

Reversed-Phase High-Performance Liquid Chromatographic Analysis of Liothyronine Sodium and Levothyroxine Sodium in Tablet Formulations: Preliminary Studies on Dissolution and Content Uniformity

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Abstract □ Levothyroxine sodium was estimated from tablet formulations of levothyroxine sodium and liotrix (liothyronine sodium-levothyroxine sodium combination tablet). The procedure consisted of the addition of 3,3',5'-triiodothyronine as the internal standard to the pulverized sample, followed by an acidic butanol extraction, evaporation, and injection onto a μ Bondapak reversed-phase high-performance liquid chromatographic column. The eluent was methanol-water-phosphoric acid (50:50:0.1), and the effluent was monitored by UV detection at 254 nm. A standard linear calibration curve was obtained for direct standard solutions equivalent to 18–225 μ g of levothyroxine sodium/tablet. The procedure is sensitive enough for single-tablet analysis. Using this procedure, content uniformity studies were performed on liothyronine sodium tablets, levothyroxine sodium tablets, and liotrix tablets. The procedure also was adapted for conducting dissolution studies on levothyroxine sodium tablets in deionized water using the rotating-paddle method.

Keyphrases □ High-performance liquid chromatography—analysis, liothyronine sodium and levothyroxine sodium in tablet formulations □ Liothyronine sodium—high-performance liquid chromatographic analysis in tablet formulations □ Levothyroxine sodium—high-performance liquid chromatographic analysis in tablet formulations

In cases of hypothyroidism, a number of medications are available for thyroid replacement therapy including thyroid tablets, thyroglobulin tablets, and tablets containing synthetic amino acids, liothyronine sodium, and levothyroxine sodium. The thyroid medications are prescribed frequently, and an evaluation of the top 100 prescribed drugs in 1978 ranked a tablet dosage form containing the sodium salt of levothyroxine¹ as the 44th most frequently prescribed drug.

BACKGROUND

The present USP procedure for the analysis of liothyronine sodium and levothyroxine sodium is based on the estimation of organic iodine (1). Procedures based on estimation of the iodine content are not specific since they do not distinguish between the various iodine-containing amino acids and, at the same time, do not distinguish between the active ingredient and the decomposition products. For the analysis of liotrix tablets (liothyronine sodium-levothyroxine sodium combination tablet), the USP method (2) consists of a laborious extraction, column chromatography, and UV determination.

Both the iodine content determination and column chromatography are insensitive and require at least 0.33–1 mg of the active ingredient for the analysis. Since the smallest dosage form of liotrix tablets contains 3.1 μ g of liothyronine sodium and 12.5 μ g of levothyroxine sodium, about 110 tablets are needed for a single analysis by the column chromatographic procedure. Although the USP (3) suggests content uniformity determination for all solid dosage forms that contain <50 mg of the active ingredient, a compendial requirement for content uniformity is absent for liothyronine sodium tablets, levothyroxine sodium tablets, and liotrix tablets. Furthermore, a compendial requirement for dissolution of these thyroid drugs is not imposed.

The Food and Drug Administration enacted its bioavailability and bioequivalence regulations to create a mechanism and means for establishing the bioequivalence and therapeutic equivalence of generic drug products. At present, bioequivalence of thyroid products or products containing the synthetic analogs and equivalence between the natural and synthetic products have not been established. Before undertaking bioequivalence studies, it was essential to develop simple and specific methods for the analysis of thyroid dosage forms. To perform content uniformity studies of the tablets, the required procedures must be sensitive and specific as compared to the present nonspecific iodine content assay. Moreover, content uniformity studies should be performed prior to dissolution and bioavailability studies since meaningful data can be obtained only on tablets that meet the criteria for content uniformity.

Few reports have appeared on the analysis of tablet dosage forms containing liothyronine sodium and levothyroxine sodium. Graham *et al.* (4) developed a procedure that can be applied to a tablet dosage formulation containing as low as 5 μ g of liothyronine sodium or levothyroxine solution. However, this method is cumbersome and nonspecific.

A literature search for analytical methodology (5–13) revealed that previously published procedures based on TLC, paper chromatography, and column chromatography are either semiquantitative or extremely laborious and insensitive. Although some reported GLC methods (14–24) seem to have the needed sensitivity, most of the studies were done on pure synthetic mixtures or the procedures have not yet been adopted for estimation from tablets.

High-performance liquid chromatography (HPLC), a powerful analytical tool, is being used increasingly in the thyroid field (25–32). Karger and Su (25) demonstrated that thyroal amino acids could be separated on a high-performance partition column. An instrument manufacturer (26) reported a reversed-phase HPLC method for the separation of liothyronine and levothyroxine in ~10 min. Hearn *et al.* (27) carried out a detailed investigation on qualitative separations following the method described in Ref. 26. Cieri and Illuminati (5) used HPLC to detect the presence of diiodothyronine in liothyronine sodium. Similarly, Smith and Graham (28) adapted the procedure described in Ref. 26 for estimation of impurities of diiodothyronine and triiodothyronine in pure levothyroxine samples.

Subsequent reports appeared in which the sensitivity of detection was improved (29) and in which the HPLC procedure was used in the analysis of thyroglobulin (30) or of the activity of an enzyme involved in thyroglobulin biosynthesis (31). The first adaptation of a procedure showing the feasibility of the HPLC procedure for the analysis of liothyronine sodium tablets appeared recently (32). In the present study, the procedure was investigated further for the analysis of levothyroxine sodium and liotrix tablets, for the determination of the content uniformity for liothyronine sodium, levothyroxine sodium, and liotrix tablets, and for dissolution studies on levothyroxine sodium tablets.

EXPERIMENTAL

Reagents and Chemicals—Liothyronine sodium and levothyroxine sodium hexahydrate were obtained commercially². 3,3',5'-Triiodothyronine was obtained as a gift³. Commercial brands of liothyronine sodium tablets USP, levothyroxine sodium tablets USP, and liotrix tablets USP were purchased from a wholesale drug distributor⁴. All chemicals were

² Sigma Chemical Co., St. Louis, Mo.

³ Nuclear-Medical Laboratories, Dallas, Tex.

⁴ The liothyronine tablets (Manufacturer A), levothyroxine sodium tablets (Manufacturers B and C), and liotrix tablets (Manufacturers D and E) were obtained from District Wholesale Co., Washington, D.C.

¹ Synthroid, Division of Travenol Laboratories, Deerfield, IL 60015.

reagent grade. Stock solutions of liothyronine sodium, levothyroxine sodium, and 3,3',5'-triiodothyronine were made in 2% ammonium hydroxide in methanol at 100 µg/ml, and appropriate dilutions were made when required.

Apparatus—The modular high-performance liquid chromatograph consisted of a constant-flow pump⁵, a loop-type injector⁶, a UV detector⁷, and a strip-chart recorder⁸ (0.25 cm/min). Stainless steel columns^{9,10}, packed with fully porous 10-µm silica particles to which was chemically bonded a monomolecular layer of octadecylsilane, were obtained commercially. A stainless steel precolumn¹¹ (2.5 × 0.25 cm) packed with a pellicular reversed-phase material was used. Co:Pel ODS was placed in line to remove unwanted material from the sample prior to passage onto the analytical column. Dissolution studies were performed on the dissolution apparatus¹² using the USP rotating-paddle method.

Chromatographic Conditions—The mobile phase was methanol-water-phosphoric acid (50:50:0.1 v/v/v). Flow rates of 1.5 and 2 ml/min were used in the analyses of liothyronine sodium tablets and levothyroxine sodium and liotrix tablets, respectively. Another mobile phase that was useful was prepared by mixing methanol and 0.1 M ammonium acetate (pH 5.0) in a 50:50 (v/v) ratio. The flow rate was 2 ml/min (Fig. 1). The mobile phase was degassed prior to use.

Procedure—All of the glass vessels were silanized, and the glassware was wrapped with aluminum foil. A single tablet of levothyroxine sodium, liothyronine sodium, or liotrix or a weighed amount of ground powder of any of these tablets was taken in an erlenmeyer flask and assayed as described previously for liothyronine sodium tablets (32).

Levothyroxine sodium concentrations were determined from a standard curve. The standard curve was obtained from standard samples, each containing 50 µg of 3,3',5'-triiodothyronine (the internal standard) and 18–225 µg of levothyroxine sodium. The standard samples were subjected to the same procedure as the tablets. The peak height ratio of levothyroxine sodium to the internal standard was plotted against the concentration of levothyroxine sodium, and a straight-line correlation was obtained ($r = 0.9993$). A standard curve was developed similarly with liothyronine sodium and 3,3',5'-triiodothyronine (the internal standard) to obtain a straight-line correlation ($r = 0.995$), and liothyronine sodium concentrations were obtained from the standard curve.

Recovery—Known aliquots from solutions containing different concentrations of levothyroxine sodium were injected onto the high-performance liquid chromatograph, and peak heights were obtained. A straight-line correlation was obtained for the peak height as a function of concentration. Similarly, peak heights obtained from experimentally processed samples gave a straight-line correlation when plotted against concentrations. The ratio of the slopes was used to estimate the percent recovery. The recoveries for levothyroxine sodium were excellent (98–100%).

Precision—The precision of the method for determining levothyroxine sodium was established by analyzing standard solutions of levothyroxine sodium of several concentrations. The samples were analyzed in triplicate as described.

Dissolution Studies on Levothyroxine Sodium—Dissolution studies were conducted only on levothyroxine sodium tablets. The USP rotating-paddle method was used with 500 ml of deionized water as the dissolution medium at 37 ± 0.5°.

Feasibility for Various Dosage Forms—Dissolution studies were done at 50 rpm on four dosage forms, i.e., 25, 50, 100, and 200 µg, of two commercial brands. A single sample of the dissolution medium was taken at the end of 40 min. The procedure for estimation of levothyroxine sodium in the dissolution sample will be described.

Time Course Study—Feasibility for the time course study was conducted on 0.5-mg levothyroxine sodium tablets. The speed of the paddles was set at 75 rpm. Samples were taken at 5, 10, 20, 40, 80, and 100 min. The dissolution samples were processed as will be described.

Estimation of Levothyroxine Sodium—The dissolution sample was filtered, and a known volume (50 ml for 25-, 50-, and 100-µg tablets, 25 ml for 200-µg tablets, and 10 ml for the time course of dissolution) was placed in the erlenmeyer flask. To this sample was added concentrated hydrochloric acid equivalent to 25% of the volume of the sample and 1.5

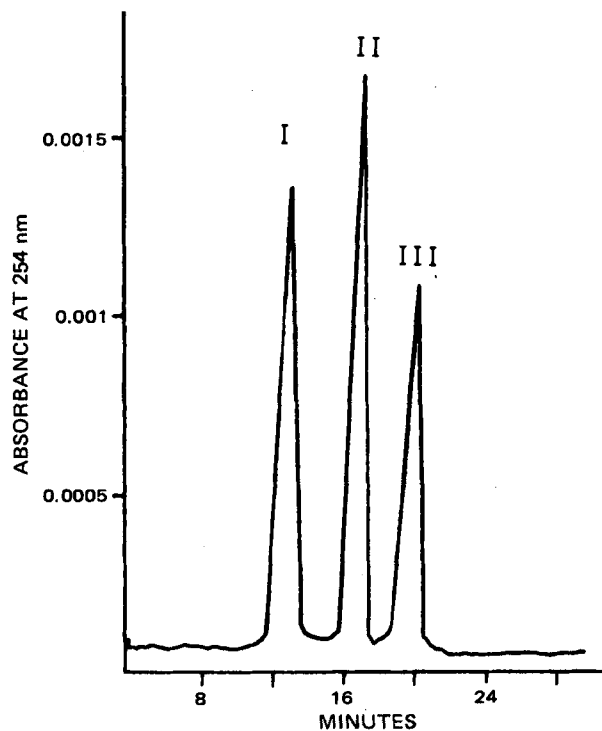


Figure 1—High-performance liquid chromatogram of liothyronine (I), 3,3',5'-triiodothyronine (II, internal standard), and levothyroxine (III) obtained on a µBondapak C₁₈ reversed-phase column using a mobile phase of methanol–0.1 M ammonium acetate (pH 5.0) (50:50 v/v).

volumes of *n*-butanol. Ten micrograms of 3,3',5'-triiodothyronine (the internal standard) also was added, and the mixture was stirred for 30 min. The mixture then was placed in a separator. The upper butanolic layer was separated and washed four times with water-saturated butanol. The washed butanolic solution was evaporated to dryness. The residue was dissolved in a known volume of 2% ammonium hydroxide in methanol (usually 200 µl), and an aliquot was injected onto the high-performance liquid chromatograph.

Levothyroxine sodium concentrations were determined from a standard curve obtained by plotting peak height ratios of levothyroxine sodium and the internal standard as a function of known levothyroxine sodium concentrations. These samples were subjected to the same experimental conditions as the dissolution samples from the tablets.

RESULTS AND DISCUSSION

Several solvent systems were investigated for the HPLC analysis of the thyroidal amino acids, and methanol and ammonium acetate gave good separation of liothyronine, 3,3',5'-triiodothyronine (the internal standard), and levothyroxine (Fig. 1). Several commercially available columns also were tried. A Partisil ODS-3¹⁰ column gave better separation than that reported by Hearn *et al.* (27) under their given conditions (Fig. 2). However, both the µBondapak C₁₈ and the Partisil ODS-3 columns were satisfactory. Hence, the analysis of liothyronine sodium and levothyroxine sodium from tablets was carried out on a µBondapak C₁₈ column⁹ and the dissolution samples of levothyroxine sodium were analyzed on a Partisil ODS-3 column¹⁰.

Table I—Precision of the HPLC Method for Levothyroxine Sodium from Standard Solutions

Total Theoretical Amount, µg	Amount Found (Range), µg	CV ^a , %
18	17.7 (17.5–17.8)	1.4
27	26.7 (26.0–27.2)	2.5
63	65.9 (64.9–66.5)	1.3
90	88.5 (87.8–89.3)	0.8
180	175.4 (173.0–177.6)	1.3
225	228.5 (226.7–231.8)	1.2

^a $n = 3$.

⁵ Model 6000A, Waters Associates, Milford, Mass.

⁶ SVOV-6, Glenco Scientific Inc., Houston, Tex.

⁷ Model 440, Waters Associates, Milford, Mass.

⁸ Direct-current analog recorder, Linear Instruments Corp.

⁹ µBondapak C₁₈ column (30 cm × 3.9 mm i.d.), Waters Associates, Milford, Mass.

¹⁰ Partisil ODS-3 column (25 cm × 4.6 mm i.d.), Whatman, Clifton, NJ.

¹¹ Whatman, Clifton, NJ.

¹² Model 72 S 115, Hanson Research Corp., Northridge, Calif.

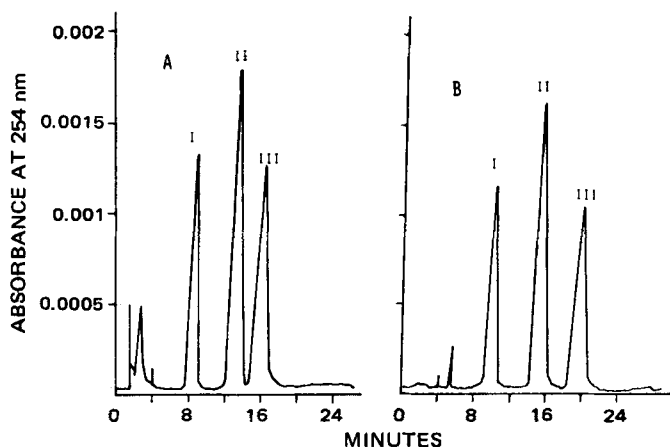


Figure 2—High-performance liquid chromatograms of liothyronine (I), 3,3',5'-triiodothyronine (II, internal standard), and levothyroxine (III) obtained on a μ Bondapak C₁₈ reversed-phase column using a mobile phase of methanol-water-phosphoric acid (50:50:0.1 v/v) (A) and of I-III obtained on a Partisil ODS-3 reversed-phase column using the same mobile phase (B).

Levothyroxine sodium tablets were assayed following the general method described for the analysis of liothyronine sodium tablets. Analysis of levothyroxine sodium tablets by HPLC (without the internal standard) revealed that 3,3',5'-triiodothyronine is not present in significant amounts (<2%) so it is a suitable internal standard. A straight-line correlation was obtained between the peak height ratios of levothyroxine sodium to 3,3',5'-triiodothyronine (the internal standard) and the concentration of levothyroxine sodium. The method gave good precision (coefficient of variation of $\leq 2.5\%$) for levothyroxine sodium in the range of 18–225 μ g (Table I). Similarly, good correlation was obtained between the peak height ratio of liothyronine sodium to 3,3',5'-triiodothyronine (the internal standard) and the liothyronine sodium concentration in the range of 10–70 μ g. The concentrations of liothyronine sodium and levothyroxine sodium from samples of unknown concentrations were determined from such standard curves.

Table II—Content Uniformity of Liothyronine Sodium Tablets

Manufacturer	Dosage Strength, μ g	Individual Tablet Assay as Liothyronine, μ g	Mean \pm SD	Percent of Labeled Strength	Analysis of Composite, μ g \pm SD ^a
A	25	22.1, 25.7, 21.2, 21.3, 21.9, 23.1, 23.2, 24.9, 22.8, 27.4	23.4 \pm 2.0	84.9–109.4	23.2 \pm 0.9
B	50	48.6, 48.1, 48.1, 50.1, 46.1, 48.1, 45.7, 40.7, 48.5, 57.0	47.9 \pm 1.8	90.9–101.3	48.0 \pm 0.4

^a Average of three determinations.

Table III—Content Uniformity of Levothyroxine Sodium Tablets USP

Manufacturer	Dosage Strength, μ g	Individual Tablet Assay, μ g	Mean (Range), μ g	Mean Percent of Labeled Strength (Range)
B	100	92.7, 97.2, 100.0, 95.7, 96.8, 101.3, 94.7, 98.2, 101.4, 102.0	98.0 (92.7–102.0)	98.0 (92.7–102.0)
B	200	188.3, 182.1, 180.8, 184.9, 201.9, 187.9, 184.9, 189.0, 196.6, 188.5	188.5 (180.0–201.9)	94.2 (90.4–100.9)
C	100	104.0, 93.6, 93.9, 94.4, 95.0, 94.6, 94.2, 89.3, 98.8, 98.9	95.7 (89.3–104.0)	95.7 (89.3–104.0)
C	200	197.5, 196.5, 187.3, 192.2, 183.2, 196.7, 196.5, 195.9, 192.3, 205.5	194.3 (183.2–205.5)	97.2 (91.6–102.7)

Table IV—Content Uniformity of Liotrix (Liothyronine Sodium–Levothyroxine Sodium) Tablets USP

Manufacturer and Labeled Dosage	Liothyronine Sodium		Levothyroxine Sodium		Ratio of Levothyroxine Sodium to Liothyronine Sodium \pm SD
	Mean (Individual Tablet Analysis), μ g	Amount Range and Range of Percent of Labeled Strength	Mean (Individual Tablet Analysis), μ g	Amount Range and Range of Percent of Labeled Strength	
D, 45 μ g of liothyronine sodium and 180 μ g of levothyroxine sodium	48.2 (47.6, 48.8, 48.8, 48.8, 48.8, 46.7, 48.8, 47.4, 47.6, 48.8)	46.7–48.8 μ g 104.9–108.4%	169.3 (162.0, 169.7, 171.5, 163.8, 174.4, 171.8, 168.3, 176.2, 169.7, 165.8)	162.0–176.2 μ g 90.0–97.88%	3.51 \pm 0.12
E, 62.5 μ g of liothyronine sodium and 250 μ g of levothyroxine sodium	64.0 (60.2, 65.2, 58.8, 66.9, 64.1, 58.2, 68.8, 69.8, 63.9, 64.2)	58.2–69.8 μ g 93.1–111.7%	235.2 (234.6, 237.0, 237.4, 245.5, 258.1, 226.2, 249.6, 221.3, 221.3, 222.6)	219.8–258.1 μ g 87.9–103.2%	3.69 \pm 0.32

Content uniformity is a general requirement in the USP for all dosage forms that contain active ingredients of 50 mg or less, provided that there is a method available for the analysis of a single tablet (3). Content uniformity assures that successive units from a given container will provide substantially equal amounts of drug. The dosage strengths that are marketed for liothyronine sodium tablets range from 5 to 50 μ g; for levothyroxine sodium tablets, they range from 25 to 500 μ g; and for liotrix tablets containing liothyronine sodium and levothyroxine sodium, they range from 3.1 μ g of liothyronine sodium and 12.5 μ g of levothyroxine sodium to 62.5 μ g of liothyronine sodium and 250 μ g of levothyroxine sodium.

Commercially available liothyronine sodium tablets were analyzed. In addition, for two of the dose levels, 20 tablets were pulverized, and composite powder equivalent to a single tablet was weighed and analyzed in triplicate. The analytical results are presented in Table II. The mean value of the composite material was in close agreement to the mean of the 10 individual analyses. The USP states that liothyronine sodium tablets contain an amount of liothyronine sodium equivalent to not less than 90.0% and not more than 110.0% of the labeled amount of liothyronine. Hence, the individual assay values of the tablets were reported for liothyronine and not for liothyronine sodium. If the general USP requirement for content uniformity is imposed, the individual analysis for the tablets for liothyronine should be in the range of 100 \pm 15%. At both dose levels, these tablets conformed to the compendial requirements.

Individual tablet analysis of 100- and 200- μ g levothyroxine sodium tablets from two manufacturers was carried out, and the results are shown in Table III. If general compendial requirements are imposed, the tablets should contain 102.5 \pm 15%, i.e., 87.3–117.5%, of the active ingredient. The tablets conformed to these requirements. Similarly, liotrix tablets of two commercial brands, at a single dose level, were subjected to individual tablet analysis. Liothyronine sodium and levothyroxine sodium were analyzed simultaneously. Both brands of tablets met the compendial assay requirement of 86.1–116.4% (101.25 \pm 15%) of the declared value for both liothyronine sodium and levothyroxine sodium contents. The results are presented in Table IV.

The fifth supplement to USP XIX also requires that the weight ratio of levothyroxine sodium to liothyronine sodium be 4:1. The ratio in the present experiments was in the range of 3.69 \pm 0.32 for tablets from one manufacturer and 3.51 \pm 0.12 for tablets from another manufacturer. Thus, both firms failed to meet the ratio requirement. If both liothyronine sodium and levothyroxine sodium are allowed by the USP to be present

Table V—Dissolution Study of Levothyroxine Sodium Tablets USP in 500 ml of Deionized Water Using 50-rpm Rotating-Paddle Method

Manufacturer	Dosage, μg	Average Percent Dissolved at 40 min	SD
B	25	31.6	5.0
	50	51.1	5.3
	100	44.2	10.2
	200	60.3	18.7
C	25	58.9	6.2
	50	47.6	13.2
	100	43.3	7.3
	200	56.2	10.4

at an amount of $101.25 \pm 15\%$, it is possible that one component may be present at the higher limit (116.4%) and the other one may be present at the lower limit (86.1%), and the tablet still may pass the content uniformity requirement. In such a case, the tablet cannot meet the 4:1 levothyroxine sodium to liothyronine sodium weight ratio requirement. For example, for a liotrix tablet containing 100 μg of levothyroxine sodium and 25 μg of liothyronine sodium, if levothyroxine sodium is present at the upper limit, 116.4% (i.e., 116.4 μg of levothyroxine sodium) and liothyronine sodium is present at the lower limit, 86.1% (i.e., 25 $\mu\text{g} \times 0.861 = 21.53 \mu\text{g}$), the levothyroxine sodium to liothyronine sodium ratio would be 5.41. At the other extreme, where levothyroxine sodium is present at the lower permitted limit and liothyronine sodium is present at the higher permitted limit, the ratio is 2.95. Hence, the levothyroxine sodium to liothyronine sodium ratio could vary from 2.95 to 5.41 while still meeting the compendial requirements for content uniformity of the individual active ingredients. This point could be important for the physician prescribing these drugs, and it is recommended that the USP look into the conflicting requirements for content uniformity and the levothyroxine sodium to liothyronine sodium weight ratio.

The applicability of this new procedure for analysis of samples from the dissolution medium was investigated. The dissolution procedure was tested only on levothyroxine sodium tablets. A straight-line correlation was obtained using standard solutions of levothyroxine sodium and 3,3',5'-triiodothyronine (the internal standard) as described under *Experimental* ($r = 0.999$; $CV = 4.5\%$ in the range of 0–20 μg of levothyroxine sodium). The feasibility of this procedure was tested on four dosage levels of levothyroxine sodium for two commercial brands, and the dissolution ranged between 30 and 60% after 40 min (Table V). The method proposed here appears to be suitable for study of the dissolution at a single time point.

This procedure also appears to be useful for time course studies of dissolution for a higher dosage of levothyroxine sodium tablets (Fig. 3). The dissolution studies and content uniformity studies were preliminary feasibility studies only, and a detailed investigation is underway.

The utility of the HPLC method for the analysis of levothyroxine sodium tablets, the determination of content uniformity of liothyronine

sodium, levothyroxine sodium, and liotrix tablets, and for dissolution studies of levothyroxine sodium tablets was demonstrated. The method is simple, specific, and sensitive.

REFERENCES

- (1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. 282, 286.
- (2) "Fourth Supplement to the United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1979, p. 106.
- (3) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, pp. xvi, 648.
- (4) J. A. Graham, D. Banes, M. E. Biesemeyer, and A. Nadakarni, *J. Pharm. Sci.*, **63**, 763 (1974).
- (5) U. R. Cieri and J. C. Illuminati, *J. Assoc. Off. Anal. Chem.*, **60**, 628 (1977).
- (6) M. A. Faircloth, A. D. Williams, and W. H. Florsheim, *Anal. Biochem.*, **12**, 437 (1965).
- (7) M. Dobias, J. Mucha, and P. Talan, *Radiochem. Radioanal. Lett.*, **33**, 179 (1978).
- (8) S. Kologlu, H. L. Schwartz, and A. C. Carter, *Endocrinology*, **78**, 231 (1966).
- (9) R. Lemieux and J. M. Talmage, *J. Pharm. Pharmacol.*, **18**, 94 (1966).
- (10) A. D. Williams, D. E. Freeman, and W. H. Florsheim, *J. Chromatogr.*, **45**, 371 (1969).
- (11) A. D. Williams, D. E. Freeman, and W. H. Florsheim, *J. Chromatogr. Sci.*, **9**, 619 (1971).
- (12) C. Peyton and C. Simon, *J. Chromatogr.*, **92**, 309 (1974).
- (13) P. Thomopoulose, *Anal. Biochem.*, **65**, 600 (1975).
- (14) F. Shahrokhi and C. W. Gehrke, *ibid.*, **24**, 281 (1968).
- (15) N. M. Alexander and R. Scheig, *ibid.*, **22**, 187 (1968).
- (16) K. Funakoshi and H. J. Cahnmann, *ibid.*, **27**, 150 (1969).
- (17) E. C. Volpert, N. Kundu, and J. B. Dawidzik, *J. Chromatogr.*, **50**, 510 (1970).
- (18) R. Bilouis and J. S. Windheuser, *J. Pharm. Sci.*, **62**, 274 (1973).
- (19) N. N. Nihei, M. C. Gershengorn, T. Mitsuma, L. R. Stringham, A. Cordy, B. Kuchmy, and C. S. Hollander, *Anal. Biochem.*, **43**, 433 (1971).
- (20) R. Docter and G. Henneman, *Clin. Chim. Acta*, **34**, 297 (1971).
- (21) B. A. Petersen, R. N. Hanson, R. W. Giese, and B. L. Karger, *J. Chromatogr.*, **126**, 503 (1976).
- (22) B. A. Petersen, R. W. Giese, P. R. Larsen, and B. L. Karger, *Clin. Chem.*, **23**, 1389 (1977).
- (23) G. J. Manius, P. Fallon, and R. Tscherne, *Anal. Biochem.*, **87**, 496 (1978).
- (24) R. O. Zimmerer, Jr., and L. T. Grady, *J. Pharm. Sci.*, **60**, 493 (1971).
- (25) B. L. Karger and S. C. Su, *J. Chromatogr. Sci.*, **12**, 678 (1974).
- (26) Application Highlight 19, Waters Associates, Milford, Mass., 1976.
- (27) M. T. Hearn, W. S. Hancock, and C. A. Bishop, *J. Chromatogr.*, **157**, 337 (1978).
- (28) D. S. Smith and J. H. Graham, *J. Assoc. Off. Anal. Chem.*, **62**, 818 (1979).
- (29) F. Nachtmann, G. Knapp, and H. Spitzzy, *J. Chromatogr.*, **149**, 693 (1978).
- (30) N. M. Alexander and M. Nishimoto, *Clin. Chem.*, **25**, 1757 (1979).
- (31) G. C. Skellern, M. Mahmoudian, and B. I. Knight, *J. Chromatogr.*, **179**, 213 (1979).
- (32) R. S. Rapaka, P. W. Knight, and V. K. Prasad, *Anal. Lett.*, **12**, 1201 (1979).

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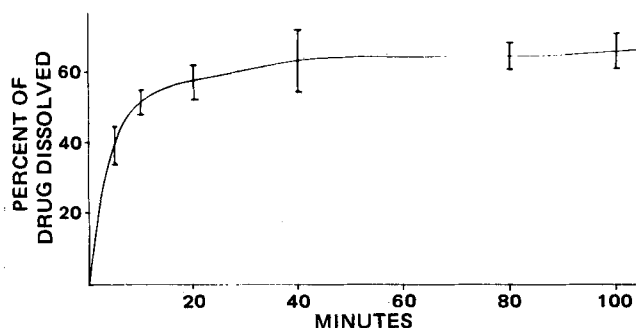


Figure 3—Dissolution profile obtained for 500- μg levothyroxine sodium tablets USP in 500 ml of deionized water at 37° using the USP rotating-paddle method at 75 rpm.