

Development and Physicochemical, *In Vitro* and *In Vivo* Evaluation of Transdermal Patches of Zaleplon

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Submission Date: 29-8-2013; Review completed: 21-9-2013; Accepted Date: 21-1-2014

ABSTRACT

The aim of the present work was to develop and evaluate matrix type transdermal therapeutic system of zaleplon (ZLP) with different ratios of hydrophilic and hydrophobic polymeric combinations. Formulations A1, A2, A3 were composed of Eudragit RL100 (ERL) and hydroxypropyl methyl cellulose (HPMC) in 1:3, 1:1, 3:1 ratios; A4, A5, A6 were composed of Eudragit RS100 (ERS) and hydroxypropyl methyl cellulose in 1:3, 1:1, 3:1 ratios. All the six formulations carried 10mg of ZLP/patch area, 6% v/w of d-limonene, 15% v/w of propyleneglycol in methanol:dichloromethane (1:1). The physicochemical compatibility of the drug and the polymers was studied by infrared spectroscopy and differential scanning calorimetry. All the prepared formulations were subjected to physicochemical studies, *in vitro* release, *ex vivo* permeation studies through rat skin, skin irritation, stability and *in vivo* evaluation. The optimized formulation, A1 was found free of skin irritation and stable at accelerated stability conditions for 3 months. Locomotor activity and rotarod methods were used to study the *in vivo* performance. The administration of ZLP through patch resulted in sustained and continued action for 24h. Hence, it was concluded that ZLP can be formulated into the transdermal matrix type patch to sustain its release characteristics.

Keywords: Zaleplon, Transdermal, Limonene, Eudragit, Hydroxypropyl Methyl Cellulose.

INTRODUCTION

Delivery of drugs into systemic circulation via skin has generated a lot of interest during the last decade as transdermal drug delivery systems (TDDS). TDDS offer many advantages over the conventional dosage forms and oral controlled release delivery systems, notably avoidance of hepatic first pass metabolism, decrease in frequency of administration, reduction in gastrointestinal side effects and improves patient compliance.¹ However, the highly organized structure of stratum corneum forms an effective barrier to the permeation of drugs, which must be modified if poorly penetrating drugs are to be administered. The use of chemical penetration enhancers would significantly increase the number of drug molecules suitable for transdermal delivery.^{2,3}

Zaleplon is a pyrazolopyrimidine hypnotic drug indicated for the short term (2 to 4 weeks) management of insomnia.⁴ It interacts with GABAA receptor and also shows some pharmacological properties of benzodiazepines.⁵

It possesses ideal characteristics, such as low molecular weight (305.54), favorable logarithmic partition coefficient (log octanol/water: 1.23), smaller dose (1mg to 10mg), short elimination half-life (1.05 ± 0.13 h) and poor oral bioavailability ($30.6 \pm 10.2\%$), for formulation as a transdermal patch.⁶

Terpenes, naturally occurring volatile oils, appear to be promising candidates for clinically acceptable enhancers.⁷ Terpenes are generally considered as less toxic and have less irritant effects compared to surfactants and other skin penetration

DOI: 10.5530/ijper.47.4.7

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enhancers, and some terpenes have been characterized as Generally Recognized As Safe (GRAS) by the US FDA (1, FDA 2006). They have high percutaneous enhancement ability, reversible effect on the lipids of stratum corneum, and minimal percutaneous irritancy at low concentrations (1–5%).⁸ Moreover, a variety of terpenes have been shown to increase percutaneous absorption of both hydrophilic and lipophilic drugs.^{9–12} *d*-Limonene, a cyclic terpene is free from toxic effects and has been used as a penetration enhancer in the transdermal delivery of several drugs.^{13,14}

Zaleplon has not been previously investigated and reported for potential administration through transdermal route. The aim of the present study was to develop transdermal matrix patches with different ratios of hydroxy propyl methyl cellulose (HPMC) and Eudragit RL 100 (ERL)/Eudragit RS 100 (ERS), containing the drug, zaleplon and optimized concentration of *d*-Limonene. To perform the physicochemical, *in vitro*, *ex vivo* and *in vivo* evaluation of the prepared patches. The purpose was to provide the delivery of drug at a controlled rate across intact skin to achieve a therapeutically effective drug level for a longer period of time from transdermal patches.

MATERIALS AND METHODS

Materials

Zaleplon, Eudragit RL 100 (ERL) and Eudragit RS 100 (ERS) were received as gift samples from Aurobindo Pharmaceuticals private limited (Hyderabad, India). *d*-Limonene was obtained from Himedia (Mumbai, India). Liquid mercury, hydroxypropyl methyl cellulose 15cps (HPMC), propylene glycol (PG), polyethylene-glycol 400 (PEG 400) were purchased from SD Fine Chemicals Ltd (Mumbai, India).

Animals

Albino mice with a weight of 20–30gm, albino rats with a weight of 150–200gm, albino rabbits each weighing 1.5 to 2.0kg were used in this study. They were fed a standard laboratory diet and had access to water *ad libitum* and kept under standard laboratory conditions in 12-hr light/dark cycle at 25±2°C. The animals were obtained from Sainath Animal Agency, Hyderabad, India. Prior approval by Institutional Animal Ethics Committee was obtained for conduction of experiment (Ref: IAEC/SUCP/07/2009).

Methods

Preparation of rat abdominal skin

Albino rats weighing 150–200gm were selected for permeation studies. The animals were sacrificed

using anesthetic ether. The hair of test animals was carefully trimmed short with a pair of scissors and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique,¹¹ which involved soaking the entire abdominal skin in water at 60°C for 45sec, followed by careful removal of the epidermis. The epidermis was washed with water and used for *ex vivo* permeability studies. The rats were obtained from Sainath Animal Agency, Hyderabad, India. Prior approval by Institutional Animal Ethics Committee was obtained for conduction of experiment (Ref: IAEC/SUCP/07/2009).

Effect of *d*-limonene concentration as penetration enhancer on the *ex vivo* permeation of ZLP across rat abdominal skin

Franz diffusion cell with a surface area of 2.64cm² was used for *ex vivo* permeation studies. The rat skin was mounted between the compartments of diffusion cell with stratum corneum facing the donor compartment. ZLP solution (5mg in 3ml of PBS of pH 7.4 containing PEG 400) was placed in the donor compartment containing different concentrations of *d*-limonene (0, 2, 4, 6% v/v). The 0% *d*-limonene served as control and PEG 400 was used to solubilize ZLP. The receiver compartment contained 13ml of 40% v/v PEG 400 in PBS pH 7.4 and the contents were stirred using magnetic stirrer. The whole assembly was kept at 37±0.5°C. Samples of 3ml were collected at preset time points up to 24h and replenished with 40% v/v PEG 400 in PBS pH 7.4. The samples were filtered through 0.45µ syringe filter (Sartorius AG, Goettingen, Germany) and drug content in the samples was measured using UV-VIS spectrophotometer at 232nm. All the experiments were performed in triplicate. The cumulative amount of the permeated drug was calculated.

Permeation data analysis and statistics

The drug concentration in the permeates was corrected for sampling effects according to the equation described by Hayton and Chen.¹⁵

$$C_n^1 = C_n \frac{V_T}{V_T - V_S} \frac{C_{n-1}^1}{C_{n-1}} \quad (1)$$

where C_n^1 is the corrected concentration of the n^{th} sample, C_n is the measured concentration of ZLP in the n^{th} sample, C_{n-1}^1 is the measured concentration of the ZLP in the $(n-1)^{\text{th}}$ sample, V_T the total volume of the receiver fluid and V_S the volume of the sample drawn.

As described by Barry (16), the steady state flux (J_{ss}) and permeability coefficient (K_p) are defined by

$$J_{ss} = \frac{dQ}{dt}_{ss} \frac{1}{A} \quad (2)$$

$$K_p = \frac{J_{ss}}{C_s} \quad (3)$$

where, A is the effective diffusion area; C_s , the concentration in the saturated solution and $(dQ/dt)_{ss}$ is the steady state slope.

The penetration enhancing effect of penetration enhancers was calculated in terms of enhancement ratio (ER) and was calculated by using the following equation (6).

$$ER = \frac{K_p \text{ with penetration enhancer}}{K_p \text{ without penetration enhancer}} \quad (4)$$

The cumulative amount permeated and flux values obtained were tested for significant differences using a one-way analysis of variance (ANOVA).

Physicochemical compatibility of drug and polymer

The physicochemical compatibility between ZLP, and polymers used in the patches was studied by using differential scanning calorimetry (DSC 821^e Mettler Toledo, Switzerland) and Fourier transform infrared (FT-IR) spectroscopy. In DSC analysis, the samples were weighed, hermetically sealed in flat-bottom aluminum pans, and heated over a temperature range of 35 to 250°C in an atmosphere of nitrogen at a constant increasing rate of 10°C/min. The thermograms obtained for ZLP, polymers, and physical mixtures of ZLP, with polymers were compared.

The infrared (IR) spectra were recorded using an FT-IR spectrophotometer (Perkin-Elmer FTIR-1605, India) by the KBr pellet method and spectra were recorded in the wavelength region between 4000 and 400 cm^{-1} . The spectra obtained for ZLP and physical mixtures of ZLP with polymers were compared.

Preparation of transdermal films

Matrix-type TDDSs of ZLP were prepared by solvent evaporation technique using different ratios of ERL/ERS and HPMC (Table 1). The polymers were weighed in requisite ratios by keeping the total polymer weight 1.2g and were allowed to swell for two hours in solvent mixture (1:1 ratio of dichloromethane, methanol). The drug solution was added to the polymeric solution while stirring. PG was incorporated as plasticizer and d-limonene as penetration enhancer. The solution was poured into a glass ring placed on the surface of liquid mercury kept in a petri plate. The solvent was allowed to evaporate for 24h. Aluminum foil was used as backing film. The polymer was found to be self-adhesive due

to the presence of Eudragit polymers along with the plasticizer. The patches were cut to give the required area and stored in an airtight container for further use.

Evaluation of physicochemical properties of patches

Thickness and weight variation

The thickness of the patches was assessed at six different points using a micrometer (Mitutoyo, Japan). For each formulation, three randomly selected patches were used. For weight variation test, three films (each 2.64 cm^2) from each batch were weighed individually and the average weight was calculated.

Folding endurance

The folding endurance was measured manually as per the reported method.¹⁷ Briefly, a strip of the film (4×3cm) was cut evenly and repeatedly folded at the same place until it broke. The thinner the film, the more flexible it is.

Flatness

Longitudinal strips were cut out from the prepared patch, the length of each strip was measured, and then the variation in the length due to the nonuniformity in flatness was measured. Flatness was calculated by measuring the constriction of strips, and 0% constriction was considered to be 100% flatness.¹⁸

Estimation of drug in polymeric films

Patch (2.64 cm^2) from each formulation was taken, cut into small pieces and was allowed to dissolve in a 100ml solution containing 15ml of methanol and 85ml of 40% v/v PEG 400 in PBS pH 7.4. The solution was filtered, diluted suitably and the absorbance of the solution was measured using UV-VIS spectrophotometer at a wavelength of 232nm against reference solution prepared with placebo films.

Percentage of moisture content

The films were weighed individually and kept in a desiccator containing activated silica at room temperature for 24h. The individual films were weighed repeatedly

Table 1: Composition of ZLP Transdermal Patches

Formulation code	ERL (mg)	ERS (mg)	HPMC (mg)	ZLP (mg)	PG (% v/w)	d-Limonene (% v/w)
A1 (1:3)	300	–	900	200	15	6
A2 (1:1)	600	–	600	200	15	6
A3 (3:1)	900	–	300	200	15	6
A4 (1:3)	–	300	900	200	15	6
A5 (1:1)	–	600	600	200	15	6
A6 (3:1)	–	900	300	200	15	6

until a constant weight was achieved. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight.¹⁹

Percentage of moisture uptake

The films were weighed accurately and placed in a desiccator containing 200ml of saturated solution of potassium chloride (84% relative humidity) at room temperature. After 3 days, the films were taken out and weighed. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.¹⁷

In vitro release studies

The *in vitro* release study was carried out using Franz diffusion cell. The drug containing film with a support of backing membrane was sandwiched in a dialysis membrane with molecular weight cut off between 12000 to 14000 (Himedia, Mumbai, India) and was further placed between compartments of diffusion cell. The dialysis membrane had been soaked for 24h in 40% v/v PEG 400 in PBS pH 7.4. The donor compartment was open at the top and was exposed to atmosphere. The donor and receptor compartment held together using a clamp and receptor compartment was provided with sampling port. The receptor compartment contained 13ml of 40% v/v PEG 400 in PBS of pH 7.4 and the contents were stirred at a speed of 400rpm. The whole assembly was kept on a magnetic stirrer and study was conducted at a temperature of $37\pm 0.5^\circ\text{C}$. The samples of 3ml were collected at preset time points up to 24h and replenished with fresh medium. The samples were filtered using syringe filter (Sartorius 0.45 μ) and drug content in the samples was estimated using UV-VIS spectrophotometer at 232nm. All the experiments were performed in triplicate. Cumulative percentage of the released drug was calculated and plotted against time.

In vitro release kinetics

Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of drug release from patches. The kinetic models used were zero order equation, first order equation, Higuchi equation and Korsmeyer-Peppas equation.²⁰

Ex vivo permeation studies

Franz diffusion cell with a surface area of 2.64cm² was used for *ex vivo* permeation studies. Excised rat skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment. The stratum corneum side of the skin was kept in intimate contact with the transdermal patch under

test. The receiver compartment contained 13ml of 40% v/v PEG 400 in PBS of pH 7.4, stirred with a magnetic stirrer. The whole assembly was kept on a magnetic stirrer and study was conducted at $37\pm 0.5^\circ\text{C}$. The amount of drug permeated was determined by removing 3ml at preset time points up to 24h and replenishing with an equal volume of fresh medium. The samples were filtered using syringe filter (Sartorius 0.45 μ) and the absorbance was measured at 232nm spectrophotometrically. The cumulative amount of drug permeated was calculated and plotted against time.

The target flux was calculated using the following equation²¹:

$$J_{\text{Target}} = \frac{C_{ss} CL_T BW}{A} \quad (5)$$

where 'A' represents the surface area of the transdermal patch (i.e., 2.64cm²); 'BW', the standard human body weight of 60kg; 'C_{ss}', the ZLP concentration at the therapeutic level (26 $\mu\text{g/L}$); and 'CLT', the total clearance (0.942L/h). The calculated target flux value for ZLP was 556.63 $\mu\text{g/h/cm}^2$.

Primary skin irritancy studies

Albino rabbits of either sex, each weighing 1.5 to 2.0kg were used in this study (n=3 in each group). They were housed in cages in the animal house under controlled temperature and light conditions. They were fed a standard laboratory diet and had access to water *ad libitum*. The dorsal surface of the rabbits was cleared and the hair was removed by shaving. The skin was cleared with rectified spirit. The control patch (without any drug, group I) and an experimental patch (A1, group II) were applied to the shaved skin of rabbits and secured using USP adhesive tape (Johnson & Johnson limited, Mumbai). A 0.8% (v/v) aqueous solution of formaldehyde was applied as a standard irritant (group III). Its effect was compared with the test. The animals were observed for any sign of erythema and oedema for a period of 7 days and scored as reported by Draize et al.²² The animals were obtained from Sainath Animal Agency, Hyderabad, India. Prior approval by Institutional Animal Ethics Committee was obtained for conduction of experiment (Ref: IAEC/SUCP/07/2009).

Pharmacodynamics

Locomotor activity and rotarod methods were used to study the *in vivo* performance of the prepared drug delivery system. The animals were obtained from Sainath Animal Agency, Hyderabad, India. Prior approval by Institutional Animal Ethics Committee was obtained for conduction of experiment (Ref: IAEC/SUCP/07/2009).

The locomotor activity can be measured using an actophotometer, which operates on photoelectric cells, which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal a count is recorded on the recorder. The more the locomotor activity the more movements are recorded by the counter and displayed on the panel. Conversely if the animal restricts its movements the count will be less. The locomotor count of normal will be much higher when compared with the activity of the same animal under the influence of ZLP. The more the effect the less the count and vice versa.

Albino mice of either sex with a weight of 20–30gm were divided into three groups including one controlled group with each group comprising of 6mice.

Sodium CMC suspension, 0.5ml (0.5%) was administered orally to group I mice, ZLP (10mg in 0.5ml of sodium CMC suspension) was administered orally to group II mice. To group III mice ZLP patch was applied. The locomotor activity at various time points was tested over a period of 24h.

Mice were pre-tested and those able to stay on the rotarod apparatus (10turns/min, diameter 3cm) for two minutes were used. These selected mice were divided into three groups with 6 mice in each group. The mice were then tested for motor coordination to record basal “fall off” time. After estimation of basal “fall off” time, 0.5ml (0.5%) sodium CMC suspension was administered orally to group I mice, ZLP (10mg in 0.5ml of sodium CMC suspension) was administered orally to group II mice. To group III mice ZLP patch was applied. After administration, mice were placed on the rotating rod and fall off time was recorded at 1h, 2h, 4h, 6h, 10h and 24h for all mice of all the three groups.

The difference in the activity was recorded considering before treatment values and after oral, patch treatment values. Finally percentage decrease in locomotor activity and fall off time was calculated.

Percent decrease in activity and fall off time were calculated for each animal using the formula,

$$\text{percent decrease in activity/fall off time} = (1 - W_a/W_b) \times 100,$$

where, W_a and W_b are average activity scores/fall off time after and before drug administration, respectively and average decrease in activity/fall off time was calculated for all groups.

Stability studies

According to ICH guidelines, sufficient replicates of the formulation A1 were wrapped in aluminum foil and stored in a petri dish at a temperature of

$40 \pm 0.5^\circ\text{C}$ and $75 \pm 5\%$ RH for 3 months. The samples were withdrawn (at 0, 30, 60, 90 days) and analyzed for drug content by UV-VIS spectrophotometry. The logarithm of percent ZLP remaining in the formulation was plotted against time (days). Degradation rate constant (K) was calculated by the following formula:

$$\text{Slope} = K/2.303 \quad (6)$$

The shelf life ($t_{0.9}$) was calculated by the following equation:

$$t_{0.9} = 0.1054/K \quad (7)$$

RESULTS AND DISCUSSION

Effect of d-limonene on permeation of ZLP

The effect of concentration of d-limonene on cumulative permeation through rat skin is shown in Fig. 1. Solution containing 6% v/w of d-limonene showed highest flux value ($64.88 \pm 2.12 \mu\text{g}/\text{cm}^2/\text{h}$) and permeability coefficient ($3.42 \pm 0.111 \text{cm h}^{-1} \times 10^{-2}$). The flux value obtained with 6% v/w of d-limonene was significantly different ($p < 0.05$) to lowest values obtained with 2 and 4% v/w d-limonene (31.55 and $46.89 \mu\text{g}/\text{cm}^2/\text{h}$) and control ($9.21 \mu\text{g}/\text{cm}^2/\text{h}$). The permeability coefficient obtained with 6% d-limonene was 7.04 times higher than that observed with control. The permeation of ZLP was enhanced by increasing d-limonene concentration from 2 to 6% v/w; hence in the preparation of patches 6% v/w d-limonene was incorporated.

The effectiveness of hydrocarbon limonene had also been demonstrated for other drugs such as ketoprofen,²³ valsartan.²⁴ The great enhancement by limonene suggests that there were possibly multiple mechanisms

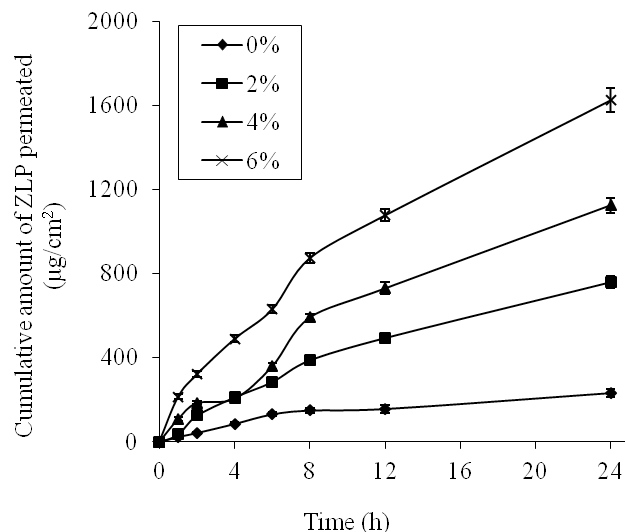


Figure 1: Effect of d-limonene concentration as penetration enhancer on the *ex vivo* permeation of ZLP across rat abdominal skin, mean \pm SD (n=3).

that could have resulted in a more permeable pathway for ZLP. They include an increased solubility of ZLP within skin, partial extraction of stratum corneum (SC) lipids,²⁵ phase separation within the SC lipid lamellae.²⁶

Investigation of physicochemical compatibility of drug and polymer

The DSC analysis of pure ZLP showed a sharp endothermic peak at 180.31°C, corresponding to the drug's melting point (Fig. 2). The DSC analysis of the physical mixture of the drug and the polymers revealed a negligible change in the melting point of ZLP in the presence of the polymer mixtures studied (Fig. 2) (180.00°C for the mixture of ZLP, ERL and HPMC, and 180.08°C for the mixture of ZLP, ERS and HPMC). The IR spectral analysis of ZLP alone showed that the principal peaks were observed at wavenumbers 2225.9, 1654.6, 1613.5, 1578.2, 1223.5 and 801.2 cm^{-1} , confirming the purity of the drug (Fig. 3) (27). In the IR spectra of the physical mixture of ZLP, ERL and HPMC, the major peaks of ZLP were observed at wavenumbers 2228.3, 1650.2, 1614.1, 1576.1, 1223.8 and 801.5 cm^{-1} (Fig. 3); for the physical mixture of ZLP, ERS and HPMC, they were observed at 2235.7, 1652.0, 1606.6, 1577.8, 1223 and 801 cm^{-1} (Fig. 3). The DSC and IR results suggest that the drug and polymers are compatible.

Preparation of transdermal films

Films were formulated with ERL/ERS and HPMC (Table 1). Many experiments were performed by varying the concentrations of polymer and it was fixed to 1.2gm so that no precipitation of drug was observed.

In addition, experiments were conducted to know optimum concentration of plasticizer to be used in all films. Plasticizer concentration of 5% v/w of film former was insufficient to form films. Plasticizer concentration at 5–10% v/w of film former yielded hard and inflexible films. Further, increasing the concentration of plasticizer above 20% v/w resulted in enormous increase in the drying time. Hence in the preparation of patches 15% v/w PG was used to get flexible films. Dichloromethane, methanol (1:1 ratio) was used as a solvent in which all formulation ingredients were soluble. All films were colourless, transparent without any air bubbles, and their surfaces were smooth.

Physicochemical characterization of patches

The results of the physicochemical characterization of the patches are shown in Table 2. The weights and thicknesses were found in the range of 66.74 \pm 4.31 to 75.24 \pm 4.42mg and 213 \pm 3.90 to 252 \pm 4.55 μ respectively. As the proportion of HPMC decreased, the

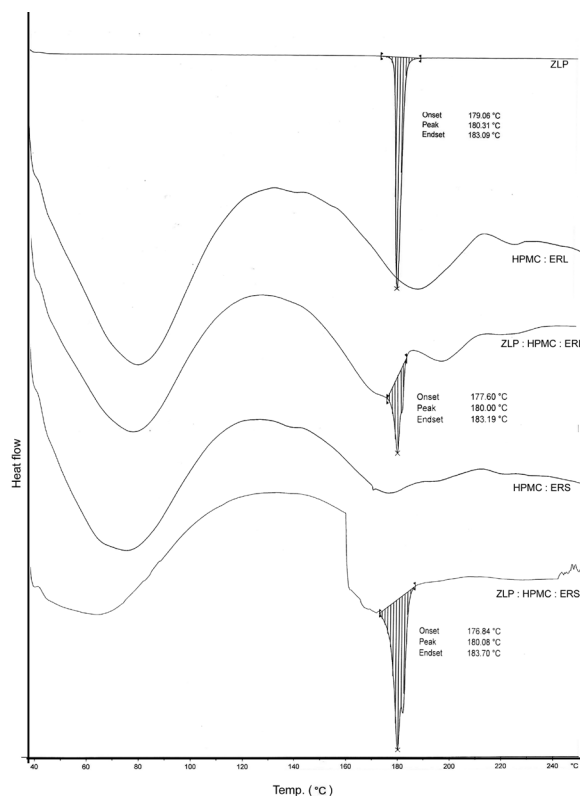


Figure 2: DSC thermogram of ZLP pure drug, ZLP, ERL, HPMC physical mixture, ZLP, ERS, HPMC physical mixture.

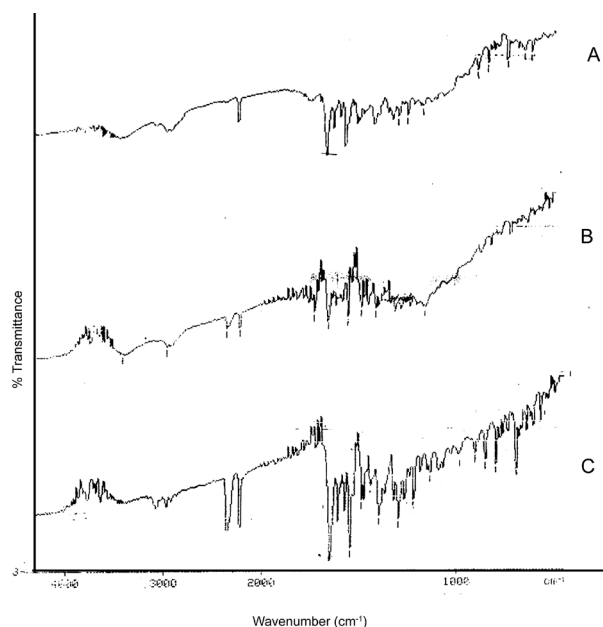


Figure 3: FT-IR spectra of ZLP, ERL, HPMC physical mixture (A), ZLP, ERS, HPMC physical mixture (B), ZLP pure drug (C).

thickness was also decreased. Drug content was uniform among all formulations and ranged from 9.91 \pm 0.66 to 10.38 \pm 0.56mg. The solvent evaporation method which was employed to prepare patches in this work was found suitable to produce patches with uniform drug content and minimal variability among formulations. The flatness study showed that

all formulations had 100% flatness as the strip length before and after their cuts was same. As there was no constriction and all patches had a smooth, flat surface; and when the patch was applied to the skin the smooth surface could be maintained. Folding endurance values were high indicating that the patches would not break and maintain their integrity with general skin folding when applied. The thinner the film was, more flexible it was.

The results of % moisture uptake and % moisture content studies are shown in Table 2. The results revealed that the increase in the concentration of hydrophilic polymer was directly proportional to the increase in moisture content and moisture uptake of the patches. The % moisture content in the patches ranged from 5.61 ± 0.51 to 8.38 ± 1.35 . The % moisture uptake in the formulations were in the range of 7.68 ± 0.27 to 10.23 ± 1.72 . The integrity of formulations wasn't changed on moisture uptake. The low moisture content in the formulations resulted in stability of patches and not giving a completely dried and brittle film.²⁸

In vitro release studies

The results of *in vitro* drug release studies from transdermal patches are shown in Table 3 and Fig. 4. Formulation A1 exhibited greatest (88.36 ± 7.30) percentage of

drug release, which was significantly ($p < 0.05$) different compared to the lowest value observed with the formulation A6 (51.36 ± 5.41). In the present study, it was observed that as the concentration of hydrophilic polymer (HPMC) increased in the formulations, the drug release rate increased substantially. The addition of hydrophilic component to an insoluble film former tends to enhance the release rates.^{29,30}

In vitro release kinetics

In order to propose a release mechanism, ZLP release data was fitted to zero-order, first-order and Higuchi model. The cumulative amount of drug released from the patches, when plotted against square-root of time, have release profiles that seem to follow the Higuchi model as evidenced by correlation coefficients ($R^2 = 0.96$ to 0.99) and zero-order ($R^2 = 0.96$ to 0.99) better than first-order ($R^2 = 0.52$ to 0.65). The data was further treated as per the Korsmeyer-Peppas equation. The n values obtained (0.521 to 0.861) by this equation indicated the amount of drug released by the non-Fickian model.

Ex vivo permeation studies

The results of *ex vivo* drug permeation studies from transdermal patches are shown in Table 3 and

Table 2: Physicochemical properties of ZLP transdermal patches, mean \pm SD.

Code	Weight (mg/2.64cm ²)	Thickness (μ)	Folding Endurance	Drug Content (mg)	Moisture Uptake (%)	Moisture Content (%)
A1	75.24 \pm 4.42	252 \pm 4.55	402.00 \pm 12.49	10.11 \pm 0.39	10.23 \pm 1.72	8.38 \pm 1.35
A2	72.03 \pm 3.80	240 \pm 3.21	387.90 \pm 15.83	9.91 \pm 0.66	10.11 \pm 1.23	7.97 \pm 1.06
A3	70.32 \pm 4.45	233 \pm 3.56	367.33 \pm 13.59	10.38 \pm 0.56	10.07 \pm 1.62	7.82 \pm 1.52
A4	68.26 \pm 3.87	235 \pm 5.49	421.22 \pm 2.34	10.04 \pm 0.23	9.40 \pm 1.05	6.51 \pm 1.12
A5	66.74 \pm 4.31	229 \pm 2.51	382.00 \pm 10.54	10.21 \pm 0.32	8.46 \pm 0.58	5.83 \pm 0.69
A6	66.79 \pm 2.55	213 \pm 3.90	345.89 \pm 8.93	9.94 \pm 0.97	7.68 \pm 0.27	5.61 \pm 0.51

Table 3: In vitro Drug Release, Ex vivo Skin Permeation, Transdermal Flux and Permeability Coefficient of Zaleplon Transdermal Patches

Formulation Code	Q24 ^a (%)	Q24 ^b (μ g/cm ²)	Jss ^c (μ g/cm ² /hr)	Kp ^d (cm hr ⁻¹ \times 10 ⁻²)
A1	88.36 \pm 7.30	3231.07 \pm 109.32	128.75 \pm 4.02	3.39 \pm 0.106
A2	74.67 \pm 8.32	2744.06 \pm 84.13	105.69 \pm 3.09	2.79 \pm 0.081
A3	70.56 \pm 7.31	2595.99 \pm 88.41	97.15 \pm 3.26	2.56 \pm 0.086
A4	71.80 \pm 9.56	2336.20 \pm 64.71	85.90 \pm 2.49	2.26 \pm 0.065
A5	63.33 \pm 7.93	2116.93 \pm 63.42	90.23 \pm 2.9	2.38 \pm 0.076
A6	51.36 \pm 5.41	1677.64 \pm 59.57	70.69 \pm 2.54	1.86 \pm 0.067

Q24^a Cumulative % drug released, results are the mean \pm SD of triplicate observations.

Q24^b Cumulative amount (μ g) of drug permeated per cm², results are mean \pm SD of triplicate observations.

Jss^c Transdermal flux, values represent mean \pm SD (n=3).

Kp^d Permeability Coefficient, values represent mean \pm SD (n=3).

Fig. 5. The formulation A1 exhibited the greatest ($3231.07 \pm 109.32 \mu\text{g}/\text{cm}^2$) cumulative amount of drug permeation, which was significantly ($p < 0.05$) different compared to the lowest value observed with the formulation A6 (1677.641 ± 59.57) in 24h. The flux obtained with formulation A1 was found to be maximum ($128.75 \pm 4.02 \mu\text{g}/\text{cm}^2/\text{h}$). The flux obtained with formulation A1 meets the required flux ($556.63 \mu\text{g}/\text{h}/\text{cm}^2$) with a minimum patch area (4.32cm^2). When the permeability coefficients of different formulations were compared, A1 was found to have highest permeability coefficient ($3.39 \pm 0.106 \text{cm h}^{-1} \times 10^{-2}$). The cumulative amount of drug permeated per square centimeter of patches through the rat abdominal skin when plotted against time, the permeation profiles of drug seem to follow Higuchi's equation as it was evidenced by correlation coefficients ($R^2 = 0.96$ to 0.99) and zero order ($R^2 = 0.96$ to 0.99) better than first order kinetics ($R^2 = 0.53$ to 0.69). As the proportion of HPMC increased in all the formulations, increased permeation was observed. As described by Rao and Diwan, initial rapid dissolution of the hydrophilic polymer occurs when the patch is in contact with the hydrated skin, resulting in the accumulation of high amounts of drug in the skin surface and thus leading to saturation of the skin with drug molecules at all times.³¹ The formulation A1 was found to be best formulation among all formulations studied and was used for primary skin irritancy studies, stability study and pharmacodynamic study in mice.

Primary skin irritancy studies

The primary skin irritancy study of the transdermal patches, placebo patch and patch A1 showed a skin irritation score (erythema and edema) of less than 2 (Table 4). According to Draize et al, (1944) compounds producing scores of 2 or less are considered non-irritant. Hence, the transdermal patches were free of skin irritation.

Pharmacodynamics

The percent decrease in locomotor activity and fall off time are shown in Fig. 6 and Fig. 7 respectively.

The oral administration of ZLP significantly ($p < 0.05$) decreased locomotor activity and fall off time initially with the maximum effect observed at 1h, but after 6h the locomotor activity and fall off time started rising gradually until it was same as the initial value at 24h. In contrast, the administration of ZLP through transdermal patch (A1) resulted in a gradual decrease in locomotor activity and fall off time, with the maximum effect observed at 4h ($p < 0.05$). Despite the fact that the patch produced a peak effect at 4h, patch decreased the locomotor activity and fall off time ($p < 0.05$) at

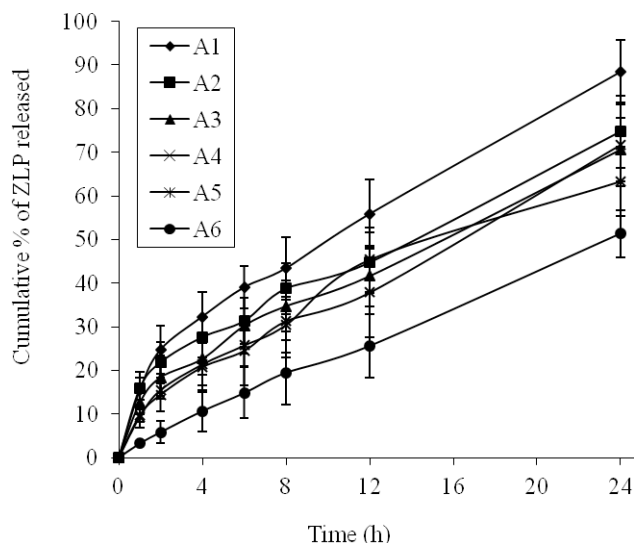


Figure 4: *In vitro* release profile of ZLP from transdermal patches, mean \pm SD (n=3).

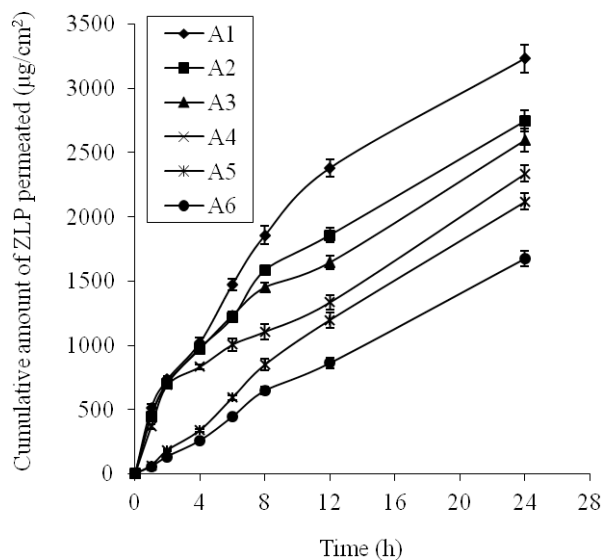


Figure 5: *Ex vivo* permeation profile of ZLP from transdermal patches through rat abdominal skin, mean \pm SD (n=3).

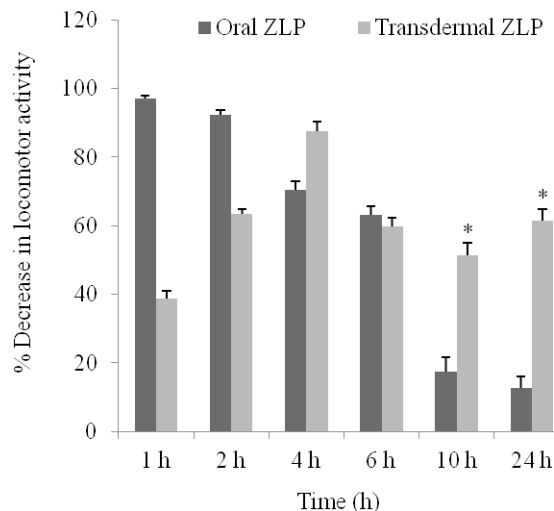
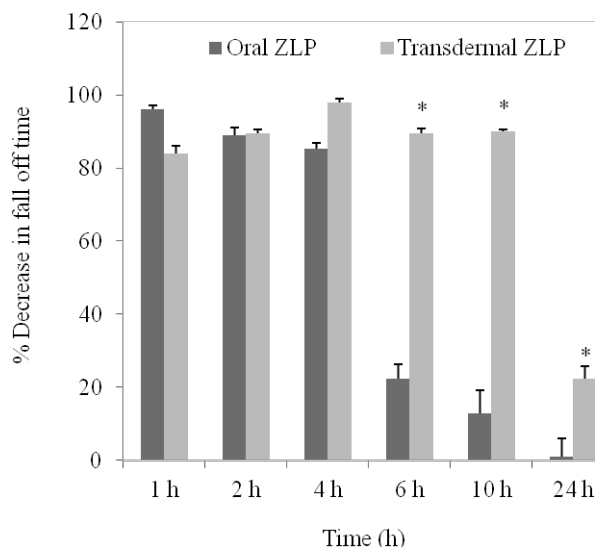


Figure 6: Percent decrease in locomotor activity of mice upon oral and transdermal administration of ZLP.

Table 4: Skin irritation scores following transdermal patch administration

Rabbit No	Group I (without any drug)		Group II (A1)		Group III (Formalin)	
	Erythema ^a	Edema ^b	Erythema	Edema	Erythema	Edema
1	0	1	0	0	3	2
2	1	0	0	0	3	1
3	0	1	1	1	3	2
Mean ± S.D	0.34±0.58*	0.67±0.58*	0.34±0.58*	0.34±0.58*	3 ± 0	1.67±0.58

*p<0.05, significant compared with formalin.
^aErythema scale: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, scar formation.
^bEdema scale: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, severe.

**Figure 7:** Percent decrease in fall off time of mice upon oral and transdermal administration of ZLP.

the first hour and the effect continued for 24h. This clearly indicate that the transdermal patch release the drug gradually over a period of time, which results in prolonged decrease of locomotor activity and fall off time for 24h. Oral ZLP acted quickly and drastically, but then its effect dropped off. The patch did not decrease the locomotor activity greatly in the initial phase when compared with the oral form, as indicated by the significant ($p<0.05$) difference between the oral and patch treated group at 1h, but the effect of oral ZLP started declining after 1h because of its short half life. Since the administration of ZLP through patch resulted in sustained and continued drug release for 24h, the patch was effective throughout the period. Clearly, the prepared transdermal patch is capable of surmounting the shortcomings of oral administration of ZLP, such as low bioavailability, short half-life, and high first pass metabolism.

Stability studies

The logarithm of % drug remaining in the formulation was plotted against time (days) and degradation rate constant was found to be $1.382 \times 10^{-4} \text{ day}^{-1}$. The shelf

life of ZLP patch was calculated as 2.12 years. The patch was found to be stable with respect to ZLP content.

CONCLUSIONS

Zaleplon can be formulated into the transdermal matrix type patches to sustain its release characteristics and the polymeric composition (ERL/HPMC, 1:3) was found to be the best choice among the formulations studied. The prepared transdermal patch is capable of surmounting the shortcomings of oral administration of ZLP, such as low bioavailability, short half-life, and high first pass metabolism.

ACKNOWLEDGMENTS

The authors are grateful to the management of the institute, Sultan-Ul-Uloom Educational Society, Banjarahills, Hyderabad, India, for providing the facilities. The gift samples of ZLP, Eudragit RL100, and Eudragit RS100 by Aurobindo Pharmaceuticals Pvt. Ltd. is highly acknowledged.

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