analysis for either of the two major sympathomimetic agents in use in topical ocular decongestants. The technique is more reproducible than the present colorimetric assay and does not suffer from the time restraints and interferences possible in colorimetric assays. The HPLC method presented can be easily automated and allows analysis of a variety of ophthalmic solutions on a single HPLC system.

REFERENCES

(1) S. C. Slack and W. J. Moder, *J. Am. Pharm. Assoc., Sci. Ed.* **46**, 742 (1957).

(2) "United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 783.

(3) "United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 543.

(4) E. M. Gindler, *Clin. Chem.*, **25**, 337 (1979).

ACKNOWLEDGMENTS

The authors thank Ms. Diane Horgen and Mr. Joseph Martin for assistance in preparation of this paper.

Stability-Indicating Assay, Dissolution, and Content Uniformity of Sodium Levothyroxine in Tablets

STEVEN L. RICHHEIMER ˣ and TAHANI M. AMER

Received July 19, 1982, from the *Stability Laboratory, Pharmaceutical Basics, Inc., Denver, CO 80223.* Accepted for publication October 14, 1982.

Abstract □ A reverse-phase high-performance liquid chromatographic (HPLC) method for determining sodium levothyroxine in tablet formulations is described. The sodium levothyroxine was extracted from tablets using a mobile phase consisting of 60% acetonitrile and 40% aqueous buffer. After centrifugation 200 μl of the solution was chromatographed on a 10-μm C_{18} column. The method gave accurate results when tested against the USP method, by the standard additions method, and by the spiked-placebo method. The method can also be used to determine content uniformity and dissolution of sodium levothyroxine tablets.

Keyphrases □ Sodium levothyroxine—stability-indicating assay, dissolution, content uniformity, tablets □ Dissolution—sodium levothyroxine, stability-indicating assay, content uniformity, tablets □ Content uniformity—sodium levothyroxine, stability-indicating assay, dissolution, tablets □ Stability—assay of sodium levothyroxine in tablets, dissolution, content uniformity

Sodium levothyroxine (I) tablets are widely prescribed in thyroid replacement therapy, with a wide range of doses available (25–300 μg/tablet). The USP XX method of assay consists of a lengthy ignition and oxidation to iodate followed by titration of the liberated iodine (1). The method is neither stability indicating nor sensitive enough to be used for content uniformity and dissolution determinations.

A number of other assay procedures based on liquid chromatography have appeared in the literature (2–13). This report describes a new reverse-phase liquid chromatographic (HPLC) assay method that adequately separates I from degradation products and can be used for the identification, content uniformity analysis, and dissolution testing of I in tablets.

EXPERIMENTAL

Reagents and Materials—Sodium levothyroxine[1] (I) was assayed by the USP XX procedure (14); sodium liothyronine[1] and 3,5-diiodo-L-thyronine[2] were used as received. Reagents used were analytical reagent grade. The levothyroxine sodium tablets (USP) were obtained commercially from four sources[3–6].

Apparatus—The high-performance liquid chromatograph[7] was equipped with a variable-wavelength UV detector[8], a strip-chart recorder[9], an electronic integrator[10], and a 200-μl loop-type injector[11]. Commercial 10-μm C_{18} columns[12] (30 cm × 4 mm i.d.) were used at am-

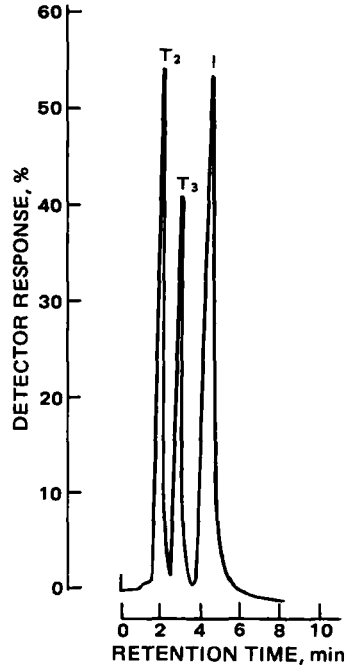

Figure 1—*Chromatogram of sodium levothyroxine (I, 20 μg/ml) with added sodium liothyronine (T_3, 10 μg/ml), and 3,5-diiodo-L-thyronine (T_2, 10 μg/ml).*

[1] Sanabo Gesellschaft, Kundle, Austria.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Armour Pharmaceutical Co., Phoenix, Ariz.
[4] Flint, Division of Baxter-Travenol, Morton Grove, Ill.
[5] Lederle Laboratories, Pearl River, N.Y.
[6] Pharmaceutical Basics, Inc., Denver, Co.
[7] Model 5020, Varian Associates, Palo Alto, Calif.
[8] Model UV-50, Varian Associates, Palo Alto, Calif.
[9] Model 1005, Beckman Instruments, Fullerton, Calif.
[10] Model CDS-111L, Varian Associates, Palo Alto, Calif.
[11] Model CV-6-UHPa-N60, Valco Instruments Co., Houston, Tex.
[12] Model MCH-10, Varian Associates, Palo Alto, Calif.; μBondapak C18, Waters Associates, Milford, Mass.

0022-3549/83/1100-1349$01.00/0

Table I—Comparison of the USP XX and HPLC Assay for a 100-μg Tablet

	USP XX	HPLC
Number of Determinations	8	8
Mean, %	101.3	100.7
Range, %	104.5–98.8	101.8–99.1
Percent *RSD*	2.21	0.85

bient temperature. A stainless steel column (4 cm × 4 mm i.d.) packed with a 40-μm C_{18} pellicular material[13] was used both as a guard column and in place of the injection loop for dissolution studies. Dissolutions were performed on a commercial dissolution apparatus[14] using the USP-rotating-paddle method.

Chromatographic Conditions—The mobile phase was a mixture of 60% acetonitrile and 40% pH 3.0 aqueous buffer containing 0.005 *M* 1-octanesulfonic acid and 0.005 *M* tetramethylammonium chloride. The acetonitrile was reduced to 50% for columns with less retentive packing material[13]. Flow rate was 2 ml/min, and the detector was at 230 nm and 0.08 AUFS.

Standard Preparation—The levothyroxine reference standard[15] (19.5 mg) or I (20 mg) was dissolved in 100.0 ml of mobile phase. The stock solution could be stored for several months in darkness at 4°. Working standards were prepared daily from the stock solution by dilution with the mobile phase.

Sample Preparation—Twenty tablets were weighed and finely powdered. A portion of powder equivalent to 200 μg of I was transferred to a glass-stoppered centrifuge tube. Mobile phase (10.0 ml) was added, and the mixture was sonicated for 5 min and then centrifuged. The supernatant was then filtered through a 0.45-μm membrane filter[16] if it was not free of particulate matter. For content uniformity analyses, one tablet was placed in a centrifuge tube and enough mobile phase was added to make the final concentration 20 μg/ml (except 25- and 50-μg tablets, 5- and 10-μg/ml were acceptable). The tablet was sonicated until completely disintegrated, and the solution was analyzed using the composite assay, reducing the standard concentration where applicable.

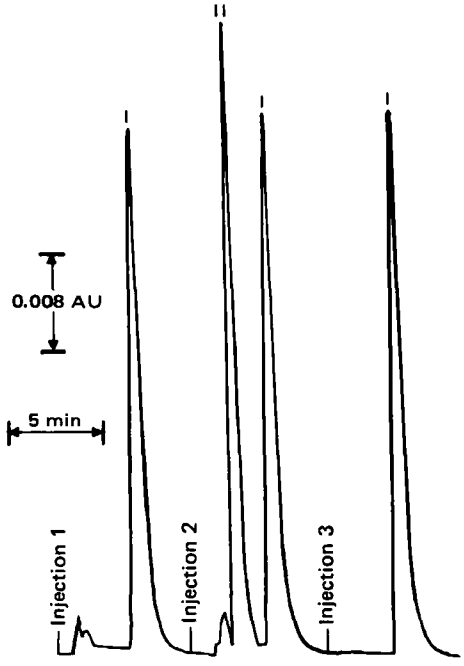

Figure 2—*Typical chromatograms for the assay of sodium levothyroxine. The mobile phase is 60% acetonitrile and 40% pH 3.0 buffer. Key: injection 1: 200-μg tablet from manufacturer B; injection 2: 100-μg tablet from manufacturer A (II is an excipient peak); and injection 3: standard, 20 μg/ml.*

[13] Vydac RP 201SC, Varian Associates, Palo Alto, Calif.
[14] Model 725115, Hanson Research, Northridge, Calif.
[15] United States Pharmacopeial Convention, Rockville, Md.
[16] Acrodisc CR, 25mm, Gelman Sciences, Ann Arbor, Mich.

Table II—Comparison of the USP XX and HPLC Assays for I in Tablets Stored at Elevated Temperatures

Manufacturer	Potency, μg	Storage Days	Temperature[a], °	USP XX, %	HPLC, %
A	100	21	60	101.3	70.9
A	300	21	60	100.0	74.0
A	100	91	40	101.0	74.3
B	100	21	60	104.8	41.1
B	300	21	60	102.4	48.0
B	100	91	40	105.2	34.3
C	100	1	80	108.3	13.4
D	100	21	60	100.0	67.0
D	300	91	40	100.7	67.1

[a] All samples stored at ambient humidity.

Assay Procedure—Standard and sample preparations (200 μl) were injected into the liquid chromatograph. Either peak areas or peak heights were measured. Compound I was quantitated by comparing the peak response of the sample to that of the standard.

Dissolution Procedure—Phosphate buffer (500 ml, pH 7.5, 0.05 *M*) at 37 ± 0.5° was used with the USP rotating-paddle method at 50 rpm. A 20-ml aliquot of the mixture was withdrawn after 30 min and filtered through a 0.45-μm membrane filter. A portion of the filtrate equivalent to 1 μg of I (0.25 μg for 25-μg tablets) was passed through a 4 cm × 4-mm guard column with C_{18} packing attached to the sampling valve. The column was rinsed with 1 ml of water and injected onto the liquid chromatograph under the same conditions specified for the assay. Similarly, 1.0 ml of a standard (1 μg/ml of I) in dissolution medium (0.25 μg/ml for 25-μg tablets) was injected. The amount of I dissolved was quantitated by comparing the peak areas obtained for the standard and sample chromatograms.

RESULTS AND DISCUSSION

Chromatography and Specificity—Various mobile phases were investigated; however, the equimolar combination of 1-octanesulfonate and tetramethylammonium chloride with acetonitrile was found to give the sharpest peak for I, as well as providing adequate separation of I from degradation products and tablet excipients. Figure 1 shows a typical chromatographic separation of I from liothyronine (T_3) and 3,5-diiodo-L-thyronine (T_2). Figure 2 shows typical chromatograms obtained in the assay of two brands of tablets and a standard. Extraction of I by the mobile phase was quantitative, as evidenced by recovery experiments using whole tablets and I in combination with individual tablet excipients. Incomplete extraction of I in the presence of magnesium stearate was observed using several other extracting solvents (100% acetonitrile, ammoniacal methanol, and 60:40 acetonitrile–water). It is possible that I forms an insoluble ion-pair complex with the stearate which is inhibited in the presence of 1-octanesulfonate and tetramethylammonium chloride but not other extracting solvents.

The specificity of the method was further tested by degrading samples of I (20–200 μg/ml) by irradiation, hydrolysis, oxidation, and heat and assaying the resulting solutions by the proposed method. Short-wavelength UV (24 hr) caused 100% degradation, while refluxing with 0.1 *N* HCl or 0.1 *N* NaOH (16 hr) caused 41.1 and 43.4% degradation, respectively. Oxidation with 0.01 *M* H_2O_2 and 0.01 *M* Fe^{2+} (16 hr) caused 97% degradation. Thermal degradation of I at 80° combined with tablet excipients was 17–87% in 24 hr depending on the formulation. In every case the unidentified degradation products eluted before the I peak and did not interfere with the analysis of I.

Comparison to the USP Method and Reproducibility—A new lot (<1 month since manufacture) of 100-μg tablets of I was assayed re-

Table III—Recovery of Sodium Levothyroxine (I) Added to Tablet Formulations

Manufacturer	Stated Potency, μg	Unspiked I/Tablet	Amt. of I Added, μg	Amt. of I Measured, μg	Recovery[a], %
A	25	24.0	12.5	35.6	97.5
A	300	281.0	150.1	429.4	99.6
B	25	22.2	12.5	33.6	96.9
B	200	195.7	100.9	297.9	100.4
C	100	89.5	50.4	140.8	100.6
D	200	196.7	100.9	297.6	100.0

[a] Percent Recovery = [measured I/(unspiked I + added I)] × 100.

peatedly by the USP XX method and by the proposed method. The results (Table I) indicate that the two methods are in close agreement for this lot and that reproducibility is good (*RSD* = 0.85%). However, samples stored at elevated temperatures (Table II) for even short periods of time gave higher assay results by the USP XX method than by HPLC due to degradation of I in the tablet formulations.

Recovery of I Added to Spiked Placebo Mixtures and Linearity—The effectiveness of the extraction step and accuracy of the method was tested by adding I in an amount corresponding to 50–125% of the label claim to several powdered placebo mixtures (25-, 100-, 200-, and 300-μg tablets). A plot of the amount of I added *versus* the amount recovered indicates that the slopes are unity within experimental error (1.000 ± 0.008), that the intercepts are near zero (0.40 ± 0.76), and that the correlation coefficients are unity (1.000). Recoveries for the 20 spiked samples tested (five for each potency) ranged from 98.1 to 100.6% (average: 100.0%; *RSD* = 0.53). In each case a placebo blank was also run which showed no interfering peaks. These data indicate that the method is linear and accurate between 50 and 125% for the tablet formulations tested. Both peak area and peak height gave equally accurate results.

The method was also tested by spiking several different commercially available tablets with additional I. Tablet potencies ranged from 25 to 300 μg, and in each case 50% of the label amount of I was added. The results (Table III) indicate that complete recovery was obtained for all formulations tested.

The linearity of the method was further tested by chromatographing a series of seven standard solutions ranging in concentration from 5 to 35 μg/ml. Plots of both peak area and peak height *versus* concentration were linear (correlation coefficients = 0.9999) and showed no bias (intercepts were 0.0 ± 0.75%). These results indicate that an external standard (equivalent to 20 μg/ml of I) can be used to analyze samples varying in concentration over a sevenfold range.

Content Uniformity and Dissolution Studies—The applicability of the method for content uniformity determinations was tested by assaying 10 individual 25-μg tablets from manufacturer A. The mean value of 95.9% agreed well with a composite assay for this lot of 96.0%, and the variation (2.60% high to 2.61% low) was close to the weight variation for the same 10 tablets (2.04–1.86%).

The accuracy of the dissolution method was tested by determining the recovery of varying amount of I added to dissolution medium (0.05 *M* phosphate buffer, pH 7.5) plus tablet excipients. The results (Table IV) indicate that recovery is quantitative in the range of 30–100% (0.3–1.0 μg); reproducibility using peak area was also good (*RSD* = 1.3%, $n = 8$). Retention of I on the guard column was complete, since washing with 10 times the normal amount of dissolution medium did not reduce the amount of I recovered (although the peak was broadened). Results using commercially available tablets (100 μg) varied from 36.2 to 68.4% dissolved using the method depending on manufacturer and were significantly lower with water (−39%) and 0.1 *N* HCl (−28%) as the dissolution media.

Several studies have shown differences in the potency and/or bioavailability of sodium levothyroxine preparations (15–17). Reported differences in bioavailability may be due to the use of subpotent tablets in the study, since several of the lots tested by the proposed HPLC method from more than one manufacturer were found to be well below the USP minimum of 90.0% of label claim. Since the stability of I in different formulations varies considerably, it is possible that the differences in potencies occur not at the time of manufacture but on aging. Unfortunately the USP method, which measures only total iodine, does not provide a specific or meaningful method for the determination of the stability of I in dosage forms. The proposed method is accurate, reproducible, and specific for I and adequately quantitates I in the presence of degradation products. In addition the method can be applied to identification, content uniformity, and dissolution of I in tablets. Official adoption of an HPLC method for the assay of sodium levothyroxine tablets will go a long way toward providing a better standardized and uniform product with a well-established shelf life.

Table IV—Sodium Levothyroxine (I) Dissolution–Recovery Study

Sample	Amount of I added, μg	Amount of I measured, μg[a]	Recovery, %
30	30.0	30.3	101.0
50	50.0	50.0	100.0
70	70.0	69.2	98.9
100	100.0	98.6	98.6

[a] 500 ml of dissolution medium was used and 5.0 ml passed through guard column prior to injection (0.3–1.0 μg injected).

REFERENCES

(1) "The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 447.

(2) R. S. Rapaka, P. W. Knight, and U. K. Prasan, *J. Pharm. Sci.*, **70**, 131 (1981).

(3) E. P. Lankmayr, B. Maichin, G. Knapp, and F. Nachtmann, *J. Chromatogr.*, **224**, 239 (1981).

(4) D. J. Smith, M. Biesemeyer, and C. Yaciw, *J. Chromatogr. Sci.*, **19**, 72 (1981).

(5) F. Nachtmann, *Acta Pharm. Tech.*, Suppl., July, 1979, p. 145.

(6) R. S. Rapaka, P. W. Knight, V. P. Shah, and V. K. Prasad, *Anal. Lett.*, **12**, 1201 (1979).

(7) D. J. Smith and J. H. Graham, *J. Assoc. Off. Anal. Chem.*, **62**, 818 (1979).

(8) M. T. Hearn, W. S. Hancock, and C. A. Bishop, *J. Chromatogr.*, **157**, 337 (1978).

(9) W. A. Dark and L. W. Grossman, Jr., *Applications Highlights* AH-139, Waters Associates Inc., Milford, Mass.

(10) B. L. Karger, S. C. Su, S. Marchese, and B.-A. Persson, *J. Chromatogr. Sci.*, **12**, 678 (1974).

(11) U. R. Cieri and J. C. Illuminati, *J. Assoc. Off. Anal. Chem.*, **60**, 628 (1977).

(12) G. G. Skellern, M. Mahmoudian, and B. I. Knight, *J. Chromatogr.*, **179**, 213 (1979).

(13) N. M. Alexander and M. Nishimoto, *Clin. Chem.*, **25**, 1757 (1979).

(14) "The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 446.

(15) S. Stoffer and W. E. Szpunar, *J. Am. Med. Assoc.*, **244**, 1704 (1980).

(16) R. W. Rees-Jones and P. R. Larsen, *J. Am. Med. Assoc.*, **243**, 549 (1980).

(17) A. Ramos-Gabatin, J. M. Jacobson, and R. L. Young, *J. Am. Med. Assoc.*, **247**, 203 (1982).