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DEVELOPMENT, OPTIMIZATION, AND PHARMACOKINETIC EVALUATION OF A NOVEL SUBLINGUAL LEVOTHYROXINE SODIUM TABLET: A PROMISING ALTERNATIVE TO CONVENTIONAL ORAL THERAPY

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ABSTRACT

Oral administration of Levothyroxine sodium tablets such as Synthroid requires strict dosing conditions to achieve consistent absorption—patients must take the tablet with a full glass of water on an empty stomach, at least 30 to 60 minutes before breakfast, and at least 4 hours apart from drugs that interfere with Levothyroxine Sodium uptake. Furthermore, dose adjustments may be needed when dosing within one hour of certain foods known to affect Levothyroxine Sodium absorption. These stringent requirements often lead to poor adherence and variable bioavailability. To overcome these limitations, a novel sublingual (SL) Levothyroxine Sodium tablet was developed using a direct compression approach optimized for rapid disintegration and mucosal absorption. The formulation was evaluated for physicochemical properties, in vitro dissolution in simulated salivary fluid, in vivo pharmacokinetics in rabbits, and stability under ICH conditions. Dissolution studies in salivary medium (pH 6.5) up to 12 minutes demonstrated 40%, 35%, and 30% Levothyroxine Sodium release for Batches #30, #29C, and #017, respectively. Pharmacokinetic evaluation in Rabbits revealed that Batches #29C and #30 were bioequivalent to Synthroid® (AbbVie Inc.), showing faster absorption (Tmax 4.0–7.0 h) and comparable systemic exposure (AUC_{0-24} 99.9–113.3%). Stability testing at 40°C/75% RH (6 months) and 25°C/60% RH, 30°C/65% RH (12 months) confirmed assay retention (95–99%) and related substances within ICH limits. The developed sublingual Levothyroxine sodium, tablet thus represents a clinically advantageous and patient-friendly alternative to conventional oral therapy, eliminating fasting and timing restrictions while maintaining bioequivalent systemic exposure and long-term stability.

KEYWORDS: Levothyroxine sodium, sublingual tablet, bioavailability, hypothyroidism, pharmacokinetics, LC-MS, direct compression.

1. INTRODUCTION

The thyroid gland plays a central role in endocrine regulation, synthesizing the iodinated hormones thyroxine (T4) and triiodothyronine (T3), which are essential for maintaining metabolic rate, growth, and overall energy homeostasis.[1] Hypothyroidism is one of the most prevalent endocrine disorders and may arise from autoimmune thyroid destruction, iodine deficiency, thyroidectomy, or impaired pituitary function. Current clinical guidelines consistently identify Levothyroxine Sodium as the therapy of choice for restoring normal thyroid hormone levels and for suppressing thyroid-stimulating hormone (TSH) in the management of differentiated thyroid carcinoma.[2]

Despite its widespread use, Levothyroxine presents notable formulation and therapeutic challenges. The drug's narrow therapeutic index, sensitivity to excipients, and dependence on gastrointestinal conditions have raised continued concerns regarding the consistent performance and bioequivalence of available products.[3] Moreover, several commonly used medications and

dietary components have been shown to interfere with Levothyroxine absorption, contributing to variability in serum TSH levels and complicating long-term disease management.[4]

The FDA Bioavailability and Bioequivalence Guidance (2018) specifies that Levothyroxine BE assessments must include baseline (endogenous) correction due to the naturally circulating thyroxine (T4) present prior to dosing. Plasma concentrations must therefore be corrected by subtracting the pre-dose baseline value to accurately quantify exposure to exogenously administered Levothyroxine. Furthermore, because Levothyroxine absorption is significantly reduced under fed conditions, the FDA recommends conducting bioequivalence studies exclusively in the fasted state to minimize food-related variability and ensure reliable assessment of systemic exposure.[5]

Given these complexities, regulatory authorities, including the U.S. FDA, emphasize stringent requirements for evaluating bioavailability and bioequivalence of Levothyroxine formulations to ensure therapeutic reliability and minimize patient risk.[5] These expectations have spurred extensive research into both conventional and emerging delivery approaches designed to enhance the predictability of Levothyroxine exposure.[6]

Among these alternatives, sublingual drug delivery has attracted increasing interest due to its unique advantages: rapid absorption through the highly vascularized sublingual mucosa, bypass of hepatic first-pass metabolism, and reduced influence from gastric pH or food intake.[7] In parallel, liquid and solution-based Levothyroxine formulations have demonstrated significant clinical benefits, particularly in patients with gastrointestinal disorders or altered gastric acidity, where they improve absorption consistency and overall treatment outcomes.[8,9] Additional evidence further supports the utility of soft-gel capsules and liquid preparations even when administered with meals, offering improved pharmacokinetic stability compared to traditional tablets.[10,11]

Pharmacokinetic comparisons have also confirmed that oral solutions provide more predictable systemic exposure relative to tablet formulations, reinforcing the need to consider alternative dosage forms for patients experiencing absorption variability.[12] Furthermore, permeation studies have shown that Levothyroxine is capable of transport across biological membranes, including skin and mucosal tissues, providing a mechanistic foundation for the development of non-oral or enhanced-absorption delivery systems.[13]

Collectively, the limitations of conventional oral Levothyroxine therapy—combined with growing evidence supporting alternative delivery routes—highlight the need for innovative formulations that can overcome gastrointestinal variability and deliver more consistent systemic exposure. Developing such approaches holds substantial promise for improving therapeutic accuracy, patient adherence, and long-term clinical outcomes in individuals requiring lifelong thyroid hormone replacement.

2. MATERIALS AND METHODS

2.1 Materials

Active pharmaceutical ingredient (API): Levothyroxine sodium

Excipients: Mannitol, SMCC HD90 (silicified microcrystalline cellulose), sodium bicarbonate, Butylated hydroxyanisole (BHA), Croscarmellose sodium, sodium alginate, Glyceryl monostearate, magnesium stearate.

2.2 Formulation Development

Sublingual tablets were prepared by the direct compression method. The active pharmaceutical ingredient (API) was geometrically blended with excipients to ensure uniform mixing and content uniformity. The final lubricated blend was compressed into tablets using optimized compression force to achieve desirable mechanical strength and rapid disintegration.

The formulations were optimized for immediate disintegration and rapid dissolution in simulated salivary fluid (pH 6.8).

Three optimized experimental batches-#017, #29C, and #30-were selected for pharmacokinetic evaluation.

Table 01: Composition of Sublingual formulations.

S.No	Component	Batch #017 (mg)	Batch #29C (mg)	Batch #30 (mg)
1	Levothyroxine sodium	0.334*	0.334*	0.334*
2	Mannitol	43.51	13.6	37.78
3	SMCC	6.0	9.2	6.0
4	Sodium bicarbonate	6.0	6.0	6.0
5	Croscarmellose sodium	1.2	3.0	3.0
6	Sodium alginate	1.8	2.0	1.8
7	Glyceryl monostearate	0.6	1.5	1.5
8	Sodium caprate	—	3.0	3.0
9	Magnesium stearate	0.36	0.55	0.5

Total tablet weight	**60 mg**	**60 mg**	**60 mg**
	*Equivalent to 0.300mg of Levothyroxine		

Manufacturing Process

1. Sifted Mannitol and SMCC HD90through #40 mesh and divide into four portions (12%, 24%, 24%, and 40%).
2. Loaded 24% portion into the blender and mix for2 minutes at 12 rpm to achieve surface saturation**.**
3. Co-sift Mannitol, SMCC HD90, and the API through #40 mesh.
   Re-sift this mixture with sodium bicarbonate and Butylated hydroxy anisole (BHA)**.**
4. Add the Step 3 mixture to the blender (from Step 2), then add the second 24% portion of Mannitol + SMCC HD90. Blend for15 minutes at 12 rpm**.**
5. Sift the remaining Mannitol + SMCC HD90, along with Croscarmellose sodium, sodium alginate, and Glyceryl monostearate, through #40 mesh**.**
6. Blend Step 4 and Step 5 materials together for 15 minutes at 12 rpm**.**
7. Add magnesium stearate (sifted through #60 mesh) and lubricate the blend for5 minutes at 12 rpm**.**
8. Compress the lubricated blend into tablets using 7.0mm round shaped D tooling punches.

Table 02: Compression parameters.

	Batch #017	**Batch #29C**	**Batch #30**
Description	**White to off white, round tablets with break line on one side and plain on the other side**		
Turret speed (RPM)	20-30RPM		
Tablet weight (mg)	60mg	60mg	60mg
Hardness kp	1.2-1.5	1.1-1.14	1.2-1.4
Thickness mm	1.50 mm	1.40-1.50	1.42-1.524
Disintegration	15sec	40sec	10sec
Wetting time	20sec	30sec	18sec
Friability	0.2	0.36	0.25

2.3 In-vitro dissolution study in saliva medium

The tablets were evaluated for hardness, friability, disintegration time, and in vitro dissolution.

Dissolution studies were conducted in simulated salivary fluid pH 6.5, paddle, 50 rpm, and 50ml.Samples were withdrawn at 2,4,6,8,10 and 12mins, filtered, and analyzed for Levothyroxine sodium content by HPLC method.

Dissolution testing was performed using 50 mL of simulated salivary fluid in a specially designed 150 mL miniature dissolution vessel to better mimic the physiological salivary volume and obtain more realistic in-vivo–relevant dissolution data.

2.4 In Vitro Dissolution Study in QC media

Dissolution studies were conducted in 0.01N HCl +0.1% SLS using paddle, 50rpm, 500ml maintained at 37 ± 0.5 °C, Samples were withdrawn at 5, 10,15,20,30, and 45 minutes, filtered, and analyzed for Levothyroxine sodium content.

2.5 Stability Studies

Stability studies were conducted in accordance with ICH Q1A (R2) guidelines under the following conditions: Accelerated – 40 °C / 75 % RH for 6 months; Long-term – 25 °C / 60 % RH and 30 °C / 65 % RH for 12 months. Samples were evaluated for appearance, disintegration time, assay, related substances (RS), water content, and dissolution performance in QC media.

The results demonstrated excellent stability: Assay values remained at approximately 95 % after 6 months under accelerated conditions and 98–99 % after 12 months under long-term storage. All measured parameters, including impurities, remained well within ICH acceptance limits (<1%), confirming the formulation's chemical and physical stability throughout the study period.

2.6 In Vivo Pharmacokinetic Study

The animal study was approved by the Institutional Animal Ethics Committee (IAEC approval) under CPCSEA guidelines.

Pharmacokinetic evaluation was conducted in male New Zealand white rabbits (n = 4 per group) to compare the bioavailability of the developed sublingual LT4 tablet administered under fed conditions while the reference marketed oral tablet (Synthroid®) was administered under fasting conditions. Animals were anaesthetized for sublingual administration and held in position for 30 min after which they were allowed free.

The study design consisted of four parallel groups:
Group I: Synthroid® oral tablet administered under fasting conditions.
Group II: Sublingual LT4 tablet administered under fed conditions.
Group III: Sublingual LT4 tablet administered under fed conditions.
Group IV: Sublingual LT4 tablet administered under fed conditions.

Each animal received a single dose (2*0.300mg=0.600mg) of the assigned formulation. Blood samples were collected from the marginal ear vein at predetermined intervals up to 24 hours post-dose. Plasma was separated by centrifugation and stored at –20 °C until analysis.

2.7 Pharmacokinetic and Statistical Analysis

Levothyroxine plasma concentrations were quantified using a validated LC–MS method, ensuring accuracy, precision, and linearity across the expected concentration range. A baseline correction was made since Levothyroxine is present endogenously.

Pharmacokinetic parameters (Cmax, Tmax, and $AUC_{0–24}$) were calculated using non-compartmental analysis (Phoenix WinNonlin®). Comparative bioavailability between formulations was assessed, with bioequivalence concluded if the 90% confidence intervals for the test/reference ratios of AUC and Cmax are within 80–125%.

3. RESULTS

A comparative dissolution study was conducted for Synthroid® and the three test formulations (Batch #017, Batch #029C, and Batch #030) using **0.01N HCl containing 0.1% SLS**, with USP **paddle apparatus at 50 rpm** and a **500 mL** medium volume.

The percentage drug release and %RSD at each sampling interval are summarized in Table 03.

Table 03: Dissolution data in 0.01N HCl +0.1% SLS, paddle, 500ml, 50rpm comparison.

Batch	Synthroid		Batch #017		Batch #029C		Batch #030	
Time mins	%Rel	%RSD	%Rel	%RSD	%Rel	%RSD	%Rel	%RSD
5	88.0	4.2	84.7	5.2	85.7	5.6	85	1.8
10	92.5	5.0	91.1	3.0	92.5	4.9	94	2.4
15	94.8	6.4	91.8	2.4	93.9	4.7	96	3.9
30	96.8	2.8	93.8	3.8	94.8	5.0	97	6.5
Recovery	100.6	2.1	99.6	2.3	97.9	3.5	101	3.0

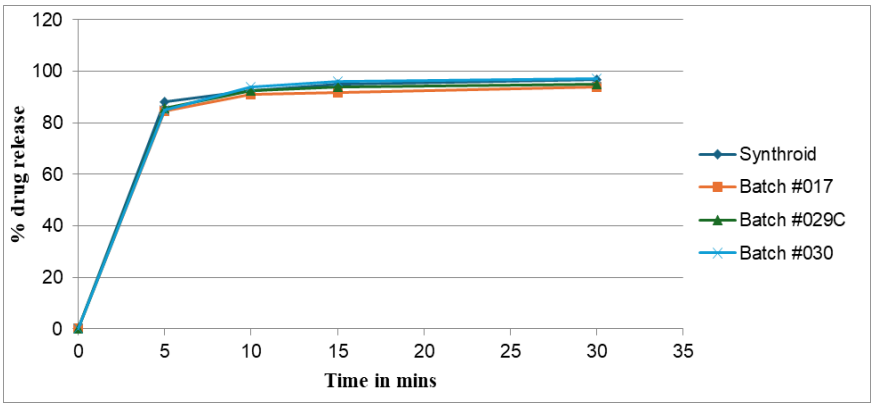


Fig. 1: Dissolution Profiles of Levothyroxine Sodium in 0.01 N HCl + 0.1% SLS (Paddle, 50 rpm, 500 mL).

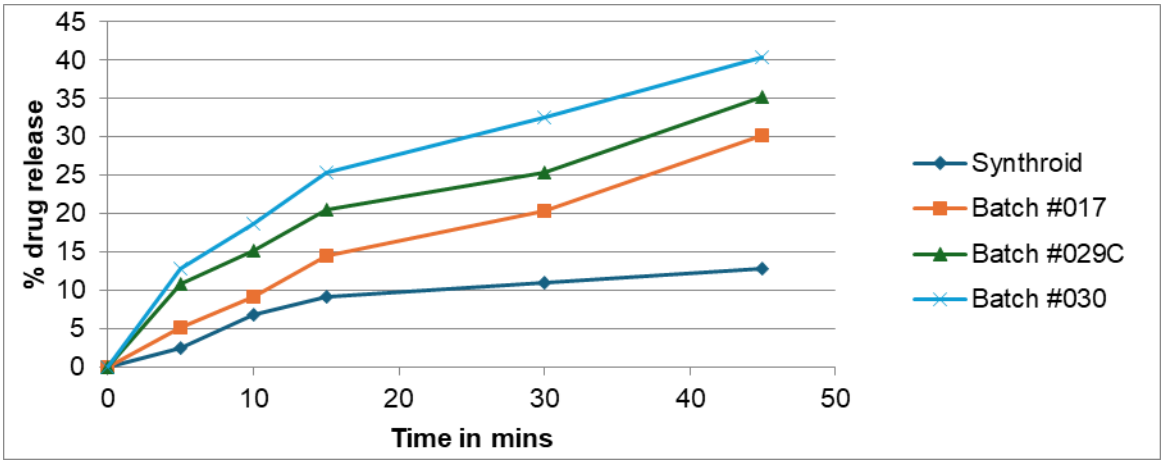
Observation: All batches—including Synthroid, batches 017, 029C, and 030—show fast and consistent dissolution in the QC release media. More than 85% drug release at 5 minutes **and** >94% by 30 minutes is seen across all batches. %RSD values remain low at all-time points, indicating good precision and uniformity. Recovery for all batches (≈98–101%) meets acceptable analytical limits.

A dissolution comparison was carried out for Synthroid® and the three test formulations (Batch #017, Batch #029C, and Batch #030) in salivary media (pH 6.5) using a USP paddle apparatus at 50 rpm with a 50 mL medium volume.

The percentage drug release and corresponding %RSD values for each time point are presented in Table 04.

Table 04: Dissolution data in salivary media pH-6.5, paddle, 50rpm, 50ml comparison.

Batch	Synthroid		Batch #017		Batch #029C		Batch #030	
Time (min)	%Rel	% RSD	%Rel	% RSD	% Rel	%RSD	% Rel	% RSD
2	2.5	11.6	5.1	11.6	10.8	11.0	12.8	10.5
4	6.8	23.1	9.2	23.1	15.2	15.8	18.6	20.5
6	9.2	20.0	14.5	20.0	20.5	11.2	25.4	15.5
10	11.0	24.7	20.4	24.7	25.4	12.8	32.6	10.8
12	12.8	7.2	30.2	7.2	35.2	8.4	40.4	5.2



Observation: Based on the dissolution data in salivary media (pH 6.5), Synthroid consistently shows the slowest drug release at every time point—for example, only 2.5% at 2 min, 6.8% at 4 min, and 12.8% at 12 min. In contrast, the test formulations release noticeably more drug over the same period. At 12 min, Batch 017 releases 30.2%, Batch 029C releases 35.2%, and Batch 030 shows the highest release of 40.4%.

These results clearly indicate that Synthroid has a slower dissolution rate in salivary media compared to the test batches. Among the test formulations, Batch 030 consistently shows the fastest and highest release, making it the most promising candidate to take forward.

3.2 Pharmacokinetic Parameters

Table 05: Pharmacokinetic data of Levothyroxine sodium in Rabbits.

Formulation	Cmax (ng/mL)	AUC_{0-24} (ng·h/mL)	Tmax (h)	Cmax Geometric mean T/R (%)	90% CI for Cmax T/R	AUC Geometric mean T/R (%)	90% CI for AUC T/R
Synthroid (R)	67.97 ± 3.4	1080.0 ± 42.1	7.0	—	—	—	—
Batch #017	64.11 ± 4.1	754.86 ± 60.5	6.0	93.1	85.7-103.8	69.9	62.9-77.6
Batch #29C	77.80 ± 3.9	1079.0 ± 55.2	7.0	111.9	105.3-124.4	99.9	92.6-107.8
Batch #30	75.30 ± 2.8	1223.3 ± 62.7	4.0	112.6	103.0-119.2	113.3	105-122.2

Plasma concentration–time profiles of Levothyroxine Sodium after sublingual administration of test formulations (Fed state) and oral administration of Synthroid® (Fasting state) in rabbits (n=4) are presented in Fig.1.

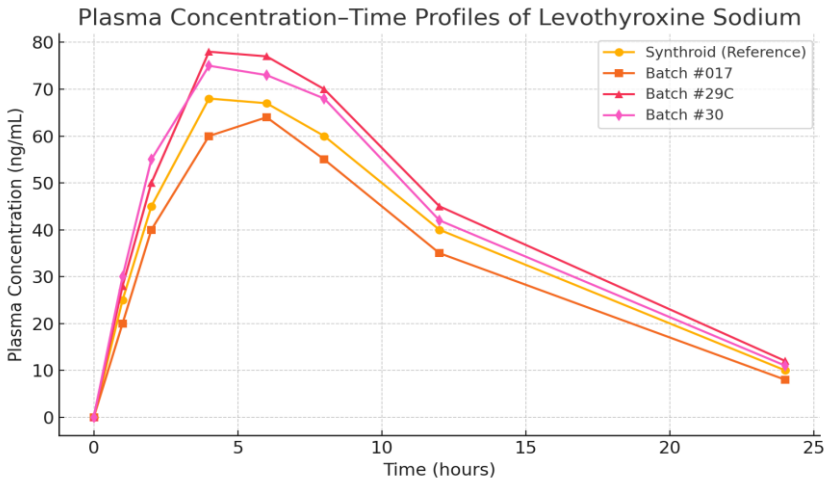


Fig. 3: Plasma Concentration–Time Profiles of Levothyroxine Sodium after Sublingual and Oral Administration.

OBSERVATION

Among the three test formulations, Batch 017 exhibits a lower Cmax and a markedly reduced AUC_{0-24}, with 90% confidence intervals falling outside the accepted bioequivalence range, indicating it is not comparable to Synthroid®. In contrast, Batches 29C and 30 show Cmax and AUC values closely aligned with the reference product, with both parameters falling within the 80–125% bioequivalence limits. Notably, Batch 30 also demonstrates a faster absorption profile with a Tmax of 4 hours, making it more suitable for the intended sublingual delivery. It is evident that absorption of Levothyroxine is not complete in mouth but continued in GIT but the initial drug absorption from sublingual route supported the better bioavailability even in fed condition.

4. DISCUSSION

The pharmacokinetic comparison between sublingual Levothyroxine sodium tablets administered in the fed state and oral Synthroid® administered under fasting conditions demonstrated that the sublingual formulation achieved bioequivalent systemic exposure despite the presence of food. This finding highlights the potential of sublingual delivery to overcome the fasting restrictions associated with conventional oral Levothyroxine sodium therapy,

The comparable PK profiles of Batches #29C and #30 emphasize the importance of excipient optimization in promoting mucosal permeability and stability. It can be expected that higher drug absorption from sublingual route compensated the drug loss from food and hence, provided bioequivalence.

Sodium caprate enhanced sublingual absorption by transiently altering lipid bilayer permeability. Sodium bicarbonate maintained Levothyroxine Sodium stability in the microenvironment, while mannitol and Croscarmellose sodium ensured rapid disintegration. The faster Tmax and bioequivalence observed support the potential of sublingual delivery for improved therapeutic consistency and adherence.

Sublingual route of Levothyroxine sodium demonstrated improved bioavailability. However, future studies in human volunteers are required to validate these pharmacokinetic trends and confirm clinical bioequivalence.

5. CONCLUSION

The novel sublingual Levothyroxine sodium tablet provides a viable alternative to oral administration, offering equivalent bioavailability, faster absorption, and enhanced patient convenience. Batch #29C demonstrated near-identical AUC (99.9%) and a slightly higher Cmax (111.9%), Batch #30 demonstrated higher AUC (113.3%) and Cmax (112.6%), than Synthroid®, fulfilling regulatory bioequivalence criteria. This platform has strong potential for improving treatment adherence, especially in patients with gastrointestinal malabsorption or difficulty in maintaining compliance for oral tablet.

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7. Abbreviations

AUC – Area Under the Curve; Cmax – Maximum Plasma Concentration; Tmax – Time to Maximum Concentration; LC–MS – Liquid Chromatography–Mass Spectrometry; SMCC – Silicified Microcrystalline Cellulose; IAEC – Institutional Animal Ethics Committee.

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