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VALIDATION ANALYTICAL TECHNIQUES FOR THE ESTIMATION OF LEVOTHYROXIN IN PHARMACEUTICALS AND BIOLOGICAL MATRICES: A COMPREHENSIVE REVIEW

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ABSTRACT

Levothyroxine is a synthetic of thyroxine (T4), a key thyroid hormone, and is widely used in the treatment of hypothyroidism and other endocrine disorders. Due to its narrow therapeutic window and the necessity for accurate dosing, the application of reliable and validated analytical techniques is critical to ensure its safety, quality, and therapeutic effectiveness. This provides a comparative evaluation a 10analytical methods employed for the quantification of levothyroxine in both pharmaceutical formulations and biological samples. The reviewed techniques include Ultraviolet (UV) Spectrophotometry, derivative UV analysis, High-performance liquid chromatography (HPLC), Ultra-high performance liquid chromatography (UHPLC), High-performance thin-layer chromatography (HPTLC), Capillary electrophoresis and Tandem Mass Spectrometric methods such as LC-MS/MS and UPLC-MS/MS. These approaches utilize various stationary phases, including C_{18}, BEH C_{18}, and silica gel, in combination with mobile phases comprising methanol, acetonitrile, formic acid, and buffers of varying pH. The detection wavelengths range

from 200 to 254 nm, with flow rates between 0.2 and 1.5 mL/min. Notably, LC-MS/MS and UPLC-MS/MS demonstrate superior sensitivity and specificity, making them highly effective for trace-level detection in complex matrices. This comprehensive summary serves as a practical guide for selecting suitable analytical techniques based on the intended application in pharmaceutical analysis or clinical evaluation.

Keywords: Levothyroxine, Analytical Techniques, UV Spectrophotometry, RP-HPLC, LC-MS/MS, Method Validation, Thyroid Hormone Analysis

INTRODUCTION:

Levothyroxine (L-T4), the synthetic levo-isomer of thyroxine, is a cornerstone in the treatment of thyroid hormone deficiency and related disorders. It is primarily indicated for hypothyroidism, a condition in which a deficiency in thyroid hormone production by the thyroid gland can cause various symptoms, including tiredness, unexpected weight gain, and reduced energy levels, cold intolerance, and cognitive impairment. Levothyroxine is also employed as a suppressive therapy in thyroid cancer, as well as in the management of goiter and autoimmune thyroiditis, particularly Hashimoto's thyroiditis.

As a prohormone, levothyroxine is converted in peripheral tissues to the active hormone triiodothyronine (T3), which binds to nuclear receptors and regulates gene expression involved in metabolism, growth, and development. Its effectiveness depends on achieving and maintaining stable serum thyroid-stimulating hormone (TSH) levels, which requires individualized dosing and routine monitoring. Various factors such as gastrointestinal pH, dietary interference, patient compliance, and drug interactions (e.g., calcium, iron, proton pump inhibitors) can affect its oral bioavailability, typically estimated to be 40–80%.

Pharmacokinetically, levothyroxine has a slow onset of action and a long half-life of approximately 7 days in euthyroid individuals, allowing for once-daily dosing. However, its narrow therapeutic index demands rigorous quality control and bioequivalence testing, particularly in generic formulations, to avoid over- or under-treatment, which can result in serious outcomes such as cardiovascular complications or osteoporosis.

Given these clinical and regulatory implications, numerous analytical

methods have been developed and validated to ensure accurate quantification and stability of levothyroxine in bulk drugs, tablets, and biological matrices. Techniques such as UV Vis spectrophotometry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), capillary electrophoresis (CE), and liquid chromatography–mass spectrometry (LC-MS/MS) are commonly used, each offering distinct advantages in terms of sensitivity, selectivity, and regulatory compliance.

This provides a comprehensive overview of the validated analytical methods reported in the literature for the estimation of levothyroxine. The focus is placed on the principles, instrumentation, method validation parameters (such as LOD, LOQ, linearity, accuracy, precision), and applications of each technique, highlighting their relevance in pharmaceutical quality control and clinical pharmacokinetics **[2][3]**.

MECHANISM OF ACTION:

The levothyroxine produce the hormone thyroxine (T4), which is called levothyroxine. Following administration, it is mostly transformed in peripheral tissues like the liver and kidneys into the physiologically active form, triiodothyronine (T3). T3 then forms a hormone-receptor complex by attaching itself to thyroid hormone receptors found in the nuclei of different cells. This complex controls the transcription of genes involved in growth, development, and metabolism by interacting with particular DNA sequences. Levothyroxine is crucial for preserving proper metabolic balance and growth because it affects a number of physiological processes through this mechanism, such as energy use, protein synthesis, and neuronal development **[1].**

ANALYTICAL TECHNIQUES:

SPECTROPHOTOMETRY:

Spectrophotometry is a commonly employed analytical technique used to determine the concentration of a substance by measuring its absorbance of light at a particular wavelength. In this process, a spectrophotometer—which typically operates in the visible (Vis) and ultraviolet (UV) regions of the electromagnetic spectrum—is employed. When molecules absorb light, the electrons are excited from a lower energy state to a higher one. Because each compound has a unique pattern of light

absorption, the resulting absorbance data can be used to identify and quantify the analyte present.

CHROMATOGRAPHY:

Chromatography, in particular High-Performance Liquid Chromatography (HPLC), is another essential method for determining and assessing the unique components in a mixture. In HPLC, the sample is moved by a liquid mobile phase through a column that contains solid stationary material. Each compound interacts differently with the stationary phase depending on its chemical characteristics, which influences its speed of travel and results in an efficient separation based on retention time.A polar mobile phase and a non-polar stationary phase—typically C18 (octadecylsilane).

REVERSE PHASE HPLC (RP-HPLC):

longer retention of more hydrophobic analytes due to their stronger interactions with the non-polar stationary material in this configuration allows for selective separation. Additionally, stationary phases like C8 or cyano can be used to alter retention time and selectivity. On the contrary.A polar stationary phase, like unmodified or amino-bonded silica, is used in conjunction with a non-polar mobile phase, like hexane or chloroform.

NORMAL PHASE HPLC (NP-HPLC):

Polar analytes have a stronger interaction with the stationary phase and are retained for a longer period of time than non-polar substances, which elute more quickly. In NP-HPLC, the separation process is driven by polar interactions like hydrogen bonds and dipole-dipole forces. A highly sensitive and specific analytical technique called Liquid Chromatography Combined with Tandem Mass Spectrometry (LC-MS/MS) blends the separation powers of HPLC with the powerful detection of mass spectrometry. chromatographic separation, the analytes are nebulized, desolvated, and ionized the charged particles in the mass spectrometer. By examining these ions based on their mass-to-charge (m/z) ratios, trace-level compounds can be precisely identified and measured, even in complex mixtures.

ANALYTICAL METHOD FOR LEVOTHYROXIN:

Levothyroxine, a synthetic thyroid hormone commonly used in the management of hypothyroidism, requires precise and sensitive analytical techniques due to its low dosage

requirements and narrow therapeutic index. Numerous validated methods have been developed to quantify this compound in pharmaceutical formulations and biological matrices.

1. UV-Visible Spectrophotometry

Levothyroxine demonstrates a distinct absorption peak around 225 nm in methanol, making it suitable for UV spectrophotometric detection. This method is simple and cost-effective, often employed in quality control for bulk drugs. The approach typically shows linearity in the range of 2–20 µg/mL and is validated according to ICH Q2(R1) guidelines. However, due to limited selectivity, it is less suitable for complex matrices like plasma or serum.

2. Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

RP-HPLC is widely used for estimating Levothyroxine in tablets and bulk materials. Commonly, a C18 column is used with mobile phases such as methanol: water (70:30) or acetonitrile: water with 0.1% phosphoric acid. The detection wavelength is typically set at 225–254 nm, and retention times generally range between 2 and 6 minutes. These methods are known for their accuracy, reproducibility, and specificity, with limits of quantification (LOQ) reaching as low as 5 µg/m.

3. LC-MS/MS and UPLC-MS/MS

Advanced chromatographic techniques such as LC-MS/MS and UPLC-MS/MS offer high sensitivity and selectivity for detecting Levothyroxine in biological samples like plasma and serum. These methods often use a C18 or BEH C18 column, with a mobile phase composed of acetonitrile and water, often with 0.1% formic acid. LC-MS-based techniques can detect analytes at nanogram levels, making them suitable for pharmacokinetic and bioequivalence studies.

4. High-Performance Thin Layer Chromatography (HPTLC)

HPTLC methods involve the use of silica gel 60 F254 plates and solvents such as methanol: chloroform (1:9) for mobile phase development. Detection is typically done at 254 nm. While less sensitive than HPLC or LC-MS techniques, HPTLC is effective for routine analysis and can be used for simultaneous estimation of multiple thyroid-related compounds.

ANALYTICAL METHOD FOR THE ESTIMATION OF LEVOTHYROXIN:

S. No.	Analytical Technique	Column/Stationary Phase	Mobile Phase	Flow Rate (mL/min)	Wavelength (nm)	Ref
1	HPLC-UV	C_{18}	Methanol: 0.05% formic acid (55:45 %v/v).	0.3	225	2
2	RP-HPLC	C_{18}	Methanol and Formic acid (0.1%) (50:50 %v/v)	1.2	254	3
3	LC-MS/MS	C_{18}	Methanol: Water (90:10 %v/v) + 0.1% formic acid	0.3	MS Mode	4
4	UV-Spectrophotometry	RP_{18}	Methanol: phosphoric acid (0.1%) (70: 30 %v/v) (pH 3)	1.5	225	5
5	HPTLC	Silica gel 60 F_{254}	Methanol: Chloroform (10:90 %v/v)	—	254	6
6	UPLC-MS/MS	BEH C_{18}	Acetonitrile: Water (80:20 %v/v) + 0.1% FA	0.2	MS Mode	7
7	NP-HPLC	Silica	Hexane: Isopropanol (90:10 %v/v)	1.0	225	8
8	Derivative UV	—	Methanol	—	239	9
9	Capillary Electrophoresis	Fused-silica	Borate buffer pH 9.2	—	200	10
10	UHPLC-UV	Acquity UPLC BEH C_{18}	Water: ACN (60:40 %v/v) + 0.1% TFA	0.3	230	11

CONCLUSION:

The estimation of Levothyroxine demands precise and sensitive analytical approaches due to its low dosage range and critical therapeutic index. This review summarizes the validated methods employed for its quantification, including UV-spectrophotometry, HPLC, LC-MS/MS, and HPTLC techniques. Among these, RP-HPLC and LC-MS/MS have emerged as the most robust and sensitive tools, particularly for analyzing biological samples and ensuring quality control in pharmaceutical formulations.

While existing methods provide adequate sensitivity, selectivity, and compliance with international validation guidelines, there is still scope for method optimization, especially in terms of eco-friendliness, cost-efficiency, and automation. Future research should emphasize the development of green analytical techniques and advanced hyphenated methods that support high-

throughput screening with minimal environmental impact. This will contribute to more sustainable and regulatory-compliant analytical practices in thyroid hormone analysis.

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CONFLICT OF INTEREST:

The authors declare that NO conflict of interest among us.

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