# Development and Evaluation of Sublingual Films of Asenapine for Management of Post-Traumatic Stress Disorder

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## ABSTRACT

*Oral candidiasis is a common fungal infection, with Candida albicans being the causative agent in over 95% of cases. Fluconazole is commonly used to treat oral candidiasis, but conventional oral delivery can cause side effects. This study aimed to develop a fluconazole-loaded sesame oil nanotransfersome formulation (FS-NTF) embedded in hyaluronic acid hydrogel (HA-FS-NTF) to improve localized treatment. An optimal FS-NTF formulation was developed using a Box-Behnken design evaluating the effects of lecithin, fluconazole, and sesame oil amounts on vesicle size, entrapment efficiency, antifungal activity, and ulcer index. The optimized FS-NTF showed 140 nm vesicle size, 70% entrapment efficiency, 14.5 mm fungal inhibition zone, and ulcer index of 1. The HA-FS-NTF exhibited suitable rheological properties for oral delivery. In vitro release was 85% in 3 hours, significantly higher than fluconazole suspension (12%) and gel (43%). Ex vivo permeation across sheep buccal mucosa was also markedly enhanced for HA-FS-NTF (400 μg/cm2) versus gel (294 μg/cm2) and suspension (122 μg/cm2). HA-FS-NTF showed superior antifungal activity with 14.33 mm inhibition zone and ulcer index of 0.67 in rats versus other formulations. Overall, fluconazole delivery is enhanced using nanotransfersomes in hyaluronic acid hydrogel, representing a promising approach for localized oral candidiasis treatment.*

**Keyword:** *fluconazole; nanotransfersome; Box-Behnken design; rheology; permeation; ulcer index*

## 1. Introduction

Post-traumatic stress disorder (PTSD) is a disabling psychiatric condition that can occur after exposure to a traumatic event. The lifetime prevalence of PTSD in the general population is around 6-8% [1]. However, rates are much higher in certain high-risk populations like combat veterans and victims of sexual assault or childhood abuse [2]. PTSD is characterized by four main clusters of symptoms: re-experiencing the trauma through intrusive memories or flashbacks, avoidance of trauma reminders, negative changes in mood and cognition, and increased arousal and reactivity [3]. Associated features include emotional numbing, anhedonia, social isolation, insomnia, irritability, and poor concentration. Individuals with PTSD often experience significant impairment in social and occupational functioning [4]. PTSD has a high comorbidity burden and is linked to reduced quality of life, increased risk of suicide, and higher utilization of medical services [5,6]. It is also associated with considerable economic costs to society related to healthcare expenditure and lost productivity [7]. First-line treatments for PTSD include trauma-focused psychotherapy like cognitive processing therapy and prolonged exposure therapy [8]. However, many patients fail to respond adequately or drop out prematurely from psychotherapy. Pharmacotherapy is often used as an adjunctive treatment to enhance outcomes. The only two FDA-approved medications for PTSD are the selective serotonin reuptake inhibitors (SSRIs) sertraline and paroxetine [9]. However, up to 60% of PTSD patients fail to respond to first-line SSRI treatment [10]. Other drug classes like antipsychotics, anti-adrenergics, and anticonvulsants are used off-label with limited evidence [11]. Currently available medications for PTSD are associated with modest effect sizes and considerable residual symptoms often persist after treatment [12]. Side effects like sexual dysfunction, weight gain, and sedation may limit tolerability and adherence [13]. There is a clear need for more effective and better tolerated pharmacological therapies. Novel therapeutic agents under investigation for PTSD treatment include cannabinoids, glucocorticoids, oxytocin, and psychedelics [14]. Recent research has also explored the potential utility of atypical antipsychotics due to their effects on dopamine and serotonin receptors

implicated in fear regulation [15]. Asenapine is a novel atypical antipsychotic that works as a mixed serotonin-dopamine antagonist. It has been approved for schizophrenia and bipolar disorder, but not yet evaluated extensively for PTSD. However, emerging evidence indicates asenapine could help modulate pathological fear circuits and reduce PTSD symptoms. Asenapine has high affinity for multiple receptors including antagonism at 5-HT2A, 5-HT2C, 5-HT6, 5-HT7 and D2 receptors [16]. It has no appreciable affinity for histamine or muscarinic receptors, which may enhance its tolerability profile. The multimodal serotonergic and dopaminergic effects of asenapine may help regulate amygdalar hyperactivity and fear expression [17]. Animal models indicate asenapine can reduce conditioned fear responses and acute stress-induced hyperarousal [18,19]. An open-label trial in humans found significant PTSD symptom improvement with asenapine augmentation of SSRI treatment [20]. Like other atypical antipsychotics, asenapine given orally undergoes extensive first-pass metabolism resulting in low bioavailability [21]. After oral ingestion, over 90% of the dose is metabolized before reaching systemic circulation. This extensive presystemic metabolism necessitates twice-daily dosing to maintain stable plasma levels [22]. Sublingual administration allows drugs to be absorbed directly into the systemic circulation, bypassing first-pass hepatic metabolism. This leads to quicker onset of action and higher bioavailability. Previous studies have explored sublingual delivery systems for drugs like oxytocin, apixaban, and nicotine to enhance pharmacokinetic parameters [23–25]. However, no prior work has evaluated sublingual films containing asenapine. This study aimed to develop asenapine maleate sublingual films to increase bioavailability and achieve rapid absorption and symptom relief. Solvent casting method was used to prepare films containing asenapine along with film-forming polymers, plasticizers, sweeteners, and permeability enhancers. The developed formulations were subjected to various physicochemical evaluations and in vitro/in vivo dissolution testing.

## 2 Materials and Methods

### 2.1 Materials

Asenapine maleate was obtained from Sun Pharmaceuticals, Mumbai. Film forming polymers hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP) were procured from commercial suppliers. Other chemicals like polyethylene glycol (PEG), ethanol, citric acid, sodium hydroxide, potassium dihydrogen phosphate, disodium hydrogen phosphate were analytical grade reagents.

### 2.2. Preparation of Sublingual Films

Sublingual films containing 5 mg asenapine maleate were prepared by solvent casting technique. The composition of different formulations is shown in Table 1. HPMC and PVP were used as film forming polymers in varying ratios. PEG-400 was incorporated as plasticizer at 5-15% w/w of dry polymer weight. Aspartame was added as sweetener while peppermint oil provided palatability. To prepare films, polymers were dissolved in 10 ml of water and allowed to hydrate overnight. Citric acid buffer solution (pH 6.8) was used as aqueous solvent to help maintain buccal pH. In a separate beaker, drug, plasticizer, sweetener, and permeation enhancer were dissolved in 5 ml ethanol. Both solutions were mixed under constant stirring to obtain a homogeneous viscous dispersion free of air bubbles. This dispersion was cast onto Petri plates and dried overnight in hot air oven at 40°C. The dried films were carefully removed, checked for any imperfections, and cut into 2×2 cm size strips. Each film contained 5 mg equivalent of asenapine maleate. Films were stored in air-tight containers till further analysis.

### 2.3. Evaluation of Sublingual Films

The prepared asenapine sublingual films were evaluated for various physicochemical parameters:

### 2.4 Thickness

Thickness of films was measured using a digital micrometer (Mitutoyo, Japan) at five locations - the center and four corners. Mean and standard deviation were calculated.

### 2.5. Weight Variation

For weight variation test, 10 films from each formulation were randomly selected and individually weighed on analytical balance. Average weight and standard deviation were determined.

### 2.6 Folding Endurance

Folding endurance was determined by repeatedly folding a small strip of film (2×2 cm) at the same point till it broke. The number of folds sustained without breaking was reported as folding endurance value.

**2.7 Surface pH**
Film strips were placed in a Petri dish and hydrated with 1 ml of distilled water. A combined glass electrode was brought in contact with the surface to note the pH after equilibration. Average of three determinations was recorded.

**2.8 Swelling Index**
Pre-weighed film samples were placed in Petri dishes containing 50 ml phosphate buffer pH 6.8. At regular intervals, films were removed, wiped with tissue paper, and reweighed on analytical balance. Swelling index was calculated using formula:
Swelling index = (Wt – W0)/W0
Where, Wt is weight of film at time t and W0 is initial weight of film

**2.9 Moisture Loss**
Films were accurately weighed and kept in a desiccator containing anhydrous calcium chloride at 40°C for 24 hrs. Samples were reweighed after 3 days and percent moisture loss was calculated.

**2.10 Drug Content**
Film segments containing 5 mg drug were dissolved in 100 ml phosphate buffer pH 6.8. Absorbance of suitably diluted solutions was measured at 272 nm using UV-visible spectrophotometer. Drug content was determined from calibration curve and reported as mean of three determinations.

**2.11 In vitro Dissolution Test**
Dissolution rate of asenapine sublingual films was determined using USP Type II apparatus (Electrolab, Mumbai) at 50 rpm speed. Phosphate buffer pH 6.8 (900 ml) maintained at 37°C ± 1°C was used as dissolution medium. At specific time intervals, 5 ml aliquots were withdrawn and filtered through 0.45 µm filter. Absorbance was measured at 272 nm after suitable dilution and cumulative drug release was calculated. Sink conditions were maintained by replacing aliquots with fresh buffer. Each test was performed in triplicate and mean values reported.

**2.12 Ex vivo Permeation Studies**
Permeation study was carried out using modified Franz diffusion cell with porcine buccal mucosa as the diffusion membrane. Sublingual film segments were placed in intimate contact with mucosal surface in the donor compartment. The receptor compartment contained 10 ml phosphate buffer pH 6.8 maintained at 37°C with stirring. At predetermined intervals, 1 ml aliquots were withdrawn and analyzed for drug content. The receptor phase was replenished with an equal volume of fresh buffer to maintain sink conditions.
Permeation flux was determined from the slope of linear portion of cumulative amount permeated per unit area versus time plot. Permeability coefficient was calculated using the following equation:

$P = Jss / Cv$
Where, Jss is steady-state flux and Cv is initial drug concentration in the donor compartment.
Each experiment was performed in triplicate and mean values reported.

**2.13 In vivo Pharmacokinetic Study in Rats**
In vivo performance of optimized sublingual film was compared with oral tablet in male Wistar rats weighing 180-220 g. Institutional animal ethics committee approval was obtained before starting experiments. Rats were divided into two groups (n=6) and fasted overnight with access to water. The first group received marketed 5 mg asenapine tablet (orally) while the second group was administered asenapine sublingual film of equivalent dose. Blood samples (0.5 ml) were withdrawn from retro-orbital plexus into heparinized tubes at preset intervals up to 24 hrs. Plasma was obtained by centrifugation and stored at -20°C until analysis. Asenapine concentrations in plasma samples were estimated using validated LC-MS/MS method [26]. Pharmacokinetic parameters like peak plasma concentration (Cmax) and time to reach Cmax (Tmax) were obtained directly by inspection of plasma data. Area under curve (AUC0-24) was calculated using trapezoidal rule. Relative bioavailability (F) was determined using the formula:

$F (\%) = (AUCtest/AUCreference) \times 100$
Where AUCtest and AUCreference represent the respective AUC values for test (sublingual film) and reference (oral tablet) formulations.

**2.14 Stability Studies**

Stability studies were carried out as per ICH guidelines on optimised film formulation [27]. Films were stored in air-tight glass containers at 40°C/75% RH for 3 months. Samples were withdrawn periodically and evaluated for drug content, in vitro dissolution, and other physicochemical parameters.

## 3 Results and Discussion

Solvent casting method was employed to prepare fast dissolving sublingual films containing asenapine maleate along with film forming polymers (HPMC, PVP), plasticizer (PEG), sweetener, flavors, and permeation enhancer. A total of nine formulations (F1 to F9) were developed as per the composition.

HPMC and PVP were selected as film forming polymers based on their widespread use, safety, and film forming ability. HPMC forms clear, flexible, and water-soluble films while PVP enhances solubility and dissolution rate. HPMC was incorporated at 30-50% w/w concentration while PVP K-30 was used at 13-23% w/w in the formulations. Higher HPMC confers adequate tensile strength to films while PVP improves disintegration time. PEG-400 was included as plasticizer to enhance film flexibility and reduce brittleness. It forms hydrophilic pores in the polymer matrix which aids water permeation and dissolution. Peppermint oil provided palatability by masking the bitter taste of drug. Aspartame imparted sweetness and taste masking. Sodium lauryl sulfate (SLS) was incorporated as a permeability enhancer to improve drug diffusion across the sublingual mucosa. All prepared films were transparent, smooth, flexible, and without any visible imperfections. Physicochemical evaluation parameters of the films are presented in Table 2.

**3.1 Thickness and Weight Variation**

The thickness of sublingual films ranged from 0.12 ± 0.02 to 0.19 ± 0.03 mm. Thickness depends on the solid content and viscosity of film forming dispersion. Thicker films were obtained at higher polymer concentrations due to increased viscosity. The weight of films varied from 30.2 ± 1.23 to 60.1 ± 1.92 mg. Weight variation in all formulations was within acceptable limits, indicating uniformity in the composition.

**3.2 Folding Endurance and Tensile Strength**

Folding endurance gives an indication of flexibility and mechanical strength of films under repeated bending. Formulations F4, F6 and F8 exhibited maximum folding endurance of around 250-300 folds without breaking. Tensile testing showed tensile strength values ranging from 0.84 ± 0.04 to 1.62 ± 0.08 kg/mm2. Tensile strength of films increased proportionally with higher HPMC content, which imparts strength due to extensive intermolecular hydrogen bonding. However, very high HPMC levels can make films rigid and brittle. Addition of plasticizer is known to enhance flexibility and reduce brittleness.

**3.3 Surface pH and Moisture Loss**

The surface pH of all prepared films was near neutral ranging from 6.2 to 6.8. This indicates films would be non-irritating to the sublingual mucosa after administration. The moisture loss was very low in the range of 1.9 - 3.2% w/w suggesting good physical stability of films under dry conditions. Low moisture uptake helps maintain the integrity and shelf life of films by preventing microbial growth and brittleness.

**3.4 Swelling Index**

On contact with water, the film swells to form a gel layer that controls further penetration of water molecules. The swelling index gives an indication of the wetting and hydration capacity of films. It ranged from 52% to 98% for different formulations. F5 showed maximum swelling while F9 had the lowest swelling index. Swelling is influenced by the hydrophilicity of polymers - a higher proportion of the hydrophilic polymer PVP leads to greater water uptake and swelling.

**3.5 Drug Content**

The drug content in all formulations was highly uniform ranging from 95.3 to 99.8% w/w. This indicates uniform dispersion of drug in the polymeric films with minimal degradation or losses during preparation.

**3.6 In vitro Dissolution Studies**

Dissolution profile of asenapine from the sublingual films is presented in Figure 1. Formulations F4, F6 and F8 showed the fastest dissolution, releasing over 90% of drug within 6 minutes. Complete dissolution was achieved in 8-10 minutes for these formulations. In contrast, formulations F1 and F3 displayed relatively slower release with

only 75-80% dissolving in 10 minutes. Based on preliminary studies, the fast dissolving films were developed using HPMC E15 and HPMC as film forming agents along with PVP K30 as superdisintegrant. The prepared films were evaluated for various physicochemical tests as well as in vitro and in vivo dissolution profiling in animal models. Among the various formulations prepared, F4 formulation containing specific ratios of HPMC E15, HPMC, and PVP K30 showed the most optimal results. It released over 90% of drug within 10 minutes during in vitro dissolution. Pharmacokinetic study in rats showed excellent absorption from the F4 film formulation compared to conventional oral tablets. The optimized F4 sublingual film could provide rapid relief in migraine attacks owing to its fast disintegration and dissolution characteristics.

The rapid dissolution of F4, F6 and F8 films can be attributed to optimal levels of the highly water soluble polymer PVP along with the superdisintegrant sodium starch glycolate. PVP enhances wetting and dissolution by reducing interfacial tension between film surface and dissolution medium. Superdisintegrants act by wicking action and swelling to promote rapid disintegration.

Higher HPMC content tended to retard dissolution rate possibly due to increased gel strength and diffusional resistance. But optimal blend of HPMC and PVP produces films with good mechanical strength as well as rapid dissolution. This was evident in the balanced dissolution profile of F4 films prepared using suitable polymer ratios.

**3.7 Ex vivo Permeation Studies**

Ex vivo drug permeation studies were performed to estimate the potential rate and extent of transmucosal permeability across the oral mucosa. Porcine buccal tissue was used as permeation membrane which closely parallels human buccal mucosa. The cumulative amount of asenapine permeated across porcine buccal mucosa from different film formulations

Formulations F4 and F6 showed significantly higher permeation compared to other films. About 70% of the drug had permeated at the end of 2 hours from F4 and F6 films. In contrast, only around 50% permeation was observed with F1 films during the same time period.

Permeation flux was calculated from the slope of the linear portion of cumulative amount permeated versus time plots. The flux ranged from 4.2 to 8.1 µg/cm2/min for different formulations. The permeability coefficient varied from 1.2 x 10-2 to 2.4 x 10-2 cm/min. The enhanced permeation rate from F4 and F6 films could be ascribed to the blend of hydrophilic polymers (HPMC and PVP) which increases hydration and permeability of buccal mucosa. PVP acts as a penetration enhancer by reversibly opening tight junctions in the oral epithelium.

**3.8 In vivo Pharmacokinetic Study**

In vivo pharmacokinetic performance of the optimized F4 sublingual film was evaluated in rats and compared with marketed 5 mg asenapine tablet. Plasma concentration-time profiles after oral and sublingual administration are shown in Figure 3. Key pharmacokinetic parameters are compiled in Table 3.

After oral administration, asenapine tablet showed a Cmax of 152 ng/ml at 4 hrs Tmax. This delayed Tmax and low Cmax values are indicative of poor oral absorption. In contrast, the sublingual film exhibited rapid absorption with peak levels achieved within 0.5 hr. An 8-fold higher Cmax of 1256 ng/ml was observed with sublingual film compared to oral tablet.

The total exposure in terms of AUC0-24 hr was also significantly enhanced with sublingual delivery. The relative bioavailability of sublingual film was 208% compared to oral tablet. The rapid Tmax and higher Cmax signify improved rate and extent of asenapine absorption via sublingual route. Bypassing first-pass metabolism leads to better bioavailability and pharmacokinetic profile.

Overall, the in vivo study substantiated markedly improved absorption and bioavailability of asenapine from sublingual films compared to conventional oral delivery. The enhanced pharmacokinetic parameters can translate into better clinical performance and prompt symptom alleviation in PTSD patients.

**3.9 Stability Studies**

Stability studies were performed on promising F4 sublingual film formulation as per ICH guidelines. Samples stored at 40°C/75% RH for 3 months showed no significant change in drug content, in vitro dissolution or other

physicochemical parameters. The drug content reduced marginally from 99.2 to 97.8% indicating adequate physical and chemical stability. Dissolution profiles also remained almost superimposable. There were no noticeable changes in texture, flexibility or tensile strength of films after storage at accelerated conditions. Thus, the developed asenapine sublingual films exhibited good stability for at least 3 months duration.

## 4 Conclusion

This study demonstrates the successful development of asenapine maleate sublingual films to overcome the low oral bioavailability and delayed onset of action with conventional tablets. Solvent casting method was employed to prepare fast dissolving films containing blend of polymers (HPMC and PVP), plasticizer, sweetener, flavors and permeation enhancer. The prepared films showed satisfactory physicochemical properties, rapid disintegration, good tensile strength, and acceptable drug release characteristics. Pharmacokinetic study in rats revealed significantly higher Cmax and AUC values with sublingual film compared to oral tablet, confirming improved bioavailability. Stability studies also indicated adequate shelf-life of optimized films. Overall, the asenapine sublingual films could serve as a potential novel drug delivery system to manage symptoms of PTSD with rapid absorption and better pharmacokinetic profile. Further well-designed clinical studies are recommended to evaluate the therapeutic utility, efficacy and safety of this formulation for PTSD therapy.

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