

## PHARMACOKINETIC STUDY IN HUMANS AND *IN VITRO* EVALUATION OF BIOENHANCED BILAYER SUBLINGUAL FILMS FOR THE MANAGEMENT OF ACUTE MIGRAINE

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### ABSTRACT

**Objective:** Long-lasting migraine pain is one of the most disabling neurological disorders and requires a quick onset of action from the administered dosage form. This study aimed to provide sublingual administration of the frequently used combination of NSAID and triptan in order to trigger their action immediately by escaping the first-pass metabolism, simultaneously improving patient compliance.

**Methods:** In the present research, sublingual bilayer films were developed by joining the two loaded layers with zolmitriptan and piroxicam, respectively. Each layer was prepared and loaded separately using the traditional solvent casting method. Mechanical support was provided by the 1:1 combination of HPMC E-15 and pullulan, which were used as water-soluble film-forming polymers with polyethylene glycol 400 as a plasticizer. Films were evaluated for various physicochemical and mechanical properties. Finally, a pharmacokinetic study was performed on six healthy human volunteers to compare the PK parameters of the best formulation, BSTF-3, with those of a commercially available formulation. Sepitrap 80 and Sepitrap 4000 were used as bio-enhancers to achieve faster systemic delivery.

**Results:** The thin, flexible bilayer films were observed to provide quick action along with increase patient compliance by preventing the first-pass metabolism and dysphagia. Sepitrap 80 successfully increased the permeation of both drugs. Approximately 92 percent of zolmitriptan was released from the formed bilayer sublingual thin films within 3 min, whereas 92 percent of piroxicam was released within 4.5 min from the best formulation. Within 30 min of the commencement of the pharmacokinetic investigation, plasma concentrations of the active component began to rise rapidly.

**Conclusion:** When compared to commercial formulations, the developed films had a greater AUC and C<sub>max</sub> with a shorter T<sub>max</sub>, indicating a faster trigger of action and higher bioavailability.

**Keywords:** Bilayer films, Bioenhancers, Migraine pain, Pharmacokinetics, Sublingual films

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### INTRODUCTION

In the present stretch of increasing stress, neurological conditions have become the major causes of disability and death at global levels [1]. Severe recurrent throbbing, pulsating headaches, and associated prodromal and postdromal symptoms such as lack of concentration, various fatigues, blurred vision, neck stiffness, hypersensitivity to light and sound, tiredness, and frequent yawning make the migraine a highly debilitating neurological condition that nearly affects 15.3% of the total world population [2]. Migraine is a huge financial burden on the world economy, costing the US 19.6 billion Dollars and the associated European countries 27 billion Euros per year [3-5]. Migraine also stands in second place in terms of the most disabling neurological conditions. According to the Global Burden of Disease Study, it was ranked as the third most prevalent disorder in the world [6]. Migraine is characterised as a highly prevalent and severely disabling frontotemporal (lasting 4–72 h) pain attack of the head that is commonly associated with nausea and vomiting [7]. It is also supported by a large literature review that suggests migraine could be a chronic, progressive brain condition [8].

Among the treatment options most commonly prescribed is the symptomatic treatment of migraine pain, which includes nonsteroidal anti-inflammatory drugs (NSAIDs) as simple analgesics and triptans as migraine-specific agents [9]. Various studies, including systematic reviews, meta-analyses, and randomised placebo-controlled trials, have reflected that the combination of triptans and NSAIDs is more effective than their individual use in meeting the primary goal of acute therapy, which is aborting the pain immediately [10–12]. Many oral therapies for migraine lack effectiveness due to deficient absorption in response to migraine-induced gastric stasis, so other routes such as IV, sublingual, and nasal are frequently utilised [13]. Zolmitriptan (ZOTP) is one of the top three triptans that have the highest pain-relief rates at two hours, whereas naratriptan is associated with

fewer adverse effects [14]. In NSAIDs, piroxicam (PRCM), with increased absorption, has been shown to have a significantly greater analgesic effect than that of naproxen and was comparable with indomethacin [15]. In another similar study of the management of acute migraine pain, sublingual piroxicam showed significant pain relief and magnificent tolerability [16]. The study was envisaged to provide quick response of the drug to migraine patient along with higher patient compliance.

### MATERIALS AND METHODS

#### Materials

Zolmitriptan (ZOTP) and Hydroxypropylmethylcellulose E-15 were obtained from Jubilant Biosciences, Piroxicam (PRCM) was collected from Mesho Pharmaceuticals. Sepitrap™ 80 (microencapsulated solubilizer) was obtained from Seppic India. Methanol and Acetonitrile of HPLC grade were purchased from High Purity Laboratory Chemicals, Mumbai. Pullulan, Polyethylene glycol 400, citric acid and mannitol were received from Sigma Aldrich Chemical Co., (St. Louis, USA.), Mint flavor, and sucralose was kindly supplied from Humed life sciences India.

#### Methods

##### Preparation of placebo films

Placebo-thin layers of the composite films were prepared using the solvent casting method. The amalgamation of HPMC E-15 and pullulan in an equal ratio of 1:1 is well known for its film-forming capabilities. They dissolve rapidly in aqueous environments and produce solutions with a pH of around 7, which lies in the salivary pH range (5.7–7.4) [17–19]. The flexibility of the films was imparted by polyethylene glycol 400 (PEG-400), which was incorporated as a plasticizer. PEG-400 does not absorb moisture when compared to glycerin [20, 21] so it gave rise to a stable finished product.

### Loading of drugs

The sublingual bilayer films of ZOTP and PRCM were prepared by the same method as utilized to prepare placebo films, and finally, two separate drug-loaded layers were joined together to form a bilayer unit. Firstly, drugs were physically mixed separately with each bioenhancer, Sepitrap 80 and Sepitrap 4000 in the mortar pestle, proven to enhance dissolution of various low-aqueous soluble drugs [22, 23]. This mixture of drug and bioenhancer was dissolved in a solvent system consisting of PEG-400 and ethanol, stirred for 1 hour to augment the solubility of drug. In a separate beaker film-forming polymer, HPMC E-15 and pullulan in a ratio of 1:1 were added in triple distilled water prewarmed at 40 °C and stirred continuously until a clear solution was obtained. After that, mannitol was added along with sucralose, citric acid, and flavoring

agent and further stirred for 1 hour. Finally, both solutions were mixed, which was then stirred again for 2 h to maintain the uniformity. The resulting solution was carefully sonicated for at least 30 min and kept aside for 1 hr to remove the air bubbles formed during stirring. Then the solution was lastly casted on the pre-lubricated glass petri dish of 9 cm in diameter and was allowed to dry in a hot air oven maintained at 45 °C for 6-8 h. After a whole night, dried films were observed for any defects and removed safely from the surface of petri dish [24]. The picture of the ready-to-join two layers of the films and the whole batch is demonstrated in fig. 1. The film, after separation from the petri-dish, was then cut to the desired size of 3×2 cm. The cut sample films were kept in a desiccator for further testing. The compositions of five different bioenhanced bilayer sublingual thin films (BSTFs) are shown in table 1.

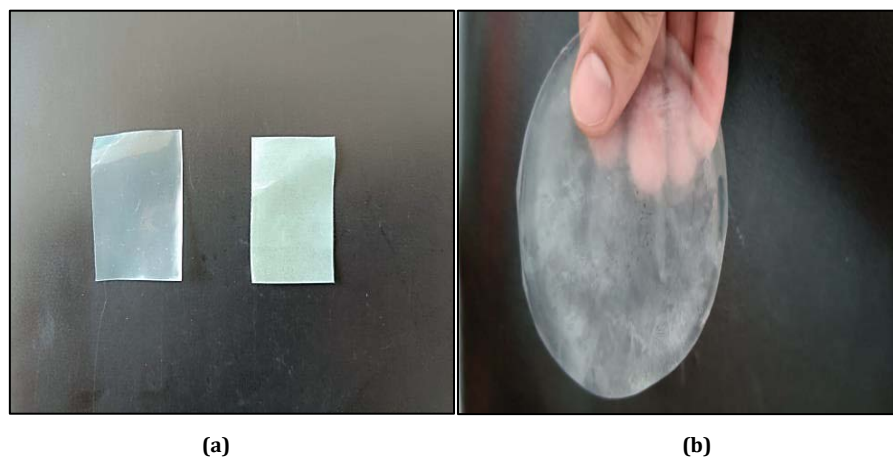


Fig. 1: Drug-loaded thin films (a) two layers of sublingual thin film loaded with zolmitriptan and piroxicam, respectively; (b) Whole batch of drug-loaded films

Table 1: Composition of bilayer sublingual thin films (BSTF) containing piroxicam and zolmitriptan

S. No.	Ingredients	BSTF1		BSTF2		BSTF3		BSTF4		BSTF5	
		L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
1.	Drug	2.5	10	2.5	10	2.5	10	2.5	10	2.5	10
2.	HPMCE15	15	15	15	15	15	15	15	15	15	15
3.	Pullulan	15	15	15	15	15	15	15	15	15	15
4.	PEG-400 (ml)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
5.	Mannitol	5	5	5	5	5	5	5	5	5	5
6.	Sucralose	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
7.	Citric acid	1	1	1	1	1	1	1	1	1	1
8.	Sepitrap 80	--	--	2.5	10	5	20	--	--	--	--
9.	Sepitrap 4000	--	--	--	--	--	--	2.5	10	5	20
10.	Mint (Flavour)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
11.	Ethanol (ml)	2	2	2	2	2	2	2	2	2	2
12.	Water	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.

1 L1-Layer 1 (zolmitriptan), L2-Layer 2 (piroxicam)

### Characterization and evaluation

#### Appearance, thickness and weight uniformity of films

Films were observed visually, such as for transparency, surface deformities and for the evaluation of their appearance [25]. A micrometer screw gauge (Baker Gauges India) was used for the measurement of the thickness of intact bilayer sublingual films. The thickness was measured after the joining of both the drug-loaded layers. The measured positions were one at the center and three different edges of the uncut film, and the calculated mean was reported [25, 26]. Three cut films 3×2 cm of a particular batch were evaluated for weight uniformity using an electronic balance (TX323L, Shimadzu Corporation, Japan) [27].

#### Folding Endurance and surface pH

The test of folding endurance was performed manually in triplicate to ensure the brittleness and toughness of the sublingual bilayer

films [28]. Films from each batch were folded repeatedly in the same place until it they ruptured. The value of folding endurance was obtained by counting the number of times a film could be folded without losing integrity [29]. After being cut into the mentioned dimensions, the films were placed in a small petri dish with 5 ml of pH 6.8 phosphate buffer. Then, after 30 min, the film pH was measured by determining the pH of the solution using a digital pH meter (Systronic, India) [30].

#### Extensibility and tensile strength

For the estimation of the mechanical strength of the sublingual bilayer films, a peel tensile tester (Model HTT-401 LABFAC, China) instrument with a maximum test force of 300 N was used. The two opposite longitudinal edges of the films were clamped, and a load of 20 N was applied at a speed of 50 mm/min. Force in N just at the breakage point and extensibility in mm of the films were recorded [31, 32]. This procedure was repeated three times with films from

each batch. The value of tensile strength was provided by the ratio of force at breakage to the cross-sectional area of the film.

#### SEM for morphological and FTIR

Using a scanning electron microscope, the surface morphology of bilayer sublingual films and drug distribution in loaded films were examined (JSM 6100 JEOL, Japan). Double-sided sticky tape was used to fix the drug-loaded film of suitable size to metal stubs. Before examination, the stubs were vacuum coated with gold using a fine coat ion sputter (JFC-1100 JEOL, Japan). Finally, at 2 KV accelerating voltage, SEM pictures were acquired at various magnifications [33]. FTIR studies of both the pure drugs and the selected formulation were also conducted through the range of 4000-550 cm<sup>-1</sup> using Perkin Elmer spectrophotometer USA and compared to assure the incorporation of drugs in both layers of the films.

#### Percentage of moisture loss

The moisture absorption capacity of the bilayer films was determined by placing the previously weighed (W1) films in a petri dish, which was then placed in a desiccator containing anhydrous calcium chloride. After the completion of 72 h, all films were weighed (W2) again [34]. The same procedure was carried out in triplicate and the percentage of moisture loss was calculated using the below-given formula.

$$\% \text{ of Moisture loss} = (W1 - W2)/W1 \times 100$$

#### Disintegration test

The slide frame method was used to measure the disintegration time (DT) of all batches of drug-loaded bilayer films. Each batch of film was placed inside a slide frame, which was put on a petri dish. A single drop of distilled water was pipette-applied to the exposed surface of the film retained in the slide frame [17, 35]. Finally, disintegration time was calculated as the amount of time it took for a drop of distilled water to penetrate the film and generate a hole.

#### Drug content uniformity

For the estimation of drug content, pieces of 3×2 cm film were cut from a different location from the uncut film of each batch. Then the films were stirred for 3 h on a magnetic stirrer with 100 ml of pH 6.8 phosphate buffer in order to extract the drug [36]. After proper staining and dilution, the drug content was determined using UV spectrophotometer (UV-1900, Shimadzu Corporation, Japan) and the results were presented as mean±standard deviation.

#### In vitro drug dissolution test

USP Type II paddle dissolution testing apparatus (LABINDIA DS 8000, LabIndia Instrument Pvt Ltd, Mumbai) was used for the in vitro drug release study [37, 38]. It was carried out using 250 ml of pH 6.8 phosphate buffer as a dissolution medium, which had the same pH as that of saliva (pH 6.8) at 37±0.5 °C. The paddle rotation per minute was maintained at 50 rpm to stir dissolution media [39, 40]. The 3×2 cm film was placed in the dissolution chamber for the study. The 5 ml sample was withdrawn at 0, 1.5, 3, 4.5, 6, 7.5, 9 and 10.5 min of time point and replaced with the same amount of buffer solution to maintain the sink condition. The samples were then passed through a membrane filter with a 0.2 µm pore diameter (Isolab, Germany). Then the samples were analyzed using a UV spectrophotometer. The studies were performed out thrice on different films for each batch and the mean value (±SD) was reported.

#### Ex-vivo permeation study

A chicken buccal membrane was used in an *ex vivo* permeation investigation [41]. The drugs from the BSTF-3 were tested for an *ex vivo* permeation study using a USP dissolution tester at 37±0.1 °C. Developed films, carrying 2.5 mg of zolmitriptan and 10 mg of piroxicam, were placed in both open-sided glass cylinder tubes (2 cm in diameter and 5 cm in length, with an area equal to 3.14 cm<sup>2</sup>), which were securely covered from one side with chicken buccal membrane and kept watertight with a rubber band (donor compartment) [42]. To replicate the impact of salivary fluid, 2 ml of phosphate buffer at pH 6.8 was added to the films. The loaded tubes were joined to the shafts of the USP dissolving tester device from the

second side. In 250 ml of phosphate buffer, pH 6.8, the shafts spun at 50 rpm. To maintain the same volume, samples were removed at specified time intervals of 5, 10, 15, 30, 45, 60, 90, 120, and 240 min and replaced instantaneously with an equivalent quantity of new phosphate buffer pH 6.8 [43]. As previously stated, the drug concentration was evaluated using an approved UV-visible spectroscopic technique.

The effectiveness of the various bioenhancers was assessed by comparing particular zolmitriptan permeability metrics in the presence and absence of the bioenhancer. The enhancement factor (EF) was derived using the following equation [44] to determine this ratio.

$$\text{Enhancement factor (EF)} = Q_p/Q_c$$

Q<sub>p</sub>-permeation in the presence of bio-enhancers

Q<sub>c</sub>-permeation in the absence of bio-enhancers

#### Stability studies

The stability of the produced sublingual bilayer films was tested in accordance with ICH recommendations at 40±2 °C and 75±5% RH [45]. After covering the optimal film formulations in butter paper and then putting them in aluminium foil, they were placed in the stability chamber for three months. The effect of 3 mo of storage on the physicochemical features of sublingual films was examined. The appearance, weight fluctuation, drug content and surface pH of the films were next assessed [46].

#### Pharmacokinetic studies in humans

Selection of healthy volunteers was done on the basis of the following criteria. Six healthy volunteers were enrolled in the study after getting their written consent and all were informed regarding the study of drugs [47-49]. The study protocol complies with the declarations of Helsinki for humans [50] and was approved by the Teerthankar Mahaveer University Institutional Ethics Committee with approval number TMMcandRC/IEC/19-20/136

#### Inclusion criteria

The current study included men and women ranging in age from 20 to 35 y, weighing 50 to 70 kg, and standing 146.30 to 172.83 cm tall. Also, the body mass index of all the participants fell within a normal range (18.5-24.9 kg/m<sup>2</sup>).

#### Exclusion criteria

Volunteers who were taking any medicines or had a history of any disease, lean or obese person, pregnant or lactating women, alcoholics and smokers, person whose Hb levels were below the normal range were excluded. The foreseeable benefits, risks or discomfort associated with the study were informed to volunteers and their representatives in written and oral form. Those volunteers who had provided their consent by signing the consent form were enrolled in the study.

#### Drug administration and blood sample collection

An *in vivo* study was conducted to compare the pharmacokinetics (PK) of the drugs from prepared films to marketed conventional formulations containing piroxicam (10 mg) and Zolmitriptan (2.5 mg). A single-dose PK study was carried out using two parallel treatment designs. After fasting overnight (>4 h) dosage forms were administered sublingually and a control sample of blood was collected from each subject. Three subjects were administered with a single dose composed of 10 mg) of piroxicam and zolmitriptan (2.5 mg) in two different layers of sublingual film. The remaining three were administered with market formulations. The blood samples were withdrawn from the veins and collected in vacutainers at 0, 0.25, 0.50, 1, 2, 3, 5, and 8 h. Then the tubes were properly sealed, stored at-20 °C and transported for further analysis of the blood sample using the HPLC-MS/MS method.

#### Blood sample analysis

The bioanalytical HPLC-MS/MS method was used for the simultaneous analysis of piroxicam and zolmitriptan in blood

plasma. A 2.5 ml aliquot of human plasma sample was mixed with 0.25 ml of the internal standard working solution (500 ng/ml of Telmisartan). To this, 2.5 ml of Milli Q water was added after vortex mixing for 10 seconds. The sample mixture was loaded onto an Oasis HLB 1 cm<sup>3</sup> (30 mg) extraction cartridge that was pre-conditioned with 1.0 ml of methanol followed by 1.0 ml of water. The extraction cartridge was washed with 1.0 ml of water. Piroxicam, zolmitriptan and telmisartan were eluted with 0.5 ml of the mobile phase. An aliquot of 20 µl of the extract was injected into the LC-MS/MS system and the total run time was 2.5 min. Detection of ions were m/z-515.2/276.2, m/z-332.1/238.1, m/z-288.2/195.1 at positive ionization mode with MRM monitoring as demonstrated in fig. 3. The various pharmacokinetic parameters C<sub>max</sub>, t<sub>max</sub>, the elimination half-life, area under the curve, area under the first moment curve and MRT were determined by using PK solver software [49, 50].

## RESULTS AND DISCUSSION

### Physical characteristics of films

The sublingual films of all batches had good flexibility, strength and homogeneity. The amount of ingredients was same in all the formulations except the amount of incorporated permeation enhancers. Hence, the average thickness of all the bilayer films from all the batches was observed closer to each other, falling in the range of 97.66±1.52 to 116.66±1.52 µm whereas the weight of the film formulations lies between 85.16±2.61 to 109.63±4.06 mg, as shown in table 2. The reflected variations in the weight of different batches were probably due to the varied amounts of bio-enhancers used. The pH of the film surfaces evaluated varied in the range from pH 6.66±0.050 to 6.90±0.030 as shown in table 2, and was observed to be closely associated with that of the normal sublingual mucosa surface. Therefore, the study suggested that there would not be any kind of noxious effect on the inner lining of the oral cavity [52].

### Mechanical properties of the films

The number of times the flexible film folded until it broke was reported in table 2 as the values of folding endurance and found to fluctuated between 155-190 times, which confirms the non-brittle feature of the films. The lowest and highest values of folding endurance were observed with the formulation BSTF1 and BSTF5, respectively. The addition of Sepitrap 80 and Sepitrap 4000 as bio-enhancers increased the folding endurance of films. The highest folding endurance value of BSTF5 could also be correlated with the presence of the higher amount of Sepitrap 4000 (microencapsulated castor oil), which is already known to increase flexibility, as reported by [54]. On the other hand, lubrication of the vessel with glycerin could be considered for varying levels of flexibility in the films. In order to analyse the mechanical properties of the sublingual bilayer films peel tensile tester was used. According to the results shown in table 2, the formulation BSTF-1 possesses the highest tensile strength as it contain higher proportion of polymers, whereas the film preparation BSTF-5 has shown the highest extensibility when compared to all other film compositions. After observation, it was concluded that with the incorporation of permeation enhancers, Sepitrap 80 and 4000 tensile strength of the prepared bilayer sublingual films were decreased while the extent of elongation of the films increases as reported in the literature that polysorbate 80 and castor oil hold the properties of plasticizer [55, 56]. The increasing amount of permeation enhancers in the films reduces the interlocking between the polymer chains and is hence responsible for decreased tensile strength. It also seems that the highest extent of elongation in the BSTF-5 formulation was due to the presence of hydrogenated castor oil in Sepitrap 4000.

### Percentage of moisture loss

The amount of solvent in the drying films is proportional to the amount of moisture in the film. Freshly made films were trimmed to size as soon as possible, and moisture controls were performed. The films' moisture loss range was found to be 7.33-8.28 percent, as shown in table 3. The formulation BSTF-1, which was prepared without permeation enhancers, had the largest moisture loss. The moisture losses of all formulations were found to be quite similar.

### Disintegration of films

It was observed from the preliminary development of the films that the *in vitro* disintegration time of HPMC-E15 decreased with the incorporation of pullulan [57]. Further disintegration time of bilayer films from all formulations varied between 26.00±2.64 to 45.33±3.51 seconds. Table 3 depicts the rapid breakdown of the films caused by the presence of low-viscosity water-soluble polymer, Sepitrap 80, and citric acid in the formulations.

### Drug content

The prepared film formulations were assayed for drug content. The observed values included in the above table 3 revealed satisfactory levels of drug holding capacity of these dosage forms. The value obtained from 95.35±1.88 to 100.62±2.57 also shows the uniformity of the drug content in the dosage forms.

### Surface morphology

The surface texture analyses of both the drug-loaded layers of bilayer films were done using a scanning electron microscope as shown in fig. 3. Both surfaces were found smooth and were not damaged. The SEM images displayed smooth surfaces with uniform distribution of material throughout the different layers of the films. In addition, no deformities and fractures were observed on both the surface of bilayer sublingual films. The results indicated proper miscibility and uniform distribution of drugs in the developed films [58]. The FTIR peaks pattern of pure drugs when compared with selected formulation BSTF-3 as shown in fig. 8, it seems both the drugs were successfully loaded in the films without any interactions.

### In vitro drug release studies

The percentage drug release of different batches of zolmitriptan and piroxicam bilayer sublingual films in the buffer solution having pH 6.8 is shown in fig. 5. For formulations BSTF2, BSTF3, BSTF4 and BSTF5 containing Sepitrap 80 and Sepitrap 4000 in different concentrations respectively, it was shown that the addition of bio-enhancers significantly affect the dissolution of the drug when compared to BSTF1 without bioenhancer. Similar abilities of Sepitrap 80 and 4000 were demonstrated by El-Setouhy *et al.* in 2015 [43]. 80 percent of zolmitriptan was released from all formulations within 9 min. However, the release of piroxicam was comparatively slow. The fastest release of both drugs was observed in the formulation BSTF-3 which contains Sepitrap 80 as a solubility and permeation enhancer at twice the weight of the drug. BSTF-3 that released approximately 92% of zolmitriptan was released within 3 min, whereas 92% of piroxicam was released within 4.5 min.

### Ex-vivo permeation studies

From the analysis of *in vitro* disintegration and dissolution tests, as formulations, BSTF-2 and BSTF-4 showed longer disintegration and drug release time; they were excluded from further *ex vivo* permeability studies. Fig. 6 shows the permeation profiles of bilayer sublingual films of piroxicam and zolmitriptan containing Sepitrap 80 (BSTF-3) and Sepitrap 4000 (BSTF-5) as bioenhancers. The permeation profile of BSTF-1 was used as control, which contains no bioenhancer. Thus, it shows the lowest permeation from the chicken buccal membrane. The permeation of both the drugs was found to be highest from the formulation containing Sepitrap 80 (BSTF-3) followed by BSTF-5. The enhancement factor (EF) of BSTF-3 and BSTF-5 compared to control BSTF-1 at different permeation time intervals. The EF of BEST-1 after 30, 90, and 240 min was 1, showing that there was no improvement in drug absorption via membrane, perhaps due to the lack of a bioenhancer in the formula. After 30, 90, and 240 min, the EFs of BEST-3 were (1.61, 1.70, and 1.78 for PRCM) and (2.05, 1.94, and 1.80 for ZOTP) whereas EFs of BEST-5 (1.40, 1.25, and 1.53 for PRCM) and (1.47, 1.55, and 1.64 for ZOTP) respectively. The greater drug penetration from Sepitrap 80 sublingual films might be attributable to a drug solubilization mechanism, a membrane contact mechanism, or both. As previously stated, all of the BSTFs showed complete drug dissolution after 9 min, indicating that the medication was totally free for absorption within 9 min. As a result, Sepitrap 80 increased drug penetration by interacting with the chicken membrane via microencapsulated polysorbate 80. Polysorbate 80 has

previously been shown to improve drug penetration via buccal mucosal membranes. Sepitrap 80 increased the polysorbate 80 qualities as a bioenhancer not only by increasing membrane interaction properties and optimizing solubilization properties owing to the large surface area, but also by allowing large amounts of polysorbate 80 to be included into bilayer films.

### Stability studies

No significant changes were observed in the visual appearance, such as color, transparency, surface texture of the selected bilayer films. On completion of the storage period, the determined contents of zolmitriptan and piroxicam were observed to fall within an acceptable range. Additionally, the weight and surface pH of the investigated films were also satisfactory as shown in table 4. Hence, the developed bilayer sublingual films presented a stable version of the sublingual dosage form.

### In vivo pharmacokinetic study

BSTF-3 had the quickest *in vitro* and *in vivo* disintegration times (26 sec) as well as the fastest *in vitro* dissolution rate (3-4 min). Furthermore, because Sepitrap 80 was included in the formulation, it demonstrated optimal bio-enhanced absorption via the sublingual membrane. As a result, BSTF-3 was chosen for *in vivo* pharmacokinetic research in comparison to the commercial formulation. Fig. 7 shows the mean plasma zolmitriptan and piroxicam concentration vs time curves after sublingual delivery of BSTF-3 and a marketed formulation to six participants. The plasma concentrations of the active ingredients started to increase significantly within 30 min, confirming that the developed dosage

form had effectively delivered the drug into the systemic circulation. BSTF-3 had a much greater C<sub>max</sub> and a lower T<sub>max</sub> than the market product, indicating that the introduction of Sepitrap 80 as a bioenhancer has resulted in improved drug absorption from the sublingual mucosa. The quick drug absorption from BSTF-3 is consistent with *ex vivo* permeation experiments, which demonstrated that zolmitriptan and piroxicam permeated better from bio-enhanced bilayer sublingual films than from the marketed formulation. All other formulation components of the films promoted the release without hindering the absorption of zolmitriptan and piroxicam through the targeted route. The calculation of the Area under the Curve AUC also supported the rationale of the study. The formulation BSTF-3 has shown its strength as a valuable addition to the faster absorption of drugs for breakthrough pain. This technique could be useful for APIs other than piroxicam and zolmitriptan with which rapid action is desirable. Pharmacokinetic parameters determined after oral administration of BSTF-3 and marketed formulation of zolmitriptan and piroxicam were recorded and shown in table 5. The observed increased sublingual systemic bioavailability of the drugs by Sepitrap 80 seems to have happened mainly via the interaction of microencapsulated polysorbate 80 with the sublingual mucosa, whereas enhanced drug dissolution could not be neglected.

On behalf of findings, the study suggested that the development of sublingual thin films might have advantages over the other conventional dosage forms, even over the ODTs with increased pharmacokinetic parameters, which provided better compliance and convenience to the entire population in varied groups i.e. geriatric, pediatric and patients with swallowing difficulties, by achieving a faster onset of analgesic action.

**Table 2: Results of evaluated thickness, weight variation and folding endurance of prepared sublingual thin films**

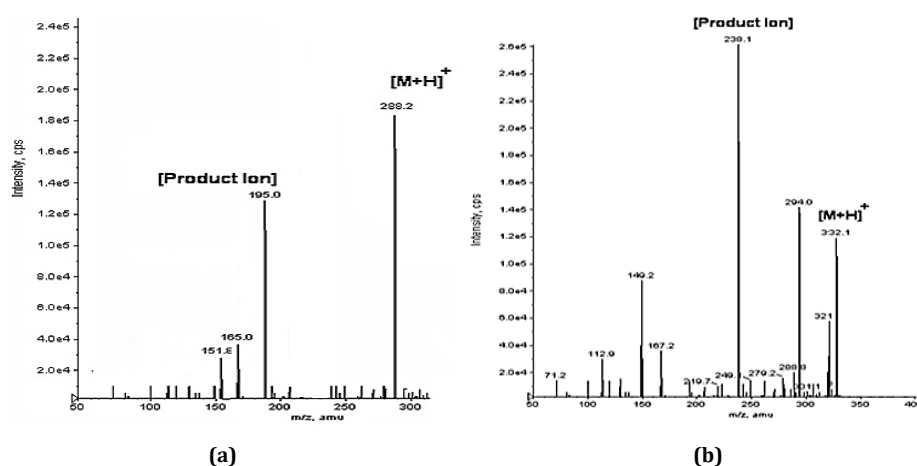
S. No.	Formulation batch	Thickness ( $\mu\text{m}$ ) mean $\pm$ SD	Weight variation (mg) mean $\pm$ SD	Folding endurance (no. of fold) mean $\pm$ SD	Tensile strength (N/mm <sup>2</sup> ) mean $\pm$ SD	Extent of Elongation (mm) mean $\pm$ SD
1	BSTF-1	97.66 $\pm$ 1.52	85.16 $\pm$ 2.61	155.33 $\pm$ 14.46	4.69 $\pm$ 0.20	1.01 $\pm$ 0.17
2	BSTF-2	106.33 $\pm$ 0.57	98.30 $\pm$ 1.10	171.3 $\pm$ 6.11	4.01 $\pm$ 0.24	1.39 $\pm$ 0.29
3	BSTF-3	110.33 $\pm$ 1.52	106.26 $\pm$ 5.40	162.3 $\pm$ 5.85	3.94 $\pm$ 0.25	1.64 $\pm$ 0.76
4	BSTF-4	107.00 $\pm$ 2.00	100.73 $\pm$ 1.97	182.3 $\pm$ 10.06	3.46 $\pm$ 0.20	2.02 $\pm$ 0.82
5	BSTF-5	116.66 $\pm$ 1.52	109.63 $\pm$ 4.06	190.0 $\pm$ 11.78	3.60 $\pm$ 0.18	2.15 $\pm$ 0.67

\*All above values represent the mean $\pm$ standard deviation and n = 5 for weight and thickness and n = 3 for all others.

**Table 3: Results of evaluated surface pH and drug content prepared sublingual thin films**

S. No.	Formulation batch	Percentage of moisture loss mean $\pm$ SD	Surface pH of films mean $\pm$ SD	Disintegration time in sec mean $\pm$ SD	Drug content (%) mean $\pm$ SD	
					ZOTP (L1)	PRCM (L2)
1	BSTF-1	8.28 $\pm$ 1.20	6.66 $\pm$ 0.050	45.33 $\pm$ 3.51	98.64 $\pm$ 2.01	96.01 $\pm$ 1.31
2	BSTF-2	7.75 $\pm$ 0.58	6.81 $\pm$ 0.045	31.33 $\pm$ 2.30	100.62 $\pm$ 2.57	95.35 $\pm$ 1.88
3	BSTF-3	7.33 $\pm$ 0.54	6.82 $\pm$ 0.036	26.00 $\pm$ 2.64	97.46 $\pm$ 1.19	97.62 $\pm$ 1.33
4	BSTF-4	8.21 $\pm$ 0.32	6.74 $\pm$ 0.025	34.66 $\pm$ 3.51	98.63 $\pm$ 0.93	94.48 $\pm$ 0.88
5	BSTF-5	8.16 $\pm$ 0.87	6.90 $\pm$ 0.030	28.00 $\pm$ 1.00	99.60 $\pm$ 1.02	97.67 $\pm$ 2.28

\*All above values represent the mean $\pm$ standard deviation and value of n = 3.



**Fig. 2: Mass spectra reflecting parent and product ion of (a) Zolmitriptan and (b) Piroxicam respectively**

Table 4: Evaluation of bilayer sublingual films (BSTF-3) during stability studies at 40 °C/75% RH

Time	Appearance	Weight (mg) mean±SD	Drug content (%) mean±SD		Surface pH mean±SD
			Layer 1	Layer 2	
0 D	Elegant	107.20±0.55	98.63±1.93	96.01±1.31	6.80±0.03
1 Mo	No Change	107.15±0.60	98.51±2.18	96.01±1.31	6.83±0.05
2 Mo	No Change	107.10±0.60	98.43±1.75	95.24±1.59	6.82±0.04
3 Mo	No Change	107.15±0.50	98.30±0.93	95.04±1.81	6.85±0.05

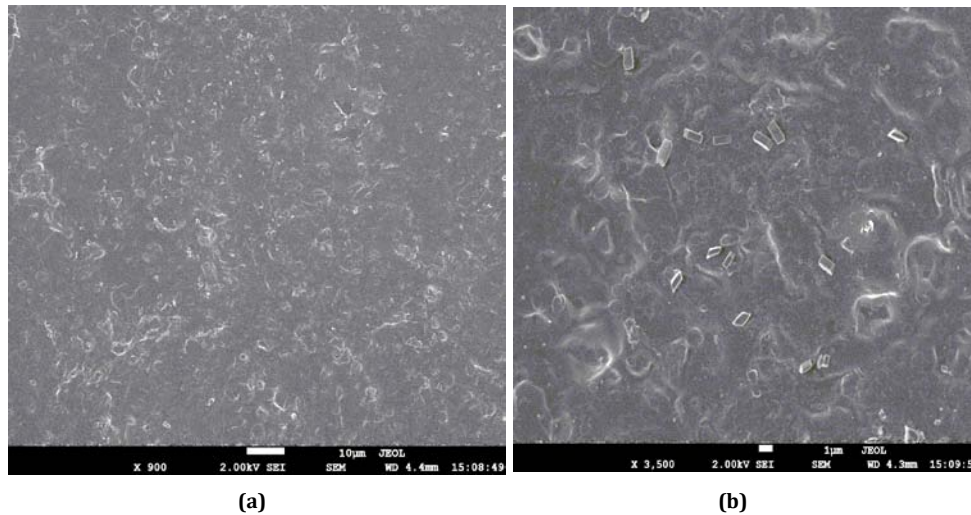


Fig. 3: SEM images of film surface demonstrating a) Uniform distribution of material at X 900 b) Material of the films at X 3500

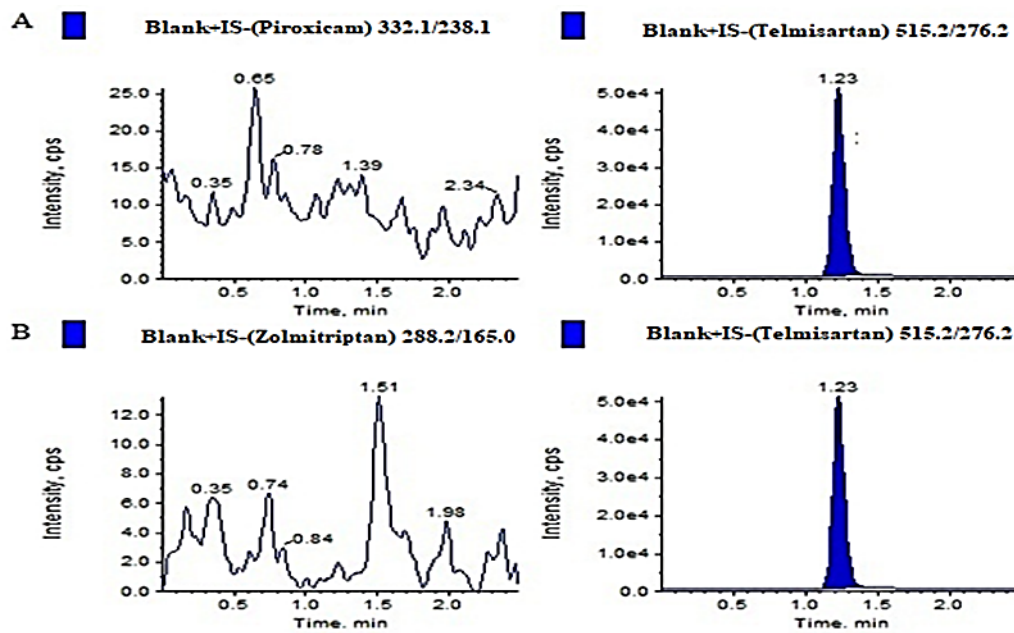


Fig. 4: Initial chromatogram of plasma with a) Blank b) IS Telmisartan

Table 5: Comparison of PK parameters of sublingual films and marketed formulation observed in humans

Parameters	Units	Zolmitriptan		Piroxicam	
		BSTF3 mean±SD	Marketed formulation mean±SD	BSTF3 mean±SD	Marketed formulation mean±SD
t <sub>1/2</sub>	H	3.76±0.29	3.41±0.11	52.11±0.82	54.06±1.62
T <sub>max</sub>	H	1.33±0.28	2.0	2.0	3.0
C <sub>max</sub>	ng/ml	2.43±0.23	1.83±0.05	428.50±14.6	341.47±8.31
AUC 0-t	ng/mlh	10.48±0.88	8.24±0.23	2541.84±70.6	2182.57±61.7
AUC 0-inf_obs	ng/mlh	13.8±1.43	10.97±0.38	27736.56±86.4	26551.36±98.6
MRT 0-inf_obs	H	6.93±0.41	5.92±0.10	79.94±4.50	71.28±7.38

(n=3)

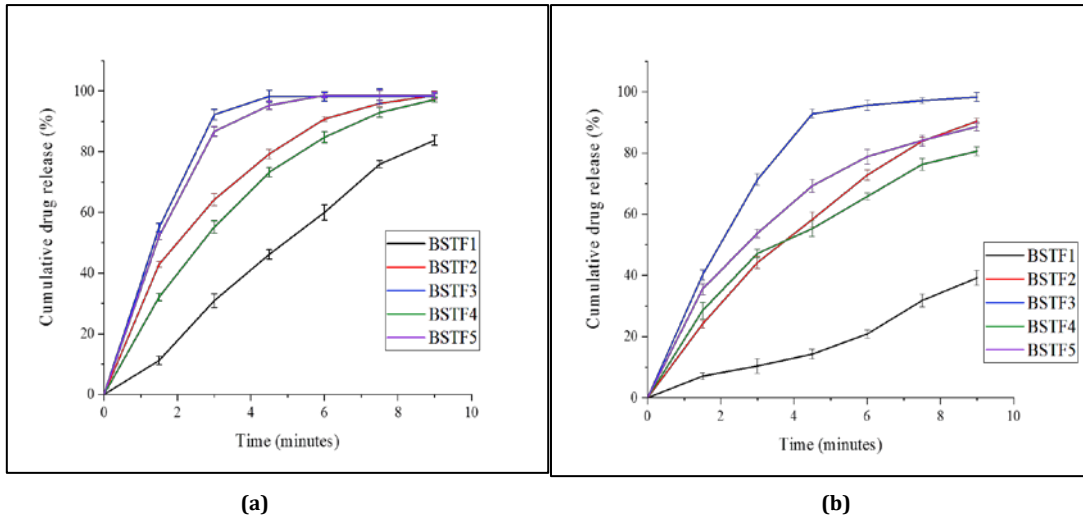


Fig. 5: Release of (a) Zolmitriptan and (b) Piroxicam from sublingual thin film

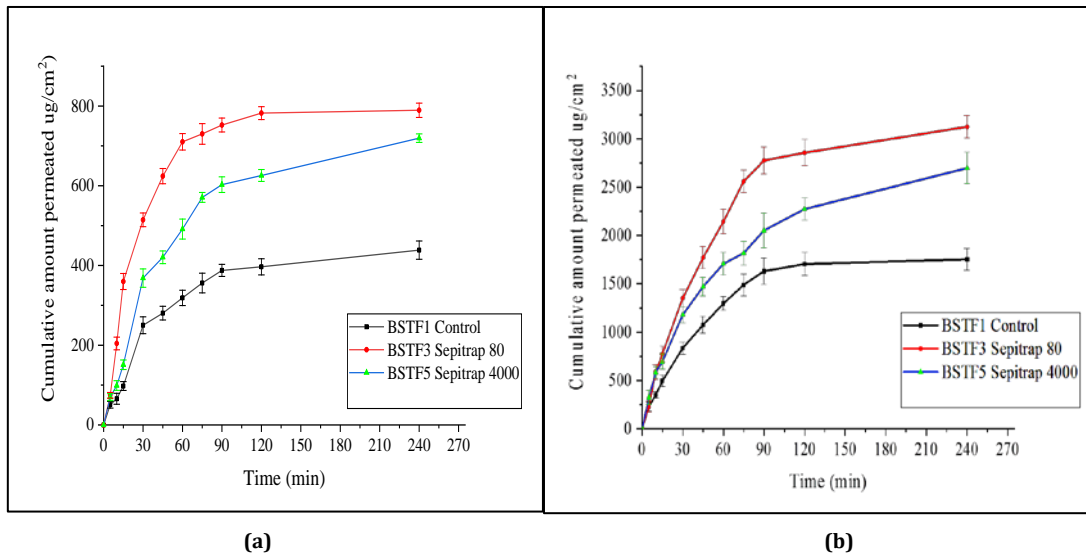


Fig. 6: Permeation of (a) Zolmitriptan and (b) Piroxicam from sublingual thin film

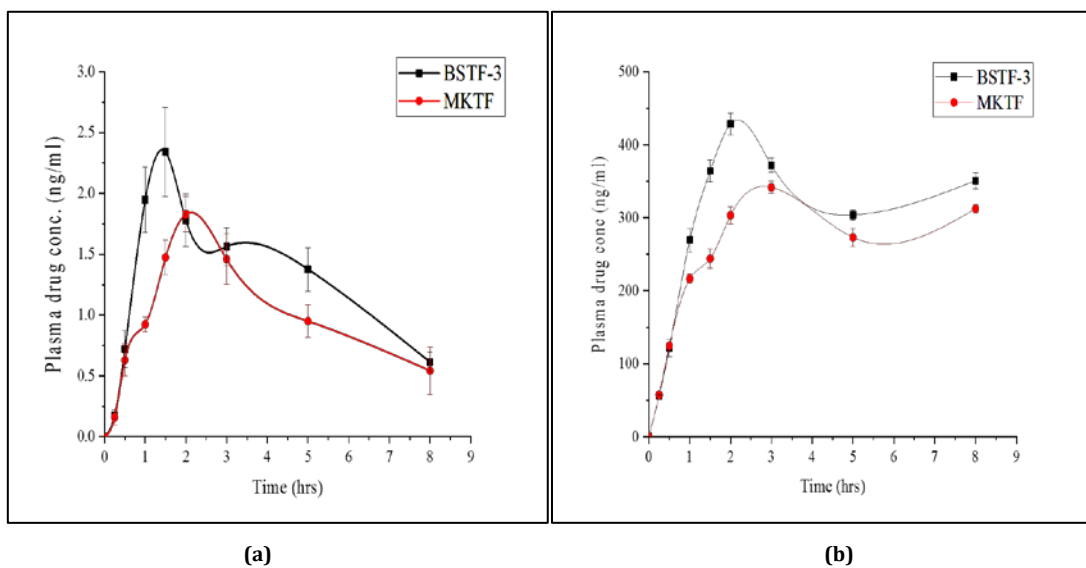


Fig. 7: The plasma concentration of three volunteers a) Zolmitriptan b) Piroxicam, versus time after administration of BSTF-3 and marketed formulation (MKTF)

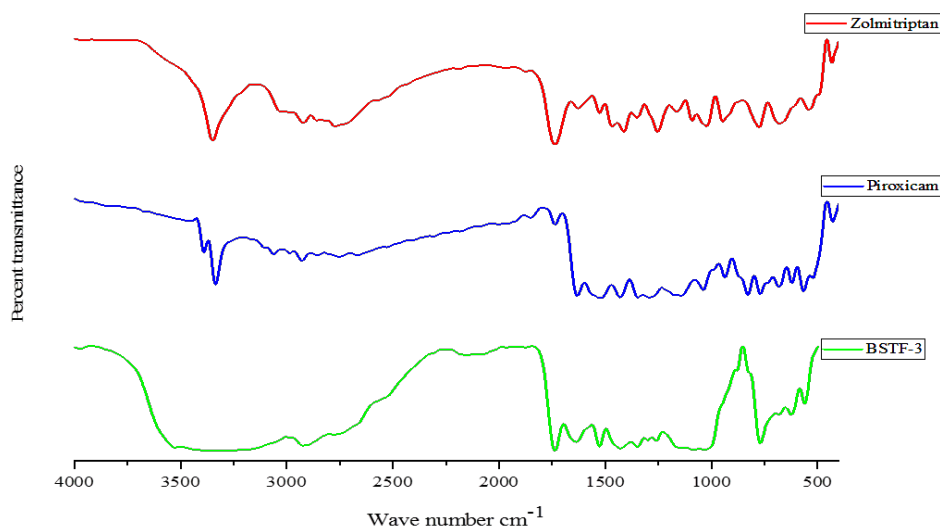


Fig. 8: FTIR of final formulation BSTF-3 compared with pure zolmitriptan and piroxicam

## CONCLUSION

Bilayer sublingual films containing piroxicam and zolmitriptan in two different layers were successfully developed with good film characteristics, satisfactory mechanical properties and drug release. The pharmacokinetic parameters of formulated films were found to be encouraging when compared to that of the marketed formulation. The plasma drug concentration-time profile indicated a slight increase in AUC values. The developed delivery system also showed higher  $C_{max}$  with lower  $T_{max}$ , resulting in significantly faster absorption of incorporated drugs, which helped in the rapid onset of action in the management of pain as required by migraine sufferers. Incorporation of Sepitrap 80 increased the dissolution of drugs along with the absorption of both active agents through the sublingual biomembrane. Although obtained results were only considered exploratory due to small number of volunteers involved in the study hence, further investigation on the developed formulation should be conducted on large number of populations. Therefore, the sublingual thin films of piroxicam and zolmitriptan may be considered suitable for clinical use in the treatment of acute migraine pain management. Such an innovative formulation could be an attractive alternative to traditional dosage forms, paving the way for huge research in this area. Few Line Deleted.

## INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki, and approved by the (TMU-IEC) Teerthanker Mahaveer University Institutional Ethics Committee of Teerthanker Mahaveer Medical College and Research Centre, TMU with approval letter no. TMMCandRC/IEC/19-20/136.

## INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

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This research received no external funding.

## AUTHORS CONTRIBUTIONS

All authors have contributed equally.

## CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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