ORAL DELIVERY OF ZOLMITRIPTAN LOADED FAST DISINTEGRATING FILM: FORMULATION DEVELOPMENT, STATISTICAL OPTIMIZATION, *IN-VITRO* AND *IN-VIVO* EVALUATION

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ABSTRACT

Introduction: Fast dissolving film technology has been developed out as a alternative drug delivery system that gives an exception advantage for taking medications. **Objective:** The aim of this study was to formulate and evaluate the Zolmitriptan loaded fast disintegrating oral film by solvent casting method. **Material and methods:** A preliminary study was conducted to select a suitable film forming polymer and plasticiser concentration. The formulation was optimized with the help of 2^2 factorial designs in which polymer and plasticizer concentration at two levels was taken as independent factors and disintegration time, tensile strength and % elongation were taken as dependent factors. The optimized formulation OP1 was subjected to stability study as per the ICH guidelines at 40 ± 0.5^0C / $75 \pm 5\%$ RH for six months. *In vivo* studies were conducted on Wister albino rats and concentration of drug in blood was analysed by HPLC technique. Various pharmacokinetic parameters for OP1 were determined and compared with reference formulation (drug sol.). **Result and Discussion:** For optimized formulation various parameters were found to be in acceptable range and it was stable under specified conditions. The value of AUC0–t (ng h/ml), AUC0–∞ (ng h/ml) of the OP1 was found to be 723.91± 84.21, 770.90 ± 104.32, respectively, for the drug sol 468.56 ± 79.36, 500.37 ± 95.43 respectively. Relative bioavailability of OP1 was 1.55 time than that of drug sol. **Conclusion:** The formulation not only increases the bioavailability of drug but also produce the quick action for the migraine patients.

Keywords: Fast disintegrating oral film, *In vitro* disintegration time, Physico-mechanical, Pharmacokinetic study, Zolmitriptan.

INTRODUCTION

Amid all the available routes, the oral route is the most opted for the administration of therapeutic agents on account of self-medication, accurate dosing, pain avoidance and effective cost. [1] Fast dissolving films are one of the safe and patient compliant dosage forms to deliver drug via the oral route. On the grounds of fast disintegration or dissolution and self-management without water or chewing, fast dissolving films are gaining popularity especially in paediatric and geriatric patients. [2]

Fast disintegrating films are novel, thin, flexible, elegant strips meant to be placed on the patient's tongue. It rapidly hydrates and disintegrates to disperse the medication for oromucosal and intragastric absorption.[3]

Fast disintegrating oral formulations are generally prepared using hydrophilic polymers enabling rapid dissolution upon contact with saliva. Some of the quality attributes of films are as follows:

a. Easy transportation, good mechanical strength & stability
b. Enhanced bioavailability due to bypassing hepatic first pass effect
c. Ease of swallowing for geriatrics and paediatrics patients with dysphasia & upper respiratory disease. [2] [4]

Oral films have established a niche in the pharmaceutical industry for the reason of possessing unique properties and fast disintegration time ranging from seconds to one minute. Migraine is a perplexing condition with an array of symptoms like spewing, disturbed vision & sensitivity to light & sound. It influences around 1 individual in 8; primarily ladies matured 30 to 50 years.[5] Zolmitriptan is a selective serotonin 5-HT1B/1D receptor agonist ('triptan') used in the treatment of migraine associated with menses and migraine with aura. It is generally well tolerated with most

adverse events being mild to moderate.[6]Zolmitriptan (4S)-4-([3-(2-[dimethylamino]ethyl)-1Hindol-5-yl] methyl)-2-oxazolidinone is a BCS (biopharmaceutics classification system) Class-3 drug with high solubility and low permeability. [7] The Recommended dose of Zolmitriptan for the acute treatment of migraine is 2.5 & 5 mg. The half life of Zolmitriptan is 2.5 to 3 hrs and it undergoes hepatic first pass metabolism resulting in poor oral bioavailability (40-50 %). [8] Since oral cavity is rich in blood vessels & highly permeable, rapid dissolving film is a suitable option to deliver drug and potentially achieving quick onset of action. Drugs absorbed through the oral mucosa enter systemic circulation directly via the jugular vein thus avoiding first pass metabolism and hence enhanced bioavailability.[9] Zolmitriptan is available as conventional tablets, nasal sprays and orally disintegrating tablets. Common problems associated with nasal sprays are unpleasant taste in mouth (20- 25%), discomfort in the nose/throat (more than 6%), skin sensitivity, numbness, etc. Migraine attacks are often accompanied by nausea (>90 % patients) and vomiting in patients (> 70 %) which makes them uncomfortable in swallowing the water. For these reasons patients preferentially avoid the intake of tablets during attacks. [10] Oral thin strips prove to be a one step ahead by providing superior patient compliance and adherence compared to the oral disintegrating tablet (undissolved particles, less friability, throat discomfort, psychological fear of swallowing, the possibility of chewing, or chocking).

MATERIAL AND METHODS

Materials

Zolmitriptan was obtained as gift sample from Azakem Labs Pvt. Ltd. (Hyderabad, India). Lycoat RS720 (pregelatinized hydroxy propyl starch) was procured from Roquette Pharma, (New Delhi, India). Hydroxy propyl methyl cellulose E-15 and N6-cyclopentyladenosine (CPA), ethyl acetate, formic acid, acetonitrile (CAN, HPLC grade) were purchased from Sun Pharma (Mumbai, India) and Sigma-Aldrich (New Delhi, India) respectively. All other chemicals and reagents used were of analytical grade.

Methods

Drug- excipients compatibility study

Compatibility between drug and excipients was determined using fourier transform infrared (FTIR) spectrophotometer (Alpha model Bruker ATR-FTIR spectrophotometer). IR spectra of Zolmitriptan and physical mixture of Zolmitriptan, HPMC E-15, Lycoat (1:1) were scanned from $4000 \, cm^{-1}$ to $500 \, cm^{-1}$ and interpreted. [11]

Preparation of Zolmitriptan loaded fast disintegrating oral film

Solvent casting method was employed to formulate oral films. [12] [13] Various placebo films were prepared using varied combinations of hydrophilic polymers by trial and error method. Polymeric mixes that exhibited smooth, uniform and flexible films were selected for preparing the drug incorporated system. Hydroxy propyl methyl cellulose (HPMC E-15), polyvinylpyrrolidone K-30

(PVP K-30), hydroxy propyl cellulose (HPC), Lycoat RS 720 was chosen for film formation in different proportions. Glycerol was used as plasticizer to induce plasticity and flexibility in films. Other excipients like citric acid as a saliva stimulating agent, aspartame as a sweetener, amaranth as coloring agent, cherry & strawberry as flavoring agent was also used for the formulation of film. The composition of selected films for preparation of medicated films is given in Table 1.

The polymer was dissolved in water (q.s) containing plasticizer (glycerol). Fixed amount of drug was dissolved in water (q.s.) along with other ingredients (citric acid, amaranth, aspartame, flavors). Both solutions were mixed and stirred for 1h. The casting solution was kept aside to remove the air bubbles. The blend was poured into a petridish and it was dried at room temperature for 24 h. Solvent evaporation was controlled by covering with funnel. Then the film was removed from the petridish and slice according to size 2x2 (4 cm²).

Optimization of Zolmitriptan loaded film by 2^2 factorial design

On the basis of preliminary screening, the combination of HPMC E15: Lycoat RS 720 was finalized for formulation. Factorial design (2^2) (Design expert 8.0.6.1 software, Stat Ease, Inc. Minneapolis, MN) with desirability function for understanding the quality and optimization of fast dissolving oral film was applied. Concentration of film former (HPMC E15 + Lycoat RS 720) and plasticizer (glycerol) at two levels was taken as independent factors and disintegration time, tensile strength & % elongation were taken as dependent factors (Table 2). Total 4 runs were obtained as given in table 3. All four formulations were prepared by solvent casting method. A Statistical model incorporating interactive and polynomial terms was used to assess the responses (Equation 1).

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 \qquad (1)$$

Where Y_i (Y_1, Y_2, Y_3) are the dependent variables, namely, disintegration test, tensile strength and percent elongation, b_0 is the arithmetic mean response of the 4 runs; b_1 and b_2 are the estimated coefficients for the factor X_1 and X_2 respectively. The main effects (X1 & X2) represent the average results of changing one factor at a time from its low to high value. The interaction term (X_1X_2) indicates how the response transit when two factors are simultaneously changed. The polynomial equations can be utilized to make determinations in the wake of considering the magnitude of coefficient and the scientific sign it conveys (i.e. positive or negative). A coefficient with positive sign speaks a synergistic effect of the factor on response, while a negative sign indicates an antagonistic effect.

Evaluation of drug loaded optimized film

The fast disintegrating oral films were evaluated for physico-mechanical attributes, *in-vitro* disintegration time, *in-vitro* drug release, stability and *in-vivo* studies.

Physico-mechanical properties

Physical appearance: All the films were inspected visually for appearance, smoothness and uniform distribution of drug in film.

Drug content: A 4cm^2 strip was sliced into small pieces to be dissolved in 100 ml phosphate buffer (pH 6.8) and shaken persistently for 24 h. Subsequently, the whole solution was sonicated by probe sonicator (Bandelin sonoplus, Germany) for 15 min. After filtration, the drug was diluted appropriately and estimated spectrophotometrically (Shimadzu 1800, Japan) at a wavelength of 224 nm. The experiment was performed in triplicate for all formulations and the average values were recorded.

Weight: The weight of the films was evaluated individually by weighing 5 randomly selected film strips (2×2 cm^2). Such determinations were performed for each formulation in triplicate. [14]

Thickness: The thickness of the film was measured by Screw gauge at five different positions. The determinations were performed in triplicate.[15]

Folding endurance: The folding endurance of the films was measured manually. A strip of film was cut 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 gave the value of folding endurance. [16]

Tensile strength and percent elongation: The tensile strength of the films was determined using an technical framework fabricated in laboratory. [17] [18] A small film strip (2×2cm) was cut and fixed to the assembly. The weight required to break the film was noted and all the while film elongation was estimated with the assistance of a pointer mounted on the assembly. Tensile strength is the maximum stress applied to a point at which the strip specimen breaks. [19] It was calculated by following Equation 2.

Tensile strength = (Force applied to repute the film (N)/Cross sectional area of file (mm^2)   (2)

Principally elongation of strip increases as the plasticizer content increases. It was calculated by the following Equation 3.

% Elongation = (Increase in length of strip/Initial length of strip) x 100   (3)

Young's modulus: Young's modulus or elastic modulus is the measure of stiffness of strip [20] and was calculated by following Equation 4.

Young's modulus = [{Force applied to break the film/Cross sectional area of the film (mm^2)} x {1/Corresponding strain}] (4)

Thumb tack test: After the preparation of the film a thumb tack test was performed to determine the tackiness by gently squeezing a thumb on a film for ~5 s and then quickly removing it. All the formulations were found to be non tacky. [21]

Physico-chemical properties

Surface pH study: The surface pH of fast dissolving strips was resolved so as to research the probability of any side effects in vivo. As an acidic or basic pH is at risk to make disturbance the oral mucosa, it was resolved to keep the surface pH as near neutral as could be allowed. A combined pH electrode was utilized for this purpose. Oral strip was marginally wet with the assistance of water. The pH was measured by bringing the electrode in contact with the surface of the oral film. The investigations were performed in triplicate, and average values were reported. [22]

Moisture uptake: The film was weighed precisely and set in a desiccator containing 100 ml of saturated solution of Aluminium chloride (79.50% RH). Following 3 days, the film was taken out and weighed. [23] The percentage of moisture uptake was calculated using the following Equation 5.

% Moisture uptake = (Final weight –Initial weight/Initial weight) x100   (5)

Moisture content: The films were weighed individually and kept in a desiccator containing Calcium chloride for three days. Afterwards the final weight was noted. [23] The percentage of moisture content was calculated as the difference between initial and final of the film with respect to initial weight as given in the accompanying condition as per Equation 6.

% Moisture content = (Final weight –Initial weight/Initial weight) x100   (6)

Disintegration time: In vitro disintegration time of all the formulations was analyzed by visual method using timer watch with decimal facility. The disintegration time limit of 30 sec or less for orally disintegrating tablets portrayed in CDER guidance can be applied to fast dissolving oral films. [24] *In vitro* disintegration time was determined visually in a petridish containing 10 ml water and swirling every 10s. The volume of disintegrating media in addition to moderate disturbance utilized amid the investigation imitates the volume of saliva and relatively static environment in the oral cavity. The disintegration time was defined as the time taken for film to completely disintegrate with no solid residue remaining.

Dissolution study: Dissolution studies were performed by employing USP dissolution type II test apparatus (paddle type, Labindia DS8000, India). In order to simulate the in vivo adhesion condition and to keep the film strips takes from drifting; each film strip was settled to a rectangular glass slide and put at the base of the disintegration vessel before beginning the test. The examinations were conducted in 200 ml of 6.8 pH phosphate buffer at a rotation speed of 50 RPM and temperature of 37± 0.5 °C. [25] Samples (10ml each) were collected at foreordained time interims (2, 4, 6, 8, 10, 15, 20, 30 min) and the same volume was renewed with fresh buffer maintained at 37±0.5 °C. The samples were filtered through a 0.45-μm membrane filter and examined for drug content with the assistance of UV spectrophotometer (Shimadzu 1800, Japan) at 224 nm. All the studies were made in triplicate to guarantee accuracy.

Evaluation of optimized film

Scanning electron microscopy (SEM) analysis

This technique was utilized to explore the morphology of the optimized film. The film was cut into size of 2x2 cm^2 and coated with gold film (thickness 200 nm). At last, the sample was visualized under reduced pressure using Quanta 200 ESEM (FEI, USA).

Differential scanning calorimetry (DSC) analysis

The thermogram of drug, polymer and optimized formulation were recorded with a DSC (Pyris 6 DSC Perkin Elmer, CT, USA) under an inert climate which was kept up by purging with nitrogen. Test (5 mg) was loaded into an aluminum pan and sealed tightly. An empty aluminum pan was utilized as a reference. Samples were heated at a scanning rate of 10 ^{0}C/min over a temperature run between 40–300 ^{0}C and the thermograms were recorded.

Stability study

The stability studies were done according to ICH Q1A (R2) guidelines for the optimized formulation (OP1). The formulations were packed in aluminium foil and stored at 40° ± 2° C/75 ±5% RH for duration of six months and assessed for any change in the appearance, drug content and disintegration, *in vitro* drug dissolution.

In-vivo studies for the determination of pharmacokinetic parameters

In this study, Albino Wistar rats (Adult/ 200-250 gm weight) were used. A protocol for the study was approved by institutional animal ethical committee (Protocol number *IAEC/09/2015).*

Allocation of groups: Rats were divided in two different lots-

> Lot A: For the administration of Optimized film formulation

> Lot B: For the administration of drug sol.

Each subgroup contained six animals. Dose for rat was calculated on premise of body weight and surface area ratio of the rat [11]. Surface area ratio calculated for 200 gm and 70 kg of human is 56.

$$70k \text{ kg}/200 \text{ gm} = \text{dose for human}/x$$

$$56 = \text{dose for human } /x$$

Where x is rat dose per 200 gm, the dose of Zolmitriptan for human is 2.5 mg. x = 2.5/56, x = 0.045 mg/ 200 mg or 0.225 mg/ kg body weight of rat.

Strategy of drug extraction from plasma: The rats were situated on a table with lower jaw bolstered in an even position. For the administration of drug stacked film, 100 μl phosphate buffer (pH 6.8) was plopped into the oral cavity of lot A animals under the light ether anesthesia. Two equal parts of the film containing 0.045 mg drug were connected to the buccal cavity bilaterally. Likewise drug sol 100 μL (carrying 0.045 mg drug) was applied to the oral cavity of lot B animals. Blood samples were gathered in EDTA coated eppendorf tubes by the intraorbital course at 0.25, 0.5, 1, 2, 4, 8, 12 h.

The blood sample was processed by high performance liquid chromatography (HPLC) reported earlier. Chromatographic separation was accomplished with a Cosmosil C18 reversed-phase column (250 mm × 4.6 mm, particle size 5 μm). The mobile phase comprised of 0.1% v/v formic acid in water: acetonitrile (95:5). It was sonicated for 10 minutes and filtered through membrane filter before utilized. The mobile phase was kept at a flow rate of 1.5 ml/min and eluents were observed at 230 nm. 20μl sample was injected using the manual HPLC injector. All conclusions were executed at ambient temperature for a run time of 10 min.

For the extraction of Zolmitriptan from plasma Liquid–liquid extraction (LLE) method was used [26]. 50 μL of 3 N NaOH and 100 μL of 100 nM N6-cyclopentyladenosine (CPA) stock solution (as internal standard, IS) were added. The samples were extracted with 900 μL of water-saturated ethyl acetate. Following centrifugation for 10 min at 12,000 rpm (4 ∘C), the organic layer was evaporated by drying. 200 μL of mobile phase (0.1% v/v formic acid in water: acetonitrile, 95:5) were added to the dried residue and centrifuged. Subsequently, 20 μL sample was injected directly into HPLC framework.

Pharmacokinetics parameters determination: A Plasma concentration–time profile of Zolmitriptan was assessed by pharmacokinetics software (PK Functions for Microsoft Excel, Pharsight Corporation, Mountain View, CA, USA). Different pharmacokinetic parameters as C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, elimination rate constant (Ke) and half life (t1/2) were determined. The relative bioavailability (BA) of Zolmitriptan stacked film to the drug sol was determined by following Equation 7.

Relative BA (%) = $AUC_{0-\infty \text{ (film)}}$ x Dose $_{(sol)}$ / AUC $_{0-\infty \text{ (sol)}}$ x Dose $_{(film)}$
(7)

Statistical analysis

Statistical analysis was accomplished using Graph pad prism 5.0 (Graph pad software San Diego, CA). All outcomes were expressed as mean ± SD. Student's *t*-test was utilized to analyze the comparison between two groups. Disparity between more than two groups was observed with the analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. P- Value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Drug -excipients compatibility study

IR spectra of Zolmitriptan and physical blend are depicted in Figure 1. For Zolmitriptan the characteristic peaks appeared at 3350 cm^{-1} (aromatic secondary amine N-H stretching), 2984 cm^{-1} (aromatic C-H stretching), 1736 cm^{-1} (C=O five members stretching) and 1259 cm^{-1} (C-N aliphatic amine stretching) . All these characteristic peaks of Zolmitriptan were seen in physical mixture at 3355 cm^{-1} (aromatic secondary amine N-H stretch), 2978 cm^{-1} (aromatic C-H stretching), 1732 cm^{-1} (C=O five members stretching) and 1262 cm^{-1} (C-N aliphatic amine stretching) stipulate no chemical interaction between Zolmitriptan and excipients.

Screening of film forming polymer and plasticizer concentration for the preparation of film

The combination of HPMC E 15: HPC was excluded because of high disintegration time. The blend of HPMC E15: PVP K30 was found to have large moisture content/uptake because of high water permeability of PVP K-30 polymer. High water content makes films prone to microbial attack and elevates bulkiness. Low folding endurance of HPMC E15: PVP K30 combination depicts poor mechanical strength, so it was also rejected for final selection. Films containing HPMC E15 & Lycoat RS 720 showed supercilious mechanical strength and least disintegration time. By virtue of the outcomes, the combination of HPMC E 15 & Lycoat RS720 was picked as a film former (Table 4 and Table 5). The low & high concentration of film forming polymer was 250 mg & 350 mg respectively. In case of plasticizer 14 and 24 % wt/wt was chosen as low and high levels. Below 14% the film may end up brittle and above 24% it becomes tacky and very much flexible not easy to handle.

Optimization of Zolmitriptan loaded film by 2^2 factorial design

2^2 factorial design was applied by fitting the value of independent variable (polymer and plasticizer concentration) in expert design software. Four sets of formulation were obtained. Formulation were prepared and evaluated for disintegration time, tensile strength and % elongation along with other mechanical and physiochemical properties to select the optimized formulation (Table 6).

The main effects (X_1 and X_2) represent the average results of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when two factors are simultaneously changed (Figure 2). The fitted equations (full models) relating the response to the transformed factors are shown in table 6.The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e. positive or negative). A coefficient with positive sign represents a synergistic effect of the factor on response, while a negative sign indicates an antagonistic effect.

A numerical optimization technique, focused on the desirability approach, was used to generate the optimum settings for the most effective formulation. As observed by literature survey, the optimum value of disintegration time, tensile strength and %

elongation should be 30 s, 1.037 to 1.235 N/mm^2 and 5 to 13.5 % respectively. The results of OP1 formulation are comparable with these values. It showed the desirability function closes to 1 as shown in Figure 3.So formulation OP1 was considered as optimized formulation (Table 6). For optimized formulation several other physico-mechanical and physico-chemical parameters were also studied as mentioned in Table 7.

Evaluation of optimized formulation

Physical appearance: After visual inspection, it was observed that all the films were flexible, smooth, thin, non-sticky and homogenous with no spot on the film surface (Figure 4)

In vitro drug release: A dissolution study is essential for ensuring drug release and the reproducibility of the rate and the duration of drug release. Cumulative % drug release from optimized formulation was found to be 98.90 % ±4.73. A comparative dissolution test was performed for optimized formulation and a marketed tablet (FDT). There was no significant (p < 0.05) difference observed between the percent drug release of both formulations (Figure 5).

SEM analysis: The SEM analysis of OP1 revealed smooth surface without any scratches or traverse striations on the films surface indicating proper miscibility with uniform distribution of drug in the whole film (Figure 6).

DSC analysis: The DSC thermogram (Figure 7) showed the melting endotherm at 140 ℃. The thermogram of film did not show any melting endotherm for Zolmitriptan indicating the presence of drug in the film in amorphous state (more soluble) rather crystalline.

Stability studies: Stability study on optimized formulation was conducted as per the ICH guidelines. Samples were withdrawn at specific time interval and evaluated (Table 8). Fast dissolving film of Zolmitriptan was found to be physically and chemically stable as no significance difference (P < 0.05) was observed in appearance, drug content and *in vitro* drug release. The disintegration time increased with time significantly (P < 0.05) but remain within the specified limit (less than 30s). The probable reason might be due to the loss of moisture from the film.

***In vivo* studies**

Albino Wistar rats (Adult/ 200-250 gm weight) were used for *in vivo* study. Figure 8, shows mean plasma concentrations and time profiles Zolmitriptan film (test) and sol (reference). The value of AUC0–t (ng h/ml), AUC0–∞ (ng h/ml) Cmax (ng/ml), Tmax (h), Ke (h^{-1}), and t1/2 (h) of the optimized film formulation was found to be 723.91± 84.21, 770.90 ± 104.32, 349.28 ± 15.93, 0.5, 0.254 ± 0.15 and 2.72 ± 0.35 respectively, for the drug sol 468.56 ± 79.36, 500.37 ± 95.43, 183.63 ± 20.26, 0.5, 0.264 ± 0.22, and 2.62 ± 0.83, respectively (Table 9). The value of AUC0–t, AUC0–∞ and Cmax of optimized film was significantly (P< 0.05) more than that of standard (drug sol.). The probable reason might be the retention of film in mouth and maximum drug released from the

film was absorbed from oral cavity only. As a result, the first pass metabolism was less than that of drug sol. which was absorbed not only from oral cavity but also from GIT. Relative bioavailability of optimized formulation (OP1) was 1.54 time than that of drug solution.
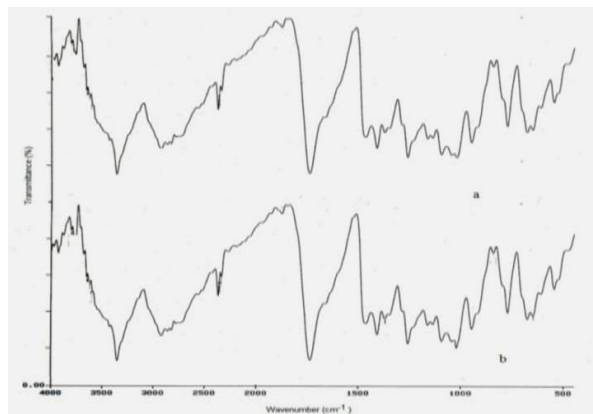

Figure 1: IR spectra of (a) Zolmitriptan (b) Physical mixture of Zolmitriptan, HPMC E-15 and Lycoat RS720.
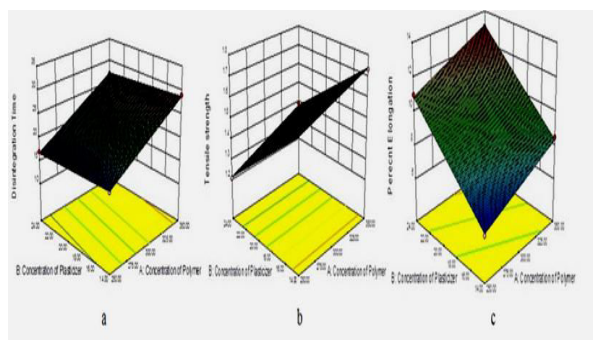

Figure 2: 3D plots showing the effect of independent variable on disintegration time (a) Tensile strength (b) percent elongation (c).

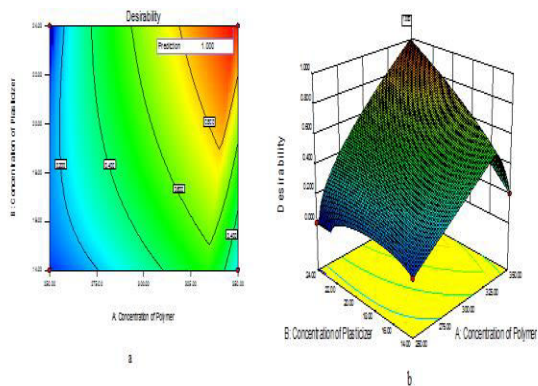**Figure 3: Desirability plot (a) Desirability Response surface area (b) between concentration of Polymer and Plasticizer.**

Figure 4: Photographic image of film.
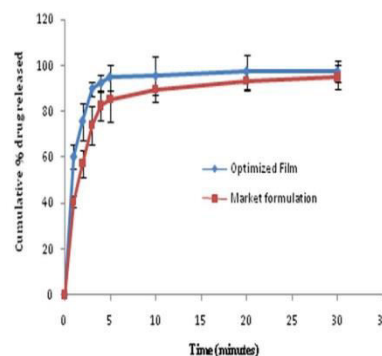

Figure 5: Cumulative % drug release of optimized films (OP1) and marketed formulation (n=3, mean±SD).
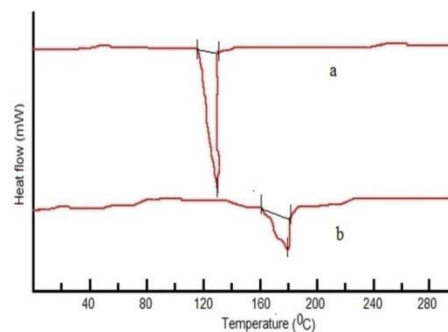

Figure 6: SEM image of optimized film.

Figure 7: The DSC thermogram of (a) Zolmitriptan and (b)Optimized film.
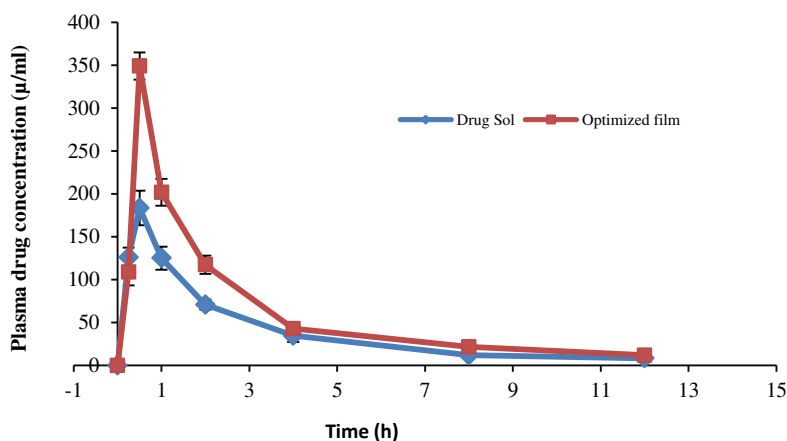
Figure 8: Means of plasma concentrations and time profiles optimized film (OP1) and Zolmitriptan sol. (n= 6, mean ±SD).

Table 1: Composition of different batches of Zolmitriptan loaded mouth disintegrating oral films for preliminary screening of film former and plasticizer

Excipients	Quantities (% w/w)					
	P1	**P2**	**H1**	**H2**	**L1**	**L2**
Zolmitriptan	100	100	100	100	100	100
HPMC E-15	166	166	200	200	214	214
PVP K-30	84	84	-	-	-	-
HPC	-	-	50	50		
Lycoat RS 720	-	-	-	-	36	36
Glycerol	14	24	14	24	14	24
Citric acid	5	5	5	5	5	5
Aspartame	3	3	3	3	3	3
Amaranth	0.1	0.1	0.1	0.1	0.1	0.1
Flavors (strawberry, Cherry)	10	10	10	10	10	10

Table 2: Variables in 2^2 factorial design

Independent Variable	Levels			
	Coded value		Actual value	
	Low	High	Low	High
X1: Amount of polymer (mg)	-1	+1	250	350
X2 : Plasticizer (%wt/wt)	-1	+1	14	24
Dependent variables				**Goals**
Y_1: Disintegration time (sec.)				Optimized
Y_2: Tensile strength (N/mm^2)				Optimized
Y_3: Percent elongation				Optimized

Table 3: Experimental matrix for the factorial design

Run	Formulation code	Coded value		Amount of polymer(mg) (X_1)	Plasticizer (% wt/wt) (X_2)
1	OP1	+1	+1	350	24
2	OP 2	+1	-1	350	14
3	OP 3	-1	+1	250	24
4	OP 4	-1	-1	250	14

Table 4: Physico-mechanical properties of Zolmitriptan fast disintegrating oral film

FC	Weight (gm)*	Drug Content (%)*	Thickness (mm)*	Folding Endurance*	Tensile strength (N/mm^2)*	Percent elongation *	Young's modulus (N/mm^2)*
P1	0.0165±0.008	96.82 ± 1.13	0.030 ± 0.0010	75 ± 3.605	1.063 ± 0.0053	6 ± 0.529	11.34 ± 0.693
P2	0.0168±0.009	95.86 ± 3.65	0.031 ± 0.0015	82 ± 3.512	1.037 ± 0.0054	7.5 ± 0.50	10.37 ± 0.437
H1	0.0182±0.005	92.42 ± 0.83	0.025 ± 0.0010	445 ± 3.511	1.143 ± 0.0150	5 ± 1.602	16.87 ± 0.634
H2	0.0190±0.010	92.35 ± 4.73	0.026 ± 0.0020	462 ± 3.050	1.151 ± 0.0168	6 ± 1.0405	16.34 ± 0.423
L1	0.0165±0.010	97.82 ± 4.43	0.025 ± 0.0020	480 ± 4.509	1.205 ± 0.0492	11 ± 1.102	9.25 ± 0.234
L2	0.0162±0.010	97.99 ± 1.82	0.026 ± 0.0022	498 ± 3.050	1.215 ± 0.0150	10 ± 0.402	9.94 ± 0.672

*FC= Formulation Code, *All data are expressed as mean ± SD, *n*=3

Table 5: Physico-chemical properties of Zolmitriptan fast disintegrating oral film

FC	Surface pH*	% Moisture uptake*	%Moisture content*	DT (seconds)*	% Drug release*
P1	6.8±0.09	7.60 ± 0.19	11.09 ± 1.12	28.42± 1.53	90.92± 5.82
P2	6.9±0.05	7.51± 0.21	11.3 ± 0.15	25.81 ± 0.57	91.66±7.25
H1	6.9±0.05	6.20 ± 1.41	9.6 ± 1.21	60.41 ± 3.54	88.85±7.82
H2	6.9±0.06	6.70 ± 1.45	9.26 ± 1.8	59.28 ± 3.14	88.56±4.29
L1	6.8±0.05	6.70 ± 0.48	7.75 ± 0.18	17.54 ± 1.77	94.43±12.39
L2	6.8±0.06	5.30 ± 0.15	6.55 ± 0.91	16.94 ± 3.55	93.06±10.52

*DT= Disintegration time, All data are expressed as mean ± SD, *n*=3

Table 6: Summary of 2^2 factorial design

FC*	Amount of polymer(mg) (X_1)	Plasticizer (% wt/wt) (X_2)	Disintegration time (S) (Y1)		Tensile strength (N/mm^2) (Y2)		% Elongation (Y3)	
			Predicted	Observed	Predicted	Observed	Predicted	Observed
OP1	350	24	27.25	27.63	1.207	1.215	13.12	13
OP 2	350	14	28.75	29.38	1.740	1.732	8.37	8.5
OP 3	250	24	16.75	17.94	1.213	1.205	10.87	11
OP 4	250	14	18.25	18.72	1.947	1.955	6.12	5
Regression equations for the responses								
Disintegration time (Y1) = 22.75 + 5.25X1 - 0.75X2 -0.25X1X2 **Tensile strength (Y2) =** 1.48 -3.2X1 - 0.27X2 +8.2X1X2 **% elongation (Y3) =** 9.63 +1.13X1+ 2.38X2 -0.13X1X2								

*FC=formulation code, **S**= Seconds

Table 7: Physico-mechanical and physico-chemical properties of optimized formulation

Weight (gm)	Drug Content (%)	Thickness (mm)	Folding Endurance	Young's modulus (N/mm^2)
0.0198± 0.012	99.99± 1.86	0.030 ± 0.0014	592 ± 3.520	9.22 ± 0.563
Surface pH	**% Moisture uptake**	**% Moisture content**	**% Drug release**	**Thumb tackiness**
6.8±0.05	7.10 ±1.42	8.56 ± 0.52	98.90±4.73	None

All data are expressed as mean ± SD, *n*=6

Table 8: Stability studies of optimized formulation at 40 ± 2^0c / 75% RH $\pm$5%.

Test parameters	Time (month)			
	0	**1**	**3**	**6**
Appearance	+++	+++	+++	++
Drug Content (%)	99.99± 1.86	97.36± 2.35	97.62± 2.94	96.89± 3.61
DT (seconds)	27.63 ± 0.20	26.35 ± 2.5	27.64 ± 3.74	31.28 ± 1.69*
% Drug release	98.90±4.73	96.32±3.83	95.31±2.47	101.28±5.12

Physical appearance: +++= Good; ++= Fair; += Poor, Data are expressed as mean ± SD, *n*=3; *P* < 0.05 (The characteristics parameters obtained at each time point (1, 3 and 6 months) were compared with parameters obtained at zero.* Significant difference.

Table 9: Pharmacokinetic parameters of optimized film (OP1) and drug solution

Parameters (unit)	Optimized formulation	Drug sol.	Test/reference
Cmax (ng/ml)	349.28 ± 15.93*	183.63 ± 20.26	1.90
Tmax (h)	0.5	0.5	1
Ke (h^{-1})	0.254 ± 0.15	0.264 ± 0.22	0.96
t1/2 (h)	2.72 ± 0.35	2.62 ± 0.83	1.04
AUC0–t (ng h/ml)	723.91± 84.21*	468.56 ± 79.36	1.55
AUC0–∞ (ng h/ml)	770.90 ± 104.32*	500.37 ± 95.43	1.54

All data are expressed as mean ± SD, *n*=6; *P* < 0.05 (Pharmacokinetic parameters after optimized film and drug sol administration were compared with each other), *Significant difference.

CONCLUSION

Oral fast disintegrating films are one of the futuristic outlooks in the field of pharmaceutical sciences. The fast disintegrating oral films of Zolmitriptan were prepared using different polymers by solvent casting method. A 2^2 factorial design was applied to select the optimized formulation. Among the various formulations, OP1 (prepared with HPMC E 15 + Lycoat RS 720) was chosen as optimized formulation as it showed 27.63 s of disintegration time. Satisfactory physico-mechanical characteristics like 1.215 N/mm^2 of tensile strength and 13 % elongation with other convincing evaluation parameters were also observed for optimized formula. Stability studies' results showed that prepared film was stable enough for the period of 6 months. *In vivo* studies also indicated that the pharmacokinetic parameters of optimized film significantly differ (P< 0.05) from drug sol (reference) exhibiting non-comparable drug plasma level-time profiles. Eventually, it can be concluded that fast disintegrating oral film approach is suitable for the delivery of Zolmitriptan. This formulation not only enhances the bioavailability of drug, but also produces quick action for the migraine patients. Oral films provide better patient compliance and a marketing advantage.

LIST OF ABBREVIATION

AUC0-∞ =Area under the curve from time 0 to time infinity, **AUC0-t**= Area under the curve from time 0 to time t, *C*max= Peak of maximum concentration, **DSC**= Differential scanning Colorimetry, **FTIR**= Fourier transform infrared, **HPC**= Hydroxy propyl cellulose, **HPMC E-15**= Hydroxy propyl methyl cellulose,

IS= Internal standard, **Ke**= Elimination rate constant, **LLE**= Liquid–liquid extraction, **SD**=Standard deviation, **SEM**= Scanning electron microscopy**, t1/2**= Half life, *T*max=Time of peak concentration, **XRD** = X-ray diffraction.

CONFLICT OF INTEREST

Authors do not have any conflict of interest.

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