Original Research Article

Design and Evaluation of Olanzapine and Risperidone Transdermal Patches Containing Vegetable Oils as Permeation Enhancers

ABSTRACT

Objective: Design and Evaluation of Olanzapine and Risperidone Transdermal Patches

Methodology: Using blends of two different polymeric combinations, Eudragit RL 100 (ERL 100) and Eudragit (ERS 100). A total of twenty-two matrix patches were prepared by using these polymers, surfactants (cationic surfactant, benzalkonium chloride (BC); anionic surfactant, sodium lauryl sulphate (SLS); non-ionic surfactant, span 20) and vegetable oils (olive oil, jojoba oil and groundnut oil) as permeation enhancers in isopropanol-dichloromethane (60:40) as a solvent system. The formulations were characterizedincluding uniformity of weight, drug content, moisture content, moisture uptake, flatness, folding endurance and thickness to study the stability of the formulations andin vitro dissolution of the experimental formulations were performed to determine the amount of Olanzapine and Risperidone present in the patches and scanning electron microscopy (SEM) of the prepared TddS were taken to see the drug distribution pattern. drug–excipient interaction studies were carried out using Fourier transform infrared (FTIR) spectroscopic technique.

Results: In vitro dissolution studies showed that the drug distribution in the matrix was homogeneous and it was found that the maximum drug release and in stability study drug content was found to be 97.03%, 97.02% and 96.32% after 1, 2 and 3 months respectively with formulation ROE3 (containing olive oil). In vitro skin permeation study was also conducted in a modified Franz's diffusion cell which shows that the maximum permeation was with the formulation ROE3 and it was maximum transdermal flux 23.14 µg/cm2/h was obtained with formulation containing olive oil as permeation enhancer respectively.

Conclusions: Optimized formulations were found to be suitable for formulating in terms of physicochemical characteristics and there was no significant interaction noticed between the drug and polymers used.

Keywords: Transdermal matrix patch, permeation enhancers, olive oil, jojoba oil and groundnut oil.

1. INTRODUCTION

Transdermal delivery constitutes one of the most important routes for new drug delivery system (NDDS). Transdermal delivery of drugs offers several advantages over conventional delivery including oral and injection methods. Transdermal delivery, that traditionally uses a patch containing drug substance pressed onto the skin, is non-invasive, convenient and painless, and can avoid gastrointestinal toxicity (*e.g.,*peptic ulcer disease) and the hepatic first pass metabolism [1].

Schizophrenia has been one of the major diseases afflicting mankindin today's scenario [2]. *Olanzapine and Risperidone*, an antipsychotic drug, is supposed to be effective in the treatment of chronic schizophrenic patients. Currently Olanzapine and Risperidone is administered orally or by injection. It is usually administeredas one or two daily oral doses, for an overall dosage of 5–20 mg per day. The drug has also been introduced in Italian market as film-coated, gastro-resistant Zyprexa® tablets [3].

The low-dose Olanzapine and Risperidone maintenance therapy is required to control the psychotic symptoms, and long-term prophylactic treatment is needed to prevent relapses. Long-acting modified dosage forms of Olanzapine and Risperidone are going to be effective in patients and can help to address the problem of poor patient compliance. The use of this drug in the lowest possible effective dosage is recommended for minimizing the risk of major side effects. Based on these hypotheses, a modified transdermal drug delivery system was developed.

Combination drug-matrix type of transdermal drug delivery system for Olanzapine and Risperidone was designed for prolonged period of maintenance therapy instead of convention oral dosage forms. Moreover, the physicochemical characteristics of Olanzapine and Risperidone also comply with the general requirement for designing a TDDS to a good extent.

This search and investigation are expected to add extensively to the existing knowledge and information in the field of proper drug regimen and maintenance therapy of schizophrenia with controlled-release TDDS of Olanzapine and Risperidone [2]. The major problem of transdermal delivery is with the barrier properties of stratum corneum. Thus, the transport across the skin membrane is a complex phenomenon. It is the cells of stratum corneum which present the primary barrier to absorption of transdermally administered drugs. Relatively recent advances in transdermaldrug delivery have enabled effective administration of a variety of drug through the skin. These advances include the development of a number of skin penetration enhancing agents, or ‘permeation enhancers’ to increase the skin permeability. Penetration enhancers

such as oils are the substances that facilitate the absorption of penetrant through the skin by temporarily diminishing the impermeability of the skin. The vegetable oils (BC, SLS, span 20, oliveoil, groundnut oil and jojoba oil) were used to study their impact as an enhancer to skin permeation of the drug olanzapine. There are number of chemical agents that enhance the permeability of the skin but weare using vegetable oils as they are easily available, cause no skin irritation and natural in origin [4]. Moreover, electron microscopic studies were also conducted in an effort to understanddrug distribution in the patches, drug release from the patches, and the permeation of the drug through skin [5].

2. MATERIALS AND METHODS

Olanzapine (Ranbaxy Labs, Ponta Sahib, India) and Risperidone (Torrent Pharmaceuticals, Badi,India) was received as a gift sample. All other chemicals used in the study were of analytical grade.

2.1 Development of transdermal system

Transdermal patches of risperidone and olanzapine were prepared by solvent casting technique in a glass mold fabricated locally. To determine the optimum combination of polymers, plasticizer and solvent, placebo patches were formulated. On the basis of preliminary studies, the optimized polymers ERL 100 and ERS 100 in different ratios were mixed to a total weight of 500 mg and dissolved in 10 ml of isopropanol-dichloromethane (60:40) solvent system using magnetic stirrer. Drugs (20% w/w of polymer weight) was added slowly to the polymer solution and mixed thoroughly to obtain a homogenous solution and di-n-butyl phthalate (30% w/w of polymer) was used as plasticizer. Different permeation enhancers (BC, SLS, span 20, oliveoil, groundnut oil and jojoba oil) were added in three different concentrations *i.e.,*1%, 5% and 10% w/w of polymer weight for each. The resulting polymeric solution was poured in circular aluminum foil cups placed in circular glass mold (internal diameter 3.57 cm and thickness 1cm) and dried at 35 ºC in dust free environment. After 24 h, the films were collected and peeled off. A circular USP adhesive tape of internal diameter 5 cm was attached on the patch. A backing film made up of aluminum was applied with the help of adhesive and a release liner (wax paper) was applied on other side of the film to complete the TDDS.

2.2 Fabrication of mould for making films

A circular mould with flat surface having internal diameter 3.57 cm was fabricated in the lab. The glass surface of the mould was labeled properly, so as to obtain films having uniform thickness.

2.3 Drug polymer interaction studies: FTIR spectra of pure drug risperidone, olanzapine, eudragit RL 100 (ERL100), eudragit RS 100 (ERS100) and mixture of both the drugs with eudragits in the same ratio as used for formulation were taken using Perkin Elmer FTIR spectrophotometer (RXIFT-IR system). One part of sample was mixed with Three parts of potassium bromide in a mortar and triturated. The triturated sample was placed in pellet maker and compressed using hydraulic press. The pellet was kept in a sample holder and scanned from 450 cm^{-1} to 4000 cm^{-1}.

2.4 Evaluation Of Transdermal Patches

2.4.1 Uniformity of weight

Ten different patches from individual batches were weighed individually and the average weight was calculated; the individual weight should not deviate significantly from the average weight [6].

2.4.2 Drug content determination

An accurately weighed portion of film (about 100 mg) was dissolvedin 100 mL of suitable solvent in which drug is soluble and then the solution was shaken continuously for 24 h in shaker incubator. Then the whole solution was sonicated. After sonication and subsequent filtration, drug in solution was estimated spectrophotometrically by appropriate dilution [7].

2.4.3. Moisture content

The film was weighed and kept in desiccator with calcium chloride at room temperature for 24 h. The film was weighed again after specified interval until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight and was reported in terms of percentage moisture content [8].

$$\% \text{ Moisture content} = \frac{[\text{Initial weight} - \text{Final weight}]}{\text{Final weight}} \times 100$$

2.4.4. Moisture uptake

The weighed film was kept in a desiccator at room temperature for 24 hours and then exposed to 84% relative humidity using a saturated solution of potassium chloride. Finally, the films were measured periodically to constant weights [8].

$$\% \text{ Moisture uptake} = [\text{Final weight} - \text{Initial weight}] \times 100$$

Initial weight

2.4.5. Flatness

Longitudinal strips were cut out from the prepared medicated patches and the lengths of each strip were measured and then the variation in the lengths due to the non-uniformity in flatness was measured. Flatness was calculated by measuring constriction of strips anda zero percent constriction was considered to be equal to a hundred percent flatness [9].

Constriction (%) = $(l_1-l_2)/l_1 \times 100$

Where l_1= initial length of each strip; l_2= final length.

2.4.6. Folding endurance

The strip of film was cut evenly 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 [10].

2.4.7. Thickness of thefilms

The thickness of the drug-loaded polymeric films was measured at five different points using micrometer. The average and standard deviation of five readings were calculated for each batch of the drug-loaded films [11].

2.4.7. Microbial studies: The potential of transdermal patch for promoting growth of micro-organisms was evaluated by bacteriological cultures. The film strips of different formulations were cut into small pieces of $1cm^2$ and aseptically transferred into each petri plate containing 25 ml of nutrient agar media. These agar plates were incubatedat 37±0.5˚C for 48 h. After incubation, sample was observed under microscope.

2.4.8. *In vitro* drug releasestudies

The *in vitro* drug release studies were performed by using a modified USP type II dissolution apparatus using 900 ml of PBS 7.4 with Tween 80 (1% w/v for risperidone and 0.75% w/v for olanzapine) as dissolution medium. A circular patch with an internal diameter of 3.57 cm was used for the study and a stainless-steel ring was employed to sink the patch at bottom of dissolution apparatus. All dissolution studies were performed at 32 ±0.5 °C (temperature of skin) at 100 rpm. Samples were withdrawn at predetermined time intervals (replaced with equal volume of fresh dissolution media to maintain sink conditions) and their concentrations were analyzed

spectrophotometrically at λ_{max} of 322 nm for risperidone and 315 nm for olanzapine [12].

To study the release kinetics, data obtained from *in vitro* drug release studies were fitted in various kinetic models: zero order as cumulative percent of drug released *vs.* time, first order as log cumulative percentage of drug remaining *vs.* time and Higuchi's model as cumulative percent drug released *vs.* square root of time. To determine the mechanism of drug release, the data were fitted into Korsmeyer and Peppas equation as log cumulative percentage of drug released *vs.* log time, and the exponent n was calculated from slope of the straight line. For slab matrix, if exponent is 0.5, then diffusion mechanism is fickian; if 0.5<n<1.0, mechanism is non-fickian; if n is 1.0, mechanism is zero order and if n >1.0, then it is super case II transport [13].

2.4.9. *In vitro* permeationstudies

Preparation of full thickness rat abdominal skin: Hairless animal skin and human cadaver skin are generally used for permeation studies. Human cadaver skin may be a logical choice as the skin model because the final product will be used in humans. But it is not easily available. So, in the present study, hairless Wistar rat abdominal skin was used. The experimental protocols were approved by IAEC (Institutional Animal Ethics Committee) as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Permeation studies: 25 rats were sacrificed by excess ether inhalation. Hairs on dorsal skin of animal were removed with animal hair clipper, subcutaneous tissue was surgically removed and dermis side was wiped with isopropyl alcohol to remove residual adhering fat. The skin was washed with PBS pH 7.4. The skin so prepared was wrapped in aluminum foil and stored in adeep freezer at -20 °C till further use. The skin was defrosted at room temperature when required [14].

The *in vitro* permeation studies were carried out in vertical Franz diffusion cell with a capacity of 35 ml, using rat abdominal skin [15]. The patch was placed on the skin with the drug matrix side towards the donor side and backing membrane on the upper side. PBS 7.4 with Tween 80 was used as receptor fluid as in release studies. The receptor fluid was agitated at 100 rpm by magnetic stirrer and temperature was maintained at 32 ± 0.5 °C. The samples were withdrawn at different time intervals and replaced with equal amounts of dissolution media. Samples were analyzed for its drug content. The drug permeated per cm^2 of patch was calculatedand plotted against time and the flux was calculatedas drug permeated per cm^2 per hour [16].

The steady state flux was determined from the slope of the linear portion of a cumulative amount permeated versus time plot. The lag time (T_{lag}) was determined by extrapolating the linear portion of the cumulative amount permeated versus time curve to the abscissa. Enhancement ratio of the flux (E_{pen}) was calculatedas:

$E_{pen} = P_{treatment}/P_{control}$

Where $P_{treatment}$ is flux of formulation containing enhancer and $P_{control}$ is flux of control group (Without permeation enhancer)

The current oral dosing regimen of risperidone is 2-8 mg/day. As bioavailability of risperidone is 70%, thus anticipated transdermal dose is 1.4-5.6 mg daily. So, the target flux requiredis 5.83-23.33 µg/cm^2/h from a patch having diameter 3.57 cm and surface area 10 cm^2. The current oral dose of olanzapine is 5-10 mg/day. Bioavailability of the drug is 60%. So,the target flux required is12.5-25 µg/cm^2/h from a patch having diameter and surface area 10 cm^2.

2.4.10. Scanning Electron Microscopy (SEM) studies: The skin sections obtained before and after permeation studies were fixedin 3% glutaraldehyde phosphate buffer (pH 7.4) and subsequently dehydratedin a series of acetone solution (50% for 20 min, 70% for 20 min, 80% for 20 min, 90% for 20 min, 100% for 50 min) in water and isoamyl acetate (100%) : acetone (100%) solutions (1:1) for 20 min followed by isoamyl acetate (100%) for 20 min. Sections were further dried using four flushes of liquid CO_2 with 100 psi pressure in critical pointdrier. The sections of film and skin before and after permeation studies were cut and mounted onto stubs using double sided adhesive tape. The sections were coated with gold palladium alloy using fine coat ion sputter to render them electrically conductive and examined under SEM (JSM 6100 JEOL, Tokyo, Japan) to observe the integrity of skin before and after permeation and distribution of drug in the film and skin [17].

2.4.11. In vivo studies

Skin irritation studies: Skin irritation studies and histopathological studies were carried out according to draize technique [18] for selected formulations (ROE3; formulations containing vegetable oil andnonionic surfactant respectively). Rabbits were used to study any hypersensitivity reaction on the skin. Rabbits were divided into 5 groups, each containing 6 animals. The animals of group I were servedas normal, without any treatment. One group of animals (group II, control) were applied with marketed adhesive tape (official adhesive tape in USP). Transdermal

patches (blank anddrug loaded) were applied on to nude skin of animals of III and IV groups respectively. A 0.8%v/v aqueous solution of formalin was applied as standard irritant (group V). The experiment was carried out for 7 days and the application sites were graded according to a visual scoring scale, by the same investigator [19]. The scores of erythema and edema were as follows: 0 for none, 1 for slight, 2 for well defined, 3 for moderate and 4 for scar formation and severe erythema and edema. After evaluation of skin irritation, skin samples were processed for histological examination.

2.4.12. *In vivo* pharmacodynamic studies: Risperidone and olanzapine usually cause a state of sedation and motor in-coordination. Rota rod and grip tests were used to assess muscular strength or neuromuscular function in rodents which can be influenced by sedative drugs and muscle relaxant compounds. Swiss mice were divided into 3 groups, each containing four animals. First group served as control *i.e.,*without drug, second group was administered with oral dose (1mg/kg for risperidone and 10 mg/kg for olanzapine) of marketed formulation (RISPId® tablet by PanaceaBioticand ONZA® tablet by Nicholas Piramal, India) in 0.5% carboxymethyl cellulose (CMC) and third group was treated with selected transdermal formulation (ROE3) containing equivalent dose as that of oral formulation [20].

For rota rod test, animals were placed on an aluminum rod; revolving at 10 rpm and the time taken to fall of animal from the rod was noted. The test was terminated at 270s [21].

For grip test, the animals were exposed to a horizontal thin metallic wire suspended about 30 cm in air which they immediately grasp with the 4 paws. The mice were released to hang on with its four limbs. Control animals were able to hold the wire with hind limbs and to climb up within 5 s. After oral or transdermal administration, the animals were not able to hold the wire with the hind limbs within 5 s or fall off from the wire and they were considered to be impaired. The test was continued for 6 h and repeated after every hour. The general behaviors were observed from selected batches in cages and observations noted. Only if their behavior and their motility in the cages seem to be normal, the disturbance of grasping reflex is consideredas caused by central relaxation.

2.4.13. Stability studies

The stability studies are conducted to investigate the influence of temperature and relative humidity on the drug content in different formulations. The transdermal formulation ROE3 was subjected to stability studies for 3 months using storage conditions 45 °C / 75% RH as per ICH guidelines. Throughout the course of aging stud, triplicate samples were taken at three sampling times (*i.e*., 0, 1 month and 3 month) and evaluated for physical texture, drug content andin *vitro* permeation studies as the indicators.

2.4.14. Statistical analysis

Graph pad prism 5 was used for statistical analysis. All studies were done in triplicates unless specified and data represents the mean ± Sd. The statistical analysis was performed using student's t-test and ANOVA. A difference below the probability level of 0.05 was considered statistically significant. Pharmacokinetic parameters were determined using Winnonlin version 5.2.

3. RESULTS AND DISCUSSION

A total of 22 formulations were prepared using ERL 100 and ERS 100 in different ratios were mixed to a total weight of 500 mg and dissolved in 10 ml of isopropanol-dichloromethane (60:40) solvent system using magnetic stirrer. Drugs (20 % w/w of polymer weight) as per formula given in Table 1. All the films were evaluated for their physicochemical parameters, and they were found to be flexible, smooth and transparent. They were also found to be uniform in their weight and thickness with low Sd values, as shown in Table 1. The transdermal patches were exposed to 84% relative humidity and the percentage moisture uptake of the formulation was determined (Figure 1). It was observed that with an increasing percentage of hydrophilic polymer (ERL 100), in the formulations, moisture uptake increased. Interestingly, with the addition of vegetable oils in the patches, little increase of moisture was observed. A similar observation was made in the case of percentage moisture content (Figure1).

Table 1 Composition and physicochemical characteristics of prepared formulations of risperidone

& olanzapine

Code	ERL 100: ERS 100 (500 mg)	Permeation enhancer (% w/w of polymer weight)	Average Weight variation (mg)**	Thickness (mm)	Drug content (%)	Folding endurance	Flatness (%)	Tensile strength (kg/mm^2)
ROA1	5:0	-	169.91±1.02	0.56±0.02	94.32±2.14	12±0.51	100±0.00	0.365±0.02
ROA2	3:2	-	170.67±1.06	0.61±0.05	95.46±1.62	11±1.00	100±0.00	0.412±0.01
ROA3	2:3	-	168.82±2.61	0.58±0.01	95.64±0.57	13±1.00	100±0.00	0.461±0.02
ROA4	0:5	-	169.15±1.9	0.61±0.01	97.82±0.62	12±0.50	100±0.00	0.401±0.02
ROB1	3:2	BC (1%)	173.23±1.92	0.63±0.02	94.07±1.72	13±2.00	100±0.00	0.444±0.02
ROB2	3:2	BC (5%)	172.48±2.63	0.62±0.02	96.38±2.08	12±0.50	100±0.00	0.361±0.01
ROB3	3:2	BC (10%)	177.29±1.27	0.67±0.02	95.55±0.49	13± 1.50	100±0.00	0.435±0.03
ROC1	3:2	SLS (1%)	172.56±2.48	0.59±0.03	97.46±1.92	13±0.50	100±0.00	0.425±0.04
ROC2	3:2	SLS (5%)	172.82±4.31	0.62±0.01	94.39±0.83	11±0.50	100±0.00	0.480±0.01
ROC3	3:2	SLS (10%)	178.36±1.21	0.67±0.02	95.78±1.37	12± 1.50	100±0.00	0.361±0.01
ROd1	3:2	Span 20 (1%)	170.19±1.81	0.62±0.02	94.53±0.72	12±2.00	100±0.00	0.381±0.02
ROd2	3:2	Span 20 (5%)	173.92±1.42	0.64±0.03	96.61±0.19	11±0.50	100±0.00	0.435±0.07
ROd3	3:2	Span 20 (10%)	177.89±1.21	0.66±0.02	97.23±0.28	13±0.50	100±0.00	0.478±0.01
ROE1	3:2	Olive oil (1%)	170.66±2.44	0.51±0.06	94.50±1.20	13±2.00	100±0.00	0.439±0.02
ROE2*	3:2	Olive oil (5%)	173.52±0.91	0.63±0.02	95.16±0.27	12±2.00	100±0.00	0.402±0.05
ROE3*	3:2	Olive oil (10%)	176.99±0.82	0.65±0.03	99.25±0.06	11±1.50	100±0.00	0.470±0.01
ROF1	3:2	Jojoba oil (1%)	172.27±1.96	0.61±0.02	97.07±1.52	12± 1.50	100±0.00	0.459±0.03
ROF2*	3:2	Jojoba oil (5%)	175.43±1.72	0.64±0.03	97.14±0.83	13±1.50	100±0.00	0.363±0.01
ROF3*	3:2	Jojoba oil (10%)	176.82±1.16	0.65±0.02	92.92±0.18	13±3.00	100±0.00	0.405±0.04
ROG1	3:2	Groundnut oil (1%)	170.61±1.22	0.63±0.04	93.47±0.06	14±1.00	100±0.00	0.422±0.02

ROG2*	3:2	Groundnut oil (5%)	173.95±1.52	0.65±0.07	93.50±1.90	14±1.50	100±0.00	0.461±0.02
ROG3*	3:2	Groundnut oil (10%)	177.41±1.53	0.66±0.02	95.28±1.59	13±1.00	100±0.00	0.428±0.04

Concentration of drugs (20% w/w of polymer weight) was kept constant in all formulations; BC is benzalkonium chloride and SLS is sodium lauryl sulphate

*Formulations, in which 20% w/w of polymer weight of dibutylphthalate was added, while in other formulations 30% w/w of dibutylphthalate was added

**n = 10 for weight; n=6 for other parameters

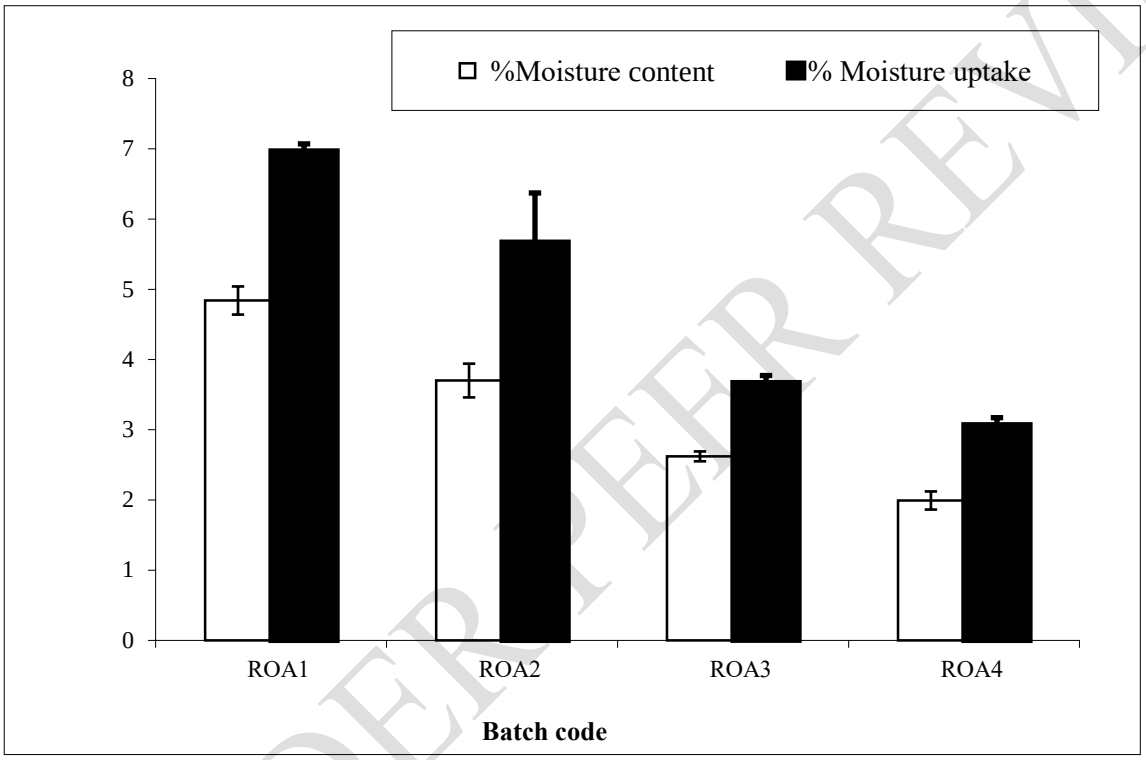

Figure 1: Moisture content and moisture uptake of batch ROA containing different proportions of polymers

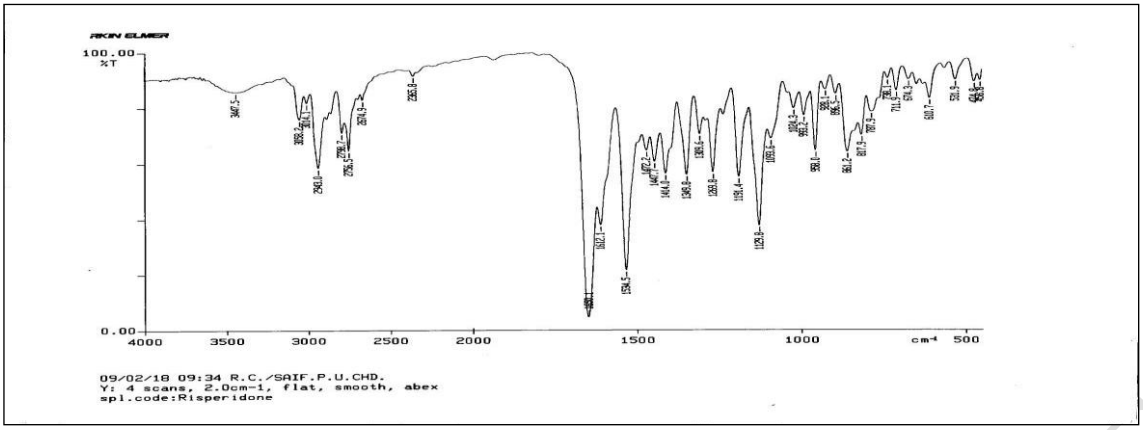

Figure 2.FTIR spectra of risperidone

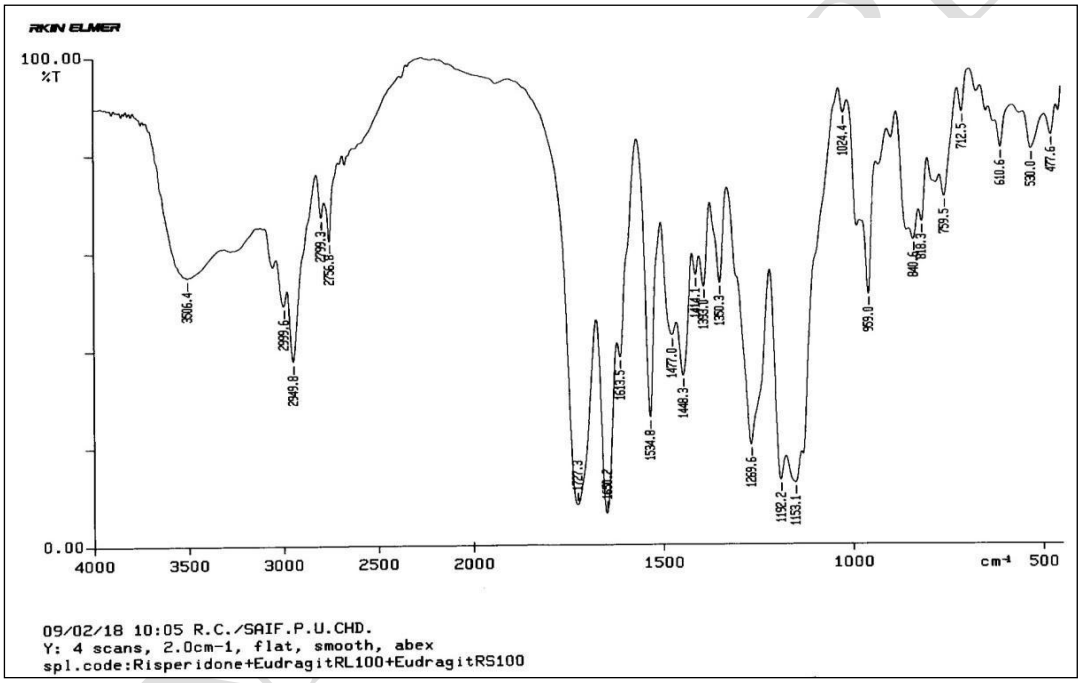

Figure 3. FTIR spectra of risperidone with ERL & ERS 100

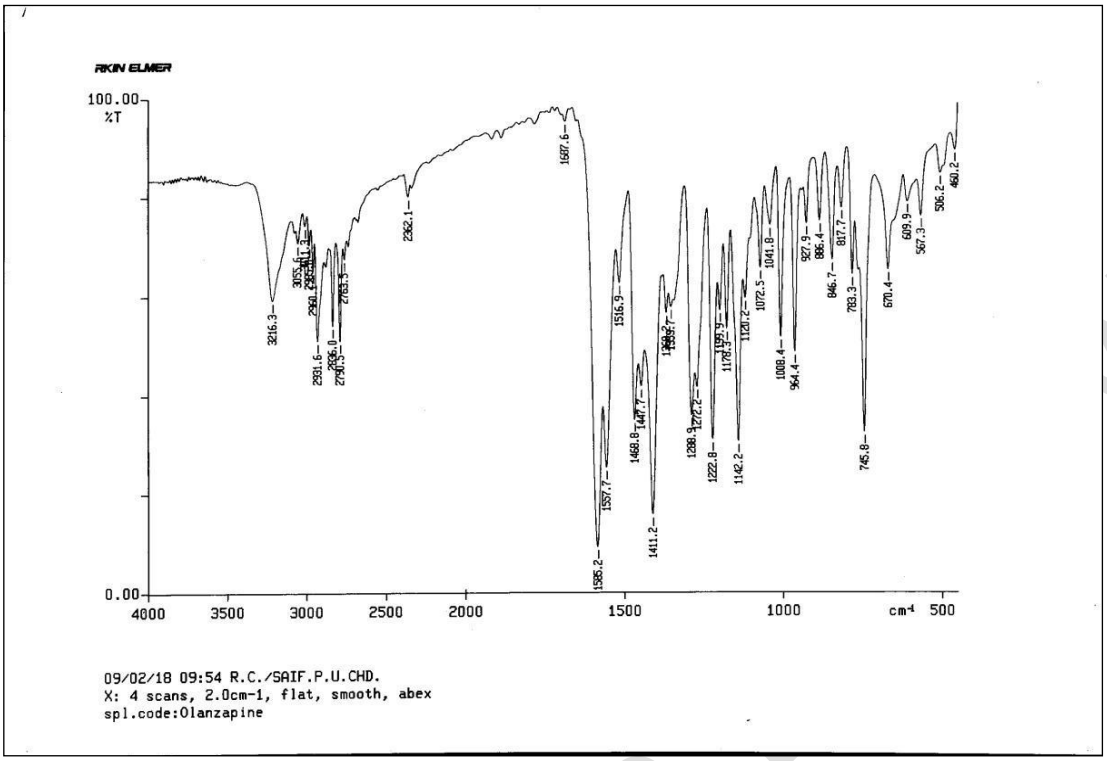

Figure 4: FTIR spectra of olanzapine

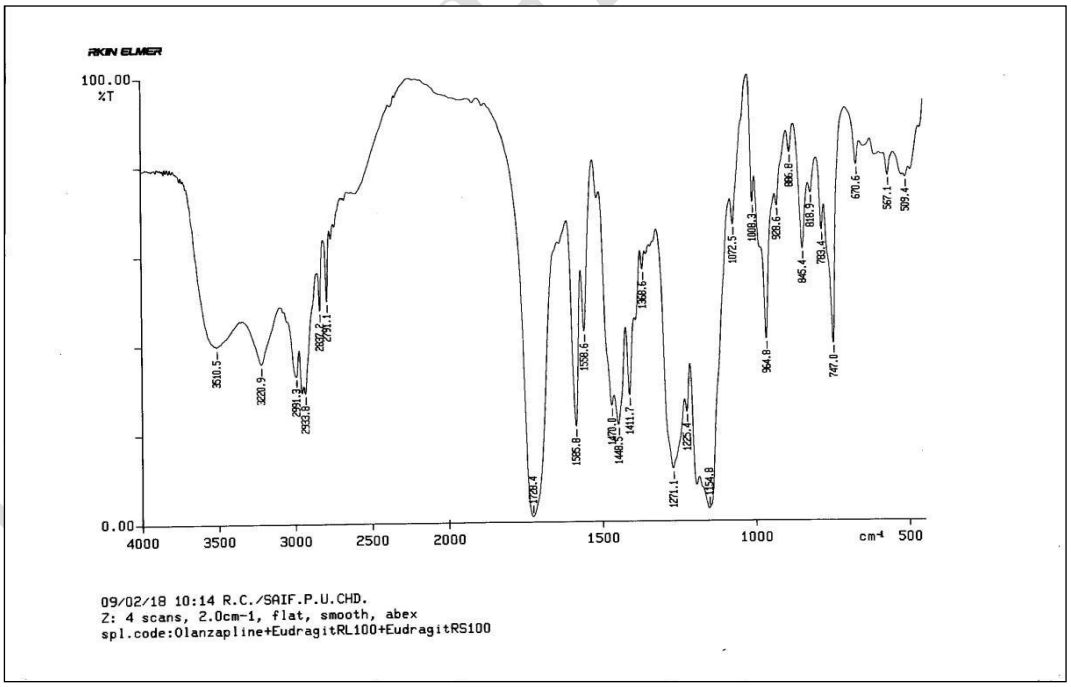

Figure 5: FTIR spectra of olanzapine with E RL100 and E RS100

A transdermal patch should possess a smooth surface and should not constrict with time, as the flatness study demonstrated. No constriction was observed in any of the prepared formulations; all of the surfaces were 100% flat (Table1). FTIR was carried out to assess the interaction between the drug and the excipients (Figures 2-5). Graphs of drug and drug-excipients confirmed that there is no interaction between the drug and excipients used.

***In vitro* release and permeation studies**:

Risperidone & olanzapine, an atypical antipsychotic is most potent and highly effective drug in treatment of psychosis. But a low dose maintenance therapy of risperidone & olanzapine is always needed for prolonged treatment of schizophrenia with lower oral side effects and enhanced patient compliance. In the present study, matrix type transdermal patches of risperidone & olanzapine were prepared using varying ratio of ERL 100 and ERS 100 as polymers to get the desired overall sustained/prolonged drug release.

The selection of suitable polymer is crucial in developing matrix type transdermal system since its film forming properties, stability and compatibility with drug have a great influence on the delivery efficacy of drug. In this study, effect of different combinations of ERL 100 and ERS 100 on release of risperidone & olanzapine from transdermal formulation was evaluated. ERL 100 and ERS 100 in the ratio of 3:2 had the highest and controlled release rate. On this basis, polymers ERL 100 and ERS 100 in the ratio of 3:2, were selected for development of optimized transdermal delivery system of risperidone & olanzapine.

Percutaneous absorption involves the passage of drug molecule from the skin surface into the stratum corneum under the influence of a concentration gradient and its subsequent diffusion through the stratum corneum and underlying epidermis, through the dermis, and into the blood circulation. It is the cells of stratum corneum which present the primary barrier to absorption of transdermally administered drugs. The incorporation of permeation enhancers seems to be a good option to enhance permeation of drug from transdermal system. In the present study, synthetic surfactants (BC, SLS, span 20) and natural vegetable oils (olive oil, jojoba oil and groundnut oil) in concentration of 1%, 5% and 10% were used as permeation enhancers of risperidone & olanzapine from patches.

Films containing ERL 100 and ERS 100 were found to be smooth, wrinkle free, transparent and with uniform color distribution. 20–30% of dibutylphthalate was found to be optimum for flexibility and uniformity

of transdermal patches. The prepared films were tested for their physicochemical parameters to evaluate the physicochemical stability of the formulation.

Films with acceptable qualities were subjected to *in vitro* release studies. *In vitro* release studies showed that the permeation enhancers in case of risperidone & olanzapine TDDS affected the order of release as: Span 20 > olive oil > BC > SLS > groundnut oil> jojobaoil.

On the basis of *in vitro* release studies, patches from each batch with maximum release *i.e.,*ROB2 (formulation containing 5% BC), ROC2 (formulation with 5% SLS), ROD3 (formulation having 10% span 20), ROE3 (formulation with 10% olive oil), ROF3 (10% jojoba oil) and ROG3 (10% groundnut oil) were selected for *in vitro* permeation studies and compared with formulation containing no permeation enhancer (ROA2). As shown in result section of *in vitro* permeation studies,(Figures 6-14) all selected enhancers increased permeation of risperidone & olanzapine through excised rat skin, but the maximum transdermal flux was obtained with formulation containing olive oil as permeation enhancer, which was due to presence of oleic acid, an unsaturated fatty acid, as the major constituent of olive oil. It has already been discussed that oleic acidincreases epidermal permeability through a mechanism involving the perturbation of stratum corneum, lipid bilayers and lacunae formation, which in turn enhances transdermal drug delivery.The results of permeation fluxes supported the data for prolongation of drug release characteristics of formulated transdermal films.

Effect of loading amount of drug in transdermal formulation investigated in study. In our experiments, cumulative amount of drug permeated increased with increase in drug concentration up to 20%. But when concentration of drug was increased to 25%, drug release gets decreased. This decrease in drug permeation can be attributed to presence of drug in super-saturation state at this concentration.(Figures 15).

The microphotographs of SEM showed presence of drug particles on dorsal and ventral side of skin during permeation studies, which confirmed permeation of drug through skin. It can be said that drug released from patches after permeation studies reached into the receptor media through skin and this permeation of risperidone & olanzapine occurred through skin appendages as indicatedin SEM images.(Figures 16).

On the basis of these observations, batch ROE3 (formulations containing 20% risperidone & olanzapine, ERL 100 and ERS 100 in the ratio of 3:2 as matrix forming polymers, 20% dibutyl phthalate as plasticizer and 10% olive oil as enhancer) was selectedas optimized batch and further evaluated by *in vivo* studies,

(Table 2-4) Rotarod test, grip test and behavioral observations were carried out to find out the tranquillizing efficacy of TDDS comparable to oral marketed formulation in mice. From the results, it can be noted that falling time in case of rotarod test and gripping time in case of grip test was almost similar to marketed oral formulations. So,it was concluded that formulation has sufficient active drug risperidone & olanzapine, which produced muscle relaxant and sedation effect due to effect on muscular strength and neuromuscular function. *In vivo* pharmacodynamic on rodents employed for assessment of neuroleptic effect indicated that risperidone & olanzapine transdermal patches gave satisfactory results regarding its therapeutic efficacy with no skin irritation.

Thus, transdermal formulations of risperidone & olanzapine using bio adhesive polymers such as ERL 100 an ERS 100 with olive oil as permeation enhancers have proved their ability to give sustained release. These results are in agreement with earlier studies of [22] who studied transdermal delivery of propranolol using mixed grades of eudragit and also similar to [23] who proved the better *in vivo* performance of glibenclamide transdermal patches in comparison with oral administration.

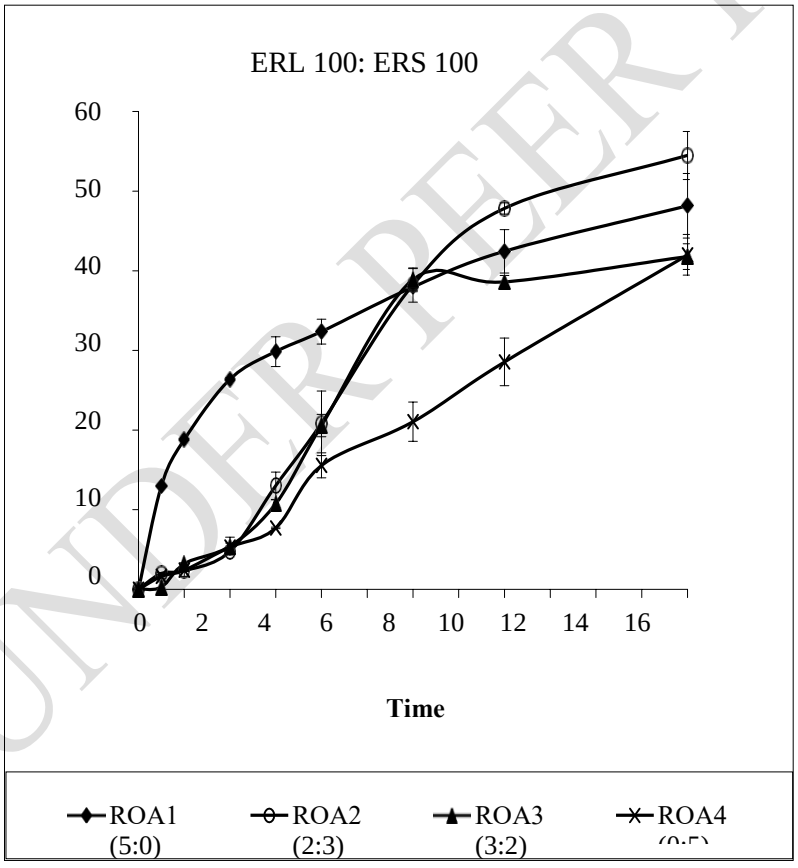

Figure 6: *In vitro* release profile of risperidone & olanzapine TDDS without enhancer

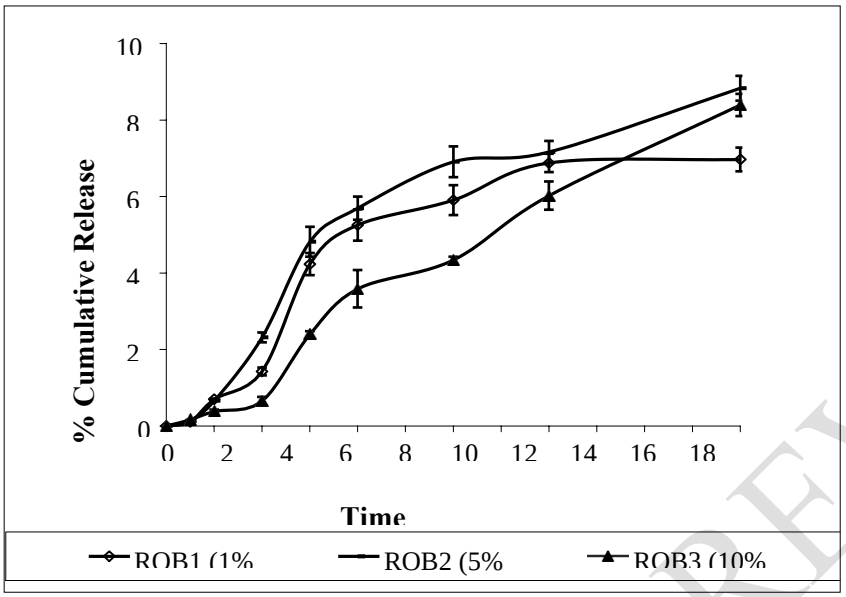

Figure 7: *In vitro* release profile of risperidone & olanzapine TDDS with BC as enhancer

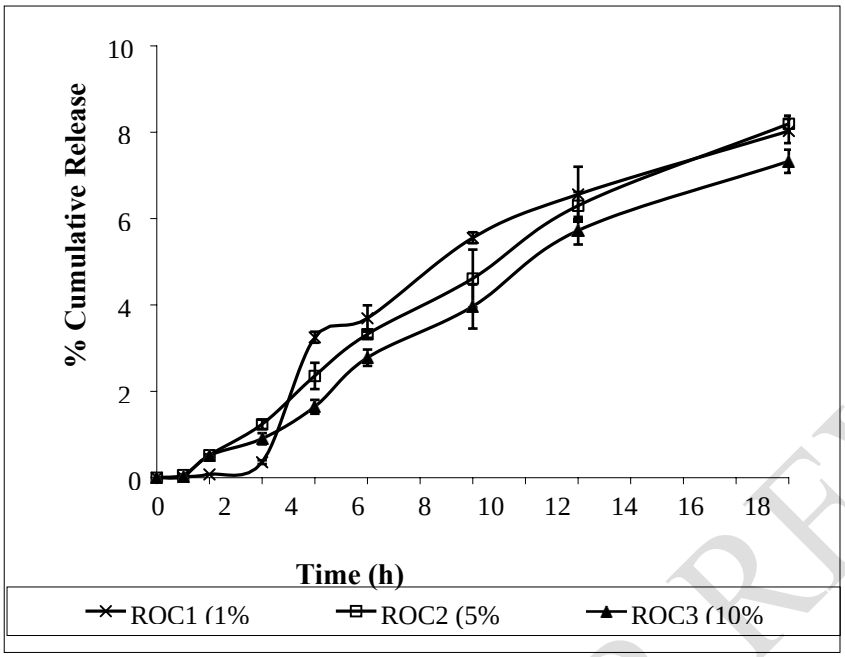

Figure 8: *In vitro* release profile of risperidone & olanzapine TDDS with SLS as enhancer

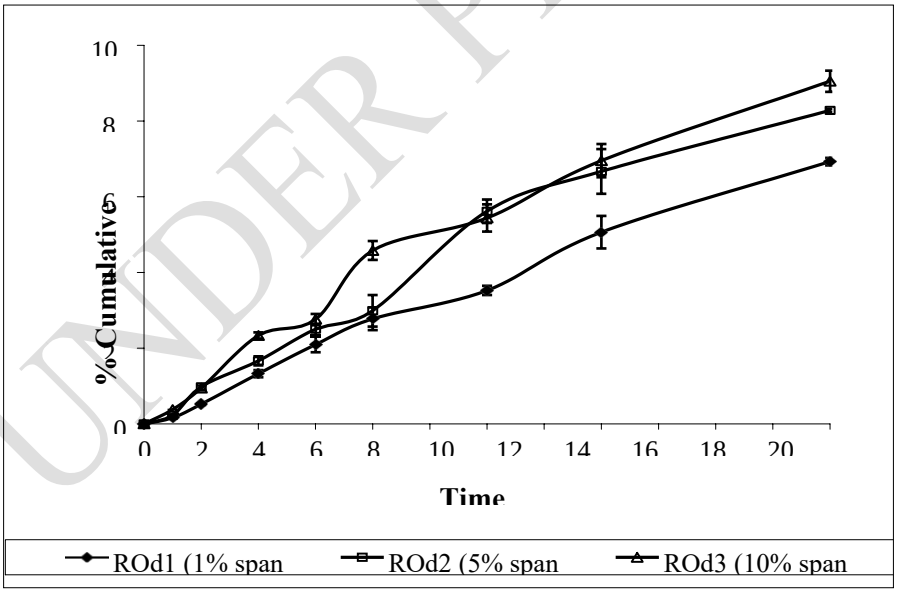

Figure 9: *In vitro* release profile of risperidone & olanzapine TDDS with span as enhancer

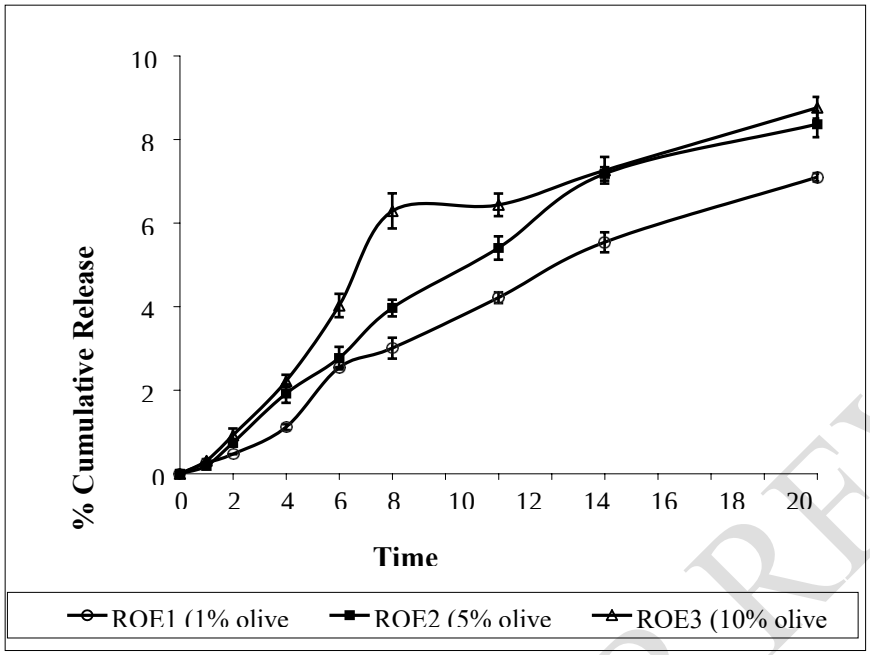

Figure 10: *In vitro* release profile of risperidone & olanzapine TDDS with olive oil as enhancer

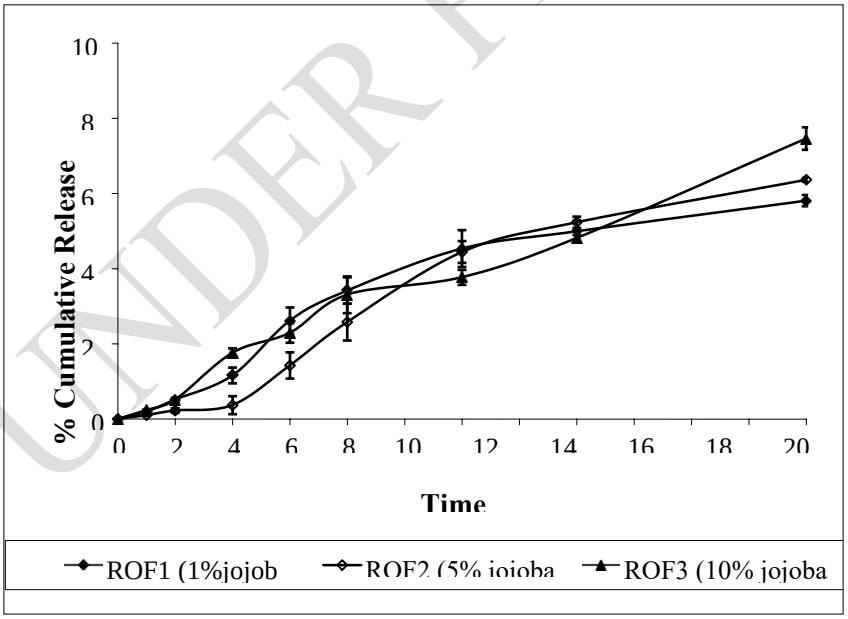

Figure 11: *In vitro* release profile of risperidone & olanzapine TDDS with jojoba oil as enhancer

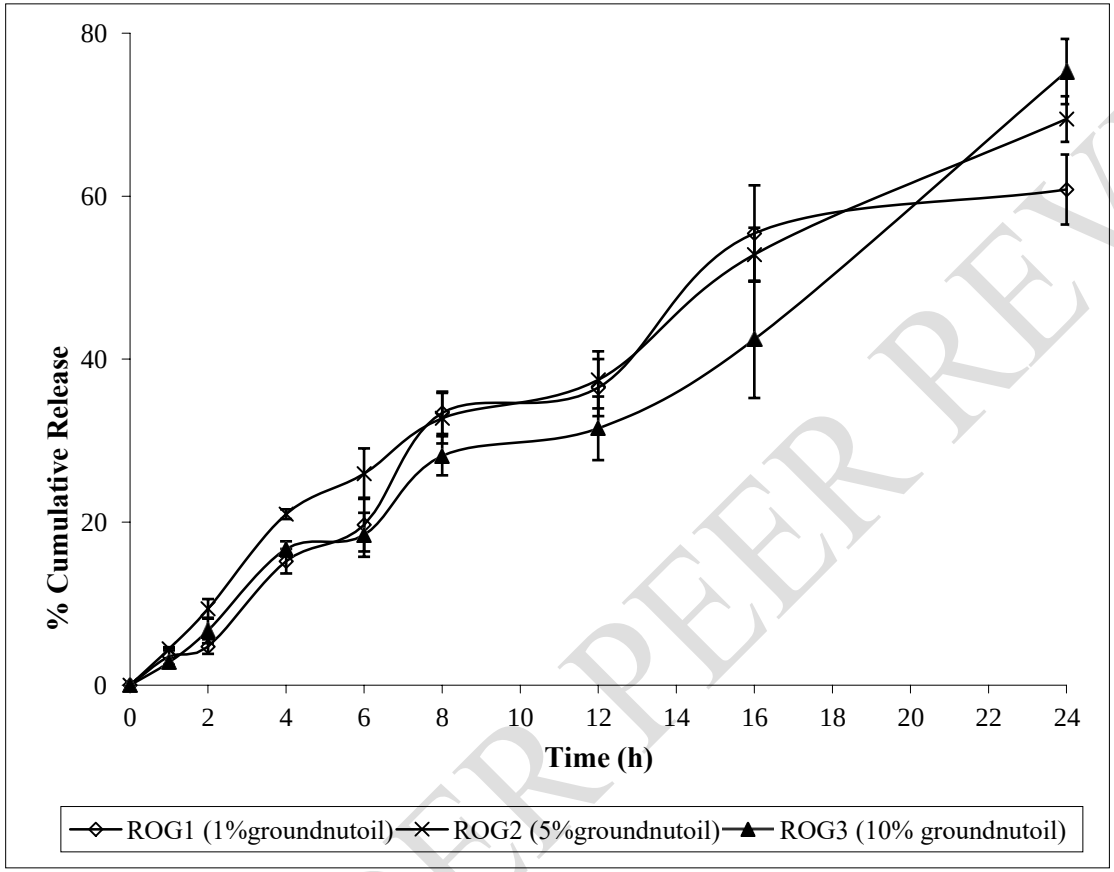

Figure 12: *In vitro* release profile of risperidone & olanzapine TDDS with groundnut oil as enhancer

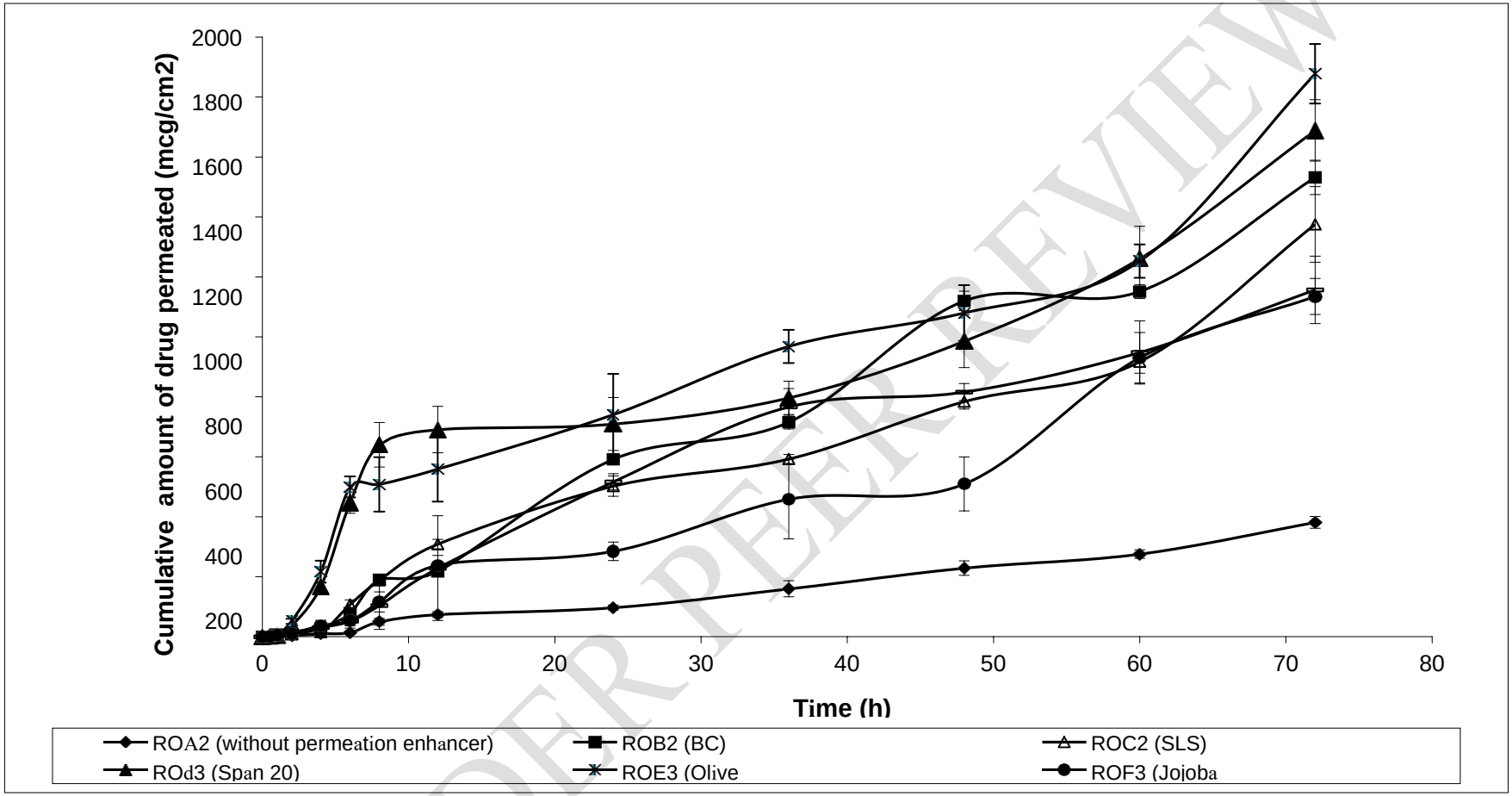

Figure 13: *In vitro* permeation profile of risperidone & olanzapine transdermal patches

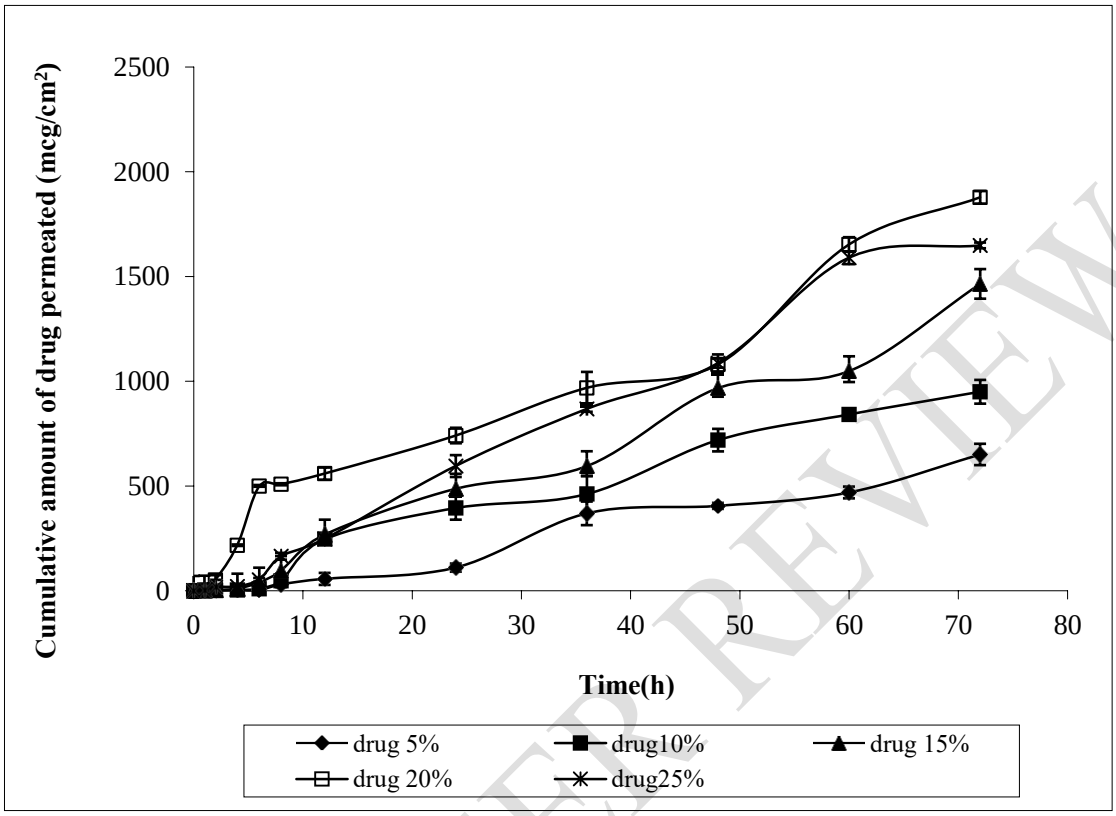

Figure 14: *In vitro* permeation profile of transdermal formulations with different drug loading of risperidone & olanzapine

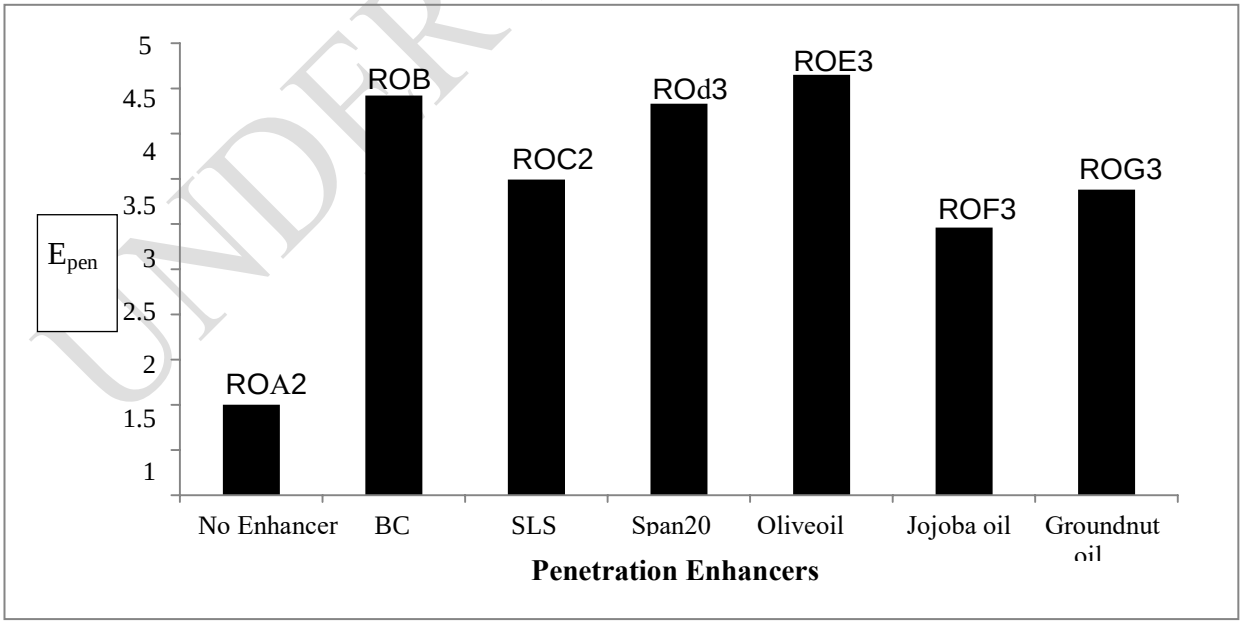

Figure 15: Relationship of E_{pen} with permeation enhancer

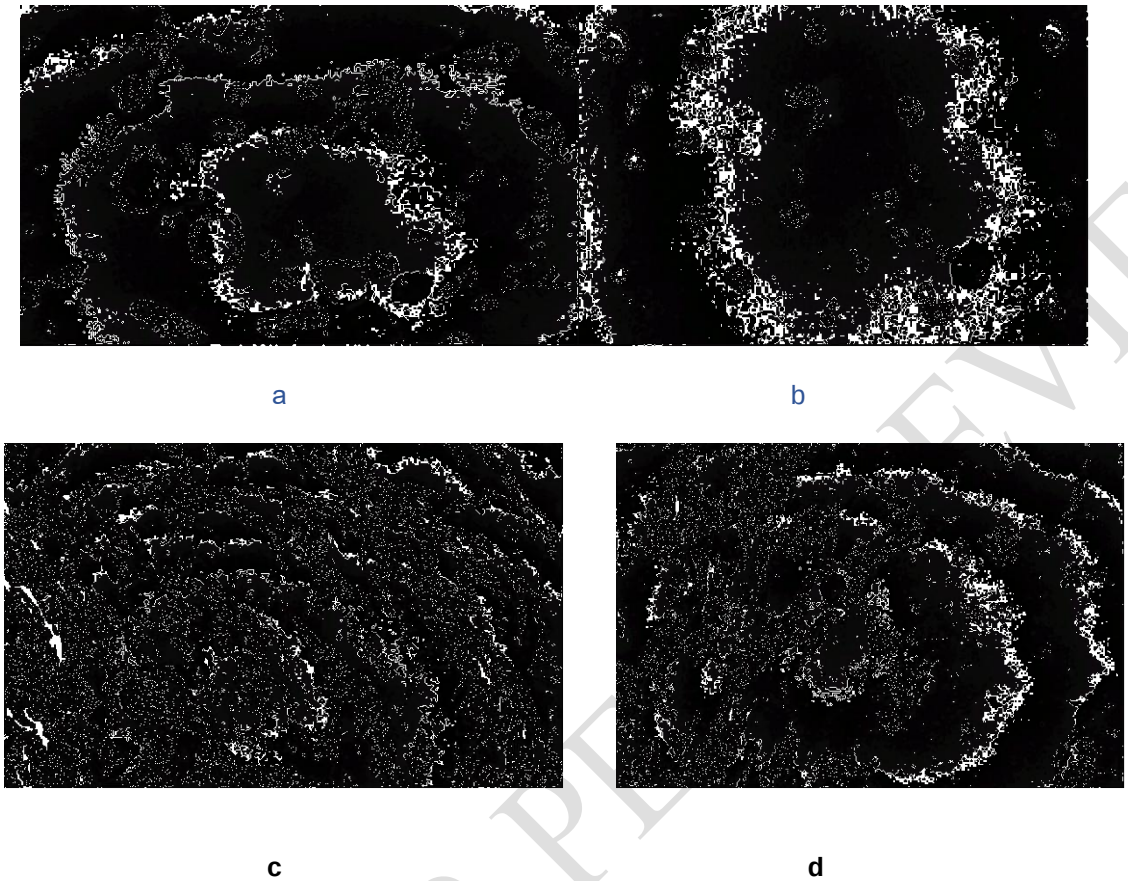

Figure 16: SEM scans of a) distribution of risperidone & olanzapine drug in matrix transdermal patch, b) transdermal patch after release of drug, c) dorsal side of skin before permeation studies d) SEM scan shows drug cluster as such reached at dorsal side from transdermal patch after release

Table 2: Visual Evaluation after skin irritation studies of risperidone & olanzapine TDDS

Rabbit No.	Control		Adhesive tape		Blank Patch		Test formulation ROE3		Formalin	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	1	0	1	1	3	3
2	0	0	0	0	1	0	1	0	2	3
3	0	0	1	0	1	1	1	1	3	3
4	0	0	0	0	0	0	0	0	3	3
5	0	0	0	0	0	0	0	0	2	2
6	0	0	1	0	1	0	0	0	3	2

Scores for skin irritation studies: 0 for none, 1 for slight, 2 for well defined, 3 for moderate and 4 for scar formation and severe erythema and edema

Table 3: Histopathological evaluation after skin irritation studies of risperidone & olanzapine TddS

Rabbit No.	Control		Adhesive tape		Blank Patch		Formulation ROE3		Formalin	
	Infarction	Edema	Infarction	Edema	Infarction	Edema	Infarction On	Edema	Infarction	Edema
1	-	-	+	-	+	+	+	+	++	+++
2	-	-	+	-	-	-	+	+	++	+++
3	-	-	+	+	+	+	+	+	++	++
4	-	-	-	-	-	-	-	-	++	+++
5	-	-	+	+	+	-	+	+	+++	++
6	-	-	+	+	+	+	-	-	+++	+++
	No ulceration								Hyperplasia,	

Scores for histopathological studies: - for none, + for slight, ++ for well defined, +++ for moderate and ++++ for scar formation and severe infarction and edema

Table 4: Tranquillizing activity of risperidone & olanzapine TDDS with rotarod apparatus

Dosage forms	Falling time (s)				
	1st hr	6th hr	12th hr	18th hr	24th hr
Control	260	260	250	260	262
Oral risperidone	20	12	20	22	27
Oral olanzapine	24	18	30	45	48
ROE3 (TddS)	23	13	14	12	12

Stability studies

Stability studies of selected formulations (ROE3) were carried out according to ICH guidelines to establish the structural integrity of matrix transdermal patch. The results revealed no changes in the physical appearance of the formulations after 3 months study. The drug content was found to be 97.03%, 97.02% and 96.32% after 1, 2 and 3 months respectively. So, there was no significant ($P>0.05$; t-test) change in drug content after storage of the formulations. Results of *in vitro* permeation studies of 3-month-old batch as compared to fresh sample (Figure 17) also confirmed no significant change in the formulation.

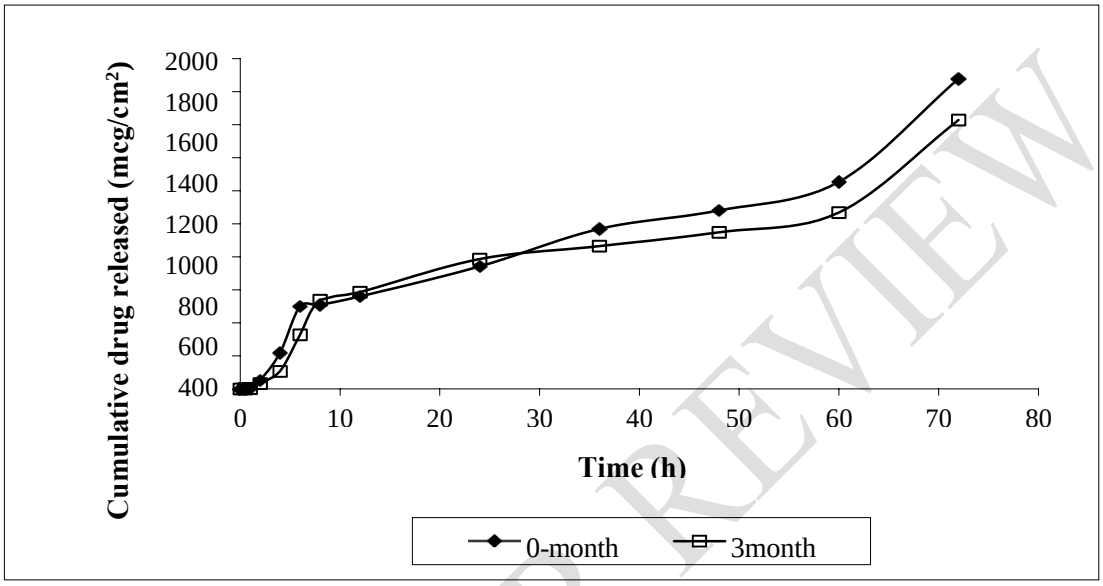

Figure 17: Permeation profile of risperidone & olanzapine TddS (ROE3) after stability studies

4. CONCLUSION

Flexible, smooth and transparent films were obtained with ERL 100: ERS100 polymers. It was found that risperidone & olanzapine TDDS showed that the most promising formulation was batch ROE3 (formulation containing ERL 100: ERS100, 3:2; risperidone 10%; olanzapine 10%; dibutylthalate 20% and 10% olive oil, all in %w/w). This formulation was able to deliver drug up to 3 days at a flux equivalent to the high dose currently marketed oral product from the patch containing surface area 10 cm^2.

Thus, optimized transdermal formulations of risperidone andolanzapine using polymers such as ERL 100 and ERS 100 with olive oil and span 20 as permeation enhancers demonstrated their ability to give sustained release, because of excellent release and permeation of drug and its influence on tranquillizing efficacy and better bioavailability data of TDDS in comparison to oral drug delivery. The developedf ormulations of these antipsychotics are expected to improve the patient compliance, form better dosage regimen and provide maintenance therapy to psychotic patients.

Ethical approval

Wistar rats were used for permeation studies ethically with the permission of institutional animal ethical committee.

DISCLAIMER:

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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