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Research Article

IN VITRO, EX-VIVO, AND DISSOLUTION KINETIC STUDIES OF MOUTH-DISSOLVING SUBLINGUAL FILMS CONTAINING LOSARTAN POTASSIUM AND HYDROCHLOROTHIAZIDE

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ABSTRACT

Objectives: Sublingual films were designed for easy administration, rapid as well as slow drug absorption for obtaining controlled release action through the oral mucosa, bypassing the gastrointestinal tract and hepatic first-pass metabolism. This can result in a faster onset of action, enhanced bioavailability, and patient convenience since water isn't required for administration. Films also offer precise dosing, enhanced drug stability, and targeted therapy for localized treatment within the oral cavity. These features make a non-invasive and painless alternative to injections, improving the patient experience. This paper focuses on *in vitro* and *ex vivo* activity using goat oral mucosa and the suitability of release kinetics using different kinetic parameters.

Methods: Films were generated using a solvent casting method where a polymer, drug, plasticizers, and excipients were dissolved in a solvent to form a homogenous mixture. This mixture was poured into a petri dish, forming a thin layer. After controlled evaporation, the resulting film was cut to the desired size and shape and stored away from light and heat.

Results: *Ex vivo* studies using goat buccal mucosa showed effective drug absorption. F4 batch was considered an optimized batch because it showed a release of 79.23±1.15% in 9 h in *in vitro* release study and *ex vivo* studies showed the highest permeation as compared to other batches and is found to be 96.54±1.23% in 9 h and the flux was calculated to be 0.975±1.15 mg $h^{-1}cm^{-2}$, 0.904±0.92 mg $h^{-1}cm^{-2}$, and 0.901 mg $h^{-1}cm^{-2}$ (Target flux 0.991 mg $h^{-1}cm^{-2}$).Two-way ANOVA suggested $p<0.05$. The drug release was best described by the Higuchi square root model, indicating a diffusion-controlled process.

Conclusions: The film containing losartan potassium and hydrochlorothiazide offers a significant advancement in hypertension treatment. This approach boosts therapeutic outcomes and patient quality of life by overcoming demands associated with traditional oral dosage forms. Further research could extend the benefits of film to other medications and conditions, enhancing the field of drug delivery.

Keywords: Hydrochlorothiazide, Losartan potassium, Goat mucosa, *Ex vivo*, Kinetic modeling, Solvent casting method.

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INTRODUCTION

Fast-dissolving films (FDFs) present several advantages compared to conventional dosage forms, including increased patient compliance, quick onset of action, precise dosing, and a better taste experience. These films show remarkable administration benefits, as they dissolve in the mouth without water or chewing. FDFs disintegrate faster and release medication directly into the oral cavity as they are composed of hydrophilic polymers. This makes them ideal for patients having difficulty in swallowing, including children, the elderly, or those with conditions like nausea, motion sickness, dysphagia, coma or mental health issues [1,2].

FDFs can be used by the sublingual route because of the high permeability and rich blood supply in this area. This leads to rapid drug absorption and quicker onset of action. This method of administration bypasses the liver, which can elevate the oral bioavailability of drugs that are extensively processed by the liver. Therefore, sublingual FDFs offer a practical approach to improve drug absorption and effectiveness [3,4].

Losartan potassium, an antihypertensive drug that acts as an angiotensin II receptor blocker (ARB), lowers blood pressure by vasodilation and enhancing blood flow to the heart. As a BCS Class III drug, Losartan typically achieves peak plasma levels within 1.5–2 h. Using a fast-dissolving sublingual film, one can improve its bioavailability by ensuring rapid disintegration and absorption before the drug reaches the stomach. Hydrochlorothiazide (HCZ), a diuretic used to treat hypertension and fluid retention, has an oral bioavailability of 45–55%. A FDFs for HCZ could address its limited bioavailability. Combining Losartan and HCZ in a single formulation can effectively manage blood pressure, reduce the risk of stroke, and enhance overall treatment outcomes compared to administering each drug separately and could be proposed to obtain synergistic action [5,6]. The main objective of current research is to formulate mouth-dissolving film and observe its release kinetic parameter by studying different kinetic models using DD solver software and to perform *ex vivo* studies on goat mucosa to manifest the permeability of the FDFs, which will help to determine how effectively the drug is absorbed through the mucosal barrier.

METHODS

Materials

Losartan potassium was supplied by Indoco Remedies Pvt Ltd, Goa, HCZ was gifted by Merck India Pvt Ltd. Hypromellose (HPMC) METHOCEL™ E3 Premium, METHOCEL™ E5 Premium, and METHOCEL™ E15 Premium were sourced from Colorcon Asia Pvt Ltd, Goa.

Method

The solvent casting method is a commonly used technique for the formulation of thin polymer films and membranes. This method involves the following steps as shown in Fig. 1 [6-8].

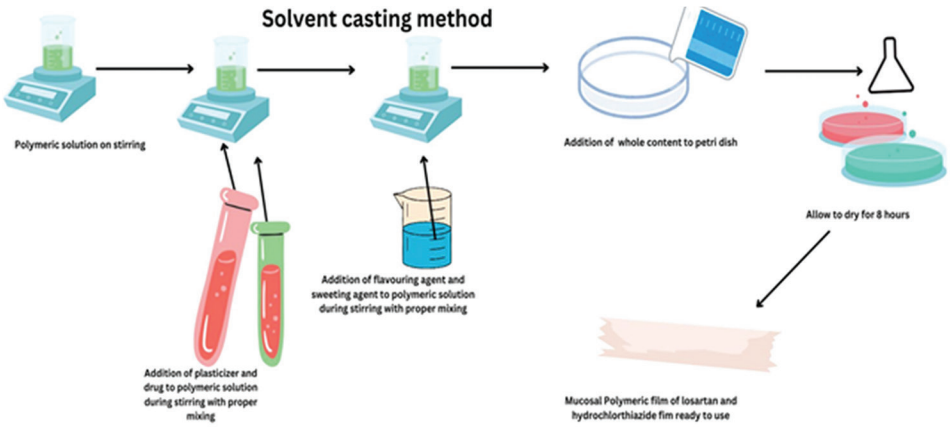

Fig. 1: Solvent casting method

Steps involved

Polymer dissolution (A)

The polymer HPMC E15 and E5 were first dissolved in water to create a polymer solution. The choice of solvent depends on the solubility of the polymer and desired film properties.

Solution preparation (B)

Drugs, PEG 600(plasticizer), sucralose (sweetener), citric acid (saliva-stimulating agent), and raspberry syrup (flavouring agent) were dissolved in water to form solution B. Both solutions A and B are mixed properly.

Casting the solution

The polymer solution is then poured on a petri dish, known as a casting substrate. The thickness of the film can be controlled by adjusting the solution volume or using techniques like spin coating.

Solvent evaporation

The solvent is allowed to evaporate, leaving behind a solid polymer film. This step can be done under controlled temperature and air.

Film removal

Once the solvent has been evaporated completely and the film is solidified, the polymer film is carefully removed from the casting substrate.

Calculation of the amount of drugs required to be added

Diameter of the petri dish=9 cm
The radius of the petri dish=9 cm/2 = 4.5 cm
Area of petri dish=πr^2=3.14×4.5×4.5=63.585 cm^2
Area of film=2 cm×2 cm=4cm^2

a. Dose of HCZ=12.5 mg
If, 4 cm^2 contains 12.5 mg HCZ
So, 63.585 cm^2 contain 63.585×12.5/4 mg HCZ
Therefore, 198.68 mg HCZ is required to be added in a Petri dish.
If 25 ml contains 198.68mg HCZ
Then, 50 ml contains 496 mg HCZ

Similarly

b. The dose is LP 25 mg
If, 4 cm^2 contains 25 mg LP
Hence, 63.585 cm^2 contain 63.585×25/4 mg LP
Therefore, 397.40 mg LP is required to be added in a Petri dish
If 25 ml contains 397.40mg LP
Then, 50 ml contains 794 mg LP
Thus, for making film, 496 mg (HCZ) and 794 mg (LP) are required.

***In vitro* drug release study**

The release of Losartan Potassium and HCZ was evaluated using a Franz diffusion cell containing 30 mL of 67 mM phosphate buffer (pH 6.8) and 0.5% Sodium Dodecyl Sulfate (SDS) at 37°C at 100 rpm for 24 h. Aliquots were collected at various intervals, filtered, diluted, and analyzed using a UV spectrophotometer (Thermo Fisher Scientific with model *Thermo Scientific™ Genesys™* 150) at wavelengths of 282 nm [9,10]. The procedure is summarized in Fig. 2.

***Ex vivo* permeation studies**

Goat mucosa was used for the *ex vivo* experiments for a number of reasons, including its morphological and physiological resemblance to human mucosa, its affordability and accessibility in comparison to human tissue, and its suitability for research. A constant and repeatable tissue model is provided by the goat mucosal membrane, which is necessary for accurate experimental outcomes. Particularly for early research, goat mucosa may be more morally acceptable than human tissue. In a controlled environment, it is more accurate to evaluate drug permeability, stability, and interaction.

Fresh goat buccal mucosa was obtained from a local slaughterhouse and transported to the laboratory under refrigerated conditions. The mucosa was carefully trimmed to remove any excess tissue and mounted using a Franz diffusion cell between the donor and receptor compartments of a Franz diffusion cell. The diffusion experiment conducted using a modified Franz diffusion cell is summarized in Fig. 3. The buccal mucosa was mounted between the donor and receptor compartments. An *ex vivo* permeation study of FDFs was conducted using goat buccal mucosa and a modified Franz diffusion cell. The buccal epithelium was carefully positioned between the two chambers of the modified Franz diffusion cell and allowed to equilibrate for approximately 1 h with pH 7.4 phosphate buffer solution in both chambers. After this equilibration period, the receiver compartment was filled with 25 ml of fresh phosphate buffer solution (pH 7.4). For comparative studies, we need two Franz diffusion cells, one Franz diffusion cell, donor compartment filled with 4 ml of a drug solution (1 mg/ml) (2 mg LP+2 mg HCZ), and another diffusion cell comprising of film cut into the appropriate size to fit into the donor compartment. The setup was then placed on a magnetic stirrer set to 50 rpm, maintaining a temperature of about 37°C. Samples of 2 ml were taken from the receptor compartment at specific time intervals (1–9 h). Every time sample is replaced by an equal volume of fresh buffer solution and stored in the refrigerator until analysis. The experiment was performed in triplicate, and the amount of drug that permeated through the buccal mucosa was measured using a UV-visible spectrophotometer. The studies were repeated in triplicate (n=3), and the mean values were calculated [9-12].

***In vitro* and *ex vivo* correlation**

In vitro and *ex vivo* correlation between the cumulative percentage of drug released *in vitro* and the percentage of drug permeated *ex vivo* was performed for optimized formulations [10].

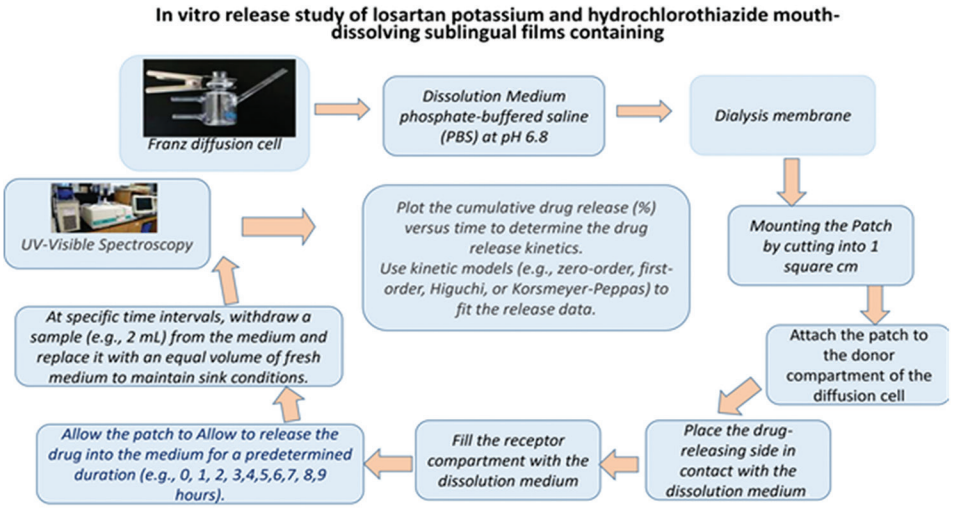

Fig. 2: *In vitro* drug release study

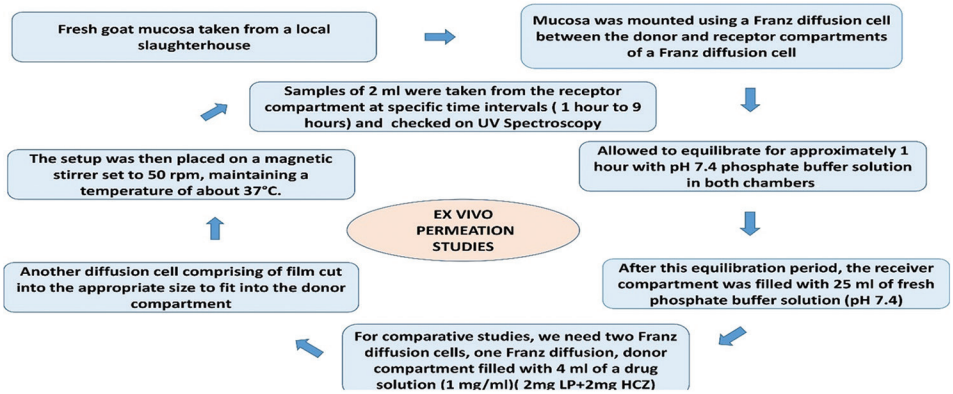

Fig. 3: *Ex vivo* permeation study

Kinetic modeling and drug release mechanism

Kinetic modeling and drug release mechanism studies are essential for the successful development, optimization, and regulation of drug delivery systems, ensuring that they are safe, effective, and reliable for patient use. The purpose of studying kinetic modeling and drug release mechanisms in pharmaceutical research is to understand and predict how a drug is released from its formulation over time. This information is crucial for developing effective and reliable drug delivery systems. One proposed mechanism for drug release from hydrophilic matrices involves water penetration, polymer swelling, drug diffusion, and the erosion of the gelatinous layer. Drug release in FDF is controlled by diffusion through the matrix, following Fick's law, and is influenced by pH, drug properties, and polymer characteristics. This was analyzed using the following mathematical models [11-15].

1. Zero-order equation

$$C=C0-K0\ t$$

Where, C=Amount of drug released or dissolved (occurs rapidly after the drug is dissolved.) C0=Initial amount of drug in solution (it is usually zero), K0= Zero order rate constant, t=time

2. First-order equation

$$Log\ C= Log\ C0-Kt/2.303$$

Where, C0=Initial concentration of drug, K=First-order constant t=time

3. Higuchi equation

$$C=[D\ (2qt-Cs)\ Cst]\ 1/2$$

Where, C=total amount of drug release per unit area of the matrix (mg/cm^2), D=diffusion coefficient for the drug in the matrix (cm^2/h), qt=total amount of drug in a unit volume of matrix (mg/cm^3), Cs=dimensional solubility of drug in the polymer matrix (mg/cm^3) t=time (h).

4. Korsmeyer and Peppas' equation

$$Ct/C\infty=ktn$$

Where, Ct/C∞=fraction of drug release at time "t", k=rate constant, n=release exponent

5. Hixson–Crowell equation

$$C01/3-Ct1/3=KHCt$$

Where, Ct=amount of drug released in time t. C0=initial amount of drug in the tablet. KHC=rate constant for Hixson-Crowell equation.

Stability studies

Stability studies were performed by utilizing ICHQ1(C) guidelines for Stability Testing for New Dosage Forms. Stability studies aimed to ensure that mucosal films maintain their efficacy, safety, and quality

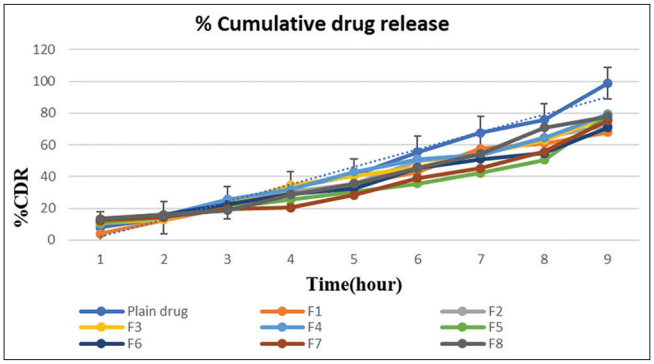

Fig. 4: Cumulative percentage drug release profiles from all formulations containing HPMCE15, HPMCE5, and PEG400. Each value represents the mean±standard deviation (n=3)

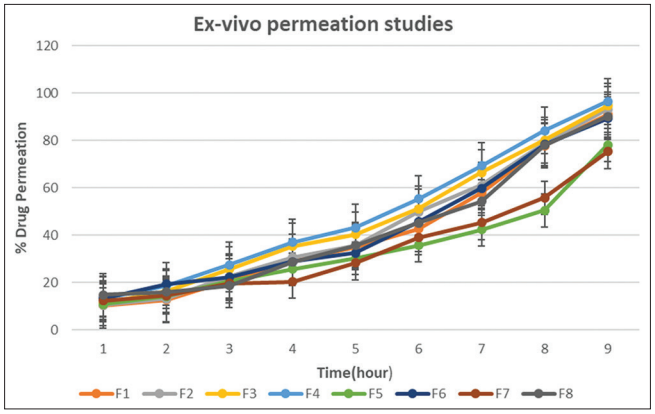

Fig. 5: *Ex vivo* permeation of films through goat buccal mucosa

throughout their shelf life. This involves assessing changes in their properties under various environmental conditions. Intermediate stability studies at 40°C and 75% relative humidity (RH) were employed where the film was stored under high temperatures and humidity to demonstrate shelf life in a shorter period. During the study, we monitored the thickness, flexibility, colour, texture, integrity, disintegration time, folding endurance, Tensile strength, and Drug release.

RESULT AND DISCUSSION

In vitro drug release study

The *in vitro* drug release study of losartan potassium and HCZ was evaluated using phosphate buffer (pH 6.8) and 0.5% Sodium Dodecyl Sulphate(SDS) at 37°C at 100 rpm for 9 h. The release of LP and HTZ from different formulations was observed to vary as shown in Fig. 4. The film was designed to obtain controlled release, which was achieved by the film's composition and structure. HPMC E15 was taken from 0.5 to 5%. HPMC E5 0.5 to 5% and PEG400 5%. The plain drug showed 98.76% release in 9 h whereas the F4 batch was considered as an optimized batch showing a release of 79.23%. In other batches, F1 showed release from 3.98 to 67.89% from 1st to 9th h similarly F2 (10.6–72.34%) F3 (10.9–77.68%) F5 (10.9–78.23%), F6 (12.89–70.90), F7 (12.42–75.32), F8 (13.56–77.90) respectively. HPMC E15 and E5, after heating form a gel and, upon cooling show film-forming capacity. Because of their capacity to produce gels that regulate the release of active pharmaceutical ingredients (APIs), they are utilized in controlled-release formulations. Due to their mucoadhesive qualities, both of the polymers included in the formulations aid in the film's adhesion to the mucosal surfaces. PEG 400 acts as a plasticizer in the formulations, improving the flexibility and texture of the resulting film. Both HPMC E15 and E5 have excellent gel-forming abilities when they come into contact with saliva or bodily fluids. The film gets hydrated and forms a dense gel layer. This gel barrier slows down the penetration of water into the film and the diffusion of the drug out of the film, thereby controlling the release rate.

Ex vivo permeation of lP and HCZ through goat buccal mucosa from films

Based on the *in vitro* drug release of all formulations, the F1–F8 formulations were selected for *ex vivo* drug permeation studies. The results of drug permeation from the films through the goat sublingual mucosal membrane revealed that the medication was released from the formulation and penetrated the goat sublingual mucosal membrane, indicating that it might potentially penetrate the human sublingual membrane. The results shown in Fig. 5 indicate that the drug permeation was slow and steady. The batch F4 was considered as an optimized batch due to the highest permeation as compared to other batches and is found to be 96.54±1.23% in 9 h and the flux was calculated to be 0.975±1.15 mg $h^{-1}cm^{-2}$, 0.904±0.92 mg $h^{-1}cm^{-2}$, and 0.901 mg $h^{-1}cm^{-2}$ (Target flux 0.991 mg $h^{-1}cm^{-2}$). Comparative analysis

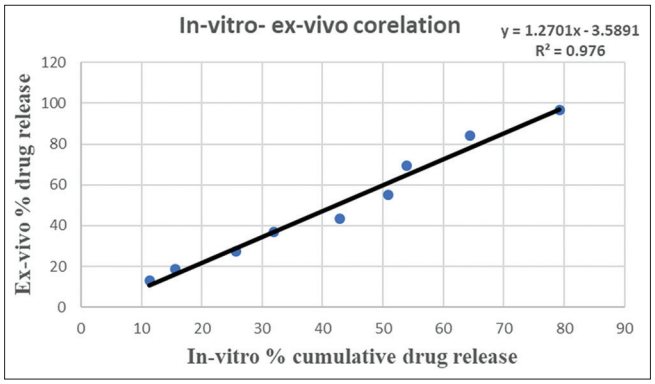

Fig. 6: *In vitro ex vivo* correlation between cumulative percentage drug released *in vitro* and percentage drug permeated *ex vivo* of optimized film

between plain drug and optimized batch found to be 0.005. Hence, the model was found to be significant.

In vitro and *ex vivo* correlation

In the study, the correlation between the cumulative percentage of drug released *in vitro* and the cumulative percentage of drug permeated *ex vivo* was manifested, for the optimized LP-HCZ film as illustrated in Fig. 6. The cumulative percentage of drug that permeated through the goat buccal membrane was compared to the cumulative percentage of drug released *in vitro* for the optimized film formulations. The data in Fig. 6 shows a strong correlation with a high R^2 value of 0.976, demonstrating a good relationship between *in vitro* drug release and *ex vivo* drug permeation across the goat buccal mucosa. This high correlation, despite the differing test conditions, suggests that the film could be an effective carrier for buccal drug delivery systems.

Kinetic modeling and drug release mechanism

Mathematical models are essential for understanding drug release kinetics. The *in vitro* and *ex vivo* drug release profile was analyzed using Zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas models and evaluated by correlation coefficient (r^2) for losartan potassium and HCZ film, as shown in Tables 1 and 2. The Higuchi square root model best fit the drug release data as r^2 values for *in vitro* as well as *ex vivo* permeation studies are closer to 1 for all formulations indicating a diffusion-controlled release.

Kinetic modeling for *In Vitro* drug release profile of (LP-HCZ film)

The results for Kinetic modeling for *In Vitro* drug release profile of LP-HCZ film are summerized in Table 1.

Kinetic modeling for *ex vivo* permeation profile

The results for Kinetic modeling for *Ex-vivo* drug permeation profile of LP-HCZ film are summerized in Table 2.

Table 1: Results of r^2 value for different models for (LP-HCZ film)

Formulation code	Zero-order model	First-order model	Higuchi model	Hixson-Crowell model	Korsmeyer Peppas model	Best-fitted model
	r2	r2	r2	r2	r2	
F1	0.8127	0.8054	**0.9625**	0.9198	0.8079	Higuchi model
F2	0.8119	0.8422	**0.9616**	0.9192	0.8085	
F3	0.8238	0.8972	**0.9646**	0.9339	0.8117	
F4	0.7976	0.958	**0.9614**	0.9467	0.8069	
F5	0.8106	0.9109	**0.9580**	0.9359	0.8060	
F6	0.8414	0.9213	**0.9656**	0.9406	0.8163	
F7	0.8393	0.9302	**0.9677**	0.9492	0.8160	
F8	0.8278	0.8962	**0.9846**	0.9339	0.8137	

Table 2: Results of r^2 value for different models for (LP-HCZ film)

Formulation code	Zero-order model	First-order model	Higuchi model	Hixson-Crowell model	Korsmeyer Peppas model	Best-fitted model
	r2	r2	r2	r2	r2	
F1	0.8117	0.9671	**0.9704**	0.9099	0.8009	Higuchi model
F2	0.8209	0.8022	**0.9716**	0.9512	0.8175	
F3	0.9036	0.8072	**0.9896**	0.9390	0.8007	
F4	0.7076	0.9581	**0.9719**	0.9807	0.8099	
F5	0.8796	0.9309	**0.9689**	0.9609	0.8060	
F6	0.8511	0.9813	**0.9750**	0.9306	0.8173	
F7	0.8613	0.9002	**0.9877**	0.9202	0.8023	
F8	0.8278	0.8162	**0.9949**	0.9009	0.8341	

Table 3: Stability test results of the optimized batch

Testing	Room temperature and ambient humidity	40°C and 75%RH
Physical appearance	Transparent, non-tacky Films	Transparent, non-tacky films
Weight (mg)	135.21±1.25	137.0±2.35
Thickness (mm)	0.210±0.005	0.22±0.02
Folding endurance	900±3.5	896±2.0
Disintegration time (s)	72.56±2.61	75.0±1.92
Tensile strength (g/cm^2)	77.46±1.02	78.02±1.76
Drug release (%) of LP	97.21±1.59	98.33±1.87
Drug release (%) of HCZ	99.09±0.81	96.20±0.71

Results are expressed as mean±SD (n=3)

Table 4: Long-term stability studies of optimized batch performed at 30±2°C/65±5% RH

Time (h)	0 day	3rd month	6th month	9th month	12th month
1	12.56	10.9	12.89	12.42	13.56
2	18.98	14.2	15.98	14.52	15.96
3	25.69	20.45	22.35	19.63	18.63
4	30.25	25.63	28.78	20.36	28.63
5	46.34	30.25	32.52	28.34	35.63
6	51.23	35.63	45.63	38.96	45.27
7	55.63	42.38	50.98	45.32	54.39
8	60.32	50.56	54.78	55.78	70.9
9	74.63	78.23	79.9	80.32	85.9

Stability study

The optimized formulation did not show any visual change in appearance and the results of the entire test conducted are tabulated in Tables 3 and 4 were found to be within limits which indicates the stability of the formulation. From the long-term stability study data, it is proved that drug release remains stable in between 74.63% from 0 da y and 85.9% of 12 month.

CONCLUSION

The conclusion of a study on LP-HCZ sublingual film demonstrated that controlled release would typically emphasize efficacy, safety, convenience, and compliance as potential applications. The findings from the study suggest that LP-HCZ sublingual film could be beneficial in treating conditions where steady drug levels are critical, potentially leading to better overall outcomes. Further studies might be recommended to explore the long-term effects of this delivery system, its applicability to other medications, and its performance across diverse patient groups.

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AUTHOR CONTRIBUTIONS

Authors Vinayata A. and Kratika D. conceived and designed the study. Vinayata A. performed the experiments, analyzed the data, and drafted the manuscript. Kratika D. contributed to experimental design, reviewed the manuscript, and provided critical feedback. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no conflicting interest

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