High-Performance Liquid Chromatographic Assay for Sodium Levothyroxine in Tablet Formulations: Content Uniformity Applications

R. L. GARNICK ˣ, G. F. BURT, D. A. LONG, J. W. BASTIAN, and J. P. ALDRED

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Abstract □ Sodium levothyroxine was quantitated in 25–300 μg/tablet formulations. The procedure consisted of pulverization of a suitable sample, extraction into acetonitrile–water (40:60, v/v) containing 0.05% *o*-phosphoric acid, and injection onto a bonded-phase cyanopropyl column; the effluent was monitored by UV detection at 225 nm. Spiked placebo recovery studies demonstrated the linearity of the method over the range of 80–120% of the label claim. Stability studies indicated that no degradation products or excipients interfered with the quantitation of the intact drug. Data demonstrating the accuracy and precision of this assay are presented, and the method was applied to the measurement of single-tablet content uniformity.

Keyphrases □ Sodium levothyroxine—assay in tablet formulations, content uniformity, high-performance liquid chromatography □ Content uniformity—single-tablet, sodium levothyroxine, high-performance liquid chromatography □ Thyroid hormones—single-tablet content uniformity, high-performance liquid chromatography, sodium levothyroxine

High-performance liquid chromatography (HPLC), an increasingly popular procedure for the quantitation of a variety of medicinally important compounds, is particularly useful for the quantitation of drugs whose active components are present in extremely low concentrations, labile, and/or possess great biological activity. The synthetic thyroid hormones levothyroxine and liothyronine are good examples, typically present at levels of 3–300 μg/tablet. Historically, analyses of these hormones have been complex, often nonspecific (based on iodine content), required tedious sample preparation, and frequently required large samples (1).

Recently, a number of GC methods have been described for the quantitation of thyroid hormones in serum and tablet dosage forms (2–9). However, none of these procedures are capable of determining the stability of the active drug in the presence of its degradation products. In this report, an HPLC assay is described for sodium levothyroxine, which possesses sufficient sensitivity for single-tablet content uniformity studies. The sample preparation procedure is simple, and the method is capable of accurately determining the amount of active drug in the presence of degradation products.

BACKGROUND

The USP XX monograph for the analysis of sodium levothyroxine is based on the estimation of iodine content (1). This method is neither specific nor stability indicating, because it is unable to distinguish between active drug and iodine-containing decomposition products. Moreover, this method is quite insensitive, requiring 3 mg of sodium levothyroxine per assay. This assay requires 120 tablets of the 25-μg dosage form and, therefore, is not applicable to the determination of single-tablet content uniformity.

A survey of the literature indicated a number of analytical methodologies for the analysis of sodium levothyroxine (1–9); however, most were either nonspecific or insufficiently sensitive. Recently, two HPLC procedures for the determination of synthetic thyroid hormones in tablets were published, both using reverse-phase C-18 columns (8, 9). These procedures, while suitably accurate and precise, were not demonstrated to be stability indicating. We found that the quantitation of sodium levothyroxine by these methods was complicated by interferences observed in aged samples.

EXPERIMENTAL

Materials—Levothyroxine and liothyronine reference standards were obtained from the U.S. Pharmacopeial Convention. 3,5-Diiodo-L-thyronine was obtained commercially[1]. All chemicals were reagent or spectrophotometric grade.

Instrumentation—A modular chromatograph was used consisting of a constant-flow pump[2], a fixed-loop injector[3], a variable-wavelength UV detector operating at 225 nm[4], and a strip-chart recorder[5]. A suitable data system, equipped with a data interface printer/plotter was also used[6]. A bonded-phase cyanopropyl column[7] was used with a mobile phase of acetonitrile–water–phosphoric acid (40:60:0.05, v/v/v) at a flow rate of 1–2 mL/min. The mobile phase was deaerated prior to use.

Procedure—The average weight of 20 tablets was determined. The tablets were then ground to a fine powder, and a sample weight equivalent to ~100 μg of sodium levothyroxine was transferred to a 16 × 125-mm culture tube (equipped with a polytef-lined cap). Two glass beads were added to serve as an internal ball mill. Mobile solvent (10.0 mL) was then added to the tube, and the solution was vigorously mixed for 5 min using a vortex mixer. The solution was then centrifuged; the clear supernatant was used for the assay.

Content uniformity was established using single tablets with the addition of 10.0 mL of mobile solvent, as stated above, for the 25–150-μg dosage forms and 20.0 mL of mobile solvent for the 175–300-μg dosage forms. Sodium levothyroxine concentrations were determined by direct comparison with a standard solution. The standard solution was prepared using levothyroxine sodium reference standard USP, the concentration of which was corrected for the sodium content.

Spiked Placebo Recovery Procedure—Placebo material was prepared containing all of the excipients found in the 25–300-μg tablet formulations. Aliquots from solutions of mobile solvent containing 80, 100, and 120% of the theoretical formulated amount of sodium levothyroxine were added to the placebo material, and the resultant solutions were carried through the sample preparation procedure. The recoveries for sodium levothyroxine were 99–100% (Table I).

The precision of the assay was determined during the spiked placebo recovery study. Relative standard deviations of ±0.5 and ±0.12% were determined for the 25- and 300-μg formulations, respectively. Replicate analyses (n = 6) of the 25- and 300-μg tablets, each from a single composite, demonstrated sample-to-sample relative standard deviations of ±0.96 and ±0.35%, respectively.

Stability-Indicating Properties—The stability-indicating properties of the method were established by subjecting sodium levothyroxine bulk material, as well as the finished tablets, to extreme stress conditions designed to produce degradation products similar, or identical to, those which might be expected to occur under various storage conditions.

Single-Tablet Content Uniformity—The HPLC method was also applied to a single-tablet content uniformity assay. The only modification necessary for this purpose was an increase in the sample volume required for the larger dosages.

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Model 110A, Beckman Instruments, Inc.
[3] Model 7125, Rheodyne.
[4] L.D.C. Spectromonitor III.
[5] Omniscribe Recorder.
[6] Sigma 10, Perkin-Elmer Co.
[7] Zorbax-CN, DuPont Co., Wilmington, Del.

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Table I—Spiked Placebo Recovery Studies for Sodium Levothyroxine Tablets

Dosage, μg	Formulated Amount Added (Nominal), %	Formulated Amount Recovered, %	Recovery, %
25	80	79.5	99.3
	80	79.9	99.9
	80	79.4	99.3
	100	99.7	99.7
	100	99.8	99.8
	100	99.6	99.6
	120	121.3	101.1
	120	119.8	99.9
	120	120.0	100.0
Mean ± *RSD*			99.8 ± 0.50
300	80	80.2	100.2
	80	79.8	99.8
	80	80.6	100.7
	100	100.1	100.1
	100	99.3	99.3
	100	99.9	99.9
	120	120.0	100.0
	120	119.9	99.9
	120	120.3	100.2
Mean ± *RSD*			100.0 ± 0.12

RESULTS AND DISCUSSION

A literature method was chosen as a starting point for the development of a stability-indicating assay for sodium levothyroxine in tablets (8). This method used a C-18 column with a mobile phase of methanol–water (50:50, v/v) containing 0.1% phosphoric acid. The method demonstrated excellent resolution between the diiodothyronine, triiodothyroxine, isotriiodothyronine, and levothyroxine peaks. However, under oxidative-degradation conditions, the method failed to resolve an unidentified impurity from the levothyroxine peak. Using several other reverse-phase columns, it was not possible to obtain results satisfactory for a stability-indicating method. A bonded phase cyanopropyl column was then investigated, as the nitrile group is of medium polarity and would be expected to afford a highly selective mode of separation. A mobile phase of acetonitrile–water (40:60, v/v) containing 0.05% phosphoric acid demonstrated excellent resolution between diiodothyronine, triiodothyroxine, isotriiodothyronine, levothyroxine, and other related compounds (Fig. 1). Significantly, the previously noted oxidative-degradation product, which was only partially resolved from the levothyroxine peak, did not interfere in this system.

It was observed that altering the acetonitrile concentration of the mobile solvent had little effect on the retention time of levothyroxine, whereas varying the phosphate ion concentration of the mobile solvent changed the retention time dramatically. This indicated that the separation mechanism was a form of ion suppression. In agreement with this was the observation that the retention time of levothyroxine decreased with increasing phosphate ion concentration.

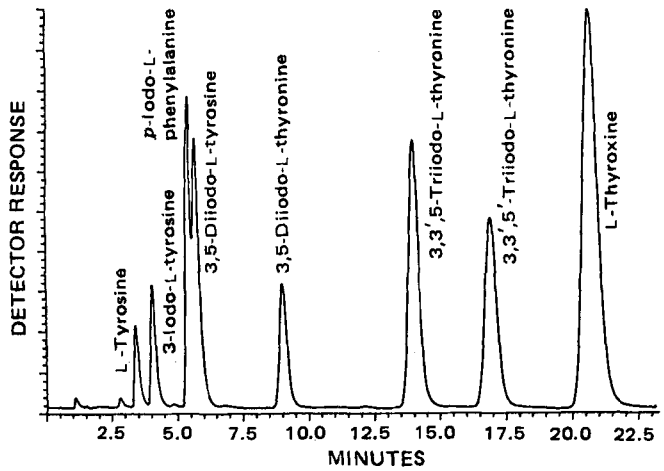

Figure 1—*Chromatogram of sodium levothyroxine and related compounds obtained on a bonded-phase cyanopropyl column using a mobile solvent of acetonitrile–water–phosphoric acid (40:60:0.05, v/v/v).*

Table II—Assay of Sodium Levothyroxine Tablets by the HPLC Method [a]

Sample	Dosage, μg	Age	Composite Tablet Assay	Percent of Labeled Strength
1	25	20 months	23.3	93.2
2	25	4 months	24.2	96.9
3	50	Initial	50.3	101
4	50	Initial	50.3	101
5	100	Initial	105.0	105
6	100	Initial	102.0	102
7	125	Initial	135	108
8	150	Initial	152.0	101
9	150	Initial	156.0	104
10	200	Initial	206	103
11	200	Initial	206	103
12	200	Initial	213	107
13	300	Initial	316	105
14	300	Initial	316	105

[a] All values were obtained for separate lots of sodium levothyroxine tablets.

Samples for assay were prepared by taking the tablet weight equivalent of 100 μg of sodium levothyroxine and extracting it into 10 mL of mobile solvent (Table II). The extract was then injected into the HPLC system, along with levothyroxine reference standard USP, at a concentration of ~10 μg/mL using a 100-μL injection loop (Fig. 2). The standard curve was constructed from eight concentration levels of standard solution. The plot of peak area *versus* the concentration of levothyroxine was linear over the range of 1–13 μg/mL (100–1300 ng/injection) as confirmed by a correlation coefficient of 0.999.

A spiked placebo recovery study was performed for sodium levothyroxine tablets at 80, 100, and 120% of the theoretical formulated amounts for both the 25- and 300-μg tablets. The results (Table I) demonstrate a mean recovery for sodium levothyroxine of 99.8% and 100% for the 25- and 300-μg tablets, respectively. Their respective relative standard deviations were ±0.50 and ±0.12%.

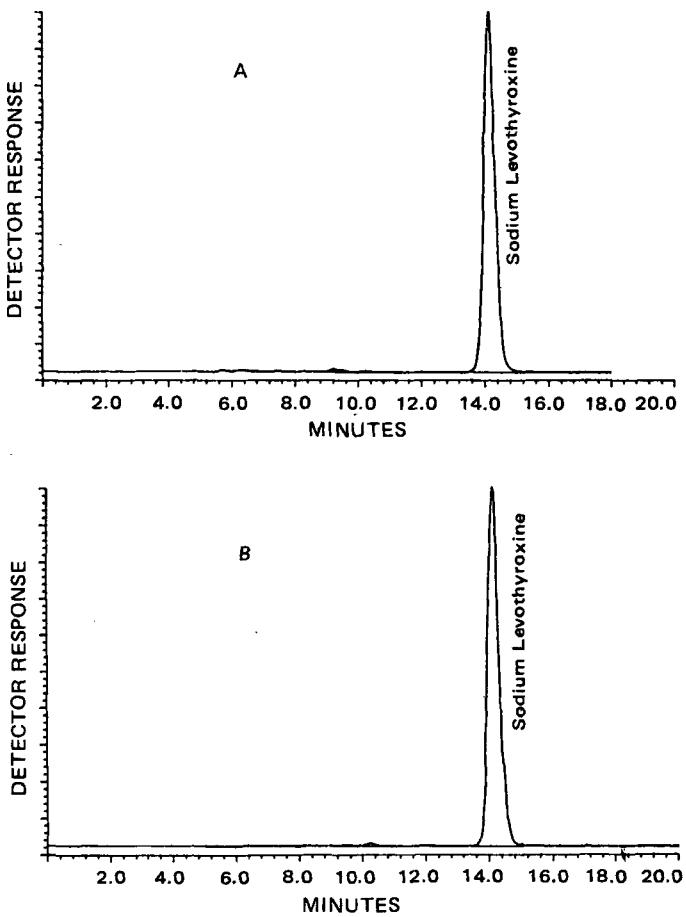

Figure 2—*Typical chromatograms of sample (A) and standard (B) preparations of sodium levothyroxine. See text for details.*

Table III—Degradation of Sodium Levothyroxine Raw Material and Finished Tablets Under Various Conditions [a]

Degradation Conditions	Reagents	Time, h	Amount Recovered, %
	Raw Material		
Thermal	80°C	168	100.8
Hydrolytic	0.1 M NaOH	87.5	90.6
	0.1 M HCl	44	92.8
Oxidative	*tert*-butyl hydroperoxide	1	37.5
Photochemical	UV light (shortwave)	144	98.9
	UV light (longwave)	48	101.4
	Intact Tablets		
Photochemical	UV light (longwave)	168	91.9
Thermal	80°C in air	168	50.0

[a] There were no HPLC interferences for any assay.

Table IV—25-μg Sodium Levothyroxine Tablet Content Uniformity *versus* Composite Assay Results

	Content Uniformity Assay	
Tablet	Tablet Wt., mg	Amount Found, μg/Tablet
1	86.09	23.20
2	86.75	24.43
3	87.63	24.25
4	88.43	25.27
5	86.80	24.26
6	87.30	24.22
7	87.12	23.52
8	87.29	23.81
9	88.35	24.15
10	85.46	23.63
Mean ± *RSD*		24.07 ± 2.41
	Composite Assay[a]	
Sample	Dosage Strength, μg	Amount Found, μg/Tablet
1	25	23.88
2	25	23.90
3	25	23.97
Mean ± *RSD*		23.92 ± 0.20

[a] All composite samples were unaged.

The degradation studies performed for sodium levothyroxine using the proposed method indicated that under oxidative, photochemical, hydrolytic, and thermal conditions, no degradation products were produced which interfered with the sodium levothyroxine peak. In these studies, there was little degradation of sodium levothyroxine *via* UV light (both short- and longwave) or *via* the thermal conditions utilized (Table III). However, oxidative degradation studies indicated that sodium levothyroxine may be subject to oxidative degradation by this means. Sodium levothyroxine was also moderately degraded under hydrolytic conditions (Table III).

Tablet recovery *versus* extraction time (1–10 min) indicated a rapid leaching of sodium levothyroxine from the tablet matrix. From these data a mean leaching time of 5 min was established for routine use.

Table V—300-μg Sodium Levothyroxine Tablet Content Uniformity *versus* Composite Assay Results

	Content Uniformity Assay	
Tablet	Tablet Wt., mg	Amount Found, μg/Tablet
1	87.68	303.27
2	88.44	299.55
3	89.21	302.25
4	89.07	305.48
5	87.49	306.48
6	87.42	303.00
7	89.27	305.08
8	90.55	311.51
9	86.60	292.96
10	88.96	301.37
Mean ± *RSD*		303.10 ± 1.60
	Composite Assay[a]	
Sample	Dosage Strength, μg	Amount Found, μg/Tablet
1	300	299.57
2	300	302.35
3	300	302.54
Mean ± *RSD*		301.49 ± 0.55

[a] All composite samples were unaged.

The assay was also used for a study of single-tablet content uniformity. The results of this study are presented in Tables IV and V for 25- and 300-μg tablets, respectively. These data demonstrate a good correlation with the tablet composite assay results also presented in the Tables. The only modification made to the assay was the use of a 20.0-mL aliquot of the mobile solvent for the 175–300-μg tablets to adjust the concentration to a suitable level.

The HPLC assay method for the determination of sodium levothyroxine in tablets has been demonstrated to be accurate, precise, and stability indicating. The method is simple, specific, and rugged, and is applicable to single-tablet content uniformity determination.

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