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Research Article

Utilization of the FTIR spectroscopic method for the quantitative determination of the narrow therapeutic index levothyroxine sodium in pharmaceutical tablets

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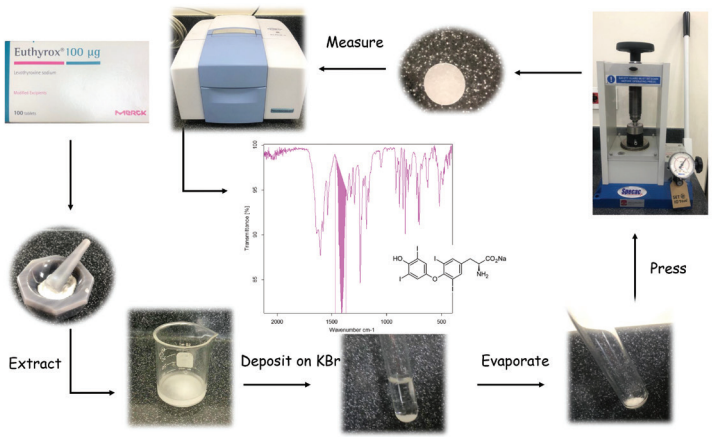
Abstract

Levothyroxine sodium is a narrow therapeutic index drug used for the treatment of hypothyroidism. The medication is marketed in tablet form with very low doses ranging from 25 to 150 µg, which requires the development of a sensitive quantitative analytical method to ensure a safe and effective pharmacological response. In the present work, a Fourier transform infrared method has been developed and validated for levothyroxine sodium determination in various pharmaceutical formulations. The proposed method involves selectively extracting levothyroxine sodium from the studied tablets using chloroform as solvent, then depositing it on a KBr pellet, followed by infrared measurements and spectra analysis. The peak band area corresponding to the C=C centered at 1409 cm^{-1} has been chosen for the quantification. The method has been validated according to ICH guidelines and was found to be simple, precise, accurate, and specific. The linearity, detection, and quantitation limits are 25–800, 8.121, and 24.545 µg/pellet, respectively. These values confer the method's sensitivity and applicability for the determination of different pharmaceutical tablets with various dosages. A statistical comparison with a reference HPLC method showed no significant difference. Accordingly, the developed method can be employed for quality control testing of levothyroxine sodium due to its simplicity and the absence of sophisticated instrumentation and procedures.

Graphical abstract:



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PENSOFT

Keywords

drug assay, FTIR spectroscopy, levothyroxine sodium, narrow therapeutic index drug

Introduction

Narrow therapeutic index drugs (NTI-drugs) constitute a group of medications that have a narrow margin between safe and lethal dosages, where small variations in the drug's plasma concentrations (Cp) can lead to an insufficient therapeutic response presented as therapeutic failure or the appearance of adverse effects (Habet 2021). One of the most commonly used NTI drugs is levothyroxine sodium (LT4), which is administered orally for thyroid hormone replacement therapy in cases of hypothyroidism, thyroid neoplasia, and thyroidectomy (Jonklaas et al. 2014). Several pharmaceutical formulations for LT4 are available on the market, with doses ranging from 25 to 150 µg of LT4 per tablet. Given its status as a NTI drug, slight dose variation outside the therapeutic window can pose a risk of severe adverse effects of hyper- or hypo-thyroidism, including harmful cardiac and/or metabolic effects (Klein and Ojamaa 2001).

According to the American Medical Association, interchangeability and switching between generics for NTI drugs should be approached with caution along with continuous monitoring and assessment to assure the desired clinical response (Holmes et al. 2011). However, several drug shortages for LT4 in Lebanon have led to switching between brands and generics. In the process of monitoring the therapeutic response, blood tests revealed a fluctuation in LT4 blood level requiring dose adjustment. This issue opened up to investigate whether all LT4 generics available in the Lebanese market contained the labeled amount that should result in the required pharmacological action.

Accordingly, a literature review was conducted to explore the available quantitative techniques for the assay of LT4. Several analytical methods have been reported, mostly using HPLC coupled to different detector types as simple as UV and fluorescence to reaching highly complex as MS and chiral (Rapaka et al. 1981; Garnick et al. 1984; Kazemifard et al. 2001; Kannamkumarath et al. 2004; Gika et al. 2005; Shah et al. 2008; Collier et al. 2011; Singare and Belamkar 2016; Islam et al. 2018; Dutt et al. 2020; Thapa et al. 2020; Y. Lee et al. 2021; Nakano-Yasaka et al. 2022; J. Lee et al. 2023; Jamal et al. 2023). Other analytical methods included derivative UV-spectrophotometry (Gregorini et al. 2013), electrospray ionization tandem mass spectrometry (ESI-MS/MS) (M.-K. Lee et al. 2008), inductively coupled plasma mass spectrometry (Pabla et al. 2009), and immunoassays (Kunst et al. 1988; Stevenson et al. 1998; Frank et al. 2004; Dhatt et al. 2006). HPLC seemed to be an appealing option for being able to separate and identify LT4. However, such a technique is expensive and time-consuming and requires the use of several solvents. Upon surfing the literature, a Fourier transform infrared (FTIR) spectroscopic method has not been used previously for the quantitative assay of LT4 in pharmaceutical preparations.

The use of Fourier transform infrared (FTIR) spectroscopy has gained a lot of interest recently. It has been widely used in the quantitative and qualitative analysis of pure pharmaceutical compounds and medications, making it a promising method in quality control testing (Rahman 2012; Fanelli et al. 2018; Song et al. 2020; Fahelelbom et al. 2023). The widespread use of FTIR in the analysis of medication is attributed to the produced spectra, as they provide a fingerprint for the analyzed sample (Silverstein 2005; Pavia Donald L. et al. 2009; Farouk et al. 2011). The mid-IR region (MIR) provides the basis for developing analytical techniques characterized by being simple, precise, accurate, and highly sensitive in the quantification process (Tripathi and Mishra 2009; Bansal et al. 2013; Bansal et al. 2021). In addition, such a technique requires minimal analysis time and little to no sample preparation steps.

Consequently, the use of IR spectroscopy seems to be a promising method for the determination of the studied NTI drug. Hence, the present work aims towards the development and validation of an innovative, simple, and sensitive IR spectroscopic method in the MIR region for the quantification of LT4 in brand and generic pharmaceutical tablets.

Experimental

Equipment

For the analysis, the Bruker ALPHA II FTIR spectrometer was used. This equipment was connected to a computer to use the "OPUS" software for analysis of the spectra.

Other equipment used is as follows: Ohaus Explorer analytical microbalance, WiseVen oven, and specac hydraulic press, where the used dies are made of 440C stainless steel with a Mohs hardness of circa 7.5 and offered in two types to prepare solid sample pellets of 13 mm diameter.

Chemicals and solvents

Levothyroxine sodium CRS of 89.2% purity (European Pharmacopeia Reference Standard) was used as a reference to measure peak band area values.

The pharmaceutical formulations used (Euthyrox50®, Euthyrox100®, Euthyrox150®, and Eltroxin®) were purchased from a local authorized pharmacy in Lebanon.

Sigma-Aldrich chloroform (99.8%) was used as a solvent. Himedia potassium bromide powder was used to prepare pellets for IR measurements.

Preparation of LT4 standards

A weight of 10 mg of standard LT4 was dissolved in 3 mL of chloroform. To the resultant solution, 1 g of KBr powder was added. Then, the solution was evaporated in an oven set at 70 °C, allowing the LT4 to deposit on the KBr fine solid particles, obtaining a LT4-KBr mixture having a concentration of 10 µg/mg.

Construction of a calibration curve

Different weights from the standard LT4 powder mixture (10 µg/mg) were diluted using KBr powder to obtain five different standard powder mixtures of concentrations ranging between 0.5 and 5.5 µg/mg, where the final weight of each mixture was kept constant equal to 150 mg to be later pressed into pellets containing concentrations ranging from 25 to 800 µg/pellet. Thereafter, each standard mixture was transferred into a mechanical hydraulic die press and compressed between two metal pistons with stainless steel faces in a cylinder with a pressure value of 10 tons, enough to form a thin, translucent KBr pellet through which the beam of the spectrometer could pass.

FT-IR spectral measurement

The obtained pellets were supported on a holder and placed in the line of the IR beam of the FTIR instrument, and the spectra were recorded in the transmittance mode at 400–4000 cm^{-1} by averaging 16 scans for each spectrum with a resolution of 4 cm^{-1}. Using the software, the % transmittance values and spectra were obtained. The standard calibration curve was constructed by plotting the peak band area (1409.27 cm^{-1}) against their respective concentrations.

Blank readings were recorded by scanning a 150 mg KBr pellet prepared under the same conditions to ensure that there are no interferences at the specified wavenumber.

Pharmaceutical application

Euthyrox (50, 100, and 150 µg) and Eltroxin (100 µg) tablets were assayed using the developed method. Ten tablets from each pharmaceutical formulation were accurately weighed and finely powdered. A weight equivalent to two tablets from each pharmaceutical formulation was dissolved in 5 mL of chloroform, stirred for 5 minutes, and then filtered. To a 3 mL volume of the filtrate, 150 mg of KBr powder were added and left to evaporate as described above. Afterwards, the 150 mg dried-up mixture was used to prepare KBr pellets, and spectral measurements were conducted as described above.

Results and discussion

IR spectroscopy is a vibrational spectroscopic analytical technique that is widely used to identify a chemical species and quantify it based on a correlation between characteristic vibrational bands formed at specific wavenumbers and the measured parameter, whether absorbance, % transmittance, or reflectance. The obtained spectrum represents a fingerprint for the drug molecule and can be used for the quality control of pharmaceuticals given that experimental parameters are optimized and chemical elucidation for the IR bands is paired (Bunaciu et al. 2015).

Conditions optimization

During the quantitative analysis of LT4 in pharmaceutical tablets, the main problem was the interference from the excipients, where those excipients represent more than 99.9% of the marketed tablet weight. Thus, during the FTIR determination, the peak bands from those excipients will mask all the bands relative to LT4 functional groups, hence interfering with its determination. To solve this, LT4 should be extracted from pharmaceutical tablets.

Pellet preparation

Usually, the classical liquid sampling technique by IR spectroscopy is to prepare the liquid samples using a volatile solvent and then deposit a few µls onto the KBr pellet, where the solvent evaporates, leaving a thin layer of the analyte on the pellet. However, this conventional KBr pellet sampling technique cannot be used in the determination of LT4 because, by extracting the active ingredient from the pharmaceutical tablet, the resulting solution has a very low concentration range for an NTI drug. So, taking a few µls from this prepared solution will result in the deposition of non-detectable amounts of LT4 on the KBr pellet.

Accordingly, a modification is required where extraction from the pharmaceutical tablets is performed. The extract was then mixed with KBr. Afterwards, the solvent was evaporated, leaving a detectable amount of LT4 as fine particles deposited on the KBr powder. The final step was to compress the KBr powder mixture into pellets for ease of measurement.

Optimal conditions were achieved by using a constant final weight of 150 mg of spectral-grade KBr powder compressed using a mechanical press at 10 tons for 5 minutes.

These conditions ensure the formation of identical translucent KBr pellets with the same path length. Thus, quantitative determination can be conducted.

Solvent characteristics

In general, the solvent to be selected to dissolve or extract the drug under study should not interact with or ruin the prepared KBr pellets. In addition, the chosen solvent should be capable of exclusively extracting the active ingredients.

Several solvents were tested (water, methanol, ethanol, and chloroform). It was found that only chloroform was able to selectively extract LT4, whereas the other tested solvents were capable of dissolving excipients along with LT4, masking LT4's IR bands and interfering with its determination.

Accordingly, the extraction of LT4 was achieved through the selective dissolution of the active ingredient using chloroform. Also, chloroform has a low boiling point (61 °C), so it can be evaporated rapidly and easily.

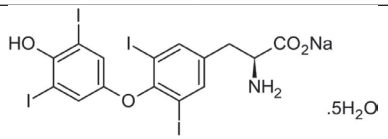
To ensure the efficiency of extraction with chloroform, standard LT4 powder was mixed with KBr powder without the use of chloroform, and pellets were prepared in the same concentration ranges (25–800 µg/pellet). The obtained results showed no difference between with or without extraction for the standard in terms of quantitation of LT4, yielding the same calibration. This demonstrates good performance and complete extraction using chloroform.

Spectral characteristics

The obtained IR reference spectrum of LT4 can be used as a fingerprint for its identification. In addition, the intensities of the functional group bands could be used for their quantification; hence, the concentration of LT4 can be derived.

By observing the position, shape, and relative intensities of the vibrational bands in the IR spectrum of LT4 (Fig. 1), characteristic peaks corresponding to different functional groups of LT4 have been assigned and compiled in Table 1.

Table 1. IR spectral analysis of levothyroxine sodium.



Band Wavenumber (cm^{-1})	Functional group
517.3	C-I
1048.8	C-O (phenol)
1184.89	C-O (ether)
1241.02	C-O (acid)
1317.88	C-N
1357.16	C-N
1409.27	C=C
1538.82	N-H
1605.09	C=O
1639.26	C=C

FTIR determination of LT4

The FTIR spectrum of LT4 (Fig. 1) reveals the presence of many characteristic peaks. Each peak represents a characteristic functional group, such as the C–I alkyl halide-stretching band, observed at 517 cm^{-1}, the C–O ether-stretching band, observed at 1184 cm^{-1}, the C=C stretch band, observed at 1409 cm^{-1} and the N–H primary amine bending band, observed at 1538 cm^{-1}. The degree to which each band contributes to the spectrum is directly related to LT4's concentration. The peaks at wavenumbers 517 cm^{-1}, 1184 cm^{-1}, and 1538 cm^{-1} were not used in the determination since, when relating peak band area to concentration, the obtained calibration graph resulted in unacceptable values of correlation coefficients (r). The best peak chosen for the quantification of LT4 is the C=C stretch band, which was observed at 1409.27 cm^{-1} (1463–1364 cm^{-1}), where the obtained peak is intense and did not interfere with other spectral peaks with very good correlation coefficients (r).

Method validation and statistical analysis

Under optimized experimental conditions, the developed method was validated by determining the following parameters: linearity, limits of detection (LOD) and quantification (LOQ), specificity, accuracy, and precision (repeatability and intermediate precision) according to the procedures described in ICH guidelines (ICH 2005).

Linearity and concentration ranges

For the construction of the calibration curve, the peak band area of the C=C stretch band at 1409 cm^{-1} was plotted against the concentration of LT4 in the range of 75–800 µg/pellet, as stated in Table 2. Linearity parameters, including correlation coefficient, intercept, slope, and the standard deviation of the residuals for the calibration data, in addition to the variance ratio (F-values) and concentration range, are summarized in Table 2.

Table 2. Assay parameters for the determination of LT4 using the proposed FTIR method.

Conc. Range (µg/ pellet)	25.0–800.0
Wavenumber (cm^{-1})	1409.27
LOD (µg/ pellet)	8.121
LOQ (µg/ pellet)	24.545
a (intercept)	0.721
b (slope)	0.022
r	0.999
S_a	0.054
S_b	1.6×10^{-4}
$S_{y/x}$	0.125
a/S_a	13.352
$(S_b)^2$	2.56×10^{-8}
S_b %	0.016
F	1.9355×10^{4}
Significance F	2.6×10^{-16}

The correlation coefficient (r) obtained was high (0.999) with high values of F (low significant F), which confirmed the linearity of the calibration curves. An important statistical parameter for indicating the random error in the estimated values of y is the standard deviation of the residuals, $S_{y/x}$. Also, the importance of $S_{y/x}$ originates from being used to calculate S_a and S_b, the standard deviation of the intercept (a) and the slope (b). These values showed the good linearity of the calibration graphs and their compliance with Beer's law. The variance test for the regression lines revealed that, for equal degrees of freedom, the increase in the variance ratio (F-values) means an increase in the mean squares due to regression and a decrease in the mean squares due to residuals (i.e., the less the scatter of experimental points around the regression line). Consequently,

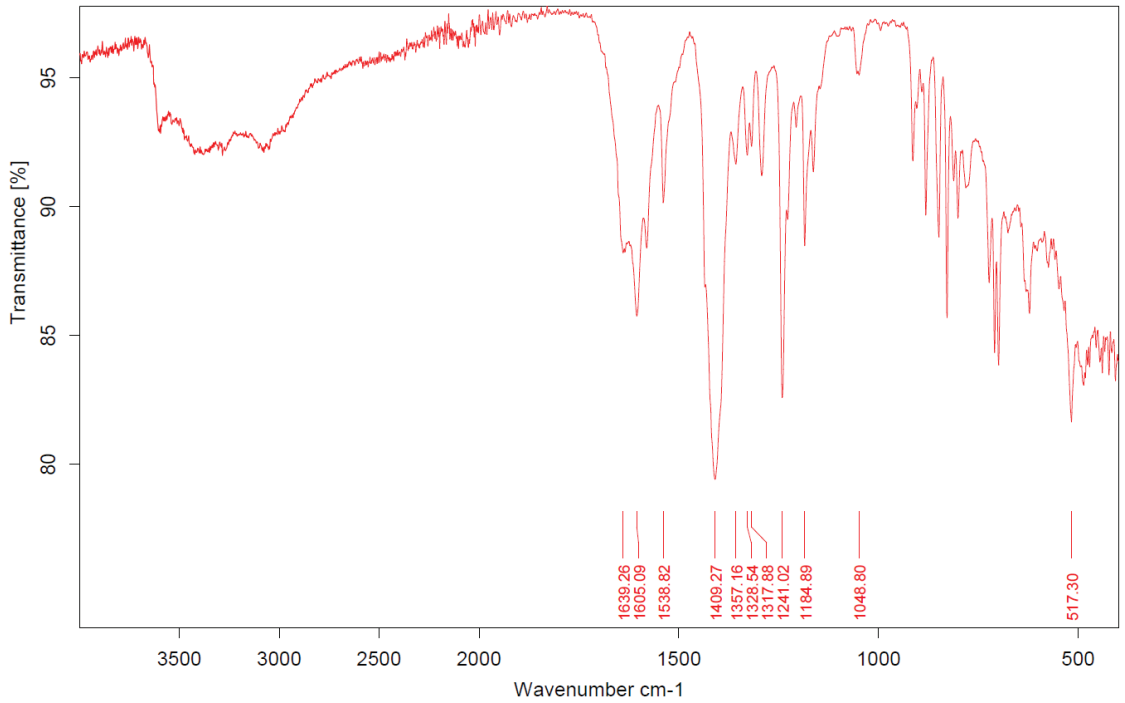


Figure 1. IR reference spectrum of standard levothyroxine sodium.

regression lines with high F-values (low significance F) are much better than those with lower ones. In conclusion, good regression lines show high values for both r and F statistical parameters (J.N. and Mileer 2005).

Limit of detection (LOD) and limit of qualification (LOQ)

The (LOD) and (LOQ) limits were calculated as (3.3 σ / S) and (10 σ / S), respectively, where σ is the intercept standard deviation and S is the calibration curve slope. The low obtained values shown in Table 2 confirm the sensitivity of the method and indicate the reliability of the method to detect and quantify LT4 by the FTIR method used.

Specificity

As per the ICH guidelines, the developed method should be specific. Specificity here refers to the ability to distinguish, identify, and quantify LT4 amidst the presence of the matrix (excipients), degradation products, or impurities, namely liothyronine sodium (ICH 2005).

The specificity of the method was determined by comparing the spectra of the pharmaceutical LT4 from tablets with those of the standard LT4. The results revealed overlapping spectra. In addition, to ensure the absence of excipient interference, the spectrum of an excipient mixture that is commonly employed in tablet formulations (starch, microcrystalline cellulose, magnesium stearate, and gelatin) was measured after being subjected to the same experimental conditions. Chloroform was used for extraction, followed by filtration, the addition of KBr, evaporation, and finally the pellet press. Comparing the spectra of the pharmaceutical LT4 from tablets with those of an excipient mixture showed no excipient interference, where there was no peak band at 1409 cm^{-1}(Fig. 3). This confirms the specificity of the developed method.

A common impurity that could be found in trace amounts in LT4 powder or tablets is liothyronine sodium. This could be due to degradation, manufacturing processes, or cross-contamination. Liothyronine sodium only differs from LT4 by having one less "C–I" bond (Ruggenthaler et al. 2017). The developed method for the quantitation of LT4 is based on C=C, which is the same in both, so it cannot differentiate. However, the difference between both would be detected in the fingerprint area (specifically seen in the C-I bond at 517 cm^{-1}). Accordingly, the complete spectrum should first be observed in order to determine whether there is the presence of LT4 or liothyronine. Also, this confirms that this FTIR method is a highly specific and selective technique for the detection of impurities or adulterations.

Accuracy

The accuracy of the proposed method was determined through a recovery study. Three different concentrations of LT4 standard pellets (100, 200, and 400 µg/pellet) prepared from independent stock powder were assayed three times. Good accuracy, expressed as % recovery, was obtained. The results, summarized in Table 3, show that the % recovery values do not exceed the accepted limits (98–102%), which demonstrate the accuracy of the developed method.

Precision

The precision of the method was evaluated as intra-day repeatability, inter-day precision, or intermediate precision. Repeatability studies (intra-day) were performed by analyzing three different concentrations of LT4 within the

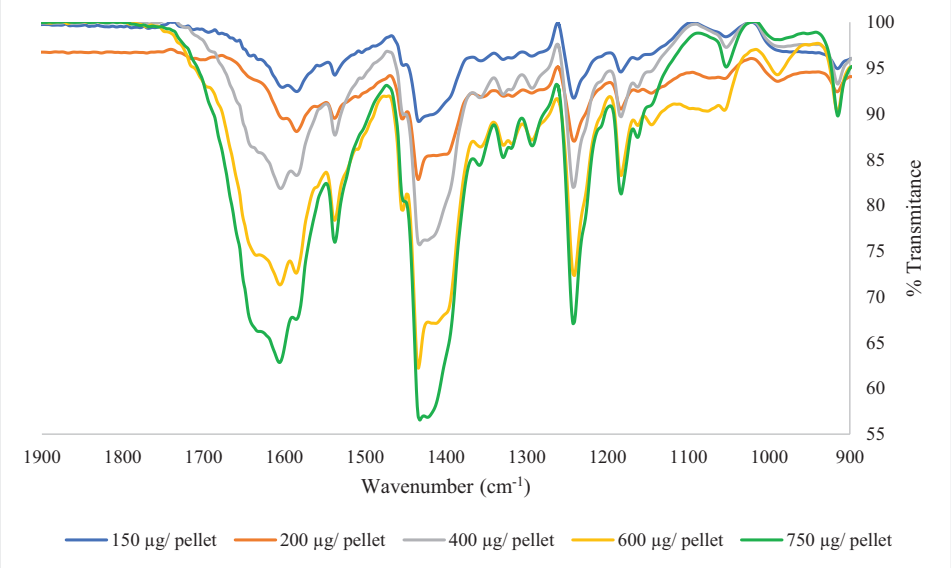


Figure 2. Overlaid IR spectra of LT4 standards.

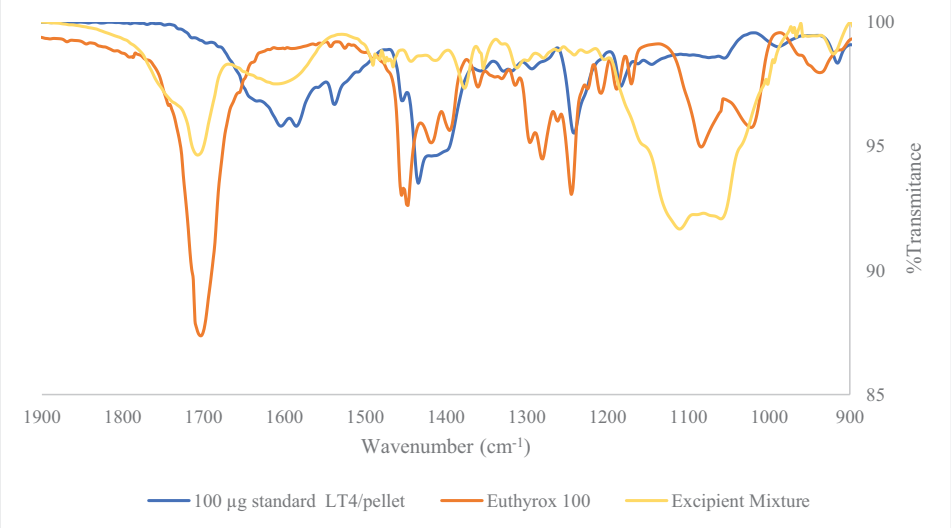


Figure 3. Overlaid IR spectra of the LT4 standard, Euthyrox 100® tablet, and excipient mixture.

Table 3. Accuracy data for the determination of LT4 using the proposed FTIR method.

LT4 quantity µg/pellet	Mean Recovery ± SD[a]	RSD %[b]	Er %[c]
100	98.00 ± 0.92	0.94	-2.00
200	99.36 ± 1.25	1.25	-0.64
400	99.18 ± 1.78	1.78	-0.82

[a] Mean ± SD for three determinations
[b] % Relative standard deviation
[c] % Relative error

calibration range in triplicate, all on the same day, using identical working conditions. Intermediate precision (inter-day) was assessed by repeating the assay on three different days under the same experimental conditions.

Good precision, expressed by the low percentage RSD such that the values did not exceed an acceptable limit, was obtained, proving the high precision of the method. The results are summarized in Table 4.

Tablet assay

The applicability of the proposed method was evaluated for two different pharmaceutical formulations: Euthyrox® tablets and Eltroxin® tablets. The results obtained from the FTIR analysis were satisfactory for the determination of LT4 in its pharmaceutical formulations and were comparable to the labeled amount expressed by high percentage recoveries with low % RSD, which indicate high accuracy and precision in the determination. Also, a statistical comparison between this method and a reference method using the student's t-test and the variance ratio F-test was performed (Jamal et al. 2023). Since the calculated t- and F-values (ICH 2005) for each drug did not exceed the theoretical ones, this indicated that there was no significant difference between the proposed methods for the determination of the drugs in commercial tablets. The results obtained are summarized in Table 5.

Table 4. Intra-day and inter-day precision for the determination of LT4 using the proposed FTIR method.

LT4 quantity µg/pellet	Intra-day precision			Inter-day precision		
	Mean Recovery ± SD[a]	RSD %[b]	Er %[c]	Mean Recovery ± SD[a]	RSD %[b]	Er %[c]
100	100.377 ± 2.794	2.783	0.377	99.574 ± 2.789	2.802	-0.426
200	100.133 ± 3.636	3.631	0.133	98.307 ± 1.399	1.423	-1.693
400	99.773 ± 2.525	2.530	-0.227	100.246 ± 2.441	2.435	0.246

[a] Mean ± SD for three determinations
[b] % Relative standard deviation
[c] % Relative error

Table 5. Determination of LT4 in pharmaceutical tablets using the proposed FTIR method.

		FTIR		Reference method (HPLC)
Euthyrox25 *	Mean Recovery ± SD[a]	99.264 ± 3.793		100.669 ± 2.372
	RSD %[b]	3.822		2.356
	Er %[c]	0.736		-0.669
	**t-test		0.535	
	**F-test		0.385	
Euthyrox50 *	Mean Recovery ± SD[a]	101.472 ± 4.003		96.332 ± 4.302
	RSD %[b]	3.945		4.466
	Er %[c]	-1.472		3.668
	**t-test		0.207	
	**F-test		0.809	
Euthyrox100 *	Mean Recovery ± SD[a]	97.842 ± 0.764		98.591 ± 4.146
	RSD %[b]	0.781		4.205
	Er %[c]	2.158		1.419
	**t-test		0.812	
	**F-test		0.328	
Euthyrox150 *	Mean Recovery ± SD[a]	100.004 ± 3.658		99.477 ± 3.257
	RSD %[b]	3.658		3.274
	Er %[c]	-0.004		0.523
	**t-test		0.816	
	**F-test		0.827	
Eltroxin100 *	Mean Recovery ± SD[a]	97.783 ±1.821		99.482 ± 3.264
	RSD %[b]	1.862		3.281
	Er %[c]	2.217		0.518
	**t-test		0.322	
	**F-test		0.410	

[a] Mean ± SD for the five determinations
[b] % Relative standard deviation
[c] % Relative error
**Theoretical values of t- and F- at P = 0.05 are 2.13 and 6.93, respectively.

Comparison to the HPLC reference method

Up until now, most cited methods for LT4 determination employ HPLC as a method for separation and quantitation in pharmaceutical tablets. As for the procedure followed, extraction from the tablets using specific solvent(s), then the HPLC machine must be operated for a certain duration of time before the injection is run. Such a technique is expensive, time-consuming, and requires the use of several solvents, making the HPLC methods inconvenient for routine and quality control analysis.

However, the proposed FTIR method provides an appealing method for determination because it neither requires sophisticated instrumentation nor several prior separation steps or the use of solvents prior to analysis. Abide the use of a non-green solvent (chloroform), where a minimum volume was used and later evaporated, this step was essential to selectively extracting LT4 from the pharmaceutical tablets, where the excipients produced very strong interference in the IR spectrum if not removed. As well, the present method reached a LOQ where the lowest available tablet dose (25 µg) was quantified, indicating the good sensitivity of the analytical method. Moreover, a statistical comparison with a reference HPLC method showed no significant difference in the results.

Conclusion

In conclusion, the present work represents an approach for the assay of LT4 in its different pharmaceutical preparations using FTIR. The developed Fourier transform infrared spectroscopic method offers an attractive alternative to classical testing techniques. This method represents

a sensitive, precise, and accurate method that does not require a sophisticated instrument or several prior separation steps. These factors encourage the use of such methods for the quality control of this investigated drug by pharmaceutical companies, as they have equal accuracy and precision compared to the other developed methods. A specific peak band area for a characteristic IR band selected from the spectrum revealed a linear relationship to the concentration of LT4, allowing the determination of the tested tablets. An application for the method revealed that there is no difference in the labeled amount between the tested brand and generic. Hence, the reason for LT4 blood level fluctuations should be further investigated.

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