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Research Article

Comparative Pharmaceutical Evaluation and Bioavailability Assessment of Levothyroxine Sodium in Different Dosage Forms

Chinna Reddy Palem, [1]*, Prashant Noolu [2], Praveen Rao Balguri [2], Varun Chilukoti [2], Dasarath Gurram [2], Nishanth Kumar Nagamalli [1], Sridhar Gumudevelli [2]

[1] R&D, Asphar Research Labs Pvt. Ltd., IDA, Balanagar, Hyderabad-500037; Telangana, India.

[2] Ascent Pharmaceuticals Inc., 400S.Technology Drive, Central Islip, NY 11722. USA.

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For Correspondence:

Dr. Palem Chinna Reddy, Asphar Research Labs Pvt. Ltd. 3rd Floor, Plot No. 47, Industrial Development Area, Balanagar, Hyderabad - 500037, Telangana, India.

Abstract

Levothyroxine sodium is recognized as a narrow-therapeutic-index drug, making its clinical performance highly sensitive to formulation variables that can influence systemic bioavailability. This study focused on the development, physicochemical evaluation, and comparative bioavailability assessment of levothyroxine sodium formulated into three distinct dosage forms: tablets, HPMC based capsules, and soft gelatin capsules. Each formulation was manufactured using optimized processing strategies to ensure stability and dose accuracy. Physical evaluation and comprehensive in vitro testing, including assay, content uniformity, dissolution profiling, and analysis of related substances, were conducted in accordance with compendial standards. All three dosage forms exhibited satisfactory predefined quality criteria, indicating acceptable pharmaceutical performance. In vivo characterization was carried out in healthy volunteers using a randomized, open-label, two-treatment, two-period crossover design to compare the test products with the reference capsule. Blood samples were collected over an extended sampling period, and levothyroxine plasma concentrations were quantified using a validated analytical method. Pharmacokinetic parameters, including C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, were derived through non-compartmental analysis and evaluated for bioequivalence using standard statistical approaches. The in vivo results demonstrated that the soft gelatin capsules exhibited bioavailability parameters closely aligned with those of the reference product, with 90% confidence intervals for both rate and extent of absorption falling within accepted regulatory bioequivalence limits. Conversely, the directly compressed tablets and HPMC-based capsule formulations displayed higher inter-subject variability and did not consistently meet equivalence criteria across all pharmacokinetic endpoints. Among the dosage forms investigated, soft gelatin capsule provided the most reliable pharmaceutical quality and bioavailability performance relative to the reference product. These findings highlight the critical role of formulation design in achieving consistent levothyroxine exposure and support the liquid-filled soft gelatin system as a promising alternative for clinical application.

Keywords: Levothyroxine sodium; Different dosage forms; Soft-gelatin capsules; Bioavailability; Bioequivalence.

INTRODUCTION

Hypothyroidism is a prevalent endocrine disorder characterized by insufficient production of thyroid hormones, necessitating lifelong hormone-replacement therapy for most affected individuals. Levothyroxine sodium, a synthetic form of the endogenous thyroid hormone thyroxine (T4), is widely prescribed for the management of hypothyroidism and various thyroid disorders and is one of the most commonly prescribed medications globally and remains the cornerstone therapy for managing hypothyroidism.[1,2] Due to its narrow therapeutic index (NTI), even minor variations in systemic exposure can result in significant clinical consequences, including subtherapeutic replacement or hormone excess. Consequently, maintaining high consistency in dosage form performance and bioavailability is essential for ensuring therapeutic efficacy and patient safety.[3,4] The physicochemical properties of levothyroxine such as poor aqueous solubility, polymorphic transitions, and susceptibility to environmental conditions pose formulation challenges that can substantially affect its stability and absorption profile. Traditional solid oral dosage forms, including tablets and hard capsules, often exhibit variability in dissolution, potency uniformity, and bioavailability, reinforcing the need for robust formulation strategies.[5,6]

Oral solid dosage forms are essential for maintaining the stability, dosing accuracy, and therapeutic performance of Levothyroxine sodium, a drug with a narrow therapeutic index and pronounced sensitivity to formulation factors. Directly compressed tablets offer a simple and cost-efficient manufacturing approach, though their performance depends heavily on excipient

compatibility, powder flow, and environmental stability, critical considerations for a molecule prone to degradation.[7,8] Liquid-filled HPMC capsules provide a more advanced option by protecting moisture and heat-sensitive drugs and enabling solubilized or dispersed systems that can enhance dissolution and reduce variability in Levothyroxine absorption. Soft gelatin capsules further support consistent drug delivery through their hermetically sealed structure, which improves chemical stability and allows the drug to remain in a pre-dissolved state, often resulting in faster dissolution and more reliable bioavailability.[9] Recent advancements in drug delivery, such as soft gelatin capsules, offer potential advantages by improving solubilization, minimizing degradation, and enhancing dose uniformity. However, comparative data evaluating different levothyroxine dosage forms under standardized pharmaceutical and pharmacokinetic conditions remain limited.[10,11]

The present study was designed to develop and systematically evaluate multiple levothyroxine dosage forms directly compressed tablets, liquid-filled HPMC based capsules, and soft gelatin capsules. Physicochemical, comprehensive in vitro characterization, including assay, content uniformity, dissolution behaviour, and related substance profiling, was conducted in accordance with compendial standards. Additionally, a controlled clinical study was performed to investigate the bioavailability /bioequivalence of each formulation relative to the reference product. This integrated approach aims to elucidate the impact of formulation design on levothyroxine performance and to identify a dosage form that provides optimal consistency and regulatory compliance.

MATERIALS AND METHODS

Levothyroxine sodium was purchased from Azico Biophore India Pvt. Ltd. (Hyderabad, India).

Butylated Hydroxyanisole from Spectrum (New Brunswick, New Jersey), Microcrystalline Cellulose, USP/NF (Avicel PH 105) and Microcrystalline Cellulose NF (Avicel PH 102) from FMC International (Co. Cork, Ireland), Sodium Starch Glycolate Type A, NF (Primojel) from Roquette (Lestrem, France), Povidone, USP (Kollidon 30) from BASF (Ludwigshafen, Germany), Colloidal Silicon Dioxide, NF (Aerosil 200 Pharma) from Evonik (Antwerpen, Belgium), Magnesium Stearate, NF from Peter Greven (Venlo, The Netherlands), Gelatin NF/USP (150 Bloom Bone) and Gelatin NF/USP (100 Bloom SRM Free Bone Gelatine) from Gelita USA Inc (Chicago, United States), Glycerin 99.7%, USP from Finar Limited (Ahmedabad, India), Medium Chain Triglycerides USP/NF (Captex 355) from Abitec Corporation (Janesville, WI, United States), Lecithin, NF (Topcithin 200) from Univar / Cargill (Hammond, IN, United States), Isopropyl Alcohol, USP from Avantor (Radnor, USA) and all other solvents are AR / HPLC grade.

DEVELOPMENT OF DIFFERENT LEVOTHYROXINE FORMULATIONS

The present study aimed to develop multiple formulations of levothyroxine sodium and to compare their in vitro and in vivo performance characteristics. Three formulations were developed such as (i) tablets manufactured by direct compression, (ii) Liquid-filled HPMC Capsules, and (iii) soft gelatin capsules in which the drug was dispersed in a liquid or semi-solid vehicle. Variations in formulation matrices, excipient composition, and physical characteristics are expected to affect the rate and extent of drug release, potentially contributing to differences in therapeutic outcomes. Detailed descriptions of the manufacturing procedures for each formulation are provided in the subsequent sections.

(i). Tablet formulation: The levothyroxine sodium tablets were manufactured by direct blending / direct compression method, in which first sifting levothyroxine sodium (active pharmaceutical ingredient), BHA (anti-oxidant), and MCC PH105 (diluent) through an 80-mesh screen twice and blending them for 30 minutes. Separately, MCC PH 102 (diluent), colloidal silicon dioxide (glidant), sodium starch glycolate (disintegrant), and colorants were screened through an 80-mesh sieve and added to the initial blend, followed by 60 minutes of secondary blending. Magnesium stearate (lubricant) was then sifted and incorporated into the mixture with a 5-minute lubrication step to prevent over-mixing. The final lubricated blend was transferred to a tablet press and compressed under controlled conditions, with routine in-process checks to maintain uniform tablet weight, hardness, and thickness.[12,13]

(ii). Liquid-filled HPMC Capsules: Liquid-filled hydroxypropyl methylcellulose capsules (LF-HPMC) were selected as an alternative dosage form to enhance the performance of poorly water-soluble and lipophilic drugs. Unlike conventional powder-filled HPMC capsules, LF-HPMC systems can improve drug solubilization, reduce dissolution variability, and facilitate faster absorption. HPMC capsules are plant-derived shells composed primarily of hydroxypropyl methylcellulose and purified water, offering a non-animal, chemically inert, and highly stable alternative to gelatin. These two-piece capsules, consisting of a cap and body, are suitable for filling with dry powders, liquids, or semi-solid formulations. Capsule sealing can be achieved through banding, heat welding, or micro-spray sealing technologies, ensuring product integrity and preventing leakage. Their low moisture content, compatibility with sensitive active pharmaceutical ingredients (APIs), and non-reactive nature make HPMC capsules well-suited for modern pharmaceutical applications. In this current study, the preparation of the liquid-fill formulation, the levothyroxine sodium was dispersed using a solvent system composed of gelatin, glycerin (as a plasticizer), and water. This mixture served as the vehicle for encapsulation, enabling uniform drug distribution and improved fill performance within the LF-HPMC system.[14]

(iii). Soft gelatin capsules: The liquid-fill formulation was manufactured by first preparing a gelatin base in which water and edetate disodium were charged into a vessel and heated with continuous mixing until the temperature reached 80 - 85°C. Glycerin and gelatin were then added, and the mixture was further heated and mixed until a clear, particle-free solution was obtained, followed by vacuum de-aeration to remove entrapped air. In parallel, the fill material was prepared by heating glycerin to 70 ± 10°C and transferring it into the gelatin base, with mixing and heating continued for an additional 50 ± 10 minutes. The drug solution was prepared separately by charging glycerin into a container, initiating mixing under argon purging, and adding levothyroxine while maintaining mixing and purging for approximately 1 hour until a clear solution was formed. This drug solution was then transferred into the vessel containing the gelatin base and fill material, and the original container was rinsed with glycerin to ensure complete drug transfer. The combined mixture was subjected to vacuum de-aeration to eliminate residual air bubbles.[15,16] Prior to encapsulation, the final fill mass was sampled for assay confirmation, after which encapsulation was performed using a fully automatic liquid-filling capsule machine.

PHYSICO-CHEMICAL CHARACTERIZATION OF LEVOTHYROXINE SODIUM FORMULATIONS:

Appearance, physical integrity, and weight variation - All the dosage forms such as tablets, liquid-filled HPMC capsules, and soft gelatin capsules were evaluated for appearance and physical integrity as part of preliminary quality assessment. Tablets and capsules were visually inspected under standard laboratory lighting for colour uniformity, surface defects, shape, and overall structural integrity. Capsules were further examined for shell uniformity, absence of leakage, and presence of air bubbles within the fill mass to ensure proper encapsulation and formulation consistency. Weight variation was determined following pharmacopeial guidelines (USP/EP). For tablets, twenty individual units were randomly selected and weighed using an analytical balance (Sartorius, Secura 225D, Bangalore, India). The mean weight was calculated to assess uniformity in mass.[17] For liquid-filled capsules (both HPMC and soft gelatin), individual filled capsules were weighed to determine total mass, then carefully emptied to measure the shell and fill mass separately. The net fill weight was calculated as the difference, and the uniformity of fill was assessed across multiple units. All evaluations were performed in triplicate.

Tablet Hardness and Friability - The mechanical strength of the levothyroxine sodium tablets was evaluated using standard pharmacopeial methods. Tablet crushing strength was measured using a digital tablet hardness tester (Tabtest 401, Coimbatore, India). Ten tablets from each batch were randomly selected and placed individually between the instrument's jaws. The force required to break each tablet was recorded in kilopond's (kp). Mean hardness values were calculated to assess uniformity and ensure sufficient mechanical integrity for handling and packaging. Tablet resistance to abrasion was determined using a rotating drum friabilator (Electrolab/EF2W, Mumbai, India). Twenty tablets, previously weighed, were subjected to mechanical stress at 25 rpm for 4 minutes (100 rotations). After the test, tablets were de-dusted, and the final weight was measured. Friability was expressed as the percentage weight loss relative to the initial weight. Values less than 1% were considered acceptable, indicating that the tablets were mechanically robust and suitable for storage, handling, and transport.[18]

Moisture Content - Moisture content in each dosage form was determined using Karl Fischer (KF) titration (Metrohm, Titrando 901, Metrohm AG, Switzerland) employing a volumetric titrator equipped with an integrated moisture analyzer. For tablet formulations, representative units were finely powdered, and an accurately weighed portion was introduced directly into the titration vessel for analysis.[19] For the liquid-filled HPMC capsules, the moisture content of the capsule shell and the fill formulation was assessed separately. The fill mass was carefully removed, weighed, and subjected to KF titration, while the emptied shells were individually analyzed to determine their intrinsic moisture contribution. Given the inherently higher water content associated with soft gelatin capsule shells, KF measurements were performed on both the intact capsule and the separated fill mass. This approach enabled differentiation between moisture originating from the gelatin matrix and that associated with the encapsulated formulation.[20] All measurements were conducted in triplicate, and results were expressed as percentage moisture by weight.

Viscosity and Rheological Characterization - The rheological behaviour of the liquid and semi-solid fill formulations intended for capsule encapsulation was evaluated using a controlled-stress rotational rheometer (TA Instruments, Model: DHR-1, Delaware 19720, USA). Measurements were performed using a cone-and-plate geometry (40mm diameter, 2°cone angle) under temperature-controlled conditions set at 37 ± 0.5°C, corresponding to physiological and processing environments. Prior to analysis, samples were equilibrated at the test temperature for 5 minutes to ensure thermal stabilization. A shear rate sweep was conducted over an appropriate range (0.1–100 s^{-1}) to characterize flow behaviour and identify Newtonian or non-Newtonian profiles. Steady-state viscosity values were recorded at each shear rate, and the dynamic viscosity at relevant shear conditions was used to assess fill flowability. These rheological measurements provided critical information on the suitability of each formulation for encapsulation into HPMC or soft gelatin shells, including flow consistency, pumpability, and the ability to maintain uniform drug dispersion throughout the filling process.[21] All analyses were performed in triplicate to ensure reproducibility.

pH Determination of Liquid Fill Formulations - The pH of the levothyroxine sodium liquid fill formulations, including HPMC-based and soft gelatin capsule fills, was measured to evaluate chemical stability and compatibility with the capsule shells. Measurements

were performed using a digital pH meter (Polmon Instruments/ LP139SA, Hyderabad, India) calibrated with standard buffer solutions at pH 4.0, 7.0, and 10.0 prior to analysis. An accurately weighed portion of each liquid fill formulation was diluted with deionized water to allow proper electrode immersion without altering ionic strength significantly. The pH electrode was immersed directly in the sample, and readings were recorded after stabilization. Measurements were carried out in triplicate, and the mean values were reported. Consistent pH values within the acceptable range confirmed the chemical stability of the drug and compatibility with the capsule shell material, minimizing the risk of shell degradation or drug instability during storage.[22]

Capsule Shell Mechanical Properties - The mechanical integrity of HPMC and soft gelatin capsule shells was evaluated to ensure robustness during manufacturing, packaging, and storage. Mechanical properties assessed included tensile strength, elasticity, and brittleness. Capsule shell segments were carefully separated from the fill mass and subjected to mechanical testing using a Tensile Testing Apparatus (Electro Force 3300, TA Instrument, DE 19720, USA) equipped with a suitable load cell and custom grips for thin-walled specimens. For tensile strength, shells were cut into standard strips, mounted in the grips, and stretched at a controlled rate until rupture. Maximum load at failure was recorded, and tensile strength was calculated based on cross-sectional dimensions.[23]

Elasticity was determined from the stress strain curves generated during tensile testing, with the Young's modulus calculated as the slope of the initial linear portion of the curve, reflecting shell flexibility. Brittleness was assessed by evaluating the elongation at break and the energy absorbed prior to fracture. All measurements were performed at room temperature (25 ± 2 °C) and controlled humidity (40 - 60% RH), with a minimum of six replicates per capsule type to ensure reproducibility.

Assay and Content Uniformity – The levothyroxine sodium content of each dosage form was determined using a validated HPLC method. The analysis was performed on an HPLC system (HPLC Water Alliance e2695, MA 01757, USA) equipped with a UV–Vis detector, autosampler, and quaternary pump. Separation was achieved on a C18 reversed-phase analytical column (250 × 4.6 mm, 5 µm) maintained at ambient temperature. The mobile phase consisted of an aqueous buffer and organic solvent mixture (90:10 v/v), delivered at a constant flow rate of 1.0 mL/min. Detection was carried out at 280 nm. Ten dosage units from each formulation batch were individually transferred to volumetric flasks, dissolved in an appropriate diluent, and filtered before injection. Each sample solution was injected in duplicate. The assay values obtained for individual units were compared against pharmacopeial acceptance criteria. Composite sample was similarly prepared for Assay determination and analyzed using the same chromatographic conditions. Quantification was performed by comparing sample peak areas to those of freshly prepared levothyroxine sodium reference standards.

Disintegration Time - Disintegration time was evaluated in accordance with the United States Pharmacopeia (USP) general chapter <701> using a USP disintegration apparatus (Electrolab /ED2SAPOX, Mumbai, India.) equipped with six glass tubes, a basket-rack assembly, and automated time recording. The medium consisted of purified water maintained at 37 ± 0.5 °C to mimic physiological conditions. The basket rack was operated at the standard frequency of 29–32 cycles per minute**.** For tablet formulations, individual units were placed in the tubes without discs, and the endpoint was defined as the complete dispersion of the tablet with no palpable core or insoluble fragments remaining. For HPMC based liquid-filled capsules and soft gelatin capsules, testing focused on two key parameters- Shell rupture time, defined as the point at which the capsule shell exhibited visible cracking or splitting; and complete release of fill mass, confirmed when the entire liquid or semi-solid content was liberated into the disintegration medium. All measurements were performed in triplicate, and mean disintegration times were reported.

In vitro dissolution Studies – In vitro drug release from the levothyroxine sodium tablets and capsule formulations was assessed following USP General Chapter <711> using a USP Apparatus II (paddle) dissolution system. Testing was performed in 500 mL of simulated gastric fluid (SGF), pH 1.2 (without enzymes), maintained at 37 ± 0.5 °C to replicate gastric physiological conditions. Paddle rotation speeds between 50 rpm were evaluated, with the optimized speed selected based on method validation criteria for discriminatory capability and hydrodynamic stability. Each dosage unit was placed at the bottom of the vessel, and dissolution was carried out under sink conditions. At predetermined time intervals 5, 10, 15, 30, 45, and 60 minutes, 5 mL aliquots were withdrawn and an equal volume of pre-warmed dissolution medium was replaced after each sampling to maintain constant volume. Collected samples were immediately analyzed using the validated HPLC method described in the assay section. Levothyroxine sodium concentration was quantified based on peak area comparison with reference standards. Dissolution profiles of the different formulations tablets, HPMC liquid-filled capsules, and soft gelatin capsules were calculated and compared.

In Vivo Pharmacokinetic Evaluation

The in vivo study was conducted to compare the bioavailability, absorption rate, and systemic exposure of levothyroxine sodium administered as directly compressed capsules, liquid-filled HPMC capsules, and soft gelatin capsules in fasted healthy volunteers. A randomized, open-label, two-period crossover design was employed, with each subject receiving a single 200µg oral dose of either the test or reference formulation according to the randomization schedule. A washout interval of 10 days separated the two dosing periods to prevent carryover effects. Participants were confined for at least 10 hours prior to dosing and remained under supervision until completion of the 72-hour post-dose

sampling. All doses were administered following an overnight fast of ≥10 hours, and fasting continued for an additional 4 hours after drug administration. In each period, 20 blood samples were collected at predetermined time points (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, 24.0, 36.0, 48.0, and 72.0 hours post-dose). Actual sampling times were recorded and used in pharmacokinetic analyses. Blood collection was performed through a dead-volume intravenous catheter to minimize repeated venipuncture; otherwise, samples were obtained by direct venipuncture. Serum was separated by centrifugation and stored at −20°C until analysis. Total serum levothyroxine concentrations were quantified using a fully validated high-performance liquid chromatography method. Method validation included assessment of accuracy, precision, within- and between-run variability, selectivity, matrix effects, and stability under various storage and handling conditions, all of which met acceptance criteria. Pharmacokinetic parameters including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and coefficient of variation (CV%) were derived to compare systemic exposure and inter-subject variability across the three dosage forms. Bioequivalence between formulations was evaluated based on standard regulatory criteria.

RESULTS AND DISCUSSION

Levothyroxine sodium was selected as the model active pharmaceutical ingredient due to its narrow therapeutic index, high sensitivity to formulation and manufacturing variables, and well-recognized challenges in achieving consistent bioavailability. As a BCS Class III/IV borderline compound with low aqueous solubility and stability concerns, levothyroxine requires precise control of dosage form performance to ensure therapeutic equivalence. Even minor differences in excipients, encapsulation matrices, or processing conditions can significantly influence its dissolution and systemic absorption.[24] Evaluating levothyroxine sodium across multiple dosage form platforms tablets, liquid-filled HPMC capsules, and soft gelatin capsules therefore provides a robust framework for understanding how formulation strategies impact bioavailability, product quality, and clinical reliability. Levothyroxine sodium is commercially available in several oral dosage forms, including tablets and capsules, and the selected formulation can markedly influence its dissolution, absorption, and overall bioavailability. The development of liquid and semi-solid oral formulations has advanced considerably in recent decades, largely in response to the growing number of drug candidates exhibiting poor aqueous solubility and limited oral bioavailability. Within this context, liquid-filled capsule systems, particularly HMPC capsules and soft gelatin capsules, have emerged as versatile platforms for enhancing the delivery of lipophilic and poorly soluble compounds. Although both dosage forms share the common objective of enclosing a liquid fill within a capsule shell, their formulation design pathways differ markedly with respect to shell composition, fill matrix compatibility, manufacturing techniques, and subsequent *In-Vivo* performance. In the present study, these distinctions are explored in detail through a comparative pharmaceutical evaluation and bioavailability assessment of levothyroxine sodium formulated in tablets and different liquid-filled capsule systems. Emphasis is placed on contrasting formulation strategies, excipient functionality, and process-related considerations that influence product quality and therapeutic performance. The discussion highlights the key advantages, limitations, and critical quality attributes associated with solid oral tablets, HPMC capsules and soft gelatin capsules, providing an integrated understanding of how these variables impact the overall behaviour of levothyroxine sodium in vivo.

The physical parameter profiles of the optimized formulations encompassing compressed tablets, liquid-filled HPMC capsules, and soft gelatin capsules are detailed in **Tables 1A and 1B**.

Table 1A: Physical characterization parameters of Levothyroxine sodium tablet formulations

Parameters	Specification	Observation	Pharmacopeial limits
Description / Appearance	White to off white, Capsule shaped, Biconvex tablets.	White to off white Capsule shaped, Biconvex tablets.	Meets the IH specification
Weight of Individual Tablets (mg)	100 mg ± 6.0% (94 – 106)	Between 98 - 102	NMT 10%
Hardness (kp)	6.0 ± 2.0 (4.0 – 8.0)	Between 5.5 – 6.5	Meets the IH specification
Thickness (mm)	3.00" (2.70" to 3.30")	2.90,2.95,3.01,2.94,2.96	Meets the IH specification
Disintegration Time (Minutes)	NMT 15	Between 6 - 8	NMT 15
Friability (%)	NMT 1.0	0.10	NMT 1.0

Table 1B: Physical characterization parameters of Levothyroxine sodium liquid filled HPMC capsules and soft gelatin capsules

Parameters	HPMC Capsule	Soft Gelatin Capsules	Pharmacopeial limits
Description / Appearance	Round/biconvex capsules containing a coloured viscous liquid.	Round/biconvex capsules containing a coloured viscous liquid.	Meets the IH specification
Weight of Fill material (mg)	80.00	80.00	Meets the IH specification
Assay (%)	97.8	100.8	90.0 -110.0
Viscosity (cP)	4352	4362	Meets the IH specification
% LOD by O'Haus	13.26	13.35	Meets the IH specification

#IH – in-house specification

These comparative data sets offer insight into the mechanical integrity, uniformity, and structural characteristics that underpin the dosage forms pharmaceutical performance. The optimized levothyroxine tablets demonstrated White to off white, Capsule shaped, Biconvex tablets in appearance, satisfactory mechanical integrity with excellent weight uniformity, with individual weights consistently falling between 98 and 102 mg and a mean weight of 100 mg, indicating good control over the compression process and minimal variability in die fill. The tablets exhibiting a hardness range of 5.5 – 6.5 kp, which is appropriate for ensuring structural robustness without compromising disintegration performance. The friability value remained below 0.1%, confirming that the tablets possess sufficient resistance to abrasion and are unlikely to incur damage during handling, packaging, or transportation. Disintegration time of 6 – 8 minutes was observed, aligning well with pharmacopeial requirements and suggesting efficient tablet breakup upon administration, which is particularly important for a drug such as levothyroxine that requires prompt dissolution for optimal absorption. Additional quality attributes including assay (between 98 - 100%), content uniformity (3.2 %RSD), moisture content (4%), and levels of related substances (ND) were within acceptable limits and are summarized in **Table 2**, further supporting the consistency and suitability of the developed tablet formulation.

Table 2: Comparative results of critical quality parameters of levothyroxine sodium formulations

Parameters	Tablets	HPMC Capsule	Soft Gelatin Capsules	Pharmacopeial limits
Assay (%)	98–102	92–99	98–102	90.0 - 110.0
Content Uniformity (RSD %)	3.2	8.5	3.2	AV: NMT 15.0 & %RSD: NMT 5.0
Moisture Content (%)	4.0	8.0	4.2	IH#
Degradation Products (%)	ND	3.5	1.0	IH#

#IH – in-house specification; ND – not detected

Both the HPMC-based and soft gelatin capsule formulations demonstrated desirable pharmaceutical quality attributes upon evaluation. The absence of leakage and the uniformity of the capsule shells indicate appropriate shell fill compatibility and effective sealing, both of which are critical for maintaining product integrity and preventing potency loss. Visual inspection confirmed that no air bubbles were entrapped within the fill mass, suggesting proper fill formulation viscosity and optimized encapsulation parameters, which together help ensure uniform dosing and stability. Weight variation across capsule units remained within acceptable pharmacopeial limits, reflecting consistent fill volume and reproducible capsule performance during manufacturing. The rheological evaluation of the liquid-filled formulations demonstrated a characteristic shear-thinning flow profile, indicating a reduction in viscosity under applied shear. This behaviour is advantageous during manufacturing, as it facilitates smooth and efficient filling through the encapsulation nozzles while minimizing mechanical stress on the dosing system. Upon return to a resting state, the viscosity increased, contributing to enhanced physical stability of the fill mass by reducing the likelihood of drug sedimentation or phase separation. Importantly, the higher resting viscosity also supported capsule integrity by preventing leakage throughout the encapsulation process. The pH of the optimized liquid-fill formulation was measured and

falling within the acceptable compatibility range for both HPMC and soft gelatin shell materials. This pH alignment is critical, as it minimizes the risk of shell deformation or hydrolytic degradation, thereby preserving capsule robustness throughout storage. Additionally, the observed pH supports the chemical stability of levothyroxine sodium within the formulation, reducing the likelihood of drug degradation or potency loss over time.

Mechanical properties of Levothyroxine sodium capsules

The mechanical evaluation of the liquid-filled HPMC capsules demonstrated tensile strength values ranging from 40–55 MPa for empty shells and 35–50 MPa for liquid-filled shells. The slight reduction in tensile strength following encapsulation is attributed to the plasticizing effect of residual moisture, which increases shell flexibility. Despite this reduction, the values remain within the optimal mechanical range required to withstand moderate to high-speed encapsulation, downstream handling, and packaging operations without excessive brittleness or risk of shell cracking. The Young's modulus of HPMC capsule shells was observed between 1.3–2.8GPa for empty shells and 1.2–2.6GPa for liquid-filled shells, indicating minor softening due to moisture equilibration with the fill mass. This modulus range suggests an appropriate balance between rigidity and flexibility, allowing the shells to deform slightly under mechanical stress such as during capsule closing, sealing, or blister compression while maintaining their structural integrity. Elongation at break values for HPMC shells were recorded as 7–13% for empty capsules and 7–15% for liquid-filled capsules. The marginal increase in elongation for filled capsules reflects enhanced ductility arising from fill shell interaction. Higher elongation values correspond to lower brittleness, which is advantageous in minimizing shell fractures during encapsulation, polishing, mechanical sorting, and packaging.

In comparison, soft gelatin capsule shells, which are inherently more flexible due to their plasticizer-rich matrix, exhibited lower tensile strength values of 20–35 MPa for empty shells and 18–32 MPa for liquid-filled capsules. These tensile characteristics are sufficient to support the stretching required during rotary die encapsulation and heat-sealing processes while maintaining adequate strength to resist deformation or leakage during storage. The Young's modulus of soft gelatin films was found to be considerably lower than that of HPMC films, with values ranging from 0.3–0.8GPa for empty shells and 0.25–0.7GPa for filled shells. These low modulus values highlight the high elasticity of soft gelatin, enabling the capsule shells to deform easily under compressive forces encountered during encapsulation and packaging without fracturing. Soft gelatin capsules also demonstrated high elongation at break, measured at 60-150% for empty shells and 80–200% for liquid-filled shells. This substantial extensibility, driven by plasticization from water and polyols, confirms the low brittleness of soft gelatin shells. Such high elongation capacity is essential to prevent cracking during rotary die processing, blister sealing, transportation, and patient handling. Overall, the mechanical property profiles of both HPMC and soft gelatin capsules confirm their suitability for liquid-fill applications, with each shell type offering distinct advantages in flexibility, tensile strength, and resistance to brittle failure.

Furthermore, chemical quality assessments including assay, content uniformity, and related substances showed compliance with established specifications, confirming the chemical stability and homogeneity of the formulations. These results, summarized in **Table 3**, collectively support the reliability and robustness of both capsule systems for delivering levothyroxine sodium effectively.

Table 3: Comparative results of mechanical properties of Levothyroxine sodium liquid filled HPMC and soft gelatin capsules

Parameters	HPMC Capsule		Soft Gelatin Capsules		Remarks
	Empty	Liquid filled	Empty	Liquid filled	
pH	-	5.0 - 8.0	-	5.0-7.5	-
Tensile Strength (MPa)	40-55	35-50	20-35	18-32	Higher more rigid; lower due to plasticization
Young's modulus (GPa)	1.3-2.8	1.2 - 2.6	0.3-0.8	0.25-0.7	Balance between rigidity and flexibility
Elasticity (%)	7-13	7-15	60-150	80–200	Low brittleness for high elasticity
Brittleness	High at low RH	Moderate	Low	Very Low	Low to very low brittleness for soft gelatin capsules

In vitro dissolution release

The comparative dissolution profiles of the optimized levothyroxine sodium formulations including tablets, liquid-filled HPMC capsules, and soft gelatin capsules are presented in **Figure 1.**

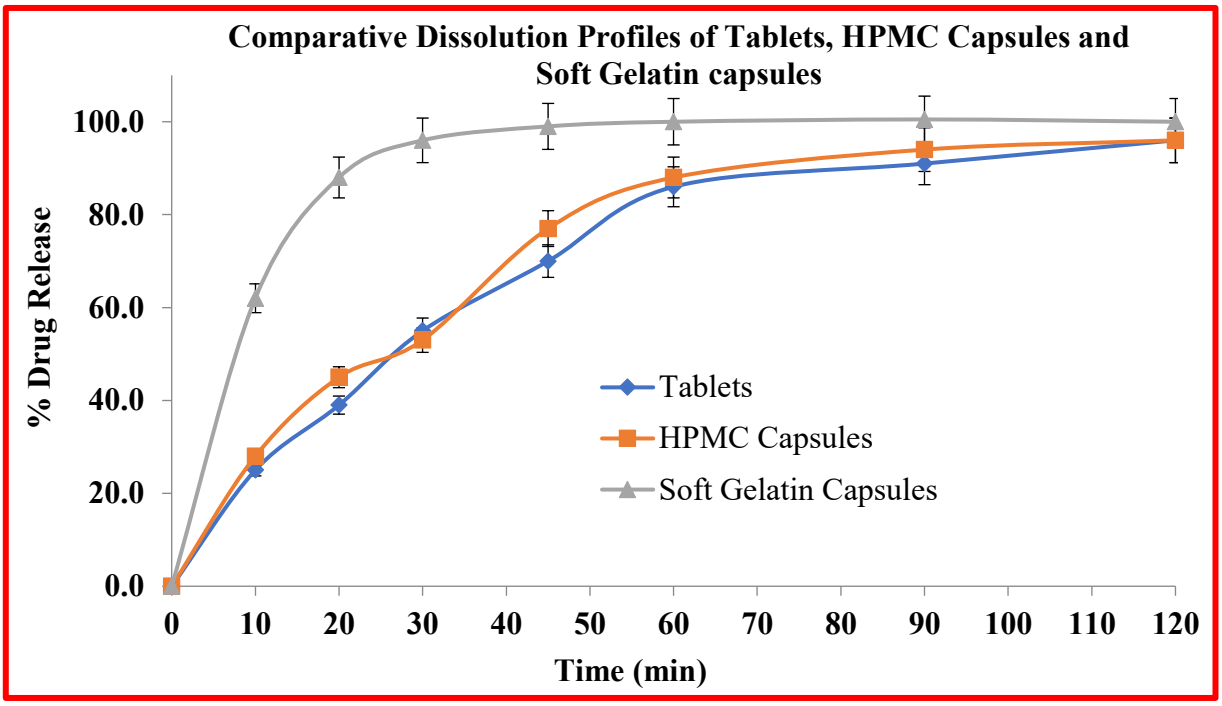

Figure 1: Comparative dissolution profiles of optimized Levothyroxine formulations from different dosage forms (tablets, liquid-filled HPMC Capsules, and Soft gelatin capsules)

The soft gelatin capsules demonstrated the most rapid drug release, achieving complete dissolution within 20 minutes. This enhanced performance can be attributed to the inherently rapid disintegration and efficient rupture of soft gelatin shells, as well as the immediate availability of the liquid fill, which facilitates faster drug diffusion into the dissolution medium. In contrast, both the tablet formulation and the HPMC based capsules exhibited comparatively slower and incomplete drug release within the same time frame. The reduced release rate may be associated with differences in formulation composition, fill matrix viscosity, shell properties, and the physical form of the drug within each dosage system. For tablets, the need for matrix disruption and particle wetting can delay dissolution, while the HPMC capsule shells, being more rigid and less moisture-permeable than gelatin, may prolong shell rupture and subsequent release of the fill mass. These dissolution outcomes are consistent with the observed disintegration times for each dosage form, further supporting the relationship between disintegration behaviour and subsequent drug release kinetics. Overall, the findings highlight the influence of dosage form design and encapsulation matrix on the release performance of levothyroxine sodium formulations.

Pharmacokinetic Assessment

Mean baseline-corrected serum levothyroxine concentration-over-time profiles for all treatments are shown in **Figure 2.** Key pharmacokinetic parameters including maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax), area under the plasma concentration–time curve ($AUC_{0-\infty}$), and inter-subject variability (CV%) are summarized in **Table 4.**

Table 4: Comparative Pharmacokinetic parameters (Cmax, Tmax, AUC) (Single dose, fasted human volunteers)

Parameters	Reference Product	Soft Gelatin Capsules	Liquid filled HPMC Capsule	Tablet Formulation
C_{max} (ng/mL)	104.21 ± 16.58	102.33 ± 14.86	74.35 ± 18.54	70.114 ± 19.28
T_{max} (h)	2.00 (1.50 - 3.50)	1.50 (1.0 – 3.50)	3.50 (1.50 – 5.50)	4.00 (2.0 – 6.0 0
AUC_{0-48} (ng·h/mL)	1854.18 ± 388.65	1886.93 ± 298.56	1214.50 ± 385.34	1149.60 ± 376.12
$AUC_{0-\infty}$ (ng·h/mL)	1874.35 ± 352.54	1898.24 ± 272.48	1226.05 ± 394.11	1154.78 ± 381.79
% CV	18.50	12.50	38.00	41.00

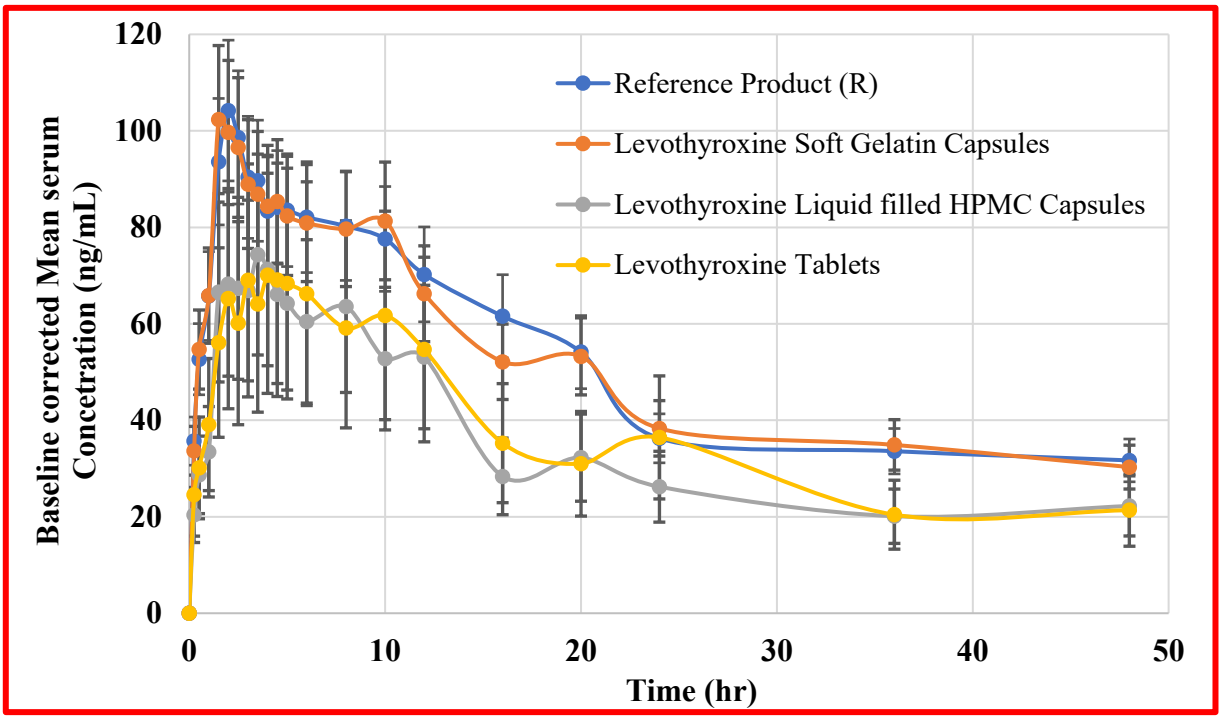

Figure 2: Comparative mean baseline-corrected serum levothyroxine concentration-over-time profiles for different dosage formulations

The in vivo pharmacokinetic evaluation revealed distinct differences in systemic exposure among the three levothyroxine sodium formulations. Among the tested dosage forms, the soft gelatin capsule formulation demonstrated significantly higher bioavailability, as reflected by increased Cmax and $AUC_{0-\infty}$ values compared with both the liquid-filled HPMC capsules and the conventional tablet formulation. The enhanced bioavailability observed with the soft gelatin capsules is likely attributable to their faster disintegration and rapid dissolution, which facilitate earlier release of the drug and improved absorption in the gastrointestinal tract. The liquid fill matrix within softgels may also promote better solubilization and reduced dependence on gastrointestinal fluid dynamics, contributing further to improved systemic exposure. In contrast, the HPMC-based capsules and tablets exhibited comparatively lower Cmax and AUC values, consistent with their slower in vitro disintegration and dissolution behaviour. Differences in the in vivo performance of the three levothyroxine sodium formulations may also be partially explained by their relative formulation stability. Soft gelatin capsules, which demonstrated superior bioavailability, typically offer enhanced protection of the drug substance due to their hermetically sealed structure and reduced exposure to oxygen and moisture factors known to influence levothyroxine degradation. In contrast, tablets and liquid-filled HPMC capsules may be more susceptible to environmental variability, leading to subtle changes in drug potency or release characteristics over time. Improved chemical and physical stability within the soft gelatin matrix likely contributes to more consistent drug release, reduced inter-subject variability, and higher systemic exposure. Thus, formulation-dependent stability differences provide an additional justification for the enhanced in vivo performance observed with soft gelatin capsules. The observed differences in Tmax also support these findings, with the soft gelatin capsules achieving peak plasma concentrations more rapidly, indicative of accelerated onset of absorption. Additionally, the relatively lower CV% for softgels suggests improved dose uniformity and reduced variability in gastrointestinal performance. Collectively, these results underscore the influence of dosage form design on the pharmacokinetic behaviour of levothyroxine sodium and highlight the superior in vivo performance of soft gelatin capsule formulations.

CONCLUSION

This study confirms that levothyroxine sodium, a drug highly sensitive to formulation and processing variables, exhibits significant differences in pharmaceutical quality and pharmacokinetic behaviour across dosage forms. Although tablets, HPMC capsules, and soft gelatin capsules all satisfied compendial quality criteria, only the soft gelatin formulation consistently demonstrated bioavailability equivalent to the reference product, characterized by higher systemic exposure, faster onset of absorption, and reduced inter-subject variability. These advantages are supported by the softgels system's rapid dissolution, enhanced formulation stability, and favourable mechanical properties. In contrast, the tablet and HPMC capsule formulations exhibited slower drug release and greater variability, resulting in suboptimal in vivo performance. Collectively, these findings highlight the decisive role of dosage-form design in ensuring reliable levothyroxine exposure and identify liquid-filled soft gelatin capsules as the most robust and clinically suitable delivery platform.

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