WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 1582-1603. Research Article ISSN 2277– 7105

"DESIGN, DEVELOPMENT AND EVALUATION OF TRANSDERMAL PATCH CONTAINING ONDANSETRON HYDROCHLORIDE AND DILTIAZEM HYDROCHLORIDE"

Assistant.Prof.Kokate Sudhir Govind.*, Prof. Tarkase Kailas, Jogdand Trimbak, Dr.Dushant Gaikwad.

VJSM'S Vishal Institute of Pharmaceutical Education and Research, Ale. Tal-Junnar, Dist-Pune, 412411, India.

Article Received on
28 March 2015,

Revised on 21 April 2015,
Accepted on 15 May 2015

***Correspondence for Author**
Assistant.Prof.Kokate Sudhir Govind.
VJSM'S Vishal Institute of Pharmaceutical Education and Research, Ale. Tal-Junnar, Dist- Pune, 412411, India.

ABSTRACT

The purpose of this research was to develop a matrix-type Transdermal therapeutic system containing drug Ondansetron hydrochloride (OSH) with using different ratio of HPMC K4M, and EC, ERLPO in combination by mercury substrate method. The polymers were weighed in requisite ratio and dissolved in Methanol. Polyethelene glycol 40% w/w total weight of polymer composition was used as plasticizer. Methanol was added 5 ml of the total weight of polymers; homogeneous dispersion was formed by slow stirring with a mechanical stirrer.F4 showing sustain release up to 24 hr with 87.21% and 93.56% drug permeation shows sustain release of Ondansetron hydrochloride and Diltiazem hydrochloride Drugs through cadaver skin,100% flatness, 51no.of fold of folding endurance test which showing good flexibility and less brittleness, moisture content was 0.851± 0.67 % and moisture uptake was 3.198± 1.87%, good tensile strength i.e. $58.1\times10^4\pm$ 0.87dynes/cm^2, so according to all parameter the F4 was the optimized formulation.

KEYWORD: Ondansetron hydrochloride, Hydrophilic and hydrophobic polymers, Sustained release, Transdermal patches.

INTRODUCTION

In the advent of modern era of pharmaceutical dosage forms, transdermal drug delivery system (TDDS) established itself as an integral part of novel drug delivery system.[1] Transdermal patches are polymeric formulations which when applied to skin deliver the drug

at a predetermined rate across dermis to achieve systemic effects. Transdermal dosage forms, though a costly alternative to conventional formulations, are becoming popular because of their unique advantages. Controlled absorption, more uniform plasma levels, improved bioavailability, reduced side effects, painless and simple application and flexibility of terminating drug administration by simply removing the patch from the skin are some of the potential advantages of transdermal drug delivery.[2] Ondansetron is a potent antagonist of Serotonin (5 HT_3) receptor which has been proved effective in prevention of chemotherapy and radiotherapy-induced nausea and vomiting. Ondansetron hydrochloride has been used by oral and injectable administration. Ondansetron hydrochloride is rapidly absorbed orally, but extensively metabolized by the liver. It should be administered 30 min before chemotherapy, and the orally administered antiemetic drug tends to be discharged by vomiting. On the contrary, intravenous administration renders rapid effect to a patient, but the onset of effect is too rapid to cause undesirable effects. In addition, it gives a local pain, and may cause an unexpected accident when it is not perfectly prepared**.**[3] In this work an attempt was made to formulate and evaluate TDDS for sustained release OSH by solvent casting method. Low molecular weight, good permeability, poor bioavailability (60%) and shorter half-life (5-6 h) of OSH made it a suitable drug candidate for the development of Transdermal patches.[4] The main objective of formulating the Transdermal system was to prolong the drug release time, reduce the frequency of administration and to improve patient compliance.

MATERIALS AND METHOD

Ondansetron Hydrochloride was obtained as a gift sample from Cipla Pvt.Ltd, Mumbai, Diltiazem Hydrochloride was obtained as a gift sample from Wockhardt, Aurangabad. Eudragit RLPO was obtained from Evonikpharma,Mumbai.Hydroxy propyl methyl cellulose K4M from Ozone Chemicals Ltd, Potassium di hydrogen orthophthalate and Sodium hydroxide from Qualigens Fine Chemicals Ltd. Mercury obtained from Abhay chemical's Ltd. All other chemicals used were of analytical grade.

FTIR- spectroscopy

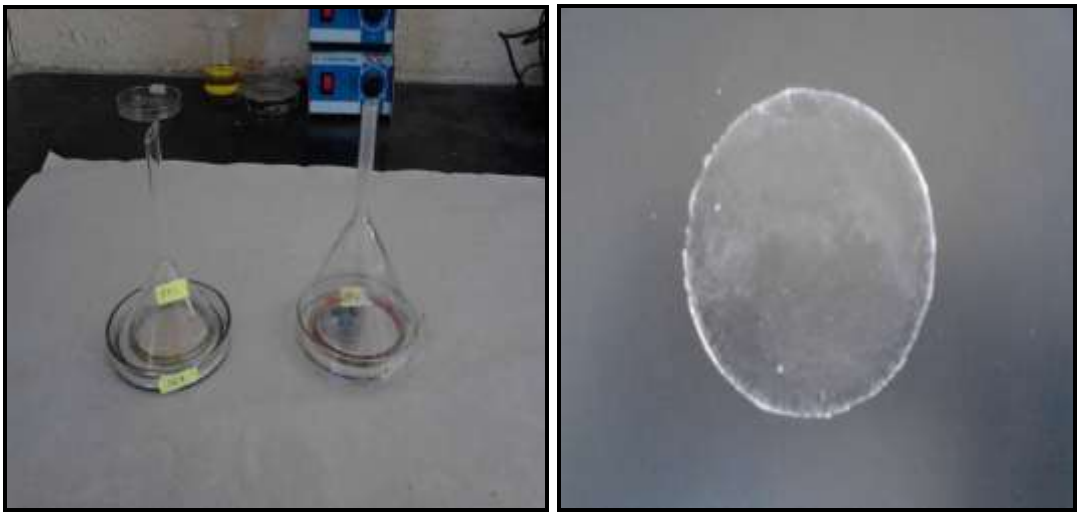
In order to investigate the possible interaction between drug and selected polymers, FT-IR spectroscopy studies were carried out. IR spectrum for pure drug and physical mixture of drug-polymers were compared. Then it was characterized for any change in the finger print-region of drug in the presence of polymer.

Formulation of transdermal patches

Matrix type Transdermal films containing ondansetron hydrochloride and diltiazem hydrochloride were prepared using different ratio of HPMC K4M, and EC, ERLPO in combination by mercury substrate method.[12] The polymers were weighed in requisite ratio and dissolved in Methanol. Polyethelene glycol 40% w/w total weight of polymer composition was used as plasticizer. Methanol was added 5 ml of the total weight of polymers; homogeneous dispersion was formed by slow stirring with a mechanical stirrer. The uniform dispersion was then poured into a glass ring mould of 6 cm diameter placed on the surface of mercury kept in a Petri dish. The solvent was allowed to evaporate under ambient conditions (Temperature: 32^{0}C) for 24 hrs. Aluminum foil was used as backing membrane and prepared film was stored in desiccators until used, preparation of film on mercury surface as
shown in figure no.1

Table No.1: Formilation of Transdermal Patch

Formulatiopn	F1	F2	F3	F4	F5
ERLPO :HPMC:EC (300mg)	2 : 1 : 0	-	-	-	-
ERLPO :HPMC:EC (300mg)	-	2 : 0 : 1	-	-	-
ERLPO :HPMC:EC (300mg	-	-	2 : 0.5 : 0.5	-	-
ERLPO :HPMC:EC (300mg)	-	-	-	2 : 0.75 : 0.25	-
ERLPO :HPMC:EC (300mg)	-	-	-	-	2 : 0.25 : 0.75
Ondansetron HCL(mg)	8	8	8	8	8
Diltiazem HCL (mg)	20	20	20	20	20
PEG-400	1	1	1	1	1
Ethanol	5	5	5	5	5



"Fig.1: Formulation of Ondansetron hydrochloride and Diltiazem hydrochloride film on mercury surface with glass ring mould."

Evaluation of Transdermal formulation

Physicochemical evaluations[5,6,7,8,9]

A) Thickness

The thickness of the patch was measured by micrometer screw gauge at five different places; an average of five values was calculated. All films of measured thickness were of low standard deviation values ensuring the uniformity of patch prepared by mercury substrate method.

B) Weight Uniformity

The weight uniformity was in the range of 105 ± 0.003 mg to 113 ± 0.003 mg. The values for all the formulations are tabulated in the Table.

C) Folding endurance

The folding endurance was found to be in the range of 44 ± 0.83 no. of folds to 55 ± 0.034 no. of folds. The data revealed that the patches had good mechanical strength along with flexibility.

D) Percent Flatness

For flatness determination, one strip was cut from the centre and two from each side of patches. The length of each strip was measured and variation in length was measured by determining percent constriction. Zero percent constriction was equivalent to 100 percent flatness. Flatness was calculated by

$$\% \, Constriction = \frac{l1 - l2}{l2} \times 100$$

Where l_1 is initial length of each strip, l_2 is final length of each strip.

E] Percent Moisture Uptake

This test was also carried to check the integrity of films at dry condition. Three films of 5 square centimeter area were cut and weighed individually and put in a desiccators containing calcium chloride at room temperature for 24 h. The films were weighed again after a specified interval until they show a constant weight. The percent moisture content is calculated using following formula.

$$\% \, Moisture \, content = \frac{Intial \, weight - Final \, weight}{Final \, weight} \times 100$$

E) Percentage elongation break test

Percent elongation break test is defined as the elongation at the moment of rupture of the specimen divided by the initial gauge length of the specimen and multiplying by 100. However percent elongation of ERL 100: HPMCK4M Patch was greater than other films, this may be due to less film flexibility.

$$Elongation \, percentages = \frac{l1 - l2}{l2} \times 100$$

Where, L1= is the final length of each strip.

L2= is the initial length of each strip.

F) Surface pH

Surface pH varied between 6.1 to 7.1 indicating that no irritation will occur on the skin after applications of the patches. All surface pH were observed in the range**.**

G) Drug content and content uniformity

This test involves the assay of individual units of a specified no. of dosage forms in order to determine homogeneity in their content. From the results it can be concluded that the film does not show significant deviation from average value. The drug content uniformity was determined for all the eight formulations by Spectrophotometric method. The drug content for prepared batches of Transdermal patches of Ondansetron hydrochloride and Diltiazem hydrochloride varies between 82.34 ± 0.45 to 97.06 ± 0.23 and93.65 ± 0.62 to 98.79 ± 0.82 ,

it was considered that the drug is dispersed uniformly throughout the film In-vitro permeation study.

H) Tensile strength

Tensile strength at break is the maximum tensile stress sustained by the specimen during the tension test, the tensile strength was found to be in the range of 31.2×10^4 dyne/cm^2 to 58.1×10^4 dyne/cm^2. The formulation F4 showed the best tensile strength.*Tensile strength = Maximum applied force/ Minimum cross sectional area= m × g / b × t kg / mm²*

Where,

m- Mass in kg

g- Acceleration due to gravity 980 cm/ sec²

b- Breath of specimen in cm

t – Thickness of specimen in cm.

In-vitro evaluations.[10]

In-vitro Drug Release Study

A modified glass disc assembly (USP Apparatus 5, paddle over disc assembly), was used for the assessment of the release of the drug from the patches. The Transdermal drug delivery system (TDDS) was mounted on the disc,The accurately weighed patches were fixed on glass discs using standard glue and placed at the bottom of the dissolution vessel. The dissolution medium was phosphate buffer of pH 6.6 and the apparatus was equilibrated to 32±0.5°C. The apparatus was operated at 50 rpm and samples were withdrawn at appropriate time intervals up to 24 hr and analyzed at 215 nm spectrophotometrically. Cumulative % drug released were calculated out and plotted against time**.**

In-vitro Drug Permeation Study

Preparation of skin

Permeation studies were carried using the modified Franz diffusion of internal diameter of 3.1 cm. Goat abdominal skin was used as the model membrane. The abdominal skin of the freshly sacrificed goat was procured from the local slaughter house. The abdominal skin was excised and trimmed evenly from the sides and then washed in isotonic phosphate buffer of pH 7.4 and used immediately. The membrane was stabilized before mounting in order to remove the soluble components.

METHOD

The in-vitrodrug permeation studies were carried out using modified Franz diffusion cell. The cell consists of two compartments, namely donor (active) and receptor. Phosphate buffer (pH 7.4) was used as receptor fluid. The polymeric films were placed in intimate contact with the stratum corneum side of the skin. The receptor fluid was agitated using magnetic bead at 50 rpm to avoid the formation of diffusant layer and the temperature of 37 ± 1^{0}C was maintained, sampling port was covered to avoid the evaporation of solvent. Aliquots of 0.5 ml sample was withdrawn at time interval of 1hr for 24 hrs and replaced with an equal volume of drug free receptor fluid to maintain the sink condition. An amount of drug permeated at each time interval was calculated spectrophotometrically at 248 & 237 nm.

In- vitro studies comprised of following constants

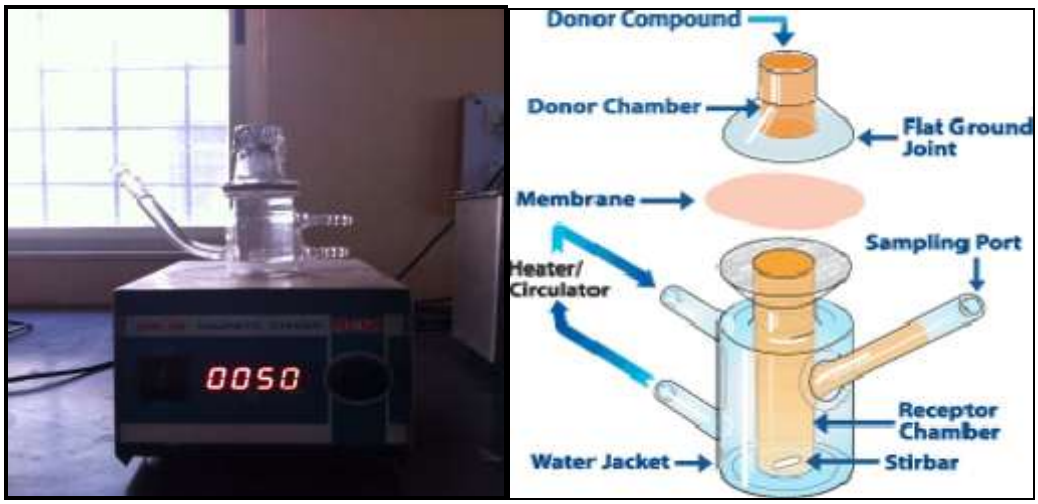
1. Volume of modified Franz cell receptor compartment - 100 ml
2. Release medium phosphate buffer - pH 7.4
3. Temperature of release medium - 37ºC ± 1ºC
4. The stirring speed of magnetic bead operated by magnetic stirrer was also kept constant.

The cumulative amount of drug permeated per unit skin surface area was plotted against time and the slope of the linear portion of the plot was estimated as the steady state flux (Jss), permeability coefficient (Kp) and enhancement ratio (ER) was calculated by using equation.

Kp = Jss / DC.

Where,DC- Donor concentration.

ER = drug flux with enhancer/drug flux without enhancer.



"Fig.2: A diagrammatic representation of modified Franz diffusion cell."

Skin Irritation study[11]

The hair on the dorsal side of Westar albino rats (200-240 gm) was removed by clipping 1 day before this portion of experiment. The rats were divided into 2 groups (n=6). Group I serves as the control; group II received Transdermal patch F4. A new patch was applied daily for 7 days. Finally, the application site was observed for erythema, edema or any skin rashes.

Stability Studies

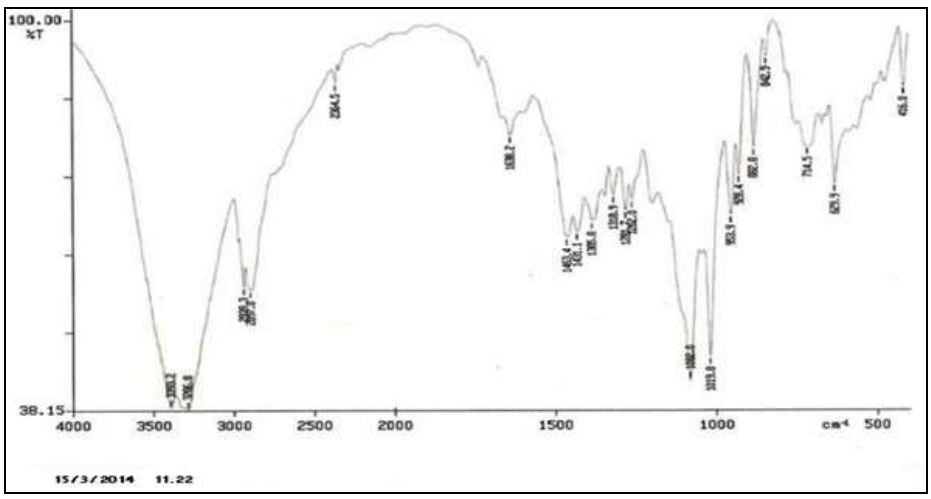
Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) specifies the guidelines for stability testing of new drug products, as a technical requirement for the registration of pharmaceuticals for human use. According to the ICH guidelines27 the stability studies of the formulation were carried out as per ICH guideline.The study was conducted at temperature of 40 ± 0.5^0 C and 75 ± 5% RH. Transdermal systems of 28.26 cm^2 area were wrapped individually in butter papers, packed in aluminum foils and placed in Petri dishes. These Petri dishes containing patches were stored in the stability chamber at 40 ± 0.5^o C and 75± 5 % RH for a period of 3 month.

RESULTS AND DISCUSSION

Spectroscopic study of drug

IR spectrum interpretation

Fourier transformed infrared (FTIR) spectra of Ondansetron hydrochloride and Diltiazem hydrochloride was taken by using the KBr disk method (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 cm^{-1} and the resolution was 1 cm^{-1}.



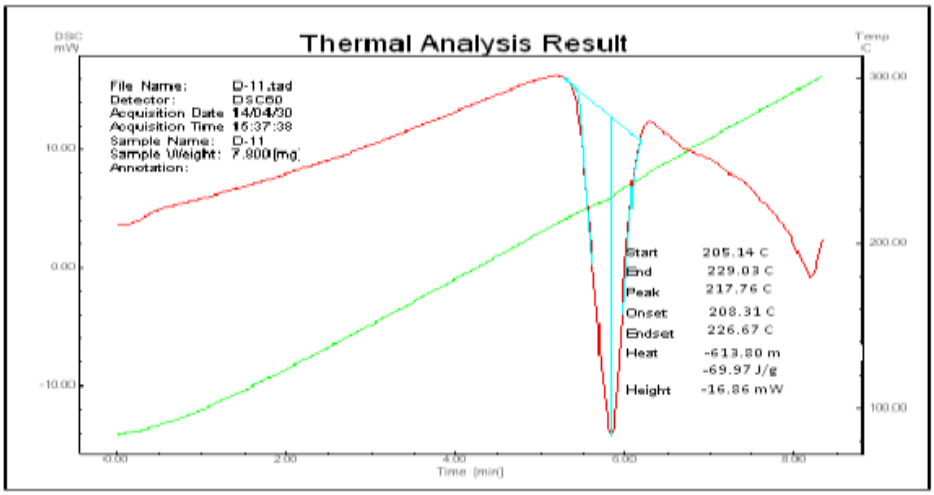
"Fig.3: IR Spectrum of Ondansetron HCL and Diltiazem HCL with Excipients."

Table.2: Frequencies Ondansetron hydrochloride &Diltiazem hydrochloride with Excipients IR spectra.

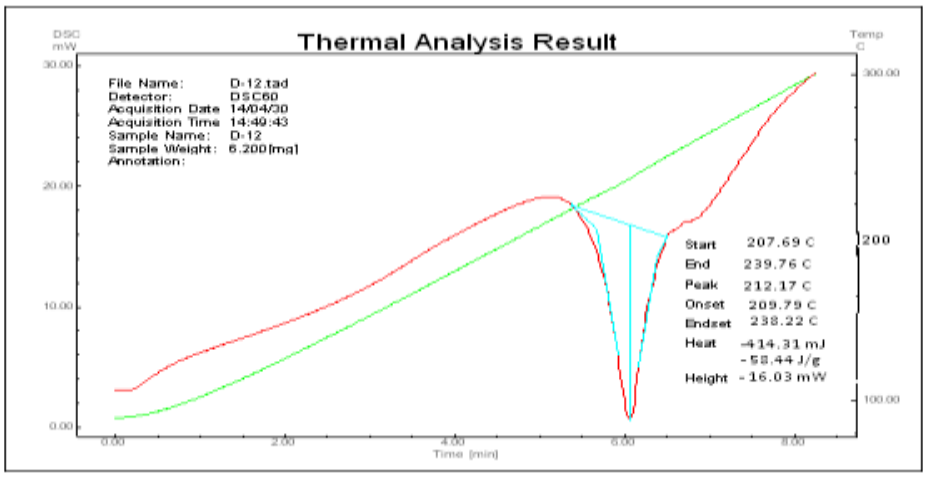
Sr. No.	Bond	Frequency (cm^{-1})
1	C-H (streching for CH_3)	2926.65
2	C=N (streching)	1637.94
3	C=C (streching)	1459.07
4	C-N (vibration)	1280
5	C=O (streching)	958.55

Differential Scanning Calorimetry (DSC)

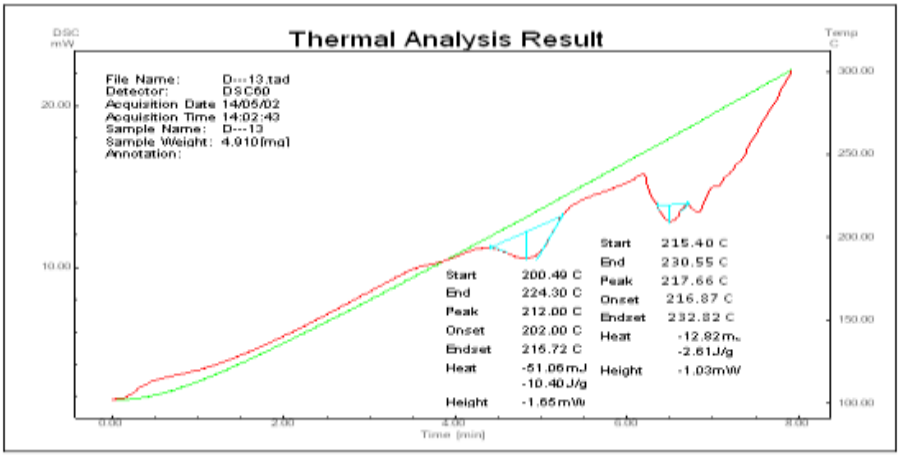
DSC studies were performed to assess any interaction between the drug and excipient. The DSC thermogram of plane drugs, drugs with ERLPO, HPMC K-4M and Ethyl cellulose.The DSC thermogram of Ondansetron hydrochloride and Diltiazem hydrochloride shows the endothermic peak onset at 217.76^{0}C and 212.17^{0}C indicated the melting point which was reported in literature. There was no sharp change in melting point of drugs.



"Fig.4: DSC Spectra of Drug Ondansetron hydrochloride"



"Fig.5: DSC Spectra of Drug Diltiazem hydrochloride"



"Fig.6: DSC Spectra of Optimized Formulation"

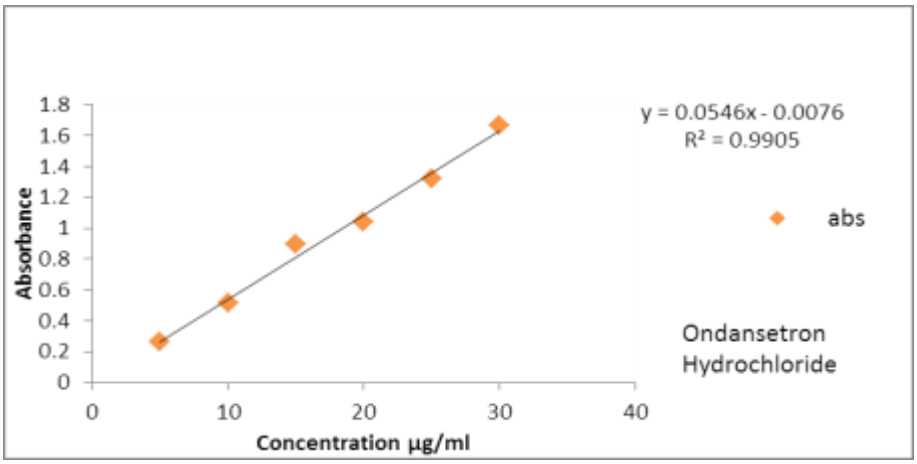
UV-VIS Spectrophotometric Method

Determination of λmax

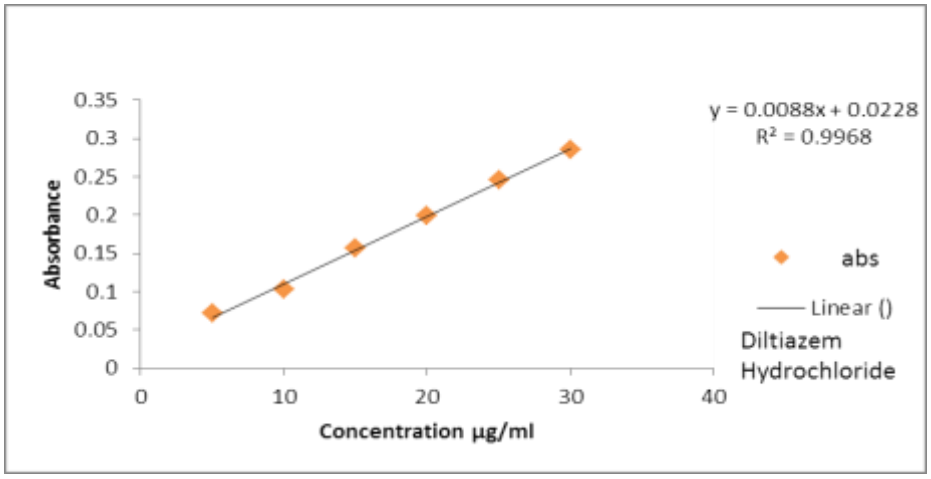
Ondansetron hydrochloride &Diltiazem hydrochloride showed maximum absorption at 248 & 237 nm respectively.

Table. 3: Dilution andabsorbance of Drugs Ondansetron hydrochloride & Diltiazem hydrochloride.

Sr. No.	Concentration (µg/ml)	Absorbance of Ondansetron hydrochloride	Absorbance of Diltiazem hydrochloride
0	0	0.00	0.00
1	5	0.2647	0.0716
2	10	0.5128	0.1023
3	15	0.8945	0.1563
4	20	1.0364	0.1983
5	25	1.3185	0.2451
6	30	1.6647	0.2849



"Fig.7: Calibration curve for the estimation of of ondansetron hydrochloride in Methanol"



"Fig.8: Calibration curve for the estimation of Diltiazem hydrochloride in Methanol."

Table.4:% Drug content of Ondansetron hydrochloride And Diltiazem hydrochloride

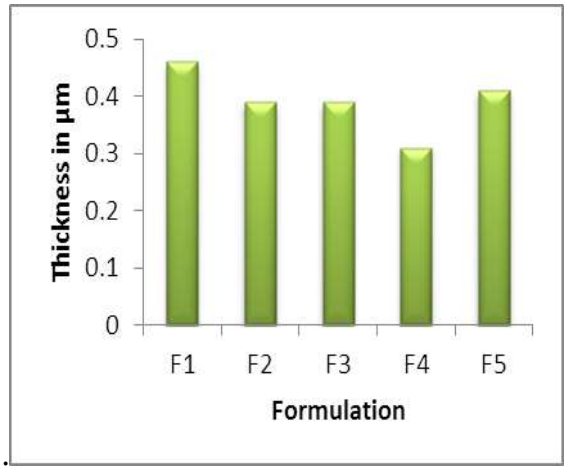
Sr. No.	Formulation code	% Drug Content Ondansetron hydrochloride	% Drug Content Diltiazem hydrochloride
1	F1	82.34 ± 0.45	94.21 ± 1.82
2	F2	91.87 ± 0.26	97.40 ± 2.33
3	F3	94.56 ±0.73	93.65 ± 0.62
4	F4	97.06 ± 0.23	98.79 ± 0.82
5	F5	88.82 ± 0.41	96.59 ± 0.54

Values in Parenthesis are expressed as ± S.D (n =3)

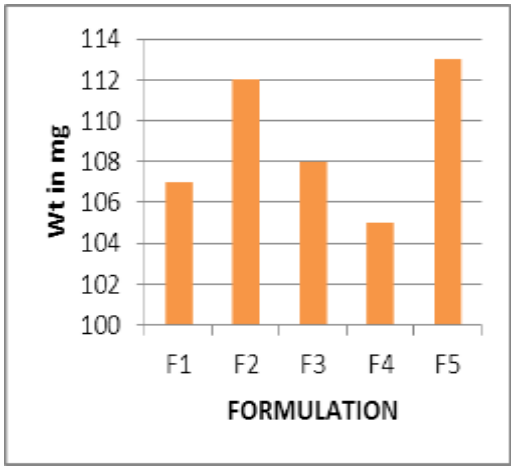
Table.5:Evaluation Parameter for all batches

SR NO.	EVALUATION PARAMETERS	FORMULATIONS				
		F1	F2	F3	F4	F5
1	Thickness(µm)	0.46 ±.017	0.39 ±.012	0.39 ±.014	0.31 ±.019	0.41 ±.027
2	Weight variation (gm)	0.107±0.002	0.112±0.004	0.108±0.005	0.105±0.003	0.113±0.003
3	Folding ndurance (no. of folds)	51 ± 0.81	45 ± 0.98	48 ±1.00	55 ± 0.34	44 ± 0.23
4	% Flatness	100	100	100	100	100
5	% Moisture content	1.934±0.63	0.892±0.94	2.925±1.45	0.851± 0.67	1.769±0.12
6	% Elongation break test	18.14±9.46	38 ±5.24	23.29±2.62	10.6 ± 8.2	15.96±5.25
7	%Drug content OND Hcl DIL Hcl	82.34±0.45 94.21±1.82	91.87±0.26 87.40±2.33	91.56±0.73 93.65±0.62	97.06 ± 0.23 98.79 ± 0.82	88.82±0.41 96.59±0.54
8	% Moisture uptake	3.338±1.38	4.220±0.98	5.231±2.56	3.198± 4.87	7.40±3.78
9	Surface pH	6.8±.0.03	6.4±0.02	6.7±0.04	7.0 ± 0.01	6.1±0.05
10	Tensile strength (dynes/cm^2)	33.6 ×10^4 ± 0.60	31.2×10^4 ± 0.53	36.8×104±0.23	58.1×10^4 ± 0.87	42.7×10^4 ± 0.32

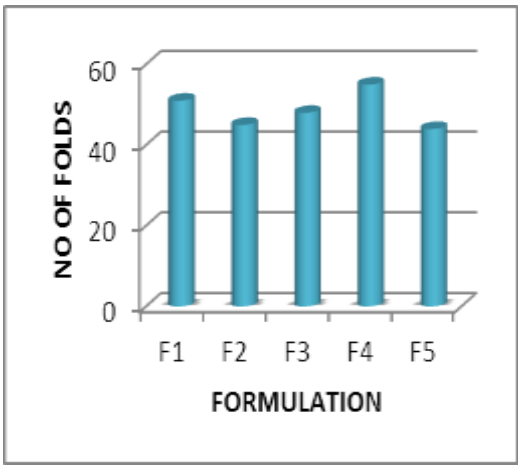
Values in Parenthesis are expressed as ± S.D (n =3)



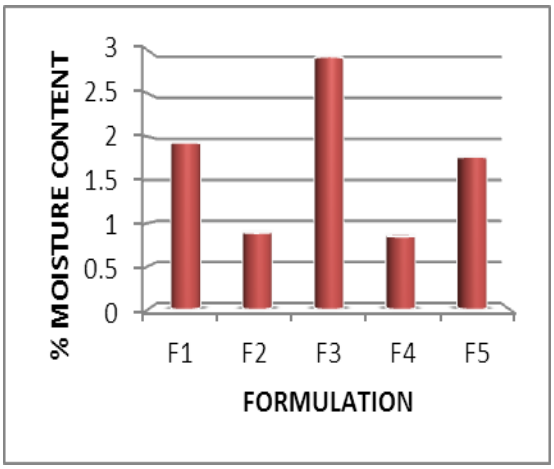
A)Thickness of the patch



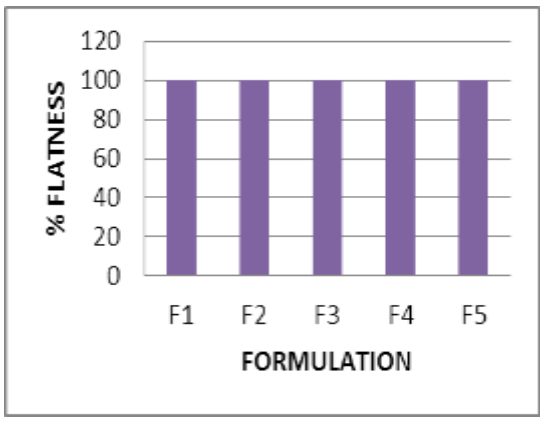
B) Weight Uniformity of patch



C) Folding Endurance of patch

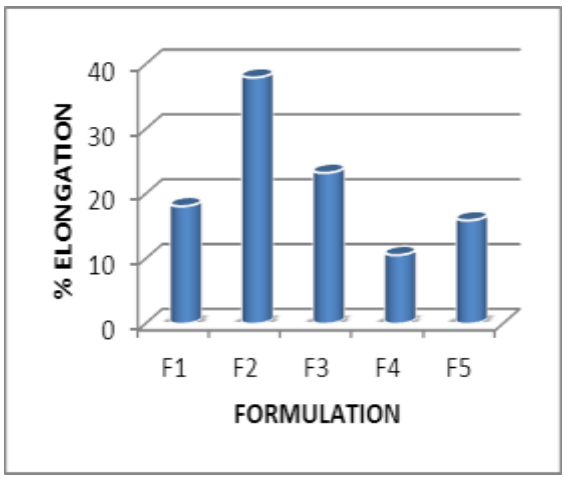


D)% Moisture content

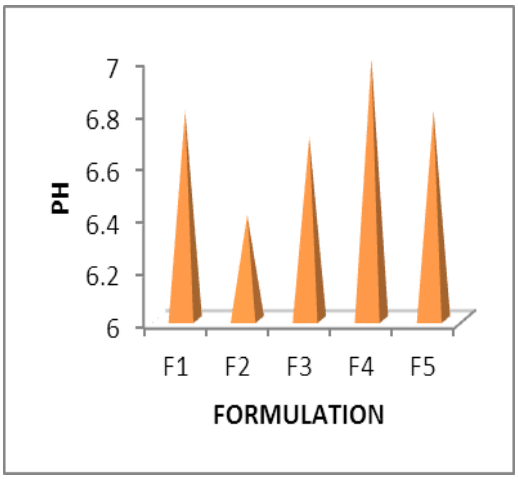


E) % Flatness of patch

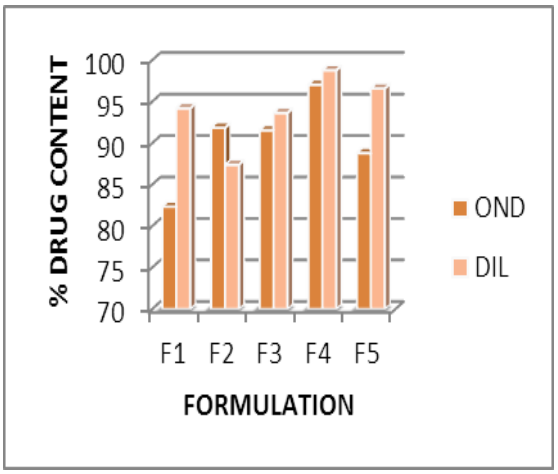
"Fig.9:Comparative study of Thickness, Weight Uniformity, Folding Endurance,%" Moisture content, %Flatness patches (F1-F5)"



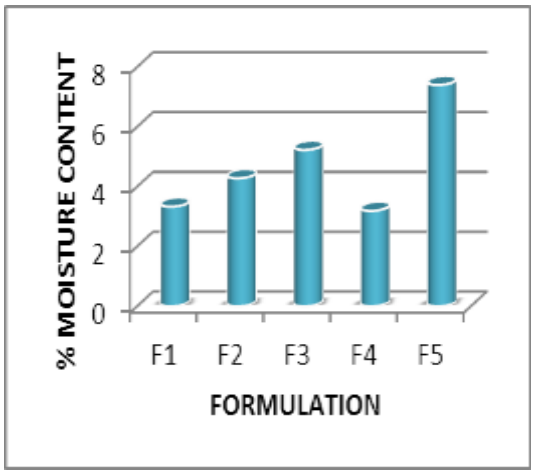
F: % Elongation break Test



G: Surface pH of Patch



H: % Drug Content



I: % Moisture uptake

"Fig.10: Comparative study of Elongation break (F), Surface pH (G) and % Drug Content (H), % Moisture uptake (I) of Patches (F1-F5)"

In-vitro Dissolution Study

The release profile of Ondansetron hydrochloride & Diltiazem hydrochloride from various Patch at pH 7.4 phosphate buffers, the release pattern of all the formulations appears to be slow release; Eudragit RLPO, HPMC K4M and Ethyl Cellulose polymers.[13] The formulations F1 to F5 have shown the 100% drug release of Ondansetron hydrochloride in the time 17, 21, 19, 23 and 18 hours and the 100 % drug release of Diltiazem hydrochloride in time 22, 20, 22, 24 and 16 hours.

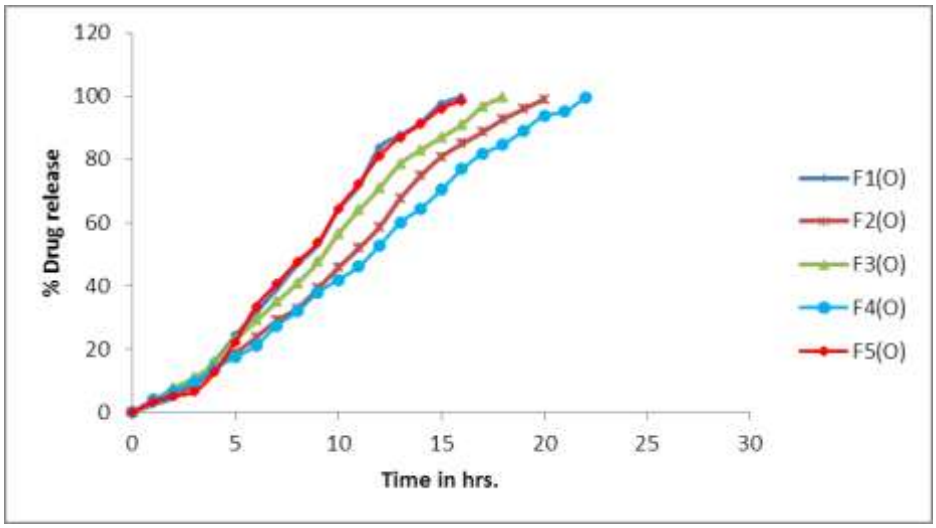
Table.6: In-vitro drug release of Ondansetron hydrochloride in F1-F5 Formulations containing Eudragit RLPO, HPMC K4M and Ethyl cellulose.

Sr. No.	Time in Hours	Average % drug release of Ondansetron hydrochloride				
		F1	F2	F3	F4	F5
1	0	00.00	00.00	00.00	00.00	00.00
2	1	2.453	3.7453	4.645	3.874	3.229
3	2	4.672	5.664	7.654	6.745	5.278
4	3	9.15	8.6453	10.864	9.645	6.425
5	4	16.125	13.764	15.635	13.874	12.835
6	5	24.195	18.645	22.765	17.524	22.331
7	6	31.673	23.674	28.974	21.104	33.589
8	7	38.983	28.974	34.876	27.412	40.671
9	8	46.742	32.844	40.763	31.871	47.671
10	9	53.161	39.086	47.435	37.853	53.631
11	10	64.129	45.763	56.423	41.908	64.417
12	11	71.673	51.867	63.876	46.152	72.356
13	12	83.671	58.634	70.875	52.743	81.096
14	13	87.45	67.543	78.543	59.917	86.952
15	14	91.612	74.764	82.863	64.431	91.043
16	15	97.391	80.756	86.946	70.312	96.053
17	16	99.627	84.842	90.908	76.842	99.526
18	17		88.543	96.654	81.751	
19	18		92.627	99.654	84.651	
20	19		95.876		89.075	
21	20		99.953		93.81	
22	21				95.023	
23	22				99.653	
24	23					
25	24					

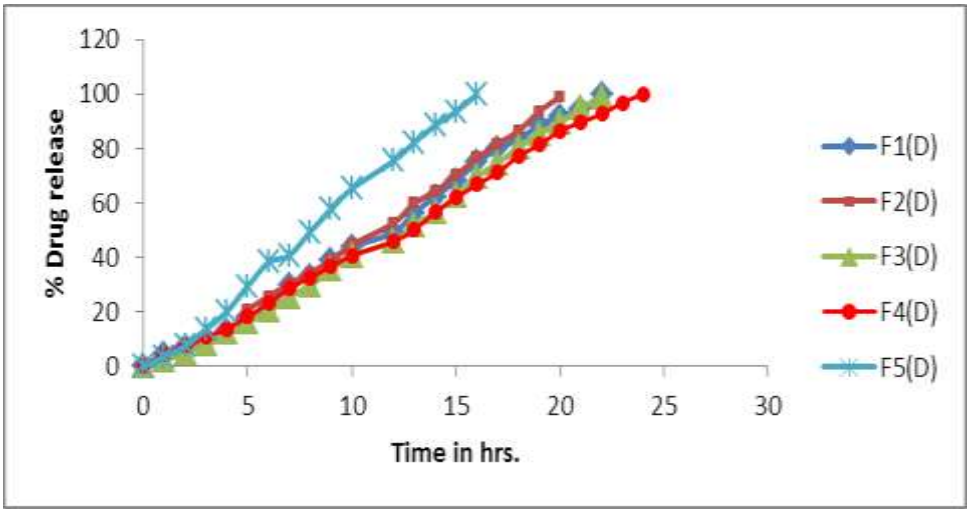
Table.7: In-vitro drug release of Diltiazem hydrochloride in F1-F5 Formulations containing Eudragit RLPO, HPMC K4M and Ethyl cellulose.

Sr. No.	Time in Hours	Average % drug release of Diltiazem Hydrochloride				
		F1	F2	F3	F4	F5
1	0	00.00	00.00	00.00	00.00	00.00
2	1	4.534	3.453	2.344	4.654	3.654
3	2	6.962	6.643	4.965	7.855	7.554
4	3	9.423	9.563	8.353	10.855	13.735
5	4	12.874	12.754	12.642	13.874	20.234
6	5	17.845	20.645	16.085	18.234	29.453
7	6	22.014	25.754	20.643	23.341	38.245
8	7	29.856	30.352	25.623	28.645	40.763
9	8	33.264	34.354	29.644	32.755	49.435
10	9	38.734	39.086	35.764	36.567	57.643
11	10	43.845	44.645	40.633	40.674	65.236
12	11	49.142	52.653	45.975	45.875	75.457

13	12	56.456	59.946	51.867	50.243	82.234
14	13	62.278	64.133	56.543	56.543	88.578
15	14	68.085	70.253	62.644	61.875	93.568
16	15	74.855	76.434	69.665	66.766	99.908
17	16	79.844	81.745	74.647	71.132	
18	17	83.453	86.454	80.533	77.234	
19	18	88.543	93.656	85.564	81.345	
20	19	91.937	98.97644	89.654	86.457	
21	20	94.463		94.765	89.675	
22	21	99.745		99.012	92.654	
23	22				96.876	
24	23				99.965	
25	24					



"Fig.11.In-vitro Drug Dissolution data of Ondansetron hydrochloride in all formulation"



"Fig.12:In-vitro Drug Dissolution data of Diltiazem hydrochloride in all formulations

In-vitro drug release kinetics"

Drug release kinetics

The release constant was calculated from the slope of the appropriate plots, and the regression coefficient (R^2) was determined. It was found that the in-vitrodrug release of F4 formulation was best explained by Matrix kinetic and Peppas as all the plots shows the highest linearity values.

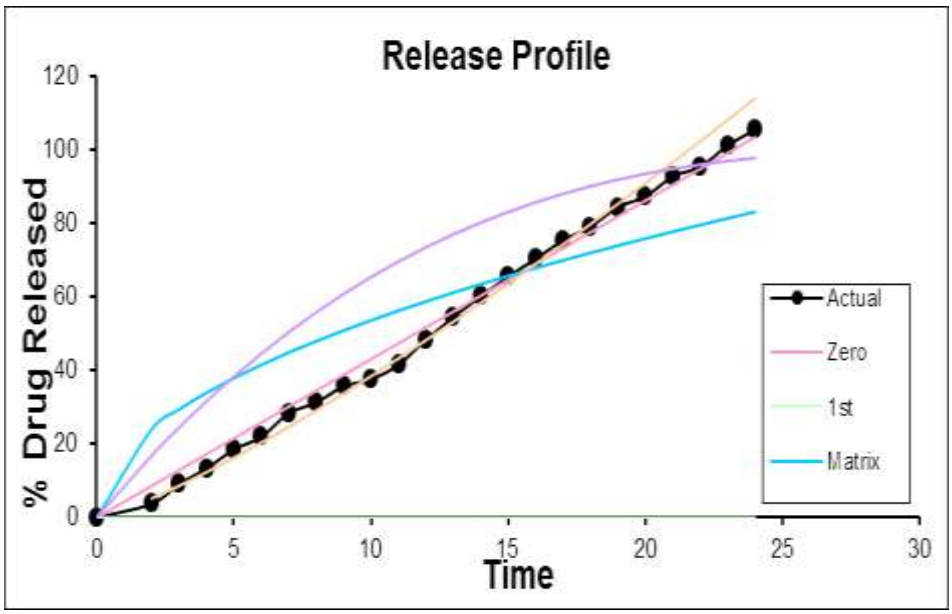
Mechanism of drug release[14, 15]

Ondansetron hydrochloride and Diltiazem hydrochloride the mechanism was found to be that the Optimized formulation i.e. F4 released the drug by non-fickian diffusion.

Table.8:Kinetic modeling of drug release of Ondansetron hydrochloride

Formulations	Zero Order Kinetics	First Order Kinetics	Matrix model	Korsmeyer-Peppas model		Hixon-Crowel model	Best fit model
	R	R	R	R	N	R	
F1	0.9210	0.9408	0.9844	0.9700	0.7240	0.9564	Matrix
F2	0.9884	0.8594	0.9924	0.9620	0.4927	0.9559	Matrix
F3	0.9272	0.8794	0.9902	0.9953	0.5231	0.9621	Peppas
F4	0.9115	0.8687	0.9951	0.9911	0.5994	0.9683	Matrix
F5	0.8861	0.8784	0.9973	0.9726	0.6442	0.9515	Matrix

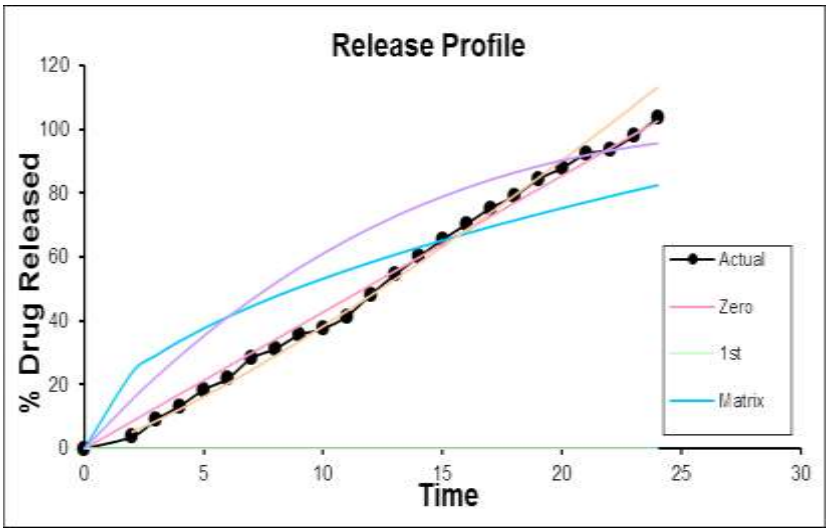
R: Regression Coefficient n: Release Exponent



"Fig.13: Drug release kinetic graph of OND HCl"

Table.9: Kinetic modeling of drug release of Diltiazem hydrochloride

Formulations	Zero Order Kinetics	First Order Kinetics	Matrix model	Korsmeyer-Peppas model		Hixon-Crowel model	Best fit model
	R	R	R	R	N	R	
F1	0.9712	0.8063	0.8425	0.9978	0.4455	0.8878	Matrix
F2	0.9739	0.9167	0.8489	0.9760	0.9048	0.9405	Peppas
F3	0.9692	0.8802	0.8409	0.9978	0.5182	0.9181	Peppas
F4	0.9712	0.8063	0.8425	0.9969	0.4441	0.8878	Peppas
F5	0.9687	0.8835	0.8406	0.9918	0.7634	0.9203	Peppas



"Fig.13: Drug release kinetic graph of DIL Hcl"

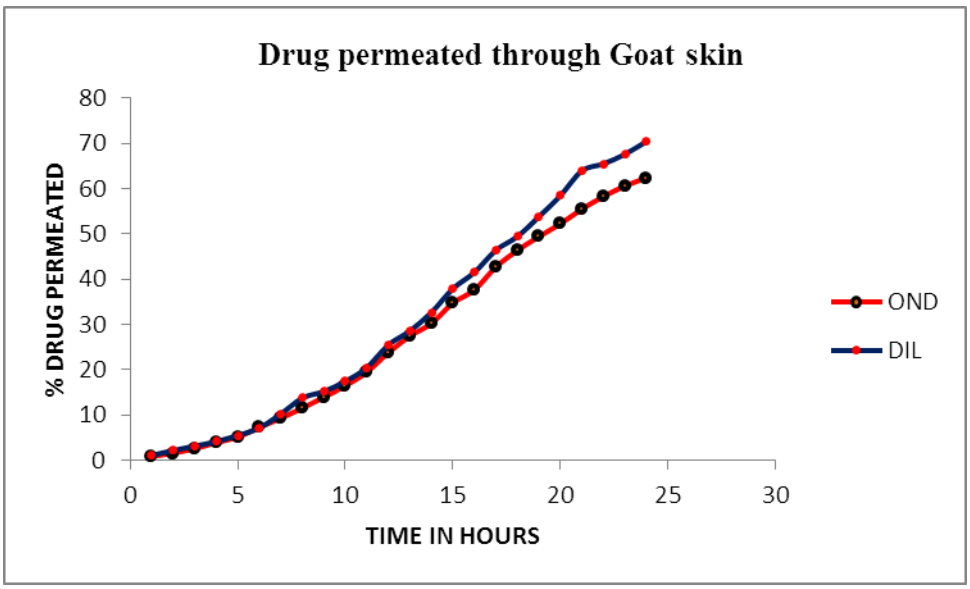
In which more amount of Eudragit RLPO which is hydrophobic in nature showing sustain release of drug and not affect the permeation of drug through the patch, so F4 is the optimized formulation which follows Matrix kinetic for Ondansetron hydrochloride and Peppas for the Diltiazem hydrochloride of drug release by diffusion mechanism.

In-vitro Drug Permeation Study

In vitro skin permeation studies are predictive of in vivo performance of drug. Permeation studies were performed for different films using phosphate buffer (pH 7.4) at 37 ± 1^{0} C. Optimized batch was only studied for permeation through goat skin.

Table.10: % Drug permeated through goat skin of F4 formulation

Sr.No	Time in Hours	% Drug permeated through goat skin of F4 formulation	
		Ondansetron Hydrochloride	Diltiazem Hydrochloride
1	0	0.00	0.00
2	1	0.917	1.129
3	2	1.581	2.195
4	3	2.593	3.168
5	4	3.927	4.163
6	5	5.172	5.492
7	6	7.301	7.182
8	7	9.296	10.234
9	8	11.534	13.756
10	9	13.951	15.221
11	10	16.455	17.451
12	11	19.519	20.487
13	12	23.764	25.462
14	13	27.455	28.568
15	14	30.224	32.634
16	15	34.735	37.882
17	16	37.621	41.496
18	17	42.683	46.372
19	18	46.342	49.412
20	19	49.399	53.782
21	20	52.210	58.423
22	21	55.481	63.892
23	22	58.230	65.452
24	23	60.564	67.563
25	24	62.349	70.381



"Fig.14. Plot of cumulative Drugs permeated Vs time (hrs) for F4 Formulation"

Human Cadaver Skin Permeation Study[16]

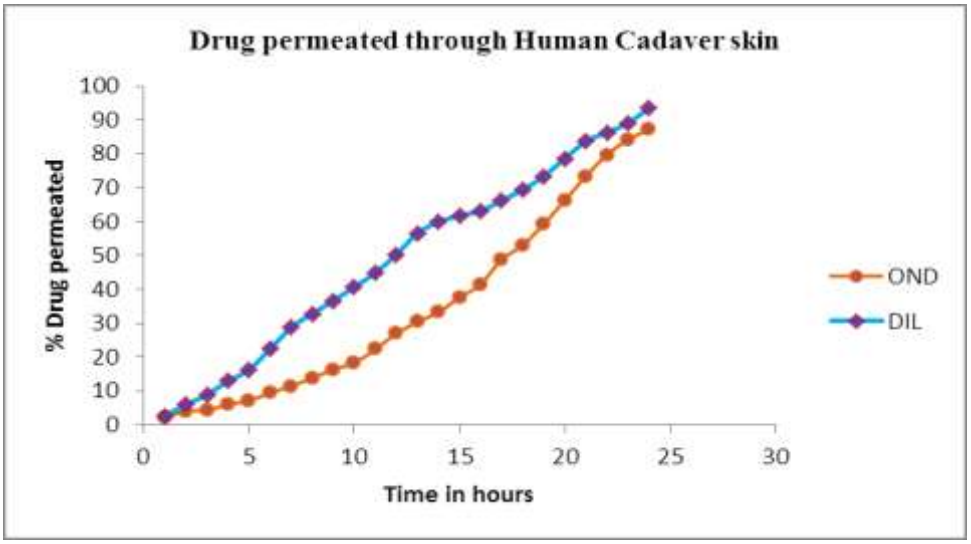
Stratum corneum was obtained from split thickness, cryo preserved cadaver skin by the heat separation technique. 0.5 cm^2 circular sections were cut from adhesive laminate, placed on stratum corneum and mounted on modified Franz cells that were magnetically stirred at ~50 rpm and maintained at 37 ^{0} C. Optimized batch was only studied for permeation through human cadaver skin.

"Fig.15:- Human Cadaver Skin"

Table.11: % Drug permeated through Human Cadaver Skin

Sr No	Time in Hours	% Drug permeated through Human Cadaver Skin	
		Ondansetron hydrochloride	Diltiazem hydrochloride
1	0	0.00	00.00
2	1	2.174	2.654
3	2	3.971	5.855
4	3	4.246	8.855
5	4	5.912	12.874
6	5	7.146	16.234
7	6	9.392	22.341
8	7	11.382	28.645
9	8	13.671	32.755
10	9	16.332	36.567
11	10	18.192	40.674
12	11	22.329	44.875
13	12	26.916	50.243
14	13	30.361	56.543
15	14	33.211	59.875
16	15	37.569	61.737
17	16	41.451	63.132
18	17	48.812	66.287
19	18	52.985	69.345
20	19	59.322	73.421

21	20	66.227	78.675
22	21	73.419	83.654
23	22	79.743	86.324
24	23	84.223	89.123
25	24	87.211	93.561



"Fig.16: Plot of cumulative drug permeated Vs time in hrs for F4 Formulation"

Skin Irritation study of formulation F4

The skin irritation study was done dorsal surface of rat abdominal skin. No erythema or edema was noticed on the skin of rat, after 24 hr and also for subsequent 7 days. Skin irritation studies were performed on Rat (Weight- 150 to 200 gm). The dorsal surface (50 cm2) of the Rat was cleaned, and the hair was removed by shaving. The skin was cleansed with rectified spirit. A representative patch (F4) was placed over the skin with the use of adhesive tape and was removed after 24 h. and the skin was observed and classified into grades on the basis the severity of skin injury.

"Fig.17.Application of Patch"

"Fig.18 After Skin irritation Study"

Table.12:Result of Skin irritation study for Optimized formulation F4.

Sr.No.	Rat	Formulation	Erythema	Edema
1	6	Control patch	-	-
2	6	F4	-	-

= - *Not observed*

= + *Observed*

Stability Studies of formulation F4

Stability study was carried out for optimized batch F4. Evaluation was carried out according to ICH guidelines; selected formulation was stored at 40± 2^0C temperature and 75±5% relative humidity (RH) for a period of 3 months. Formulations were evaluated at periodical intervals of one month for Flexibility, Appearance, Colour, Clarity, Surface Texture, % drug content Evaluation parameters do not show any major difference and all were in acceptable limit.

Table.13: Results of Stability study for Optimized formulation F4.

Parameters		**Observation**		
		1 Month	**2 Month**	**3 Month**
Changes in Appearance	Colour	No change	No change	No change
	Clarity	No change	No change	No change
	Flexibility	No change	No change	No change
	Surface Texture	No change	No change	No change
Drug Content (%)	OND Hcl	91.41 ± 0.6	90.12 ± 0.8	92.34 ± 1.2
	DIL Hcl	94.32 ± 0.42	95.85 ± 0.62	94.91 ± 0.95

REFERENCES

1. Tortara G. S., Grabowski S. K.,Principles of Anatomy and Physiology, John wiley and sons publication, 11th edition, 2000; 140-194.
2. Meheta R.,Topical and transdermal drug delivery: What a pharmacist needs to know, Inte CE221-146-04-054-H01.
3. Delivery Times, You can't Tech an old patch new tricks or can you, an Alza Technologies publication, volume - I, Issue III, URL : http/www.Alza.com.
4. Wilkosz M. F.,Transdermal Drug Delivery, Part I: CURRENT STATUS U. S. Pharmacist, 2003;Volume – 28, URL: http//www.U_S_Pharmacist.htm..
5. Patel N.B., , Sonpal R. N., Mohan S., Selvaraj S, 2010, Formulation & evaluation of iontophoretic transdermal delivery of Diltiazem Hcl, International journal of pharmaceutical sciences, 2010; 1(3): 338-344,.

6. Bhavnayadav , Kamal saroha, Benika Sharma, ,Transdermal patch: a discrete dosage form International Journal of Current Pharmaceutical Research, 2011; 0975-7066.
7. Dipen M. Patel, Kavitha K,Formulation and Evaluation Aspects of Transdermal Drug Delivery System International Journal of Pharmaceutical Sciences Review and Research, Feb- 2011; Vol-6.
8. Nisha M. Patel, Akash D. Patel, D.A. Modi, Dr. P. D. Bharadia, June,Review on Transdermal Drug Delivery System International Journal of Universal Pharmacy and Life Sciences, 2012; 2(3).
9. VandanaYadev, SipaiAltafBhai. M, Mamatha. Y, PrasanthV.V Transdermal drug delivery Technical Writeup Journal of Pharmacutical and Scientific Innovation, 5-12.
10. Posina Anitha, SundraapandiyanRamkanth, Mohamed T S Saleem, Preparation, in-vitro And in-vivo characterization of transdermal patch containing glibenclamide and atenolol: A Combinational Approach Pak. J. Pharm. Sci, April 2011; 24(2): 155-163.
11. P. verma, A. ram,Effect of different penetration enhancers on skin permeation of drug using ethosomal carrier systems Journal of Current Pharmaceutical Research, 2011; 5(1): 42-44.
12. Williamas RO, Reynolds TD, Cabelka TD, Investigation of type of excipients and level on drug release from controlled release tablets containing HPMC. Journal of Pharma Dev Tech, 2007; 19-81.
13. Shalin A. Modi1, P. D. Gaikwad,V. H. Bankar1, S. P. Pawar.,Sustained release drug delivery system,A review International Journal of Pharma Research and Development, 2011; 2(12): 16.
14. Srikanth A. Permeation Studies Of Few Antihypertensive Drugs for Transdermal Delivery Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore, 2005; 49.
15. Aggarwal G. Development, Fabrication and Evaluation of Transdermal Drug Delivery- A Review, 2009.
16. Srikanth A. Permeation studies of few antihypertensive drugs for transdermal delivery. Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore, 2005; 6-8.