ACKNOWLEDGMENTS AND ADDRESSES

Received September 24, 1973, from the *School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64110*

Accepted for publication January 10, 1974.

Supported in part by a grant from the School of Graduate Studies, University of Missouri-Kansas City, Kansas City, Mo., 1973.

The authors express their appreciation to Jerome W. Kinnison, Warner-Jenkinson Co., St. Louis, Mo., for samples of colorants and valuable suggestions.

[x] To whom inquiries should be directed.

PHARMACEUTICAL ANALYSIS

Analysis of Sodium Levothyroxine or Sodium Liothyronine in Tablets

JOSEPH H. GRAHAM[x], DANIEL BANES*, M. E. BIESEMEYER, and ASHOK NADKARNI‡

Abstract □ A new procedure for the analysis of sodium levothyroxine tablets and sodium liothyronine tablets is proposed. The active ingredient is isolated by partition chromatography. The thyronine is degraded by bromine oxidation, directly converting its iodine content to iodate, which is subsequently reduced by excess iodide and determined spectrophotometrically as the triiodide ion. The method is extremely sensitive and may be applied conveniently to dosage levels as low as 5 μg of sodium liothyronine. A suitable procedure for individual tablet analysis is also presented.

Keyphrases □ Sodium levothyroxine or sodium liothyronine tablets—analysis, partition chromatography and triiodide spectrophotometry □ Levothyroxine or liothyronine tablets—analysis, partition chromatography and triiodide spectrophotometry □ Liothyronine or levothyroxine tablets—analysis, partition chromatography and triiodide spectrophotometry

The USP XVIII (1) monographs for sodium levothyroxine, sodium levothyroxine tablets, sodium liothyronine, and sodium liothyronine tablets incorporate assays that require an alkali fusion of the drugs and subsequent conversion of the alkali iodide thus produced to elemental iodine. The latter is determined titrimetrically with sodium thiosulfate.

This analytical scheme is satisfactory for the drug substances themselves, but difficulties arise when it is applied to the dosage form, particularly those containing less than 100 μg of drug. The assays stipulate that a portion of powdered tablet composite equivalent to 3 mg of sodium levothyroxine or 1 mg of sodium liothyronine be ignited; these amounts theoretically yield 0.09 and 0.03 mEq of iodine, respectively. When these quantities of iodine are titrated with 0.01 *N* sodium thiosulfate, the true end-point is extremely difficult to judge and thus the accuracy of the analysis is doubtful. Furthermore, the great mass of the charge that must be ignited may itself lead to serious errors. The analysis of tablets containing 5 μg of sodium liothyronine would require 200 tablets and about 33 g of potassium carbonate as the fusion mixture. The mass of carbonized material often generated in such an ignition interferes with the quantitative recovery of inorganic iodides produced in the fusion. These difficulties strongly emphasize the need for a more accurate and sensitive assay procedure for tablets of both sodium levothyroxine and sodium liothyronine.

In an investigation of possible new methods, particularly for the assay of sodium levothyroxine, an attempt was made to utilize the reaction between ceric ion and arsenious acid under the powerful catalysis of submicrogram quantities of thyroxine. However, this reaction is subject to so many variables that it was abandoned as an analytical approach. The isolation of thyroxine from tablets by forming an extractable ion-pair with bis(2-ethylhexyl) hydrogen phosphate gave a final sample preparation that could be analyzed by UV spectrophotometry, but the procedure lacked the necessary sensitivity. A colorimetric method utilizing the chromophore produced by oxidizing the product of the reaction between thyroxine and 3-methyl-2-benzothiazolinone hydrazone hydrochloride was successful in determining as little as 80 μg of thyroxine. This procedure also afforded a degree of specificity in that the related compounds, diiodothyronine and diiodotyrosine, did not undergo the reaction and liothyronine yielded a color about one-fifth as intense as that obtained from thyroxine under the same conditions. Unfortunately, erratic results frequently occurred, probably because of variations in tablet excipients, traces of

organic solvents, and the pH of the reaction medium. Although this approach lacked the ruggedness desired for a standard assay procedure for sodium levothyroxine tablets, it may prove to be useful as an auxiliary test in examining thyroxine specimens directly.

In contrast, the reactions that form the basis for the official assay are rugged, reliable, and well understood. The reactions in the sequence leading to the production of elemental iodine from organically bound iodine are stoichiometric and can be conducted with ease. Therefore, the sensitivity ranges of these reactions were investigated when applied in conveniently performed quantitation procedures adaptable to the problem.

In an earlier report from this laboratory, Moody *et al.* (2) utilized the absorption of iodine in benzene solution at 295 nm for spectrophotometric determination of the free element. Custer and Natelson (3) had shown earlier that the triiodide ion in potassium iodide solution absorbed UV and visible light even more intensely than the iodine–benzene complex. It was determined that microgram quantities of thyroxine and liothyronine can be degraded to iodate by treatment with boiling, dilute acetic acid–sodium acetate–bromine solution. The iodate thus produced can be stoichiometrically converted to the triiodide ion in acetate buffer with excess potassium iodide.

These observations form the foundation for the method described here for the determination of either sodium levothyroxine or sodium liothyronine when each compound appears as the sole iodine-containing substance in tablets.

EXPERIMENTAL

Tablet Composite Assay

Reagents—*Standard Potassium Iodate*—Dilute 0.05 *M* potassium iodate (4) stepwise to 1 in 2500 to obtain a solution equivalent to 2.538 μg I_2/ml.

Bromine-Sodium Acetate Test Solution—Prepare as directed in USP XVIII (5).

Column Eluant—Dilute a solution of 30 ml of acetic acid and 200 ml of 2-propanol to 1 liter with chloroform.

In addition, the following reagents are also required: acetate buffer, pH 3.9 (1.0 *M* sodium acetate in 5.5 *M* acetic acid); potassium iodide, 15% (w/v) aqueous (freshly prepared); aqueous 0.1 *M* sodium hydroxide; methanolic 0.1 *M* sodium hydroxide; 5 *M* acetic acid (29.8 ml of acetic acid/100 ml); silicon carbide boiling chips; and chromatographic siliceous earth.

Sample Preparation—Thoroughly wet, with warming on a steam bath, an amount of powdered tablet composite (not exceeding 1.1 g) equivalent to 25–500 μg of sodium liothyronine or sodium levothyroxine with 4 ml of 5 *M* acetic acid in a 150-ml beaker. Mix thoroughly with 6 g of chromatographic siliceous earth and transfer quantitatively to a glass chromatographic column containing a glass wool plug. Compress the mass moderately tight. Dry rinse the beaker with 1–2 g of chromatographic siliceous earth, transfer it to the column, and hold the mass in place with glass wool. Elute the column into a 125-ml conical flask with 120 ml of column eluant, using a portion of it to rinse the sample beaker. Evaporate the eluate to dryness with an air jet on a steam bath. Rinse the walls of the flask with 5 ml of methanolic sodium hydroxide and reevaporate to dryness. Add 15 ml of aqueous sodium hydroxide to the residue and warm to effect complete solution. Add a few coarse silicon carbide boiling chips, chill the solution in an ice bath, and swirl vigorously to disperse finely any gelatinous precipitate. Quantitatively transfer the supernate through a filter into a 50-ml volumetric flask, completing the transfer with cool water. Equilibrate to room temperature and dilute to volume with water.

Oxidation—Pipet an aliquot of the sample preparation equivalent to 25–50 μg of sodium levothyroxine or sodium liothyronine to a 125-ml conical flask. Add 1 ml of bromine–sodium acetate test solution, water to a total of 40–50 ml, and silicon carbide boiling chips. Boil the solution, avoiding spattering, until its volume is reduced to about 10 ml. Cool the solution to room temperature and transfer quantitatively (through a small filter if the solution is hazy) to a 25-ml volumetric flask with 10 ml of acetate buffer, completing the transfer with water. Do not dilute to volume; leave space for the addition of 1 ml of potassium iodide solution.

Potassium Iodide–Potassium Iodate Color Development—Into separate 25-ml volumetric flasks containing 10 ml of acetate buffer, pipet a 10-ml aliquot of the standard preparation and 10 ml of water to serve as a blank. Add 1 ml of 15% potassium iodide to each flask (sample, standard, and blank), dilute to volume with water, and mix well. Record the spectrum of each solution relative to the blank in 1-cm cells from 460 to 340 nm. Correct the absorbance at about 352 nm by subtracting the absorbance at 450 nm. Calculate the quantity of anhydrous sodium levothyroxine or sodium liothyronine in micrograms per tablet by Eq. 1:

$$(A_u/A_{std}) \times C \times V_{std} \times (50/Q) \times (\text{average weight/weight}) \times GF \quad \text{(Eq. 1)}$$

where A_u is the corrected absorbance of the sample at about 352 nm, A_{std} is the corrected absorbance of the standard at about 352 nm, C is the equivalent iodine (I_2) concentration of standard potassium iodate (KIO_3) (micrograms per milliliter), V_{std} is the volume of the standard aliquot, average weight is the average weight per tablet (grams), GF is the gravimetric factor (1.574 for sodium levothyroxine and 1.768 for sodium liothyronine), Q is the aliquot volume of sample, and weight is the weight of sample (grams).

Shake-Out Procedure for Individual Tablet Assay

Reagents—In addition to the reagents described under *Tablet Composite Assay*, sodium sulfate, 10% (w/v) aqueous solution, is required.

Sample Preparation—Disperse the tablet in a 125-ml separator containing 10 ml of 5 *M* acetic acid. Extract vigorously for about 1 min with 20 ml of column eluant. Quantitatively transfer the lower phase to a second separator containing 30 ml of sodium sulfate solution. (Add a few milliliters of column eluant to the separator and drain it into the second separator.) Rinse the stem of the separator with water. Shake the second separator vigorously for about 30 sec, allow the phases to separate completely, and quantitatively transfer to a 50-ml volumetric flask[1]. Rinse the stem with column eluant. Repeat these serial extractions and transfers, using a second 20-ml portion of column eluant. Add a few drops of column eluant to the second separator and draw off into the flask. Rinse the stem, dilute the solution to volume with the column eluant, and mix well.

Oxidation—Pipet an aliquot of the sample preparation equivalent to 20–50 μg of sodium levothyroxine or sodium liothyronine into a 125-ml conical flask. Evaporate to dryness on a steam bath with the aid of a current of air. Rinse the walls of the flask with 5 ml of methanolic sodium hydroxide and evaporate to dryness. Add 10 ml of aqueous sodium hydroxide and warm to effect complete solution. Chill the solution in an ice bath. If a precipitate appears, filter quantitatively into another 125-ml flask, using water to complete the transfer. Add 2 ml of bromine–sodium acetate test solution, silicon carbide boiling chips, and sufficient water to make about 50 ml. Continue as directed under "Oxidation" in the *Tablet Composite Assay* procedure, beginning with "Boil the solution . . ." to the conclusion of the procedure.

Special Procedure for 5-μg Tablets: Potassium Iodide–Potassium Iodate Color Development

Proceed as directed in the *Tablet Composite Assay* procedure, but use a 1.0-ml aliquot of the standard preparation and record the spectrum using 5-cm cells. Calculate the quantity of sodium levothyroxine or sodium liothyronine in micrograms per tablet by Eq. 2:

[1] In the case of tablets containing 50 μg or less, transfer the extract to a 125-ml conical flask and use the entire extract for the determination.

Table I—Assays of Sodium Levothyroxine Tablets by Proposed Procedures

Sample	Manufacturer	Declared, μg/Tablet	Tablet Composite Assay, Column Procedure		Individual Tablet Assay, Shake-Out Procedure	
			Found, % of Declared[a]	Average, % of Declared	Found, % of Declared	Average, % of Declared
1	A	25	81.4, 81.1	81.3	—	—
2	A	25	83.4, 84.4, 84.3	84.0	84.7, 80.7, 83.3, 82.1, 80.5	82.3
3	A	100	91.5, 93.3	92.4	91.3, 90.9, 89.0, 89.8	90.3
4	A	100	91.8, 91.1	91.5	—	—
5	A	150	92.6, 94.4	93.5	94.5, 91.2, 98.1, 96.6	95.1
6	A	200	95.7, 97.5	96.6	—	—
7	A	500	91.3, 93.2	92.3	91.7, 95.6, 92.8, 93.0, 96.7	94.0
8	B	25	97.4, 99.6	98.5	97.1, 97.6, 94.8, 96.8	96.6
9	B	100	94.0, 91.0	92.5	97.3, 98.1, 101.7, 104.0	100.3
10	B	300	94.5, 96.7	95.6	106.3, 100.6, 101.7	102.9
11	B	500	101.2, 101.4	101.3	—	—
12	C	100	91.3, 91.2	91.3	—	—
13	C	200	79.3, 79.1	79.2	—	—
14	C	200	90.6, 89.4	90.0	—	—
15	C	200	87.6, 89.6	88.6	—	—
16	D	200	98.7, 103.1, 102.3, 103.2, 100.7	101.6	113.7, 116.8, 114.4, 111.3, 116.1	114.5

[a] Each entry represents a complete, separate assay.

Table II—Assays of Sodium Liothyronine Tablets by Proposed Procedures

Sample	Declared, μg/Tablet	Tablet Composite Assay, Column Procedure		Individual Tablet Assay, Shake-Out Procedure	
		Found, % of Declared[a]	Average, % of Declared	Found, % of Declared	Average, % of Declared
1	5	99.8, 96.2, 102.4	99.5	—	—
2	5	99.7, 99.9	99.8	101.2, 93.0, 116.0, 100.0, 96.0, 98.6, 116.4	103.0
3	25	105.3, 99.9, 98.0	101.1	—	—
4	25	91.0, 93.1	92.1	115.6, 95.2, 126.4 91.2	107.1
5	50	99.9, 97.1	98.5	93.2, 91.2, 93.2	92.5

[a] Each entry represents a complete, separate assay.

$$(A_u/A_{std}) \times C \times V_{std} \times (50/Q) \times GF \qquad \text{(Eq. 2)}$$

The factors have the same function ascribed in Eq. 1.

RESULTS AND DISCUSSION

Table I gives the assay results for sodium levothyroxine tablet composites and individual tablets, as obtained by the column extraction technique and the shake-out procedure, respectively, followed by bromine oxidation. Results of the assay of tablet composites indicated an overall precision of ±2.1%.

Results of the assays on various dosage levels of a single brand of sodium liothyronine tablets are given in Table II. The procedures were the same as those applied to the assay of sodium levothyroxine tablets except that the appropriate gravimetric factor, 1.768, was used in the calculations. The sodium liothyronine tablets had considerably more excipient material than the sodium levothyroxine samples analyzed; this material appeared as a gelatinous substance and was somewhat difficult to rinse thoroughly after the filtration step. The overall precision of the assays of composite tablets was ±1.7%.

During the development of the proposed method, several approaches to the extraction of thyroxine from tablets were studied to investigate the convenience of application and the effect on the iodine determination of tablet excipients entering the extract. Experiments with pure thyroxine showed that iodine can be quantitatively recovered after a 30-min fusion with alkali at 450–500° prior to oxidation with bromine and that fusion above 600° resulted in losses of iodine. Extracts of thyroxine from tablets that were essentially filtrates of their aqueous suspensions contained large amounts of carbonizable excipient material and were frequently difficult to handle, since they were incompletely carbonized at 450–500°. Butanol extracts of acidified aqueous suspensions of powdered tablets in separators are somewhat tedious to prepare; they require several washes and transfers and are prone to form emulsions. However, they do yield residues of thyroxine that are sufficiently free of interfering excipient material to be amenable to the low temperature fusion. The mechanical deficiencies of the shake-out technique are largely eliminated by the proposed column procedure (although filtration of a gelatinous precipitate is necessary), and the final assay preparation is free of interfering substances. The fact that boiling thyroxine or liothyronine in a dilute acetic acid–sodium acetate–bromine solution quantitatively yields iodate was the basis for replacement of the fusion technique.

The linearity of the spectrophotometric determination was established using a series of aliquots of a standard potassium iodate solution equivalent to 2.565 μg I_2/ml. A computer-calculated least-squares treatment of the eight data points gave the following: *(a)* linear range from 0.0 to at least 1.5 μg I_2/ml, *(b)* intercept −0.009, *(c)* slope 0.567, *(d)* standard error 0.003, and *(e)* correlation 0.999. The absorbance of the triiodide ion generated from an aliquot of the standard diluted to an equivalence of 1.026 μg I_2/ml was 0.573 at 352 nm. It was necessary, for the purpose of

this study, to buffer the iodate–iodide reaction in the 3.4–4.0 pH range; in this range the reduction of iodate is almost instantaneous and no oxidation of the excess iodide by atmospheric oxygen occurs.

The ratio, thyroxine/$2I_2$, was determined on a series of six aliquots of a standard thyroxine solution ranging from 50 to 200 μg with the proposed oxidation and spectrophotometric procedures. The average (1.5131) of the determinations was 98.7% of the theoretical value (1.5304), and the standard deviation of the series was 0.0074.

Sixteen sodium levothyroxine tablet composites were extracted with butanol in separators, and aliquots of the extracts were analyzed by the low temperature ignition and the bromine oxidation procedures. The data obtained established that the two procedures yield concordant results; the greatest discrepancy between the two procedures (3.7%) was of the same order of magnitude as the greatest discrepancy (4.0%) within either individual procedure. On this basis, the bromine oxidation procedure was concluded to be as reliable as the ignition procedure and it is more convenient and simpler.

The scheme presented here for individual tablet analyses of sodium levothyroxine or sodium liothyronine tablets is an outgrowth of the more elaborate butanol shake-out procedure developed earlier in this investigation. By substituting the chloroform–2-propanol–acetic acid system for butanol and eliminating several washes, the procedure was shortened considerably and in no case did an emulsion form even after prolonged vigorous extractions in separators. However, because of attempts to keep the procedure as short as possible, difficulties were encountered during development of the individual tablet assay. The averages of the individual tablet analyses compared satisfactorily with those obtained by using the column assay of composites and weighed amounts of these composites equivalent to single tablets for all dosage levels of sodium levothyroxine tablets from Manufacturer A, the one brand of sodium liothyronine assayed, and, fortuitously, the two lots of sodium levothyroxine tablets from Manufacturer B that were initially assayed. The one dosage level of sodium levothyroxine tablets from Manufacturer D gave a substantial discrepancy between the averaged individual tablet analyses and the column assay of the composite sample. When additional dosage levels and lots from Manufacturer B were studied, more discrepancies were noted. Several attempts to remedy the situation were made without success until a sodium hydroxide treatment of an aliquot of the extract was included, as in the column assay. Since all of the samples analyzed produced some quantity of a gelatinous precipitate after sodium hydroxide treatment in the column assay, the discrepancies may have arisen as the effect of another excipient which is denatured by the sodium hydroxide treatment. In only two cases was there sufficient precipitate from the aliquot taken to warrant filtration in the individual tablet analyses.

This procedure, coupled with the extraction and spectrophotometric techniques, provides a very convenient method feasible for the determination of sodium levothyroxine or sodium liothyronine in individual tablets or composites in the range of 5–500 μg. The essential advantages of this procedure over the USP XVIII procedure (1) are realized in its sensitivity, precision, accuracy, and simplicity. Preliminary studies indicated that the proposed oxidation technique may be advantageous for the assay of certain other organic iodo compounds, and more definitive studies are in progress.

REFERENCES

(1) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, pp. 642–646.

(2) J. E. Moody, J. R. Hohman, and G. B. Kaplan, *J. Pharm. Sci.*, **57**, 634(1968).

(3) J. J. Custer and S. Natelson, *Anal. Chem.*, **21**, 1005(1949).

(4) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 1036.

(5) *Ibid.*, p. 1024.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 9, 1973, from the *Division of Drug Chemistry, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC 20204*

Accepted for publication November 29, 1973.

* Present address: The United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852

‡ Present address: Drugs Control Laboratory, Bombay 51, India.

ˣ To whom inquiries should be directed.

Liquid Chromatography in Pharmaceutical Analysis: Determination of Cough–Cold Mixtures

I. L. HONIGBERG ˣ, J. T. STEWART, and A. P. SMITH

Abstract □ Operating conditions are described for the qualitative and quantitative analysis of antihistaminic, antitussive, and analgesic compounds in cough–cold mixtures by high-pressure liquid chromatography. Twenty-one drugs were investigated. Determinations can be made in less than 30 min with an accuracy of 1–5%.

Keyphrases □ Antihistaminic, antitussive, and analgesic drugs—retention times, high-pressure liquid chromatography, application to analysis of cough–cold mixtures □ Analgesic, antihistaminic, and antitussive drugs—retention times, high-pressure liquid chromatography, application to analysis of cough–cold mixtures □ Antitussive, antihistaminic, and analgesic drugs—retention times, high-pressure liquid chromatography, application to analysis of cough–cold mixtures □ Cough–cold mixtures—analysis, high-pressure liquid chromatographic retention times of drug components □ High-pressure liquid chromatography—analysis, cough–cold mixtures, retention times of various antihistaminic, antitussive, and analgesic drugs

The recent introduction of commerically available, high-pressure liquid chromatography (HPLC) systems suggested a reinvestigation of the analysis of multicomponent pharmaceutical dosage forms. The cough–cold preparations which contain several therapeutic classes are exemplary of this type. The separation and quantitative analysis of these products present difficult problems to the pharmaceutical analyst. Mario and Meehan (1) reviewed the problems involved and used an all-glass GLC system because