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A simple HPLC method for the dissolution studies on levothyroxine sodium tablets

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Summary

A simple method for the analysis of dissolution samples of levothyroxine sodium was developed. The procedure consists of filtration of the dissolution sample and direct injection of the sample onto the HPLC column. Although levothyroxine sodium is present at low concentrations (20–50 ng/ml) in the dissolution samples, its detection and quantitation was possible due to the large volumes of injection onto the HPLC column of up to 2 ml and also due to the enhanced sensitivity of up to 6 times by UV monitoring at 225 nm instead of at 254 nm. Large volumes of injection of up to 2 ml are possible due to 'sample enrichment' of the amino acid due to the strong adsorption property of the amino acid on the reverse-phase material of the column or precolumn packing material. The usefulness of the procedure was demonstrated by the analysis of various brands and dosage strengths of the above tablet.

Introduction

A number of pharmaceutical preparations are currently available for the treatment of hypothyroid conditions. These pharmaceutical preparations range from products such as thyroid tablets, (whose composition is not defined properly) to tablets that contain pure synthetic amino acid sodium salts, such as liothyronine sodium and levothyroxine sodium (United States Pharmacopeia, 1980). Presently the U.S.P. (1980) does not have either a content uniformity or a dissolution requirement for liothyronine sodium, levothyroxine sodium and liotrix tablets, although the above tablets contain synthetic amino acids. Moreover, the bioavailability and the bioequivalence of the thyroid medication is not established presently. Prior to

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conducting such a study. it is essential to develop analytical methodology for conducting content uniformity, dissolution and plasma analysis. With the publication of recent reports on the lack of bioequivalence among different manufactures of levothyroxine sodium (Hansen, 1980; Ingbar et al., 1980; Jacobsen et al., 1980; Stoffer and Szpunar, 1980) it became apparent that it is necessary to develop precise chemical methodology for dissolution studies, and further examine the bioequivalence of the above products.

First reports of the adaptation of an HPLC procedure assay for the tablet content-uniformity and dissolution (Rapaka et al., 1979, 1981) have appeared recently from this laboratory. This procedure has certain advantages, i.e. processing of large volumes to achieve the needed sensitivity and isolation of the amino acids in a pure state that is suitable for derivatization; however, it is time consuming. Recently in the literature, there are reports on a direct and simple extraction procedure described for the determination of liothyronine sodium and levothyroxine sodium from tablets by electrochemical detection (Jacobsen and Fonahn, 1980) and also a slightly modified version of the above procedure with HPLC-UV detection (Smith et al., 1981) or HPLC-amperometric detection (Hepler et al., 1980). In general, the direct injection procedure has the disadvantage for tablet analysis that the recoveries may not be quantitative and erroneous results are possible, especially if an internal standard is not used (Smith et al., 1981; Jacobsen and Fonahan, 1980). This is principally due to the unusual solubility properties and strong adsorption properties of the thyroidal amino acids (Petersen et al., 1976, Rapaka et al., 1980). However, a direct injection procedure may be utilized for dissolution studies, since in this case the value determined is the amount that is recovered into the solution from the tablet or capsule formulation. Hence, unlike the tablet assay, recovery is not a problem in analyzing dissolution samples. In this report a simple procedure for dissolution studies based on direct injection coupled with trace enrichment technique is described.

Materials and methods

Materials

L-Thyroxine sodium salt pentahydrate (3,3',5,5'-tetraiodo-L-thyronine) was purchased from Sigma Chemicals. Levothyroxine tablets were purchased from District Wholesale Drugs, Landover, MD. Methanol and acetonitrile were of HPLC grade and were obtained from Burdick and Jackson. All other chemicals were of reagent grade.

Apparatus

The high-performance liquid chromatograph consisted of a Waters Associates Flow pump, Model 6000A, a Waters Associates UV detector Model 440 and a Varian Model 9176 strip chart recorder. A Tracor 970 variable wavelength absorbance detector was connected in series with the Model 440 UV detector. A Waters Associates μBondapak C_{18} reverse-phase stainless column (3.9 mm × 30 cm)

was used. A Brownlee Lab precolumn packed with a reverse-phase material (4.6 mm × 3 cm, RP-18, 10 mm) was used to remove interfering materials from the sample prior to passage onto the analytical column. Dissolution studies were conducted on an apparatus designed by Federal Engineering Laboratory in Winchester.

Chromatographic conditions

The mobile phase used in the analysis of levothyroxine sodium was methanol–water–phosphoric acid (57.5:43.5:0.1, v/v). The flow rate was 2 ml/min. The eluate was monitored at two wavelengths 225 nm and 254 nm by two absorption spectrophotometers connected in series.

Precision and linearity

Levothyroxine sodium pentarhydrate was dissolved in a solution of 2% ammonium hydroxide in methanol and diluted, as needed, to obtain solutions of 100 μg/ml and 10 μg/ml of levothyroxine sodium. Aliquots of this solution were added to a 500 ml volumetric flask and made to volume with distilled water (same volume as of the dissolution medium) to obtain desired concentrations of levothyroxine sodium. At each concentration, the samples were analyzed in triplicate. Within and between run variability was determined by analyzing standard solutions.

Known volumes of a solution of levothyroxine sodium (200 ng/ml) from 0.1 to 2.0 ml (20–400 ng) was injected and peak heights obtained were plotted against volumes of injection (concentration).

Procedure for dissolution study

Dissolution studies were conducted using the U.S.P. rotating paddle method with 500 ml of deionized water as the dissolution medium at 37 ± 0.5°C. The paddle speed was set at 100 rpm.

Levothyroxine sodium studies

Five dosage levels, i.e. 25, 50, 100, 200 and 300 μg, were studied with 500 ml of distilled water. A 30 min sample was taken for each dosage level and at each dosage 3 samples were analyzed.

Procedure for analysis

Dissolution samples were pipeted and filtered through fine fritted disc filters. A solution of 50 μl of 2% ammonium hydroxide in methanol was added to 10 ml of the filtrate and mixed well. The filtrate was injected onto the HPLC column. All the glassware used was silanized. The dissolution vessels were silanized as well.

Results

Standard aqueous solutions of levothyroxine sodium were injected from 0.1 to 2.0 ml and plotted against peak heights; a linear relationship is obtained (Fig. 1).

TABLE I

PRECISION OF THE HPLC METHOD FOR LEVOTHYROXINE SODIUM FROM STANDARD SOLUTIONS

Eluate monitored at 225 nm				Eluate monitored at 254 nm			
Total theoretical amount (ng)	Amount found mean, range (ng)	CV [a]	Relative error (%)	Total theoretical amount (ng)	Amound found mean, range (ng)	CV [a]	Relative error (%)
10	9.3 (9.1–9.8)	4.3	7.0	10	Cannot be measured	–	–
20	21.9 (20.5–23.3)	6.39	9.5	20	–	–	–
50	49.8 (48.9–51.7)	3.3	0.4	50	41.8 (33.2–50.4)	20.7	13.6
80	82.1 (80.2–83.0)	2.0	1.1	80	67.6 (63.3–72.0)	6.4	15.5
100	100.1 (96.6–105.5)	1.5	0.1	100	89.2 (84.9–93.5)	4.8	10.8
200	197.2 (195.3–198.1)	0.8	1.4	200	189.8 (184.1–197.1)	3.5	2.0
400	400.9 (399.9–402.8)	0.4	0.3				

[a] n = 3.

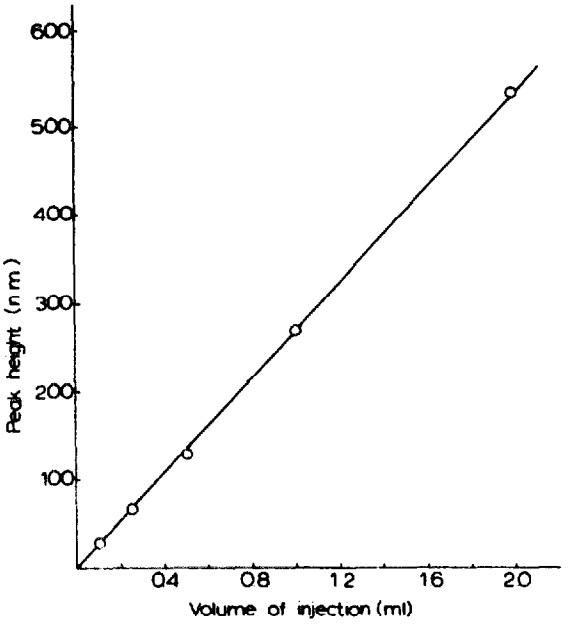

Fig. 1. A plot of peak height in nm versus volume of injection of levothyroxine sodium (200 ng/ml).

Usually, in the analysis of dissolution samples not more than 0.5 ml of a filtered dissolution sample was needed. As the peak heights were linear with volume in the range of volume studied, a standard (peak height versus concentration) plot was developed usually at a single volume of injection (by generally injecting 1.0 ml) at various concentrations. The coefficient of correlation was around 0.998.

The wavelength of detection was 225 nm. Utilizing large volumes of injection and selected wavelength monitoring, concentrations as low as 10 ng/ml could be determined with a coefficient of variation of 4.3% and a relative error of 7% and at higher concentration, the procedure has excellent precision (0.4% CV at 400 ng/ml) (Table 1). However, utilizing 254 nm for detection, even with large volumes of injection, only dissolution samples containing above 100 ng/ml sodium levothyroxine could be determined accurately (Table 1). However, the method using 225 nm for detection was found to be precise over the concentration range of 10–400 ng/ml. The within run variability was around 3–5% and between run variability is around 4–7%. Regression coefficients were 0.99 or better.

The dissolution samples are filtered through a silanized fine grade sintered glass funnel prior to injection onto HPLC to eliminate the interfering peaks. HPLC chromatogram of a sample not filtered as above (filtered through a filter paper) is presented in Fig. 2, along with the chemical structure of thyroxine. This HPLC chromatogram was obtained by monitoring the eluate both at 225 and 254 nm and demonstrates the increase in sensitivity by monitoring at 225 nm. Filtration of the dissolution sample through a sintered glass vessel results in the elimination of the interfering peaks and such HPLC chromatograms are presented in Fig. 3A and B.

Using the procedure developed, dissolution samples from 4 brands of levothyro-

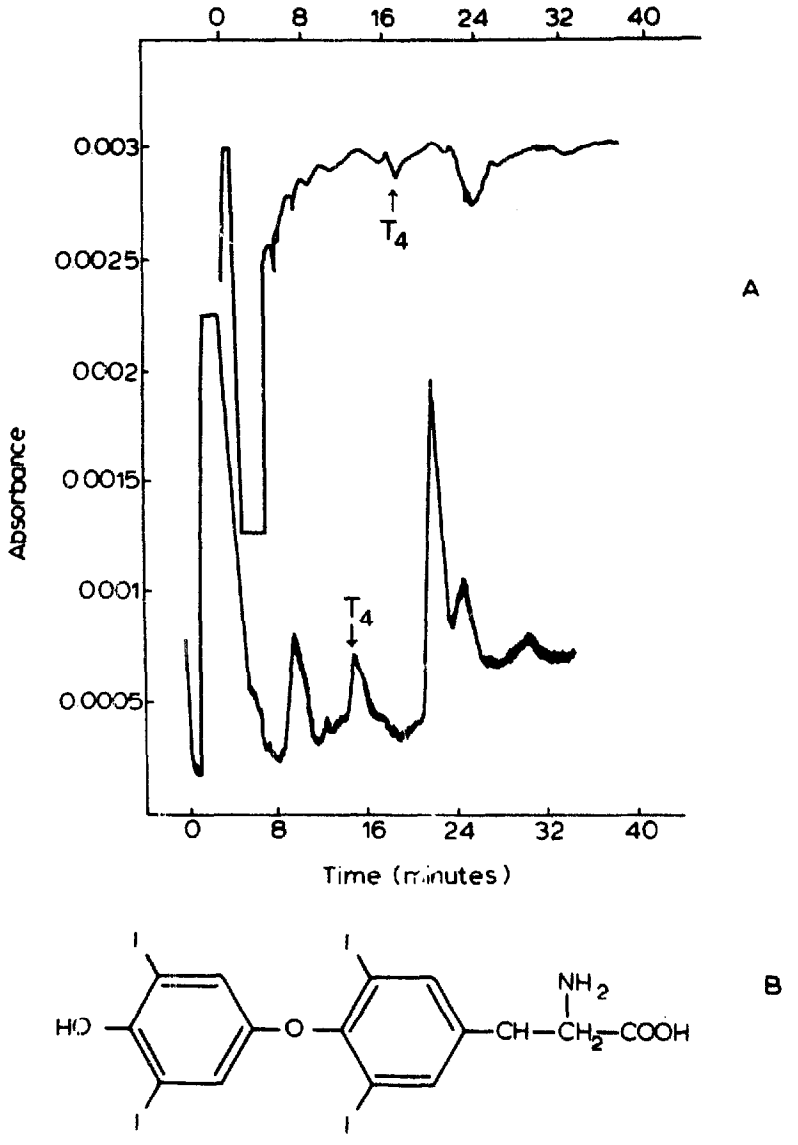

Fig. 2. A: HPLC chromatogram obtained after injection of 1 ml of a dissolution sample of a 50 μg levothyroxine sodium tablet and the sample was filtered through a filter paper. The top portion of the chromatogram is obtained by monitoring the eluate at 254 nm and levothyroxine peak is indicated by an arrow. The bottom chromatogram is obtained by monitoring the eluate at 225 nm. HPLC conditions are as described in text. B: chemical structure of thyroxine.

xine sodium (ranging from 25 μg to 300 μg) were analyzed and the results are presented in Table 2.

Discussion

The earlier published method (Rapaka et al., 1981) is useful both for tablet assay and dissolution studies. However, a simpler and faster method is required for

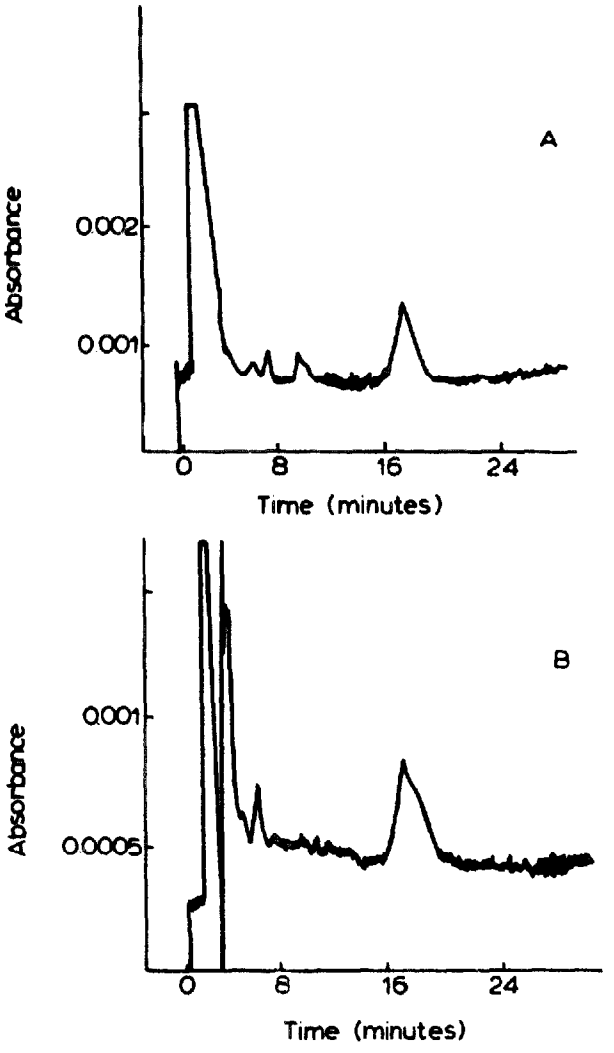

Fig. 3. A: HPLC chromatogram obtained from a filtered dissolution sample (filtered through a fritted glass disc of fine grade) and other conditions are as described for Fig. 2A. B: HPLC chromatogram obtained as described above (Fig. 3A) of a generic brand of levothyroxine sodium tablet.

analysis of dissolution samples. The method described meets the above requirements. As the tablet formulations contain levothyroxine sodium only in small amounts, a very sensitive detection system is required. Two simple ways of enhancing sensitivity for levothyroxine sodium were achieved by selected wavelength monitoring and utilizing large volumes of injection. As shown in Table 1, selected wavelength monitoring increased the sensitivity of detection.

The thyroidal amino acids have hydrophobic aromatic residues and iodine atoms. These properties make the amino acids particularly suitable for 'trace enrichment'. A number of publications have appeared relating to 'trace enrichment' (Frei, 1978; Ishii et al., 1978; Otsuke, 1977; Schauwecker et al., 1977; Wolkoff and Creed, 1981) where the chemical to be analyzed is present in only small concentrations. The

TABLE 2

DISSOLUTION STUDY OF LEVOTHYROXINE SODIUM IN 500 ml OF DEIONIZED WATER AT 37°C USING 100 rpm ROTATING PADDLE METHOD [a]

Manufacturer brand	Dosage (μg)	Mean percent drug dissolved at 30 min ± S.D. [b]
30.6 ± 2.5	A	100
27.4 ± 2.5	B	50
38.4 ± 4.1	C	25
36.7 ± 4.9	D	100
32.9 ± 2.3	D	200
24.0 ± 6.9	D	300

[a] The eluate was monitored at 225 nm.
[b] n = 3.

technique for a non-polar compound consisted of a preconcentration step on a precolumn where the compound is deposited as a 'plug' on the top of a column by injecting the compound in a relatively polar solvent followed by elution with solvents of decreasing polarity. Thyroidal amino acids have poor solubility in water and aqueous buffers. Hence attempts were made to solubilize levothyroxine sodium in a polar solvent like water (by dissolving the amino acid in a small volume of 2% ammonium hydroxide in methanol, followed by dilution with water) and injecting on a reverse-phase column. When aqueous solutions of levothyroxine sodium was injected in volumes ranging from 0.2 to 2.0 ml, peaks with good chromatographic characteristics resulted and more significantly, peak heights were linear with concentration, irrespective of the volume of injection. Dissolution medium is usually water and frequently buffers were used as well. Our preliminary experiments in various buffers of pH 2.4–7.4 indicated that large volumes of buffers could also be injected similarly. The linearity of response with large volumes of injection, in a solvent of either water or aqueous buffers, made this procedure particularly attractive. Another advantage of large volumes of injection is that use of an internal standard is not required if large volumes are injected. However, if the internal standard is desired, either 3,5-diiodo-3',5'-dibromothyronine (Rapaka et al., 1979) or metathyroxine (a pentaiodo analog of thyroxine, Shiba et al., 1964) may be utilized.

A number of mobile phases were investigated for the HPLC analysis of the amino acid and finally it was decided to use a system comprising of methanol–water–phosphoric acid (Hearn et al., 1980). However, a slightly modified mobile phase developed by Alexander and Nishimoto (1979), consisting of acetonitrile–water–phosphoric acid may also be used.

This dissolution method consisted of filtration of the dissolution sample and injection of a known volume of up to 2 ml onto the HPLC column. Here it must be pointed out that filtration is a critical step. Initially the dissolution samples were filtered through pre-pleated filters and the filtrate injected onto the HPLC column

and the eluate monitored at 225 nm and 254 nm. Although at 225 nm a peak for compound II could be detected, there are other peaks possible resulting from the excipients (Fig. 2). However, filtration through a fritted disc, results in a better chromatogram (Fig. 3) and hence all dissolutions were filtered through a fine grade sintered glass funnel. A UV scan of the HPLC peak revealed (instrument equipped with an auto scan) that there are no other interfering materials present in the peak. It must be pointed out here that a point of major concern in the direct injection method is whether any of the dyes or excipients from the formulation are interfering and the method can be used only if it is established that there are no interferences.

Sodium salt of levothyroxine is slightly soluble in water or buffers of pHs ranging from 2 to 7. The reported solubility for sodium levothyroxine is 15 mg/100 ml of water (Merck Index, 1977), and in our experiments it was found that solubility of sodium levothyroxine was 12 mg/100 ml. As the solubilities of these compounds are low, the filtered dissolution samples were analyzed at different time periods to examine if the compounds precipitated from the dissolution medium. Levothyroxine sodium was precipitating from water and as much as 30% loss from solution was observed after one hour and about 70% loss from solution after 24 h. Hence, after filtration of the amino acid, 50 μl of 2% ammonium hydroxide in methanol was added to 10 ml of dissolution medium. Analysis of the samples immediately, and after 24 h, showed that no precipitation of amino acids occurred after the addition of ammonical methanol.

A simple procedure for analysis of dissolution samples for all dosage levels of levothyroxine sodium is presented. The success of the method was due to the large volumes injected directly onto the HPLC column and by enhanced sensitivity by UV detection at 225 nm. However, when employing a direct injection method, it should be thoroughly verified that there are no interferences in the chromatogram either by scanning the eluting peaks or by the standard addition technique.

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