each sampling, equal amount of plain buffer was added to the receptor compartment. The amount of ASP in the sample solutions was determined by HPLC. After the permeation experiments, the skin was removed from the diffusion cells and the skin surface was washed thoroughly with water. The skin integrity was verified by means of digital multimeter. The skin was then cut into small pieces, and the drug was extracted overnight using methanol. The amount of drug in the sample was determined by HPLC [22, 23].

Application of Ultrasound (US)

Low-frequency ultrasound (LFU) was applied using a 130-W ultrasonic processor (VibraCell™, VC 130, Sonics and Materials Inc., Newton, CT, USA). The probe tip was inserted into the donor compartment and maintained at 3–5 mm from the surface of the skin (Fig. 1). The US parameters varied were duration of US application (10–30 min), amplitude of US (20–40), and mode of US application (simultaneous application or pretreatment). In simultaneous application (SA) mode, US was applied along with the drug suspension in the donor compartment (for the respective duration). After that time duration, US application was ceased and permeation studies were continued for 24 h with the drug dispersion in the donor compartment. In pretreatment (PT) mode, the donor compartment was initially filled with plain buffer and US was applied for the respective duration. Following this, the buffer was discarded and the donor compartment was filled with drug suspension. The permeation studies were then continued for 24 h. In both modes of US application, the time of addition of drug dispersion to the donor compartment, was taken as time 0 h. Temperature rise during US application was prevented by pulsed application (1 s “on,” 1 s “off”; 50% duty cycle) and replacement of the donor drug dispersion every 2 min [22, 23].

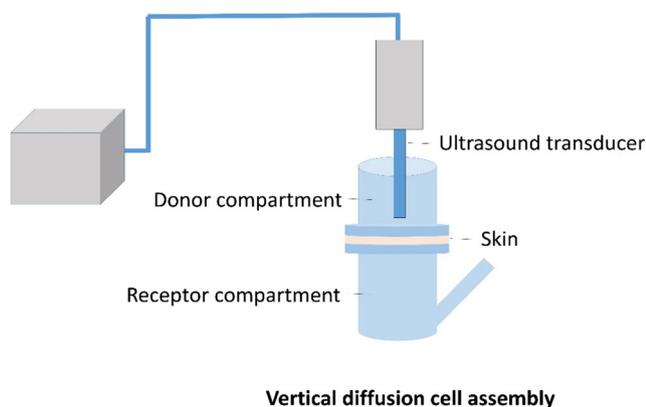


Fig. 1 Vertical diffusion cell assembly for permeation studies with ultrasound application

Skin Permeation Parameters

The steady-state flux (J_{ss} : $\mu\text{g}/\text{cm}^2/\text{h}$) of ASP was calculated from the slope of the linear portion of the graph of drug permeation with respect to time. Following this, the enhancement ratio (ER) was calculated as enhancement factor = (enhanced flux)/(unenhanced flux). The amount of drug permeated through skin in 24 h (Q_{24}) was also determined [9, 22].

Experimental Design

In this study, the input (or independent) variables were a combination of numeric and categoric factors. Therefore, I-optimal design under response surface methodology was selected and run using Design Expert software (version 9.0.3.1; Stat Ease, Minneapolis, USA). US parameters viz., duration of US application (A), amplitude of US (B), and mode of US application (C) were chosen as the input variables. The output (or dependent) responses studied were transdermal drug flux (R1) and amount of drug retained in skin at the end of 24 h (R2). The input variables and their ranges were selected based on initial single-factor trials. I-optimality was chosen to lower the average variance of prediction in the region of experimentation. The design included 19 runs with five replicates and five lack-of-fit points to give the output responses (Table 1). Diagnostic case statistics and polynomial equations for each response variable were generated. The values of $p < 0.05$ were considered statistically significant.

Degradation of ASP in Skin

The degradation of ASP was assessed in dermal, epidermal, and skin extracts. Briefly, the excised skin was mounted on the vertical diffusion cell, with the stratum corneum towards the donor compartment. Both the donor and receptor compartments were filled with pH 7.4 phosphate buffer. The setup was maintained on a magnetic stirrer. After 24 h, the solutions from donor and receptor compartments were collected separately (epidermal and dermal extracts, respectively). To prepare skin extract, 1- cm^2 area of skin was homogenized in pH 7.4 phosphate buffer (10 mL), centrifuged, and the supernatant collected. To 5 mL of each of these extracts, a standard solution of ASP in pH 7.4 phosphate buffer was added. These mixtures were maintained on a shaking water bath at 32 °C for 8 h, after which they were analyzed by HPLC. Plain ASP standard solution in buffer was taken as the control. These studies were performed in triplicate. This experiment was conducted to determine if ASP undergoes degradation in skin, which could confound the results of permeation and retention studies [8, 24].

Table 1 Diagnostic case statistics for the response variables

Run No.	A	B	C	R1		R2	
				Actual value ($\mu\text{g}/\text{cm}^2/\text{h}$)	Predicted value ($\mu\text{g}/\text{cm}^2/\text{h}$)	Actual value ($\mu\text{g}/\text{cm}^2$)	Predicted value ($\mu\text{g}/\text{cm}^2$)
1	1	-1	Level 2 of C	23.27	20.17	564.7	496.25
2	0	-1	Level 1 of C	51.03	52.13	873.21	902.12
3	0	0	Level 1 of C	76.53	75.66	1126.5	1067.24
4	-1	1	Level 2 of C	13.02	12.21	182.3	196.95
5	1	-1	Level 1 of C	73.6	80.15	1056.91	1007.10
6	1	1	Level 2 of C	36.07	36.65	860.9	872.46
7	-1	1	Level 2 of C	14.43	12.21	198.7	196.95
8	0	-1	Level 1 of C	57.32	52.13	905.67	902.12
9	1	1	Level 1 of C	127.68	128.61	1495.6	1499.76
10	0	0	Level 2 of C	23.86	25.73	520.12	499.75
11	0	-1	Level 2 of C	18.07	18.19	230.13	392.85
12	1	0	Level 1 of C	111.89	106.93	1121.8	1224.35
13	0	0	Level 2 of C	21.4	25.73	478.96	499.75
14	0	1	Level 1 of C	94.18	94.10	1370.4	1290.51
15	-1	0	Level 1 of C	35.3	36.90	656.3	649.95
16	-1	-1	Level 2 of C	8.18	8.70	99.07	29.27
17	-1	1	Level 1 of C	50.49	52.10	769.83	821.10
18	0	0	Level 2 of C	27.02	25.73	549.08	499.75
19	-1	0	Level 1 of C	37.58	36.90	637.98	649.95

A: Duration of US application, -1 = 10 min, 0 = 20 min, 1 = 30 min; B: Amplitude of US, -1 = 20, 0 = 30, 1 = 40 amplitude; C: Mode of US application, Level 1 = simultaneous application mode, Level 2 = pretreatment mode

Results and Discussion

In the case of passive diffusion studies, the transdermal flux of ASP was found to be $2.575 \mu\text{g}/\text{cm}^2/\text{h}$ ($Q_{24} = 53.8 \pm 4.5 \mu\text{g}/\text{cm}^2$). The amount of drug retained in skin at the end 24 h was $46.22 \pm 5.8 \mu\text{g}/\text{cm}^2$. Figure 2 shows the stability of ASP in plain buffer solution, epidermal, dermal, and skin extracts. More than 90% drug was found remaining in all the extracts,

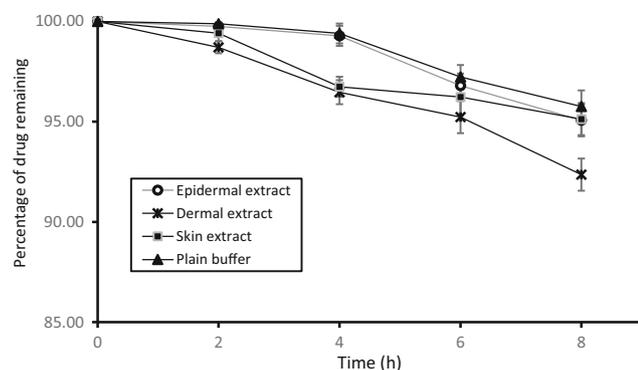


Fig. 2 Degradation of ASP with respect to time in epidermal, dermal, and skin extracts and plain pH 7.4 phosphate buffer, expressed as percentage of drug remaining in solution. At the end of 8 h, more than 90% of drug was found remaining in all extracts. The values are presented as mean \pm SD, $n = 3$

indicating sufficient stability in skin. This confirms the suitability of ASP for transdermal administration. With the application of US, a total of 19 experiments were executed in analogy with I-optimal design. The independent variables (coded) and output responses (actual and predicted) are given in Table 1.

The range of values of transdermal drug flux was found to be $8.18 \mu\text{g}/\text{cm}^2/\text{h}$ ($Q_{24} = 158.30 \mu\text{g}/\text{cm}^2$, ER = 3.19) (run 16) to ($Q_{24} = 2265.80 \mu\text{g}/\text{cm}^2$, ER = 49.58) (run 9)). The range of values of drug retention in skin at the end of 24 h was found to be $99.07 \mu\text{g}/\text{cm}^2$ (run 16) to $1495.6 \mu\text{g}/\text{cm}^2$ (run 9). US application parameters, viz., duration (10–30 min.), amplitude (20–40), and mode (SA or PT), were taken as the independent variables. For response R1, the chosen model was found significant ($p < 0.0001$) and lack-of-fit was insignificant ($p > 0.05$). For response R2, the model was found significant ($p < 0.0001$). The corresponding adjusted and predicted R^2 values were 0.9588 and 0.8650, respectively. Therefore, the model was taken as representative of the data and response surface. All the tested input variables (A, B, and C) were found to be statistically significant for both output responses, with p values < 0.0001 . Additionally, for response R1, AB, BC, and AC interactions were also found significant ($p < 0.0002$). The highest values of drug flux through skin and retention in skin were both found at +1 levels of factors

A and B, and level 1 of factor C (i.e., SA mode). On the other hand, the lowest levels of drug flux and retention in skin were found at -1 levels of A and B, and level 2 of C (i.e., PT mode). The results of ANOVA and model adequacy parameters are given in Table 2. The second-order polynomial equations for R1 and R2 were given by

$$R1 = 50.6980 + 21.9975A + 12.9916B - 24.966C + 3.2411AB - 13.0182AC - 7.9962BC - 3.7522A^2 - 2.5478B^2 \tag{1}$$

and,

$$R2 = 783.49356 + 286.4100A + 165.0863B - 283.7481 + 52.1302BC - 0.7892AC - 29.1132 BC - 130.0879A^2 + 29.0735B^2 \tag{2}$$

In the above equations, positive values indicate synergistic effect and negative values indicate antagonistic effect of the input variable on the output response. The results indicated that both independent variables A and B had a positive effect on the dependent variables, R1 and R2. In the case of variable C, the effect on R1 and R2 was negative, as given by the above coded equations. This indicates that the responses R1 and R2 decreased when the level of C was changed from SA (level 1) to PT (level 2).

Effect of Duration of US Application

In this study, the durations of US used were 10 min (coded as -1), 20 min (coded as 0), and 30 min (coded as +1). After US application for the respective duration, permeation studies were continued up to 24 h. As clearly indicated by Eq. (1) and response surface plots (Fig. 3a, b), duration of US application has a significant positive effect on the drug permeation through skin. The highest transdermal flux of drug across skin was found to be 127.68 μg/cm²/h (Q₂₄ = 2265.80 μg/cm²). With both the modes of US application (SA or PT), it was found that increasing the duration of US also enhanced the drug permeation in skin. Also, it was observed that duration of US application had significant positive effect on drug retained in skin at

the end of 24 h. This is indicated in Eq. (2), and corresponding contour plots (Fig. 3c, d). The highest amount of drug retained in skin was found with run 9 (1495.6 μg/cm²).

Various studies have used varying durations of US application, from a few seconds up to 4 h [13]. It has been demonstrated that for ultrasound-assisted transdermal drug permeation to occur, there is a “threshold dose” of energy, below which there may not be significant permeation enhancement [16, 25]. In the current study, we have taken the upper limit of US application duration, as 30 min, to be feasible in a clinical setting. As demonstrated in previous reports, the energy applied during sonophoresis is given as: energy = intensity × application time × duty cycle. Thus, the applied energy is directly related to the time duration of US application and would translate into higher skin permeation of drug [16].

Ultrasound has been shown to enhance drug uptake in various tumors, reduce the antibiotic resistance of bacterial films, facilitate delivery of drugs into brain, deliver DNA intracellularly, dissolve clots in treatment of stroke, and trigger drug release from carriers [26]. Moreover, it has been found to enhance the toxicity of chemotherapeutic agents to cancer cells and also facilitate wound healing. With respect to transdermal drug delivery, ultrasound has been shown to enhance the skin permeation of both low-molecular-weight agents like ketoprofen, diclofenac, salicylic acid, and high-molecular-weight agents like heparin, insulin, interferon-γ, erythropoietin, and vaccines like tetanus toxoid and also nanoparticles [10, 13, 26, 27].

Effect of Amplitude of US

The amplitude of US used in this study were 20 (coded as -1), 30 (coded as 0), and 40 (coded as +1), corresponding to output wattage of ~4, 7, and 10 W/cm², respectively. As indicated by Eqs. (1) and (2), and corresponding response surface plots (Fig. 3a–d), amplitude has a direct effect on both, the transdermal flux and skin retention of drug. Amplitude is a measure of the power or intensity of US. When amplitude increases, the intensity (and power) of the US also increases. Mitragotri et al. [25] have shown that skin permeability is directly proportional to the US intensity. This would translate into higher values of drug permeation observed at the end of 24 h. However, as the amplitude of US was increased, it was observed that the skin retention of drug also increased.

Table 2 Regression analysis for output responses R1 and R2

Response	Model	R ²	Adjusted R ²	Predicted R ²	SD	% CV	Adequate precision
R1	Quadratic	0.9934	0.9882	0.9699	3.78	7.96	46.1313
R2	Quadratic	0.9771	0.9588	0.8650	81.39	11.29	26.2525

R1 = Transdermal flux of drug μg/cm²/h, R2 = Amount of drug retained in skin at the end of 24 h (μg/cm²)
SD standard deviation, CV coefficient of variation

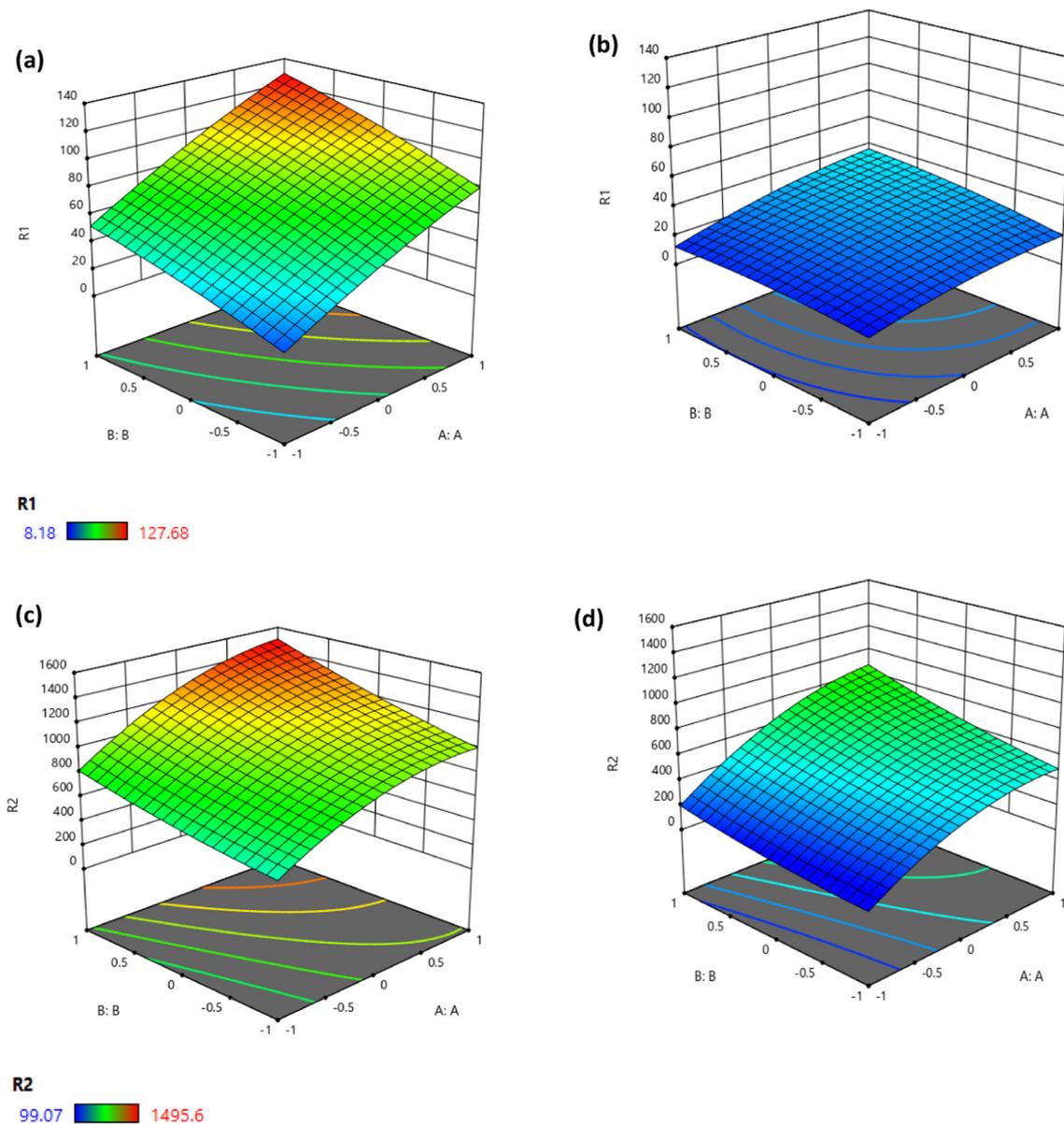


Fig. 3 Response surface plots demonstrating the effect of independent variables (A and B) on output responses R1 and R2. **a, c** At level 1 of C. **b, d** At level 2 of C. A: Duration of US (min.), B: Amplitude of US, C:

Mode of US application (simultaneous application—level 1 or pretreatment—level 2); R1: Transdermal flux of ASP ($\mu\text{g}/\text{cm}^2/\text{h}$), R2: Amount of ASP retained in skin in 24 h ($\mu\text{g}/\text{cm}^2$)

Effect of Mode of US Application

Ultrasound has been applied on skin in mainly one of two ways, i.e., (a) simultaneous application, wherein US is applied through a coupling medium containing the drug, or (b) pretreatment, in which the skin membrane is treated with US prior to contact with the drug [13]. The former mode is said to enhance drug transport across skin through two ways—enhancement of skin permeability by convection-related mechanisms and structural changes in the stratum corneum. The latter mode is said to increase skin permeability only through induction of structural changes. Convection-related mechanisms like acoustic

streaming can cause the bulk fluid to move in the direction of the applied US field and also induce rigorous mixing of the medium. This causes greater availability of the drug or permeant at the skin surface and has been implicated by some researchers in the greater permeability of mannitol, sucrose, and lidocaine observed with simultaneous application of US [28, 29].

In the current study, we have studied the application of US using both modes. Analysis of the responses clearly indicated that the values of transdermal drug flux and drug retention in skin were considerably higher with SA mode of US application than with PT mode of US application (Table 1). This was also indicated in Eq. (1), wherein changing the level of C from

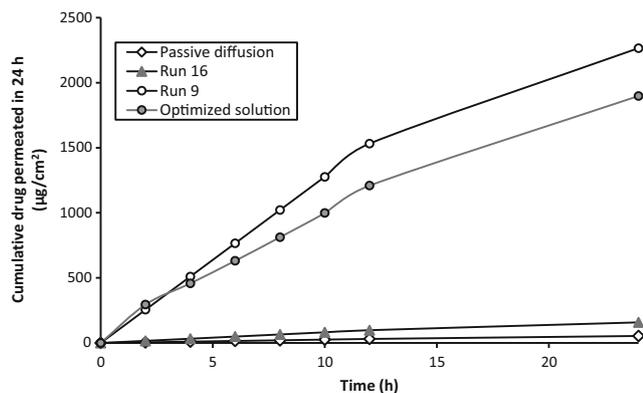
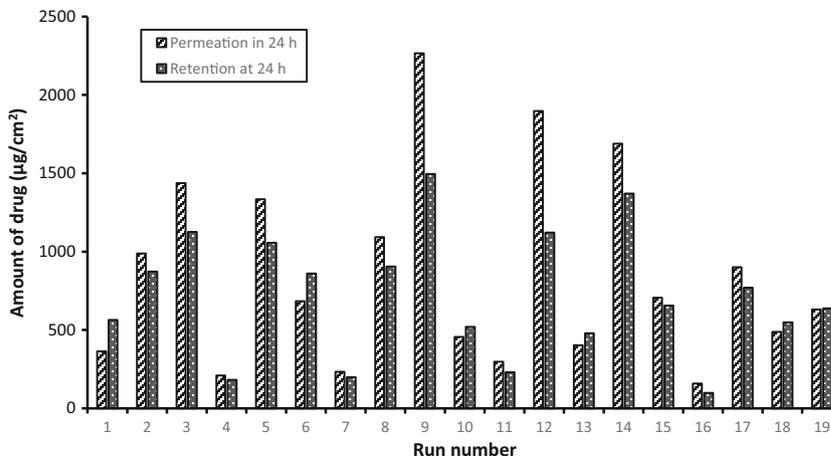


Fig. 4 In vitro skin permeation profile of ASP with passive diffusion and ultrasound treatments (Run 16, Run 9 and optimized solution)

level 1 (SA mode) to level 2 (PT mode) decreased both the drug flux and retention values. The same was again indicated in the response surface plots, where the values of responses R1 and R2, obtained with level 1 of C (Fig. 3a, c), were greater than those obtained with level 2 of C (Fig. 3b, d).

Tezel et al. studied the skin permeability brought about by the combination of surfactants and US [30]. They noted that US application could enable diffusion of surfactant into and homogenous distribution within the skin, allowing greater interaction of skin lipids with surfactant. Similarly, in our study, the SA of drug along with US, could be driving the drug molecules into the skin in the duration of the US application. With the skin as a depot, the drug molecules could be diffusing out slowly into the receptor medium. Even at the end of 24 h, the amount of drug retained in the skin could still be higher than the amount of drug that diffused into the receptor medium. In contrast, with PT mode of application, the US could be creating structural changes in skin, which enhance drug permeability. However, unlike SA experiments, the amount of drug molecules getting driven into skin layers may be lesser, as evidenced by the lesser amount of drug retention in skin at the end of 24 h.

Fig. 5 Amount of ASP permeated through skin in 24 h (Q_{24}) and amount of ASP retained in skin at 24 h in the sonophoretic permeation and retention studies (Runs 1–19)



Interaction Effects

Interestingly, as shown in Eq. (1), the duration of US application (A) demonstrated interaction with the amplitude of US (B) and mode of US application (C), for output response R1. Moreover, the amplitude of US (B) demonstrates interaction with mode of US application (C). With increase in the amplitude of US, the increase in transdermal flux and retention values are lesser with SA, than with PT. This means that the effect of increasing US amplitude and consequently, the US intensity, is less pronounced in SA mode than in PT mode. As demonstrated by earlier reports, with SA of drug and US, higher US intensity induces growth of bubbles at the surface of the US transducer, thereby decreasing the efficiency of the acoustic streaming process. This would contribute to the lesser increase in drug permeation and retention, with higher US amplitude (and intensity).

Optimization of Skin Permeation Parameters

The target skin permeation rate of ASP can be given as [24, 31, 32]

$$J_{ss} = \frac{Cl \times Cp \times W}{A}$$

Here, J_{ss} is the transdermal drug flux ($\mu\text{g}/\text{cm}^2/\text{h}$), Cl is the rate of clearance of drug, Cp is the plasma concentration of drug, W is the weight, and A is the area of application (cm^2). The clearance rate and plasma concentration of drug as obtained from literature are 52 L/h and 4 ng/mL [1, 33]. From these values, the target permeation rate for ASP as calculated from the above equation was found to be 208 $\mu\text{g}/\text{cm}^2/\text{h}$. From the results of transdermal flux obtained with our study, it was observed that the desired transdermal flux values could be obtained with an appreciable area of application of $\sim 2 \text{ cm}^2$. As the goal was also to maximize the transdermal drug flux and minimize the skin retention drug of drug, the predicted

optimum solution consisting of duration of US application 30 min, amplitude of US 27–28, and mode of US application: SA mode gave desirability value of 0.830. Herein, predicted value of drug flux was $102 \mu\text{g}/\text{cm}^2/\text{h}$. However, skin retention of drug was on the higher side, at $1176.44 \mu\text{g}/\text{cm}^2$. The experimental values for this trial were $93.92 \mu\text{g}/\text{cm}^2/\text{h}$ ($Q_{24} = 1897.95 \mu\text{g}/\text{cm}^2/\text{h}$, $\text{ER} = 36.47$) and $1260.07 \mu\text{g}/\text{cm}^2$, respectively. The permeation profiles of ASP and the amount of drug permeated across skin in 24 h (Q_{24}) and drug retained in skin at 24 h are given in Figs. 4 and 5. Therefore, it was concluded that the target flux of ASP could be achieved by optimizing the US application parameters. However, as the drug flux increased, the value of drug retention in skin also increased.

Conclusion

The present study reported the use of response surface methodology in the optimization of sonophoretic transdermal permeation of asenapine maleate, *in vitro*. The model and the effect of independent variables on the output responses were found to be statistically significant. Ultrasound-assisted transdermal delivery was found to considerably enhance the drug flux. By adjusting the ultrasound parameters, considerable drug permeation with slightly lower drug retention values was found to be achieved.

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Compliance with Ethical Standards

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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