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Formulation and *in-vivo* evaluation of theophylline transdermal patch

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Abstract

This was an analytical study to evaluate the transdermal patch of theophylline. Theophylline transdermal patch was prepared by solvent casting method using hydroxyl propyl methylcellulose (HPMC) as polymer in a ratio of 1:3 (drug: polymer), propylene glycol as a permeation enhancer and 0.05M sodium hydroxide solution as solvent. Physicochemical analyses such as color, clarity, flexibility, smoothness, thickness, folding endurance, weight uniformity, percentage moisture uptake, percentage moisture loss, flatness and determination of drug content were performed. The *in-vivo* study was performed using six Wistar rats, 8-10 weeks old weighing 100-180 g, maintained at a temperature of 30.4 ± 1°C and relative humidity of 84% for the study. The patch was removed from the animals and analyzed for drug content using High Performance Liquid Chromatography (HPLC) at 30 min, 1 h and 2 h. The urine of the animals was collected at the same time interval to assay drug content. Mean weight uniformity (mm) was 0.0433 ± 0.0013, mean thickness uniformity (mm) was 0.34 ± 0.018, folding endurance was 198.67 ± 3.09,% moisture loss and uptake was 12.46 ± 2.98 and 2.12 ± 1.92 respectively. Drug content (%) was 105 ± 0.37. Drug concentration in patch after administration ranged from 89.99% to 97.44% for males and 89.74% to 97.14% for females. Urine drug concentration was highest after 2 h (10.71%) for males and 4.08% for females. The results indicate the feasibility of formulating a theophylline transdermal delivery system.

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Introduction

The transdermal drug delivery system appears to have surpassed the oral dosage form of drug administration as the most successful innovative research area in drug delivery (Ruela *et al.,* 2013). A transdermal drug delivery system (TDDS) is defined as a self-contained discrete dosage form that, when it is applied to intact skin, delivers the drug(s) through the skin layers at a controlled rate into the systemic circulation over a period of time (Amandeep and Alka, 2016). The transdermal device is a membrane moderated system, which may be of an active or passive design providing an alternative route for administering one or more active ingredients (Seema *et al.,* 2014). Delivery of drugs via the transdermal system is gaining prominent importance nowadays across the world (Seema *et al.,* 2014).

A transdermal patch consists of a polymer matrix, which is the main part of the delivery system and drug release can be controlled by varying its composition. Such polymers are expected to be biocompatible with the skin (Jhawat *et al.,* 2013), chemically non-reactive with the drug and the excipients, must be stable on storage, and the physiochemical properties and molecular weight of the polymer must allow diffusion of the drug substance (Gaikwad, 2013).

Suitable drug candidates for transdermal delivery should possess a molecular weight of less than approximately 1000 daltons (Latheeshjlal *et al.,* 2011), the substance should have a relatively low melting point of less than 93°C, the drug should possess an affinity for both lipophilic and hydrophlic phases (Prabhakar *et al.,* 2013), skin permeability coefficient should be less than 0.5 × 10-3 cm/h (Dhiman *et al.,* 2011; Patel *et al.,* 2011).

Theophylline is excreted unchanged in the urine (up to 10%) and evaluating its concentration using the urine becomes feasible. Conventional oral dosage forms are administered in multiple doses at particular time intervals in a given amount in order to achieve effective therapy. Although, this administration of multiple doses is known to be effective, several limitations in drug therapy abound, such as inconvenient administration, reduced patient compliance, the effect of first pass metabolism, and alteration of drug plasma level. In order to overcome such complications, transdermal drugs delivery systems are designed

Material and methods

Reagents and Equipment

Theophylline was received as a gift sample from Vitabiotics Nigeria limited, Lagos Nigeria. Propylene glycol and hydrochloric acid were obtained from (BDH Chemicals Ltd., Poole, England), Anhydrous calcium chloride (Guangdong Guanghua Sci-Tech CO., Ltd, China); Hydroxyl propyl methyl cellulose (HPMC) (SD Fine Chemicals, India). Sodium hydroxide pellet (Burgoyine & Co, India). Acetonitrile (Sigma, USA). All chemicals used were of analytical grade, High performance liquid chromatography; HPLC (Cyber lab, USA); analytical weighing balance (Shimadzu Corporation, Philippines); micrometer screw gauge (Control Company, USA); metabolic cage (Tecniplast, USA) and hygrometer (Thermo Fisher Scientific company, USA).

HPLC Instrumentation

The HPLC system consisted of a LC100 liquid chromatographic pump, a high pressure gradient mixer (1500µL mixer), Capell Pak C18, HPLC packed column SHISEIDO, Injector pump (20µL) and an injector pin 7725i

Chromatographic condition

Detection was carried out at 272 nm which is the wavelength closest to a major transition of theophylline (Nirav and Kaushal, 2011). The mobile phase was a mixture of Acetonitrile, Methanol and water in a volume ratio of 40:30:30. The injected volume was 20µL, and the overall analysis time was 20min. The system was operated at a flow rate of 0.5mL/min (Fernàndez *et al.,* 1996).

Validation studies of the HPLC method

Linearity and range: A stock solution of theophylline (0.01mg/ml) was prepared in 0.05 M Sodium hydroxide solution. Five standard solutions (10µg/ml to 0.625µg/ml) were diluted from the stock solution using the mobile phase for the assessment of linearity (Zhan *et al.,* 2015).

Linearity is evaluated by a plot of peak areas as a function of analyte concentration, and the test results were evaluated by appropriate statistical methods (Firdose *et al.,* 2013).

Accuracy

The accuracy was determined by recovery studies which were carried out by preparing a solution of the patch in sodium hydroxide and acetonitrile (50:50 v/v). The sample was analyzed thrice to determine the accuracy of the method (Rios *et al.,* 2017).

Formulation of transdermal patch

The transdermal patch was formulated based on the formula in Table 1. The patch was formulated by solvent casting method with theophylline as the test drug and hydroxyl propyl methyl cellulose (HPMC) as polymer in a ratio of 1:3 using propylene glycol as permeation enhancer and 0.05 M sodium hydroxide solution as solvent.

The polymer (2100 mg) was weighed and dissolved in 25mL of distilled water; the solution was mixed carefully and to this, 0.7mL of propylene glycol was added. Theophylline powder (710 mg) was dissolved with 35mL of 0.05 M sodium hydroxide solution and added to the homogenous dispersion with slow stirring.

The uniform dispersion formed was cast on to a petri dish of defined area (70.89cm²) and allowed to air dry for 72 h to obtain a dry patch. After drying, the formulated patch was removed from the petri dish and cut into small patches (1cm²). About 10 patches were obtained. They were stored in a desiccator before further evaluation.

Table 1. Composition of theophylline transdermal patch

Ingredients	Quantity Used
Drug (mg)	710
HPMC (mg)	2100
Propylene glycol (ml)	0.7
0.05M NaOH (ml)	35

*Formulation table; HPMC: Hydroxyl propyl methyl cellulose

Evaluation of Transdermal Patches

The patches were evaluated for physical properties, thickness (Patel *et al.,* 2014), folding endurance (Amjad *et al.,* 2011), weight uniformity (Gajanan *et al.,* 2011), percentage moisture uptake (Kumar *et al.,* 2013), percentage moisture loss (Amjad *et al.,* 2011), flatness determination (Rajendran and Ruckmani, 2011), drug uniformity (Prabhu *et al.,* 2011; Shirisha *et al.,* 2017), skin irritation study (Soujanya *et al.*, 2014)

In-vivo Drug Release Study

Six wistar rats of 8-10 weeks old weighing 100-180g were obtained from Delta State University Laboratory animal center Abraka, Delta State and maintained at a temperature of 30.4 ± 1°C and a relative humidity of 84% for the study.

The animals were housed in stainless metabolic cages and provided with standard diet and water. The urine obtained from the test animals (control group) after 24 h was centrifuged at 2000 rpm for 30 min to obtain a clear solution.

About 2mL of the sample was measured into a sample container containing acetonitrile and sodium hydroxide in ratios 3:2. The solution was filtered and analyzed using HPLC. Patch samples were applied to the shaved site on the dorsal surface. At 30 min, 1 h and 2 h interval, the patch was removed from the animals and analyzed for drug content left in the patch using HPLC. Both the used and unused patches were separately crushed in a mortar containing 10mL of 0.05 M NaOH to dissolve the theophylline. The polymer (HPMC) was precipitated out of the solution using 10mL acetonitrile. The mixture was filtered and analyzed with HPLC for drug content.

Results

Physical Appearance of the Formulated Patch

The colour, texture and odour of the patch were judged by three independent panelists and found to be white, brittle and odourless respectively

Physicochemical evaluation of the transdermal patch

The results of the physicochemical evaluation of the formulations are presented in Table 2.

Table 2. Physicochemical properties of the formulated transdermal patch.

Parameter evaluated	Values
Mean weight uniformity (g) + SD	0.043 ± 0.001
Mean thickness uniformity (mm) + SD	0.340 ± 0.018
Flatness determination (%) + SD	100.000 ± 0.001
Folding endurance	198.670 ± 3.090
Percentage moisture loss (%) + SD (RH 84%)	12.460 ± 2.980
Percentage moisture uptake (%) + SD (RH 84%)	2.120 ± 1.920
Percentage drug content (%) + SD	105.000 ± 0.370

RH: Relative Humidity, SD: Standard deviation

Linearity and range

A linear plot was obtained in the concentration range of 0.625 µg/ml to 10 µg/ml with a correlation value of 0.992. The accuracy was 95.93 ± 0.34% (Fig. 1)

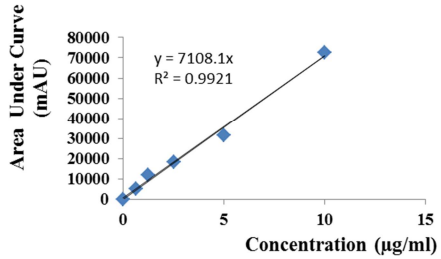


Fig. 1. Calibration curve of theophylline in 0.1N HCL at 272nm.

Chromatograms of in-vivo analysis

The results of chromatographic analysis of the patch are presented in Fig. 2 to 4. From the chromatograms, the results obtained indicate an increase in the peaks areas of the chromatograms with increasing time of administration. At 30 min, the area of the chromatogram was 37,023.7 compared to 145,019.9 at 2 h. The greater the area obtained, the higher the drug concentration in the urine. This means that the theophylline excreted unchanged in the urine increased gradually with time. Highest drug concentration was obtained in 2 h in both sexes.

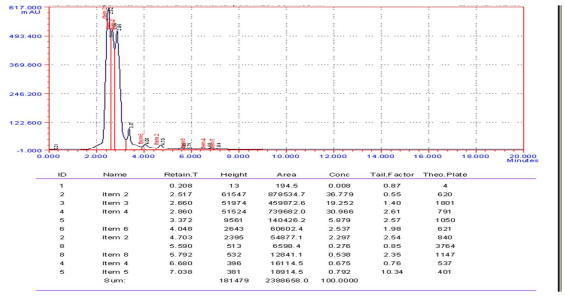
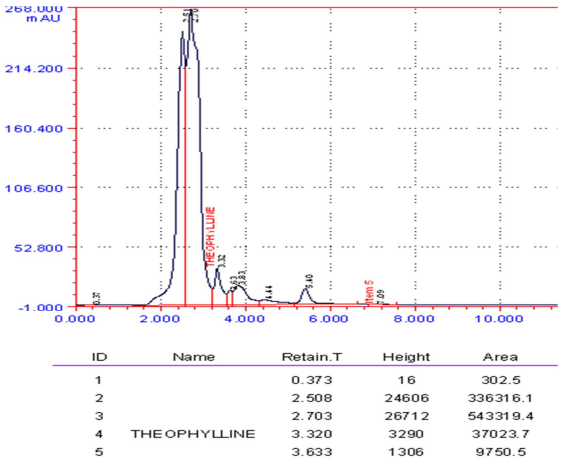


Fig. 2. Chromatogram of the urine of control groups after 30 minutes.

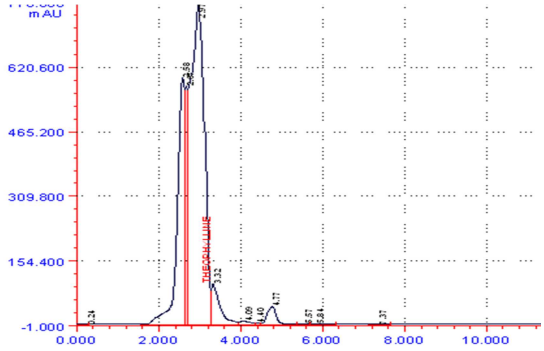
The control was not administered the drug.



ID	Name	Retain.T	Height	Area
1		0.373	16	302.5
2		2.508	24606	336316.1
3		2.703	26712	543319.4
4	THEOPHYLLINE	3.320	3290	37023.7
5		3.633	1306	9750.5

Fig. 3. Chromatogram after 30 min of drug administration.

The urine sample after 30 minutes of drug administration was analyzed and the area obtained for theophylline was 37,023.7 with retention time of 3.320 min at a flow rate of 0.5mL/min at 272 nm



ID	Name	Retain.T	Height	Area
1		0.235	9	132.1
2		2.582	59507	731530.6
3		2.665	57358	227837.4
4		2.965	77430	1794919.9
3	THEOPHYLLINE	3.322	9856	145019.9
6		4.089	835	17604.4
7		4.395	463	3206.2

Fig. 4. Chromatogram after 2 hours of drug administration.

The urine sample after 2 h of drug administration was analyzed and the area obtained for theophylline was 145,019.9 with retention time of 3.322 min at a flow rate of 0.5mL/min at 272 nm

Skin Irritation Study

The skin irritation study was carried out to assess the degree of erythema (superficial redness of the skin) upon administration of the transdermal patch (Table 3). Degree of erythema was slight and moderate.

Table 3. Results of skin irritation study.

Test animals	Indication of erythema
A_1	No reaction
A_2	Slight, patchy erythema
A_3	Moderate erythema
B_1	Slight, patchy erythema
B_2	Moderate erythema
B_3	Moderate erythema

A_1: Male at 30 min; A_2: Male at 1 h; A_3: Male at 2 h; B_1: Female at 30 min; B_2: Female at 1 h; B_3: Female at 2 h.

Discussion

There has been an increased interest in the development of transdermal drug delivery system (TDDS) for some drugs. This specialized delivery has peculiar advantages over oral route of drug administration for both topical and systemic drug administration. The transdermal delivery can also eliminate pulsed entry into the systemic circulation, which might often cause undesirable side effects (Garala *et al.,* 2009). Drug stability in transdermal drug delivery are also of paramount importance to ensure that the drug remain intact and clinically efficacious. This is assessed by evaluating the concentration of the drug in the plasma or conversely the concentration excreted through the kidneys for some drugs. This study was initiated to formulate transdermal patch of theophylline and also evaluate its physicochemical properties. The physical properties of the dosage form were void of any degradation and remained same through-out the study. This is very important as it is an indicator of dosage form stability and safety. The result of the folding endurance test measured manually, showed that the cut patches have good folding endurance which is a measure of flexibility and mechanical strength. From the result obtained, there was no constriction of patches. Moisture content is an indicator of the extent to which the patches can either absorb moisture from body tissues or from the environment or release moisture. This is a contributory mechanism in drug diffusion to the skin (Patel *et al.,* 2012). The formulation had low moisture uptake and low moisture loss values. It is well documented that age, ethnicity, certain disease state and more importantly skin hydration had been found to affect drug absorption through the skin (Carpentieri-Rodrigues *et al.,* 2007; Souto *et al.,* 2022).

The moisture content and moisture uptake of transdermal formulations provide that the formulation remains stable and not a completely dried and brittle film. However, studies had shown that increasing the concentration of polyvinyl pyrrolidone (PVP) increases the percentage moisture content and moisture uptake (Arora and Mukherjee, 2002). A low moisture is needed to avoid microbial contamination and bulkiness of the patches. The formulated patches retained their 100% flatness. This means that such formulations can maintain a smooth and uniform surface when they are attached onto skin. Therefore the polymers used are suitable for transdermal formulations in terms of their physical stability. The linearity plot obtained from the HPLC assay is indicative of the efficiency of the assay method to detect drug levels in a concentration manner giving a linearity of approximately 1 ((Arora and Mukherjee, 2002).

In vitro release profile is an important tool that predicts in advance how the drug will behave when in a biological system (Katayose and Kataoka, 1997). Thus, we can eliminate the risk of hazards of drugs because of direct experimentation in the living system. *In vitro* skin permeation experiments are known for their value in studying the rate and mechanism of percutaneous absorption of drugs as first observed by Chien, (1987). The drug concentration was evaluated using the urine of albino rats (animal model). The results show a decrease in the volume of urine on the administration of the formulated patch after specified time intervals. This occurred due to the overdose of the drug from the patch into the systemic circulation of the test animals causing a decrease in urine output. Conversely, as drug concentration increases in the urine, the drug left in the patch would decrease. However, the stratum corneum is not homogenous for all animals of the same species and may have structural differences which are due to differences in lipid content (Rajendran and Ruckmani, 2011). The process of drug release in most controlled release devices including transdermal patches is governed by diffusion and the polymer matrix has a strong influence on the diffusivity as the motion of a small

molecule is restricted by the three-dimensional network of polymer chains (Anderson *et al.,* 2000). As the time lags, the concentration of the drug in the urine increases while conversely it decreases in the plasma. This is evidenced in the increase in the area of the curve with time (urine concentration). This therefore posits that the concentration of the drug in the formulated patch would also decrease with time. This phenomenon had also been documented in other studies. The implication of skin permeation of drug on release-rate profiles and corresponding drug levels in the plasma or body fluids of the experimental formulations cannot be ignored, because the skin is known to have a substantial role in the variation of release kinetic (Johnson *et al.,* 1997). The irritation study is an indicator of how safe the medication is when given through the skin. The degree of erythema is a function of the drug's compatibility with the skin and its tendency to cause irritation when placed on the skin. All the formulations had zero to moderate erythema similar to other studies (Kawahara and Tojo, 2007). This is an anticipated phenomenon in transdermal drug delivery. However, Kawahara and Tojo, (2007) recommended that the residual API in the skin be removed promptly after application of the transdermal formulation. This would reduce erythema and improve compliance.

Conclusion

Theophylline is a methyl xanthine used in the management of respiratory disorders. To overcome the side effects of oral therapy, the transdermal patch of theophylline was formulated using sodium hydroxide solution as the solvent system and HPMC polymer by solvent casting technique. The test animals used excreted a considerably high percentage of theophylline unchanged in the urine which was recorded by the HPLC. This present study indicated the feasibility of formulating a theophylline transdermal drug delivery system. The mechanism of drug release from the dosage form was not evaluated and it was thought to be by diffusion. Further study is required to evaluate this dosage form in terms of *in-vivo* activity in animal models.

Declaration of interest

The authors declare no conflict of interest

References

Amandeep S, Alka B. 2016. Formulation and characterization of transdermal patches for controlled delivery of duloxetine hydrochloride. Analytical Science and Technology **7(25),** 2-13.

Amjad M, Ehtheshamuddin M, Chand SH, Sabreesh M, Asia R, Kumar GS. 2011. Formulation and evaluation of transdermal atenolol. Advance Research in Pharmaceuticals and Biologicals **1(2),** 109-119.

Andersson TL, Stehle B, Davidsson B, Hoglund P. 2000. Bioavailability of estradiol from two matrix transdermal delivery systems: Menorest and Climara. Maturitas **34,** 57-64.

Arora P, Mukherjee B. 2002. Design, development, physicochemical, and *in vitro* and *in-vivo* evaluation of transdermal patches containing diclofenac diethylammonium salt. Journal of Pharmaceutical Sciences **91(9),** 2076-2089.

Carpentieri-Rodrigues LN, Zanluchi JM, Grebogi IH. 2007. Percutaneous absorption enhancers: Mechanisms and potential. Brazilian Archives of Biology and Technology **50(6),** 949-961

Chien YW. 1987. Development of transdermal drug delivery systems. Drug Development and Industrial Pharmacy **13,** 589-651.

Darwhekar G, Jain D, Patidar V. 2011. Formulation and evaluation of transdermal drug delivery system of clopidogrel bisulfate. Asian Journal of Pharmacy and Life Science **1(3),** 269-277.

Dhiman S, Singh TG, Rehni AK. 2011. Transdermal patches: A recent approach to new drug delivery system. International Journal of Pharmacy and Pharmaceutical Sciences **3(5),** 26-34.

Fernàndez P, Lafuente N, Bermejo AM, López-Rivadulla M, Cruz A. 1996. HPLC determination of Cocaine and Benzoylecgonine in plasma and urine from drug abusers. Journal of Analytical Toxicology **20(4),** 224-228.

Firdose S, Prashanth P, Srikalyani V, Madhavi S, Nalluri BN. 2013. Analysis of Rivastigmine in *in vitro* transdermal permeation studies by RP-HPLC-PDA method. Journal of Chemical and Pharmaceutical Research **5(11),** 436-442.

Gaikwad AK. 2013. Transdermal drug delivery system: Formulation aspects and evaluation. Comprehensive Journal of Pharmaceutical Sciences **1(1),** 1-10.

Garala KC, Shinde AJ, Shah PH. 2009. Formulation and *in-vitro* characterization of monolithic matrix transdermal systems using HPMC/Eudragit S 100 polymer blends. International Journal of Pharmacy and Pharmaceutical Sciences **1(1),** 108-120

Jhawat VC, Saint V, Kamboj S, Maggon N. 2013. Transdermal drug delivery systems: approaches and advancements in drug absorption through skin. International Journal of Pharmaceutical Sciences Review and Research **20(1),** 47-56.

Johnson ME, Blankschtein D, Langer R. 1997. Evaluation of solute permeation through the stratum corneum: Lateral bilayer diffusion as the primary transport mechanism. Journal of Pharmaceutical Sciences **86(10),** 1162-1172.

Katayose S, Kataoka K. 1997. Water soluble polyion complex associates of DNA and poly(ethylene glycol)-poly(L-lysine) block copolymers. Bioconjugate Chemistry **8(5),** 702-707.

Kawahara K, Tojo K. 2007. Skin irritation in transdermal drug delivery systems: a strategy for its reduction. Pharmaceutical Research **24(2),** 399-408.

Kumar SS, Behury B, Sachinkumar P. 2013. Formulation and evaluation of transdermal patch of Stavudine. Dhaka University Journal of Pharmaceutical Sciences **12(1),** 63-69.

Latheeshjlal L, Phanitejaswini P, Soujanya Y, Swapna U. 2011. Transdermal drug delivery systems: An overview. International Journal of PharmTech Research **3(4),** 2140-2148.

Nirav PM, Kaushal KC. 2011. Method development, validation and stability study for simultaneous estimation of Etofylline and Theophylline by RP-HPLC chromatography in marketed formulation. Journal of Chemical and Pharmaceutical Research **3(3),** 597-609.

Patel DM, Kavitha K. 2011. Formulation and evaluation aspect of transdermal drug delivery system. International Journal of Pharmaceutical Sciences Review and Research **6(2),** 83-90.

Patel VH, Patel SS, Raj MM, Patel NK, Raj LM. 2014. Development and characterization of matrix-membrane controlled transdermal drug delivery system of Amlodipine Besylate. Indo American Journal of Pharmaceutical Research **4(11),** 5306-5313.

Prabhakar D, Sreekanth J, Jayaveera KN. 2013. Transdermal drug delivery patches: A review. Journal of Drug Delivery and Therapeutics **3(4),** 213-221.

Prabhu P, Shah S, Gundad S. 2011. Formulation development and investigation of domperidone transdermal patches. International Journal of Pharmaceutical Investigation **1(4),** 240-246

Rajendran V, Ruckmani K. 2011. *Invivo* and *Invivo* characterization of transdermal delivery of sertraline hydrochloride films. DARU Journal of Pharmaceutical Sciences **19(16),** 424-432.

Rios F, Alberola A, Melendez J, Muedra G, Trigo F. 2017. A validated HPLC method for the determination of vanillyl butyl ether in cosmetic preparations. Cosmetics **4(1),** 1-5.

Ruela ALM, Figueiredo EC, Perissinato AG, Lima ACZ, Araujo MB, Pereira GR. 2013. *In vitro* evaluation of transdermal nicotine delivery systems commercially available in Brazil. Brazilian Journal of Pharmaceutical Sciences **49(3),** 579-588.

Seema A, Nida A, Shiv SB. 2014. Transdermal delivery a preclinical and clinical perspective of drugs delivered via patches. International Journal of Pharmacy and Pharmaceutical Sciences **6(5),** 26-38.

Shirisha S, Joshi G, Sahoo SK, Rao YM. 2017. Preparation and evaluation of matrix type transdermal patches of domperidone maleate: *in-vitro* and *ex-vivo* characterization. Indian Journal of Pharmaceutical Education and Research **51(4),** 517-524.

Soujanya C, Satya B, Reddy M, Manogna K, Prakash P, Ramesh A. 2014. Formulation and *in vitro* and *In-vivo* evaluation of transdermal patches of lornoxicam using natural permeation enhancers. International Journal of Pharmacy Pharmaceutical Sciences **6(4),** 282-286.

Souto EB, Fangueiro JF, Fernandes AR, Cano A, Sanchez-Lopez E, GarciamL, Severino P, Paganelli MO, Chaud MV, Silva AM. 2022. Physicochemical and Biopharmaceutical aspects influencing skin permeation and role of SLN and NLC for skin drug delivery. Heliyon **8,** 1-16

Zhan X, Mao Z, Chen S, Wang G. 2015. Formulation and evaluation of transdermal drug-delivery system of isosorbide dinitrate. Brazilian Journal of Pharmaceutical Sciences **51(2),** 373-382.