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Review on Analytical Methods Devlpopment on Liothyronine and Levothyroxine in Pharmaceutical Dosage Form.

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ABSTRACT:

Levothyroxine (LT4) and liothyronine (LT3) are synthetic hormones commonly prescribed for the treatment of thyroid disorders such as hypothyroidism. This review comprehensively evaluates analytical methods employed for the quantification of levothyroxine and levothyroxine in pharmaceutical dosage forms. Various techniques, including chromatographic methods such as HPLC, LC-MS, spectroscopic methods like UV-Vis, IR, and electrochemical methods, are discussed in detail. The review outlines the advantages, limitations, and applications of each method, providing insights into their suitability for pharmaceutical analysis. Emphasis is placed on the importance of robust and validated analytical methods to ensure the quality, safety, and efficacy of formulations containing levothyronine and levothyroxine. Additionally, future perspectives and challenges in this field are highlighted to guide further research and development efforts.

Keyword: Liothyronine, Levothyroxine, HPLC, RPHPLC, UV Spectrophotometry, UPLC-MS/MS.

INTRODUCTION:-

HPLC is commonly used due to its high sensitivity and specificity. It involves separating the analytes based on their interactions with the stationary phase and mobile phase in a chromatographic column. Detection is often achieved using ultraviolet (UV) or mass spectrometric detection.(2)Spectrophotometric methods, such as UV-visible spectroscopy, are simpler and less expensive compared to chromatographic techniques.(1)These methods rely on the measurement of the absorbance of the analyte at a specific wavelength. However, they may lack the specificity and sensitivity of chromatographic methods.(3)Liothyronine sodium and levothyroxine sodium are widely used in thyroid replacement therapy (4).Studies comparing levothyroxine (LT4) therapy with LT4+ liothyronine (LT3) or desiccated thyroid extract (DTE) did not detect consistent superiority of either treatment. Here, we investigated these therapies, focusing on the whole group of LT4- treated hypothyroid patients, while also exploring the most symptomatic patients (5).Hypothyroidism is a state of deficiency of endogenously produced thyroid hormone and affects nearly 5.0% of the world population(6).According to The Wickman Survey. hypothyroidism, which is the most general endocrine disorders, observed an annual diagnosis of the disease at a rate of 4.1 per 1000 for women and 0.6 per 1000 for men. T3 is the most potent thyroid hormone, and its affinity for the thyroid hormone receptor is 10 to 20 times that of thyroxine.(7)Iodine is an essential trace element to animals and humans. It is utilized by the thyroid gland for the biosynthesis of the thyroid hormones 3,3',5,5-tetraiodothyronine (T) and 3,3,5-triio- dothyronine (T3). On the other hand, an excess of iodine can produce goiter and hypothyroidism as well as hyperthyroidism.(8)Levothyroxine Sodium (L-T_1, Fig. 1) is a synthetic hormone widely used for the treatment of hypothy- roidism, with effects identical to those of the natural hormone produced by the thyroid gland. The biological action of thyroid hormones is to increase the basal metabolic rate, and thus entailing an increase of substrates use, enzymes activity and other hormones secretion. In addition to this, it contributes to the general development of organs and tissues; fetal and postnatal thyroxine is essential for the correct development of neurons and growth of their extensions. Thyroid hormones deficiency in childhood and adults involves a progressive reduction in nervous system reactivity, affecting both the motor function and the intellectual aspects.(9)Diagnosis and treatment of hypothyroidism is often considered simple and is mostly carried out in a primary care setting. However, studies continue to show problems in the management of this condition. Many patients on thyroid hormone replacement are either under-replaced or over-replaced and a significant number of patients on thyroid hormone replacement report not feeling well despite having thyroid function tests within the healthy reference range." In this review, we discuss current approaches to the management of primary hypothyroidism and explore potential future developments..(10).An early study In hypothyroid patients compared treatment with the usual daily L-thyroxine dose, consisting of two or three 100-µg tablets, versus the same number of tablets, each containing 80 µg of 1-thyroxine and 20 µg of liothyronine (11).

DRUG PROFILE:

Table no.1 – Drug Profile on levothyronine and Levothyroxine.

Drug Name	**Levothyronine**	**Levothyroxine**
Category	**Anticancer**	**Anticancer**
Chemical name	**(2S)-2-amino-3- [4-(4-hydroxy-3-iodo-phenoxy)-3,5-diiodo-phenyl]propionic acid**	**(2S)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propanoic acid**
Chemical formula	**C15H12I3NO4**	**C15H11I4NO4**
Molecular weight	672.95g/mol .	776.87 g/mol.
Melting point	205-207°c and 235-236°	235-236°c
Solubility	**Water and alkali hydroxide, methanol**	**Water and alkali hydroxide, methanol**
Storage Temperature	**Below 25 c**	**Below 25 c**

Fig 1: Structure of Levothyronine

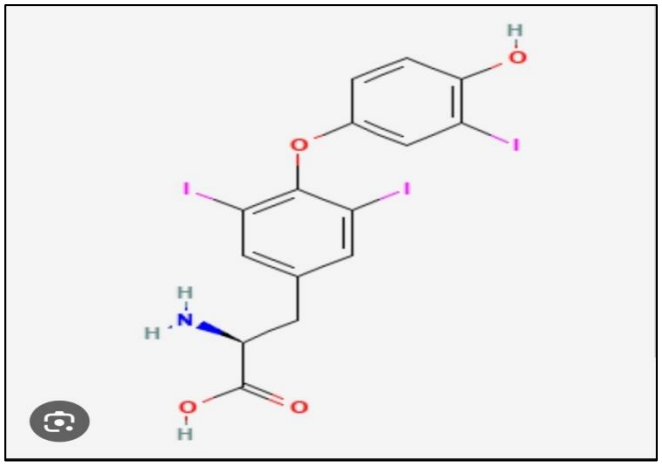
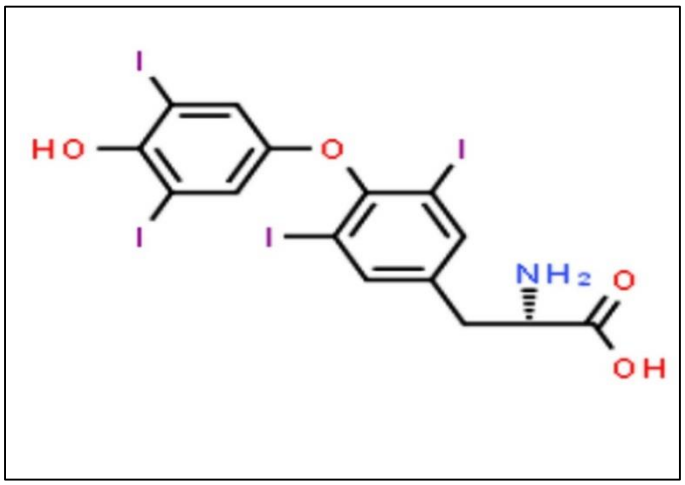


Fig 2: Structure of Levothyroxine



METHOD DEVELOPMENT AND LITERATURE STUDY IN BULK AND PHARMACEUTICAL DOSAGE FORM:

1) High Performance Liquid Chromatography (HPLC).

High-Performance Liquid Chromatography is a term that is commonly used to describe liquid Chromatography, which consists of a liquid mobile phase that is mechanically pumped through a stationary Phase-containing column. An HPLC system consists of an injector, a pump, a column, and a detector The pump is in charge of regulating the flow of solvent through the system. After leaving the pump, the Solvent passes through the injector, then through the section, and then through the optical unit of a detector. HPLC columns are made up of spherical silica gel beads coated with the hydrophobic stationary phase And packed into the column. C4 (butyl), C8 (octyl), C18 (octadecyl), phenyl (phenylpropyl), and nitrile (cyanopropyl) columns are common stationary phases .(12)

Table no.2 : Summary of HPLC method for determination of levothyroxine and levothyronine.

Sr.no	Drug	Analytical method	Description	Reference
1	**Levothyroxine and levothyronine**	**Hplc**	**Results show that optimal sepurations of these compounds standard solution can be achieved using a X Bridge C18 column (150 50m) maintained 4.6 pm. Sampletemperature 15°C by using pH 2.0 buffer solution. Mobile phase and methanol with a flow rate of 1.0 ml/min at 225 of detection and a gradient time of 38 minutes.**	13
2	**Levothyroxine**	**Hplc**	**Mobile phase:-acetate buffer sol, acetic acid.** **wavelength:-252mm** **Flow rate:-1.2 ml/min** **retention time of 3.03 minutes on a NH2** **Column at C18**	14
3	**Levothyroxine**	**HPLC-UV-ICP-MS**	**Mobile phase:-Acetonitrile, Nitric acid** **Flow rate :-0.3ml/min** **Wavelength:-2 to 5** **Retention time25min** **Column C18.**	15
4	**Levothyroxine and levothyronine in tablet.**	**Rphplc**	**reversed phase (RP-18) column p- octadecylsilane .** **mobile phase: consisting of potassium dihydrogen phosphate, methanol, and water at 44°C.** **Flow rate:-1ml/min.**	16

			Wavelength:-254nm **Retention time:3min**	
5	**Levothyronine sodium and Levothyroxine sodium in tablet.**	**Rphplc**	**Mobile Phase:-Methanol, water phosphoric acid.** **Flow rate :-2ml/min** **Wavelength:-254** **Retention time: 10min** **Column RP18**	17

2) Comparative Effectiveness of Levothyroxine and levothyronine.

Studies comparing levothyroxine (LT4) therapy with LT4 + liothyronine (LT3) or desiccated thyroid extract (DTE) did not detect consistent superiority of either Treatment. Here, we investigated these therapies, focusing on the whole group of LT4-Treated hypothyroid patients, while also exploring the most symptomatic patients.(18)LT3 Monotherapy: Levothyronine is the active form of thyroid hormone and is more rapidly absorbed and metabolized than LT4. Some studies suggest that LT3 monotherapy may provide more rapid symptom relief compared to LT4, particularly in patients with genetic variations affecting thyroid hormone conversion. However, LT3 has a shorter half-life and requires more frequent dosing, which can lead to fluctuations in hormone levels and potentially undesirable effects.(19)LT4 Monotherapy: Levothyroxine is the standard treatment for hypothyroidism. It's considered the preferred option due to its stable pharmacokinetics, long half-life, and consistent efficacy. Most patients with hypothyroidism achieve normal thyroid function and symptom relief with LT4 monotherapy.(20)In conclusion, while LT4 remains the standard treatment for hypothyroidism, some patients may benefit from alternative approaches such as LT3 monotherapy or combination therapy. However, further research is needed to better understand the comparative effectiveness, safety, and long-term outcomes of these treatment options. Treatment decisions should be individualized based on patient characteristics, preferences, and response to therapy.(21)

3) Ultra-high performance liquid chromatography MS/MS.

UPLC is highly effective for determining the concentration of a medication in a biological sample.Bioanalysis is a technique for estimating concentrations of metabolites or endogenic components in biological samples such as blood, plasma, serum, CSF, and urine, as well as saliva. Hypothyroidism is a state of deficiency of endogenously. Produced thyroid hormone and affects nearly 5.0% of the world population. Hypothyroidism management is done. with monotherapy of levothyroxine (LT4). Levothyroxine is a synthetic analogue of thyroxine hormone and is used in concentration equivalent to thyroid stimulating hormone. (TSH) present in the body. Although, treatment with LT4achieve normal TSH levels, but 5-10% of individuals with hypothyroidism are not satisfied with monotherapy, and do not consider their previous well-being to be restored +. The more potent thyroid hormone is Triiodothyonine (T3), 20% of which is produced by the thyroid gland and rest 80% is produced by conversion of thyroxine (T4) to T3 in the liver (22)

Table no.3:summary of uplc Ms/Ms method for determination of levothyroxine and levothyronine.

Sr.no	**Drug**	**Description**	**Reference**
1	**Levothyroxine and levothyronine**	**Mobile phase-** **Glacial acetic** **acidin water, Acetonitrile.** **Flow rate:- 0.8 ml/min.** **retention time: of 2.65, 1.67 and** **2.56. respectively.** **The total run time was 4min.** **Column C18**	3

4) UV Spectrophotometry Method.

Principle:-

UV spectrophotometry relies on the absorption of ultraviolet (UV) light by molecules in a sample. Levothyronine and levothyroxine exhibit characteristic absorption spectra in the UV range, allowing for their quantification based on the Beer-Lambert law, which states that absorbance is directly proportional to concentration and path length.

The aim of the present work was to develop and validate a derivative UV spectrophotometry method that could be used in routine quality control for the determination of LT4 in tablets containing different amounts of the drug. In order to bring more confidence to the method, it was compared with an HPLC one, modified from the USP 31 proposed method.(23,24)

Table no.4: summary of uv Spectrophotometry method for determination of levothyroxine sodium.

Sr.no	Drug	Description	Reference
1	**Levothyroxine Sodium**	**Mobile phase-methanol : water (50:50; v/v** **(pH 11.2)** **RP-18 column (125 × 4 mm, 5 µm) phosphoric acid (0.1%) (70 : 30, v/v) (pH 3) as mobile phase. Flow rate was set at 1.5 mL/min, and detection was performed at 225 nm.**	7

CONCLUSION:

It can be concluded that there are various effective techniques available for their analysis, including chromatographic methods such as HPLC and LC-MS, spectroscopic methods like UV-Vis and IR, as well as electrochemical methods. Each method has its advantages and limitations, and the choice of method depends on factors such as sensitivity, selectivity, cost, and availability of instrumentation. Overall, the development of robust and validated analytical methods is crucial for ensuring the quality, safety, and efficacy of pharmaceutical formulations containing levothyroxine and levothyroxine.

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