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Simultaneous Estimation Method Development and Validation of Levothyroxine and Liothyronine by HPLC Method

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Abstract:

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay has been developed for simultaneous estimation of various tablet formulations. The separation was achieved by using C-18 column (Phenomenax, 250 x 4.6mm i.d.) coupled with a guard column of same material, in mobile phase Acetonitrile: Water (25:75). The pH of mobile phase was adjusted to 6.0 ± 0.1 with 50% ortho phosphoric acid. The flow rate was 1.0 mL.min-1 and the separated drugs were detected using UV detector at the wavelength of 300 nm. The retention time of various formulations was noted to be 2.08 and 5.05 min, respectively for Levothyroxine and Liothyronine, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent. It was successfully applied for the analysis of these drugs in marketed formulations and could be effectively used for the routine analysis of formulations containing any one of the above drugs, or a combination, without any alteration in the chromatographic conditions.

Keywords:

Introduction: Hypothyroidism is a state of deficiency of endogenously produced thyroid hormone and affects nearly 5.0% of the world population [1]. Hypothyroidism management is done with monotherapy of levothyroxine and Levothyroxine is a synthetic analogue of thyroxine hormone and is used in concentration equivalent to thyroid stimulating hormone present in the body [2]. Levothyroxine sodium (l-3,5,3′,5′-tetraiodothyronine sodium salt) pentahydrate is the sodium salt of the levo-isomer of thyroxine, and is the primary hormone secreted by the thyroid gland to regulate metabolic processes and physical development [3]. Levothyroxine sodium serves as a replacement therapy for the inadequate secretion of T4 in the body and is commonly used to treat hypothyroidism, simple non-endemic goiters, and chronic lymphocytic thyroiditis, thyroxine, a prohormone and iodothyronine the more active form produced from T4, are solely responsible for the normal development of the central nervous system in infants, and the regulation of the normal functioning of multiple organ systems in adults [4]. Liothyronine Sodium (L-triiodothyronine or LT3Na) is typically used to treat patients with hypothyroidism, a condition wherein the thyroid gland does not produce enough thyroid hormone. It is also used to help decrease the size of enlarged thyroid glands (goiter) and treat thyroid cancer [5]. As we mentioned above, Liothyronine Sodium is a white or slightly coloured, hygroscopic powder, practically insoluble in water, slightly soluble in ethanol (96 %) and dissolves in dilute solutions of alkali hydroxides. Liothyronine Sodium has the chemical structure depicted below, and it is chemically described as O-(4- hydroxy-3-iodophenyl)-3, 5-diido-monosodium salt (1:1) (Figure 1). The molecular weight of Liothyronine Sodium is 672.96 g/mol and molecular formula is C15H11I3NNaO4 [6].

Experimental

Chemicals and Reagents Pharmaceutical grade were obtained from Intas Pharm. Pvt. Ltd. India. Water and Acetonitrile (HPLC grade) were obtained from Rankem, Ranbaxy Fine Chemical Limited, New Delhi, India. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard volumetric glassware.

Instrumentation and Chromatographic Conditions

The instrument used was a Shimadzu chromatographic system (Japan), equipped with an LC-10 AT vp solvent delivery module, SPD-10A UV-Visible detector, and a Rheodyne model (7725i) injector valve fitted with 20µL volume sample loop. The samples were injected through a Hamilton, Bonodaz AG microliter syringe. Chromatographic separation was

performed on C-18 column (Phenomenax, 250 x 4.6mm i.d.) coupled with a guard column of the same material. The mobile phase was composed of Acetonitrile: Water (25:75 v/v) and pH of mobile phase was adjusted to 6.2 with phosphate buffer solution. The flow rate was maintained at 1.0 mL.min-1. The column effluent was monitored on UV detector set at 300 nm [7].

Preparation of Stock and Working Standard Solution:

Individual stock solutions of the both drugs (600 µg.mL-1) were prepared by dissolving 6 mg of individual drug in 10 ml of mobile phase. The mobile phase standards containing mixture of both drugs were prepared by appropriately diluting the stocks in the range of 15–150 µg.mL-1 using mobile phase.

Preparation of Sample Solution

Mixture of sample solutions were prepared from formulations containing:

A) Levothyroxine (100mg)

(B) Liothyronine (100mg)

Twenty tablets of formulations containing A and B were weighed and crushed to a fine homogenous powder. Quantity equivalent to 25 mg was accurately weighed and taken individually in a 25 mL volumetric flask. The powdered mixtures were dissolved in the mobile phase and volume was made up. The supernatants of both the solutions were taken, mixed thoroughly and diluted with the mobile phase (final concentration, 70, 30, 80 and 95 µg.mL-1 for all formulations, respectively) and 100 µL of this solution was injected for HPLC analysis [8].

Quality Control Standards:

The quality control (QC) standards for both drugs were prepared from stock solutions by dissolving 6mg each of individual drugs in 10 mL of mobile phase. The working solutions of the mixture of both drugs were prepared in the concentration ranges of low (40 µg.mL-1), medium (80 µg.mL-1), and high (150 µg.mL-1) concentrations using mobile phase as a solvent.

Method Validation:

The method was validated in terms of stability, linearity, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ)

Results and Discussion

Optimization of Chromatographic Conditions: Spectroscopic analysis of compounds showed that the drugs have maximum UV absorbance (λmax) at 275 nm and 320 nm respectively. Therefore, the chromatographic detection was performed at 285 nm using a UV-Visible detector. It was observed that when a combination of both drugs was injected, Levothyroxine and Liothyronine together gave a single peak. Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in the mobile phase. Different experiments were performed to optimize the mobile phase but adequate separation of drugs could not be achieved. By altering the pH of mobile phase, a good separation was achieved. The optimized mobile phase was determined as a mixture of acetonitrile: pH 1.2 buffer (20:80) at a flow rate of 1.0 mL.min-1. Under these conditions were eluted at 2.08 and 5.05 minutes respectively for Levothyroxine and Liothyronine with a run time of 10 min [9].

Method Validation

Linearity and Calibration standards: A number of various concentrations of a mixture of both drugs were prepared for linearity studies and response was measured as peak area. The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 10 to 100 µg.mL-1 for both drugs. The best fit for the calibration curve could be achieved by a linear regression equation of Levothyroxine and Liothyronine found to be $y = 11.135x – 25.032$ and $y = 14.1015x + 31.8022$, respectively and the regression coefficient values (r2) were found to be 0.9999 and 0.9998 respectively indicating a high degree of linearity for both drugs.

Specificity: The specificity studies revealed the absence of any other excipient interference, since none of the peaks appeared at the same retention time of both drugs. The interaction study of both drugs in standard solution was also carried out by comparing peak of each drug, individually Vs peaks of drug mixture. Interaction studies indicated that the analytes did not interact with each other and were well within the acceptance level of ± 2.0% [10].

Accuracy and precision: The accuracy of method was determined and calculated as % bias. The low value of % bias showed that the method is accurate within the acceptance limit of 2%. The precision (repeatability and intermediate precision) of the method was established by carrying out analysis of the analytes using proposed method. The low value of % CV

showed that the method is precise within the acceptance limit of 2%. The results are shown in Table 1.

LOD and LOQ: The determination of limit of detection (LOD) and limit of quantitation (LOQ), the method based on the standard deviation and slope was adopted. The limit of detection for both drugs were 0.35 µg.mL-1 and 0.352 µg.mL-1, respectively and the limit of quantitation (LOQ) was 0.37 µg.mL-1 and 1.03 µg.mL-1, respectively [11].

System suitability parameters: The system suitability parameters were done by three replicate injections of mixed standard solution injected to HPLC. The parameters such as the Resolution, Capacity factor, Tailing factor, Theoretical plate, Retention volume and Asymmetry factor of the peaks were calculated.

Conclusion

An RP-HPLC method for simultaneous estimation of Levothyroxine and Liothyronine were developed and validated. The amounts attained by the proposed method are between 99.13% and 101.07%, within the acceptance level of 90% to 110%. The results obtained specify that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 10 to 100 µg.mL-1 for all both drugs. It can be used for the routine analysis of formulations covering any one of the above drugs or their groupings without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method decreases the time mandatory for switch over of chromatographic conditions, equilibration of column and post column blushing that are typically related when different formulations are analyzed.

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Table 1. Accuracy (% Bias) and Precision (%RSD) of Levothyroxine and Liothyronine

Drugs	Concentration	Accuracy % Bias	Precision % RSD

		Intra day	**Inter day**	**Intra day**	**Inter day**
Levothyroxine		0	0	0	0
	10	0.6	0.9	0.2	0.5
	20	0.4	0.7	0.2	0.4
	30	0.4	0.1	0.1	0.2
	40	0.2	0.5	0.3	0.5
	50	0.2	0.6	0.5	0.6
	60	1.2	1.3	0.5	0.9
	70	1.4	1.6	1.2	1.2
	80	0.2	0.5	0.6	0.7
	90	0.3	0.8	0.7	0.9
	100	0.5	0.8	0.4	0.8
Liothyronine	0	0	0	0	0
	10	0.9	1.2	0.9	1.2
	20	1.1	1.2	1.1	1.4
	30	0.9	0.9	0.6	0.8
	40	0.7	1.1	0.4	1.1
	50	1.1	1.2	0.4	0.7
	60	0.9	1.2	0.9	1.2
	70	0.8	1.1	0.4	0.2
	80	1.2	1.3	0.5	0.9
	90	1.4	1.6	1.2	1.2
	100	1	1.1	0.6	0.8

Table 2. System suitability parameter of the HPLC method

Parameters	**Levothyroxine**	**Liothyronine**
Resolution	4.03	6.42
Capacity factor	2.1	1.05
Tailing Factor	1.01	1.25
Theoretical plates	7956	8424
Asymmetry factor	1.3	1.2

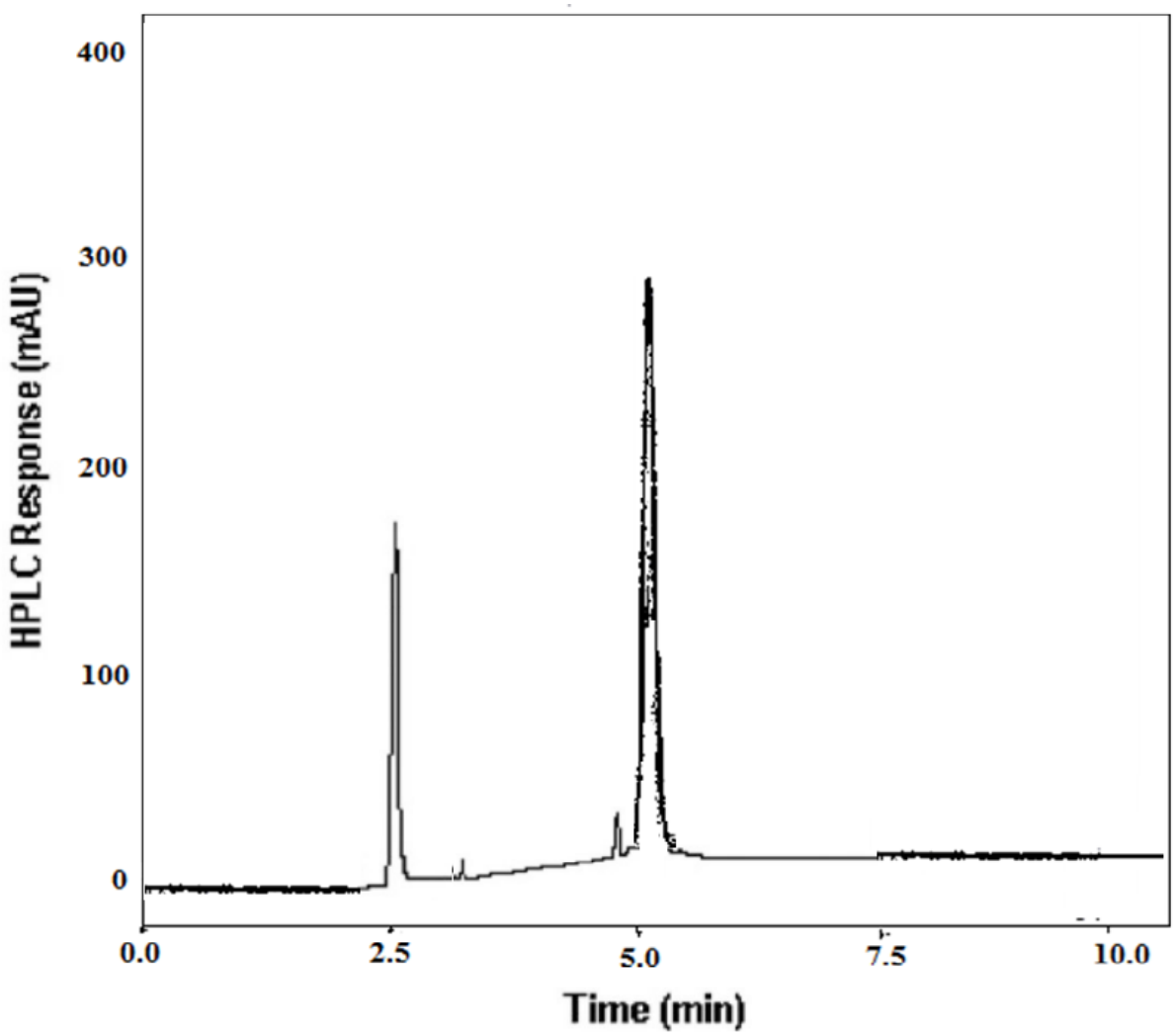

Figure 1: RP-HPLC Chromatogram of both drugs Levothyroxine and Liothyronine