(5) R. B. Bruce and W. R. Maynard, Jr., *Anal. Chem.*, **41**, 977 (1969).
(6) L. J. Dombrowski, P. M. Comi, and E. L. Pratt, *J. Pharm. Sci.*, **62**, 1761 (1973).
(7) G. R. Wilkinson, *Anal. Lett.*, **3**, 289 (1970).
(8) T. Walle and H. Ehrsson, *Acta Pharm. Suec.*, **8**, 27 (1971).
(9) D. J. Edwards and K. Blau, *Anal. Biochem.*, **45**, 387 (1972).
(10) J. C. Lhuguenot and B. F. Maume, *J. Chromatogr. Sci.*, **12**, 411 (1974).
(11) L. Neelakantan and H. B. Kostenbauder, *J. Pharm. Sci.*, **65**, 740 (1976).
(12) H. Kinsun, M. A. Moulin, and E. C. Savini, *J. Pharm. Sci.*, **67**, 118 (1978).
(13) W. D. Mason and E. N. Amick, *J. Pharm. Sci.*, **70**, 707 (1981).
(14) B. M. Farrell and T. M. Jefferies, *J. Chromatogr.*, **272**, 111 (1983).
(15) F. T. Noggle, Jr., *J. Assoc. Off. Anal. Chem.*, **63**, 702 (1980).

ACKNOWLEDGMENTS

We thank Dr. Pieter Bonsen for critically reviewing this manuscript and Ms. Rose Wright for her extensive literature research.

Determination of Sodium Levothyroxine in Bulk, Tablet, and Injection Formulations by High-Performance Liquid Chromatography

JAMES F. BROWER ˣ, DUCKHEE Y. TOLER, and JOHN C. REEPMEYER

Received July 15, 1983, from the *National Center for Drug Analysis, Food and Drug Administration, St. Louis, MO 63101.* Accepted for publication September 28, 1983.

Abstract □ Sodium levothyroxine was determined in bulk drugs, tablets, and injections by high-performance liquid chromatography (HPLC). Levothyroxine was separated from excipients and impurities on a 10-μm cyanoalkyl column using an acetonitrile–water–phosphoric acid mobile phase. The HPLC method is shown to be linear, accurate, and precise, and the results obtained by the HPLC and USP XX methods are compared.

Keyphrases □ Sodium levothyroxine—HPLC, determination of bulk, tablet, and injection formulations □ HPLC—sodium levothyroxine, determination of bulk, tablet, and injection formulations

In a survey[1] of sodium levothyroxine products and formulations, 63 samples of tablets, representing 20 formulations from 5 manufacturers, 9 samples of injections from 2 manufacturers, and 6 samples of bulk sodium levothyroxine from 5 manufacturers, were analyzed in this labroatory. The purpose of the survey was to evaluate the quality of sodium levothyroxine products on the market and the adequacy of present compendial standards and methods. Methodology was developed for content uniformity analysis of sodium levothyroxine by high-performance liquid chromatography (HPLC). This method, unlike the official compendial method (1), differentiates levothyroxine from iodinated impurities and degradation products.

Previously developed methods for the determination of sodium levothyroxine (2–13) were evaluated and tested. A modification of the HPLC procedure described by Garnick *et al.* (13), using a cyanoalkyl bonded phase column, was selected. This method offers advantages over those already in the literature by avoiding buffers in the mobile phase, no sample derivatization, faster analysis times, greater sensitivity due to shorter retention times, lower flow rates, and 229 nm detection. A sample solvent was selected that readily dissolved sodium levothyroxine without degradation from tablet formulations. This method and the results obtained on the survey sample are reported here.

[1] This study was a national survey for the Food and Drug Administration.

EXPERIMENTAL SECTION

Apparatus—A modular high-performance liquid chromatograph[2] (HPLC) was equipped with a fixed-wavelength (229 nm) cadmium lamp UV detector[3], an automated injector[4], a microprocessor controller[5], and a recorder–integrator[6]. A stainless steel column (3.9 mm × 30 cm) was packed with irregular 10-μm silica particles to which a layer of cyanoalkyl silane was chemically bonded[7].

The mobile phase consisted of acetonitrile–water–phosphoric acid (350:650:1). This solution was passed through a 0.45-μm filter[8], deaerated, and then pumped through the HPLC system at a rate of 1 mL/min.

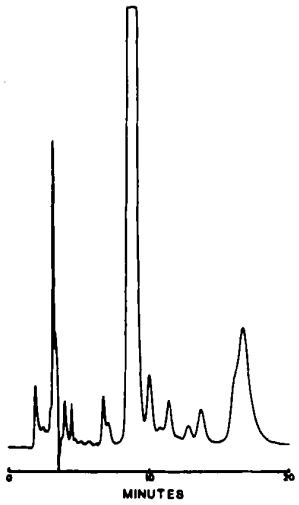

Figure 1—*Chromatogram of sodium levothyroxine bulk drug decomposed by heating in air; detector at 229 nm and 0.02 AUFS. Key: (1) sodium levothyroxine at a level of ~100 μg/mL.*

[2] Model ALC 204; Waters Associates.
[3] Model 441; Waters Associates.
[4] Model 710B WISP; Waters Associates.
[5] Model 720 System Controller; Waters Associates.
[6] Model 730 Data Module; Waters Associates.
[7] μ-Bondapak-CN; Waters Associates.
[8] Durapore UVLPO4700; Millipore Corp., Bedford, Mass.

0022-3549/84/0900-1315$01.00/0
© 1984, American Pharmaceutical Association

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

Table I—Sodium Levothyroxine Determined by HPLC and USP XX Procedures

Manufacturer[a]	Dosage Form, mg	Label Claim, % C.U.[b]	Label Claim, % Composite Assay HPLC	Label Claim, % Composite Assay USP
A	Tablet, 0.025	96.7 (1.2) 30		
	Tablet, 0.025	97.6 (1.6) 30		
	Tablet, 0.050	90.8 (1.8) 30		
	Tablet, 0.050	94.3 (2.1) 30		
	Tablet, 0.100	96.7 (1.8) 30		
	Tablet, 0.100	95.8 (1.3) 30		
	Tablet, 0.100	97.2 (1.9) 30		
	Tablet, 0.100	97.5 (2.5) 30		
	Tablet, 0.125	97.6 (1.7) 30		
	Tablet, 0.125	98.4 (0.8) 30		
	Tablet, 0.150	97.8 (2.0) 30		
	Tablet, 0.150	104.4 (2.0) 10		
	Tablet, 0.150	95.0 (1.8) 10		
	Tablet, 0.175	95.0 (1.8) 30		
	Tablet, 0.200	97.4 (1.3) 10		
	Tablet, 0.200	100.0 (2.5) 30		
	Tablet, 0.200	100.6 (1.9) 30		
	Tablet, 0.200	98.5 (1.7) 10	96.5	104.9
	Tablet, 0.300	102.0 (1.4) 10	97.8	109.2
	Tablet, 0.300	102.5 (2.6) 10	100.8	106.3
	Bulk drug		100.2	98.4
	Tablet, 0.050	80.7 (1.2) 10	Past expiration date	
	Tablet, 0.100	99.1 (1.7) 10	Past expiration date	
	Tablet, 0.175	88.3 (2.2) 10	Past expiration date	
	Tablet, 0.300	90.9 (1.9) 10	Past expiration date	
B	Tablet, 0.100	92.4 (2.6) 10	94.3	97.4
	Tablet, 0.100	89.5 (6.7) 10	95.7	96.6
	Tablet, 0.200	99.1 (5.2) 10	102.5	105.2
	Bulk drug		100.9	96.8
C	Tablet, 0.100	99.4 (3.2) 20		
	Tablet, 0.100	99.6 (2.1) 20		
	Tablet, 0.200	94.7 (1.8) 10	97.4	113.6
	Tablet, 0.200	90.2 (2.5) 20	95.6	111.7
	Bulk drug		100.7	96.4
D	Tablet, 0.100	63.9 (2.5) 20	62.3	101.8
	Tablet, 0.300	100.8 (1.5) 10	101.7	104.3
	Bulk drug		98.1	97.1
E	Tablet, 0.025	99.7 (3.7) 10		
	Tablet, 0.025	99.1 (2.9) 10		
	Tablet, 0.025	97.8 (2.7) 10		
	Tablet, 0.025	97.8 (2.1) 10		
	Tablet, 0.050	102.0 (2.1) 10		
	Tablet, 0.050	101.5 (3.7) 10		
	Tablet, 0.050	106.1 (1.4) 10		
	Tablet, 0.050	102.6 (2.3) 10		
	Tablet, 0.100	94.5 (1.4) 10		
	Tablet, 0.100	104.3 (2.3) 10		
	Tablet, 0.100	105.1 (2.7) 10		
	Tablet, 0.100	104.3 (1.9) 10		
	Tablet, 0.100	104.8 (2.0) 10		
	Tablet, 0.100	105.5 (2.0) 10		
	Tablet, 0.150	102.1 (1.5) 10		
	Tablet, 0.150	102.9 (1.2) 10		
	Tablet, 0.150	105.6 (1.2) 10		
	Tablet, 0.150	103.7 (1.4) 10		
	Tablet, 0.150	105.2 (1.6) 10		
	Tablet, 0.150	104.4 (1.8) 10		
	Tablet, 0.200	88.7 (1.5) 10		108.2
	Tablet, 0.200	106.3 (3.0) 10		106.6
	Tablet, 0.200	104.4 (2.7) 10		
	Tablet, 0.200	105.8 (2.3) 10		
	Tablet, 0.200	103.6 (3.3) 10		
	Tablet, 0.200	105.0 (2.7) 10		
	Tablet, 0.200	105.1 (1.8) 10		
	Tablet, 0.300	102.0 (1.8) 10		
	Inj., 0.100	84.1 (1.5) 5	86.5	
	Inj., 0.100	94.2 (2.9) 5		
	Inj., 0.100	89.0 (4.8) 5	94.4	
	Inj., 0.200	94.4 (4.7) 5		
	Inj., 0.200	95.5 (2.8) 5		
	Inj., 0.200	97.5 (1.3) 5		
	Inj., 0.500	95.5 (0.5) 5		
	Inj., 0.500	93.4 (3.6) 5		
	Bulk drug		97.8	95.4
	Bulk drug		99.2	98.0
F	Inj., 0.500	83.8 (9.4) 3		

[a] (A) Armour Pharmaceutical Co., Scottsdale, Ariz.; (B) Chelsea Laboratories Inc., Inwood, N.Y.; (C) Generic Pharmaceutical, Palisades Park, N.J.; (D) Western Research Laboratories, Denver, Colo.; (E) Travenol Laboratories, Deerfield, Ill.; (F) Carter-Glogau Laboratories, Glendale, Ariz. [b] Content uniformity by HPLC, mean; *RSD* in parentheses; number of units tested.

Reagents—Methanol[9], acetonitrile[9], phosphoric acid[10], and sodium hydroxide[11] were used as received. Deionized water was supplied by a commercial[12] water system. Samples and standards were prepared in 0.01 M sodium hydroxide in 50% methanol.

Standard Preparation—About 10 mg of USP levothyroxine reference standard was accurately weighed into a 50-mL volumetric flask, dissolved, and diluted to volume with 0.01 M methanolic sodium hydroxide. (This stock solution is stable for several weeks when refrigerated.) Two milliliters of this solution was pipetted into a 100-mL volumetric flask and diluted to volume with 0.01 M methanolic sodium hydroxide.

Sample Preparation—*Tablets*—One tablet was placed in a glass-stoppered flask and accurately diluted with 0.01 M methanolic sodium hydroxide to ~4 μg/mL. The flask was placed in an ultrasonic bath[13] until the tablet disintegrated; the tablet was then mechanically shaken[14] for 30 min. The solution was then filtered[15] into a vial for injection.

Powdered Composites and Lyophilized Injections—An accurately weighed portion of the powdered sample, equivalent to ~400 μg of sodium levothyroxine, was placed in a 100-mL volumetric flask. Fifty milliliters of 0.01 M methanolic sodium hydroxide was added, and the flask was placed in an ultrasonic bath for 1 min. The flask was shaken for 5 min; the contents were diluted to volume with 0.01 M methanolic sodium hydroxide and mixed well. The solution was then filtered[15] into a vial for injection.

Bulk Drug Substances—A sample of bulk drug substance was prepared in the same manner as the standard.

[9] Matheson, Coleman and Bell, Cincinnati, Ohio.
[10] NF Grade; Mallinckrodt Inc., St. Louis, Mo.
[11] AR Grade; Mallinckrodt Inc.
[12] Milli-Q; Millipore Corp.
[13] Model SC400T; Randall Mfg. Co., Inc., Hillside, N.J.
[14] Oscillating shaker; Eberbach Corp., Ann Arbor, Mich.
[15] Nylon-66, 13-mm diameter, 0.45-μm pore size; Rainin Instrument Co., Woburn, Mass.

Determination—Fifty microliters each of the standard and sample solutions were injected into the liquid chromatograph, and the chromatograms were recorded. Sodium levothyroxine was calculated on the basis of peak areas.

RESULTS AND DISCUSSION

Linearity, Reproducibility, and Recovery—A series of validation tests were performed on the HPLC method. A linear response was obtained when four standard solutions containing from 1 to 8 μg/mL were tested. Placebo samples, based on the batch formulation of the manufacturers, were spiked with various levels of the standard. The recoveries ranged from 99.5 to 100.6%. The reproducibility of the method was determined by consecutively injecting 10 aliquots of standard solution. The *RSD* was 0.2%.

Decomposition and Stability Studies—In the course of the survey, it was found that the bulk drug substances were sensitive to the conditions of the USP drying procedure (60°C in a vacuum over P_2O_5). If the vacuum was not maintained below 10 mm Hg, sodium levothyroxine decomposed rapidly (10–15% in 4 h). Undried bulk drug samples were used in the analyses because of this problem, and corrections were made for moisture content. Figure 1 shows a chromatogram of sodium levothyroxine bulk drug dried in the presence of air. Some decomposition products can be seen in the chromatogram; these were not observed in the chromatograms of an unheated sample of sodium levothyroxine measured at the same sensitivity. In addition, there are probably other decomposition products in the heated sample which are not eluted from the column. The identity of these decomposition products will be investigated at a later time.

To test the stability of sodium levothyroxine in the sample solvent (0.01 M methanolic sodium hydroxide), solutions of the bulk drug samples were stored at 5°C and assayed periodically (*versus* freshly prepared reference standards) over a six month period. The assay values remained constant over the entire testing period, indicating little or no decomposition.

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

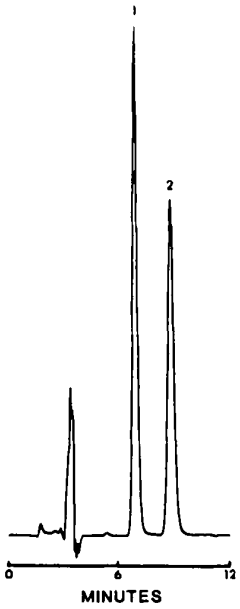

Figure 2—*Chromatogram of sodium levothyroxine and sodium liothyronine; detector at 229 nm and 0.02 AUFS. Key: (1) sodium liothyronine; (2) sodium levothyroxine, each at a level of ~4 μg/mL.*

The chromatographic system parameters were adequate to separate sodium levothyroxine from sodium liothyronine for testing the bulk drug substances for impurities; Fig. 2 shows a chromatogram of this separation. Liothyronine was found at levels of 0.04–0.96% in five sodium levothyroxine bulk drug samples analyzed by HPLC.

Sample Analysis—Table I lists the results obtained from survey by the HPLC procedure. A comparison of results for composite samples, obtained with the HPLC and the USP XX methods, shows that the latter gave a higher result in practically every case. This is not surprising since the USP XX assay is nonspecific for levothyroxine and measures total iodine content. However, the difference in assay values could not be totally accounted for by a total area summation of HPLC peaks. The major impurities and degradation products probably are not eluted from the column with this mobile phase.

Low assay values were a problem experienced by most manufacturers; this problem would not be recognized if the assays were based only on the results from the USP method. Some tablet composite samples gave suitable assay values for total iodine by the USP method, but gave extremely low assays for sodium levothyroxine by HPLC. All samples which gave low assay values by HPLC gave suitable assays by the USP method. This fact indicates that the problems of low assays of marketed sodium levothyroxine are, in all probability, attributable to sodium levothyroxine instability.

REFERENCES

(1) "The United States Pharmacopeia XX," U.S. Pharmacopeial Convention, Rockville, Md., 1980, pp. 446–447.

(2) B. L. Karger and S. C. Su, *J. Chromatogr. Sci.*, **12,** 678 (1974).

(3) F. Nachtmann, G. Knapp, and H. Spitzy, *J. Chromatogr.*, **149,** 693 (1978).

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

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

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

(7) E. P. Lankmayr and K. W. Budna, *J. Chromatogr.*, **198,** 471 (1980).

(8) D. J. Smith and J. H. Graham, *J. Assoc. Off. Anal. Chem.*, **62,** 818 (1980).

(9) B. R. Hepler, S. G. Weber, and W. C. Purdy, *Anal. Chim. Acta*, **113,** 269 (1980).

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

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

(12) I. D. Hay, T. M. Annesley, N. S. Jiang, and C. A. Gorman, *J. Chromatogr.*, **226,** 383 (1981).

(13) R. L. Garnick, G. F. Burt, F. R. Borger, J. P. Aldred, J. W. Bastian, and D. A. Long, in "Hormone Drugs, Proceedings of the FDA-USP Workshop on Drug and Reference Standards for Insulins, Somatropins, and Thyroid-Axis Hormones," U.S. Pharmacopeial Convention, Rockville, Md., 1983, pp. 504–516.

ACKNOWLEDGMENTS

The authors thank Dr. Thomas P. Layloff and Donald P. Page for assistance during the survey.

Determination of Isosorbide 5-Mononitrate in Human Plasma by Capillary Column Gas Chromatography

P. STRAEHL and R. L. GALEAZZI ˣ

Received February 22, 1983, from the *University of Bern, Department of Medicine, Inselspital, CH-3010 Bern, Switzerland.* Accepted for publication August 25, 1983.

Abstract □ An electron-capture gas chromatographic method for the determination of isosorbide 5-mononitrate in human plasma using a capillary column is described. Isosorbide 5-mononitrate and the internal standard (isosorbide dinitrate) are extracted from the alkalinized plasma with ether. The lower limit of detection for isosorbide 5-mononitrate is 1 ng/mL of plasma.

Keyphrases □ Gas chromatography—isosorbide 5-mononitrate, human plasma □ Isosorbide 5-mononitrate—GC, human plasma

Isosorbide 5-mononitrate [1,4:3,6-dianhydro-D-glucitol 5-nitrate (I)] is the primary metabolite of isosorbide dinitrate [1,4:3,6-dianhydro-D-glucitol dinitrate (II)] which has been used for many years in the treatment of angina pectoris and congestive heart failure. Recent studies of the hemodynamic effect of the mononitrate (1) indicate that, after acute administration, cardiac work load decreases at rest and during exercise. Pharmacokinetic studies (2–4) showed that the mononitrate is rapidly and completely absorbed from the GI tract without undergoing first-pass elimination. The maximum concentrations were reached within 1 h after oral administration, and the substance was eliminated with a half-life of ~4 h. Thus, the mononitrate has a half-life which is at least four times as long as the half-life of the dinitrate (5). Published gas chromatography (GC) assays (2, 6, 7), using older column

0022-3549/84/0900-1317$01.00/0
© 1984, American Pharmaceutical Association

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.