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Analysis of Sodium Liothyronine in Tablets[1)]

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A study on the determination of sodium liothyronine in tablets by colorimetry is described. The method is based on the iodine–starch (potato) reaction after ignition of the sample with potassium carbonate. For the ignition procedure, effective mixing of the sample and potassium carbonate, each previously pulverized, in an agate mortar was essential if reproducible results were to be obtained. The conditions of the iodine–starch reaction were optimized for application to a dosage level as low as 5 μg of sodium liothyronine in a tablet. The accuracy and recovery of the proposed method are satisfactory for practical pharmaceutical analysis.

Keywords——sodium liothyronine; pharmaceutical analysis; iodine–starch colorimetry; liothyronine sodium tablets; iodoamino acid determination; liothyronine tablet ignition-spectrophotometry

A number of attempts have been made to develop determination methods for sodium liothyronine and related iodoamino acids. These include column chromatography,[2–4)] spectrophotometry,[5–8)] gas chromatography,[9–14)] and high-performance liquid chromatography.[15–18)] However, these methods could not be satisfactorily applied to pharmaceutical preparations, particularly dosage formulations containing as little as 5 μg of sodium liothyronine per tablet. Difficulties were often encountered during pretreatment prior to assay, such as clean-up procedures or solvent extractions of the tablets. Consequently, reproducible results were not obtainable. The USP XX method is based on the titration of iodine with sodium thiosulfate after ignition of the tablets with potassium carbonate and subsequent conversion of iodide to iodine. As the amount of sodium liothyronine in tablets is too low to titrate, the analysis requires that 1 mg of sodium liothyronine, that is, 200 tablets for a tablet labelled as containing 5 μg of the drug, be ignited. When such a large amount of sample is ignited, an incompletely charred mass is often produced and this can cause serious errors. Wachholz and his co-worker[8)] reported a colorimetric procedure using the iodine–starch reaction after the extraction of sodium liothyronine with alkali. The method involves the oxidation of organically bound iodine to iodate by permanganate, the reduction of excess oxidant by sodium nitrite, the addition of starch and iodide, and the development of the color in the dark. However, in the reduction step, brownish discoloration still remained in the solution and resulted in excessively high values, because of the extraction of considerable amounts of excipients from the tablets. Furthermore, a large amount of nitrite was consumed and the volume of the final solution was therefore over the stipulated 10 ml. In this work, we utilized both the above analytical schemes, with modifications, and found suitable conditions for applying the reaction to the determination of a small amount of sodium liothyronine in tablets. The reaction schemes of this work are as follows:

$$\text{sodium liothyronine} + K_2CO_3 \xrightarrow{\text{ignition}} KI + \text{inorganic material}$$

$$KI + KMnO_4 + H^+ \xrightarrow{\text{oxidation}} IO_3^- + Mn^{2+}$$

$$\text{excess } MnO_4^- + NaNO_2 + H^+ \xrightarrow{\text{reduction}} Mn^{2+} + NO_3^-$$

$$\text{excess } NO_2^- + NH_4OSO_2NH_2 + H^+ \xrightarrow{\text{destruction}} NO_3^- + SO_4^{2-}$$

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O \xrightarrow{\text{potato starch}} \text{colorimetry}$$

Experimental

Reagents——1) Sodium liothyronine was purchased from Sigma Chemical Co., and the assay value obtained by the JP X method was 97.1%.

2) Potato starch reagent: A mixture of 1 g of potato starch (Wako Pure Chemical Industries, Tokyo, chemical grade) and 10 ml of H_2O was added to 200 ml of boiling H_2O, and the resulting mixture was boiled for 15 min. The solution was prepared just before use.

3) Soluble starch reagent: One g of soluble starch (Kokusan Chemical Co., reagent grade) was treated in the manner described for the potato starch reagent.

4) One percent sodium nitrite and 0.4% potassium iodide were prepared before use. All other reagents were of reagent grade.

Measurement——Absorbance was measured with a Shimadzu UV-200 spectrophotometer.

Preparation of Sample——A powdered pharmaceutical sample containing about 0.05 mg of sodium liothyronine was mixed with 1 g of pulverized anhydrous potassium carbonate in an agate mortar, and the mixture was transferred to a crucible. The residue adhering to the mortar was further mixed with an additional 1.5 g of pulverized anhydrous potassium carbonate, and the mixture was transferred to the same crucible. The charge was ignited at between 675 and 700°C for 30 min. The crucible was allowed to cool, then small portions of H_2O was added. The mixture in the crucible was heated at 50—60°C for 2—3 min and filtered through a glass filter into a 20-ml volumetric flask. The crucible was washed with a small portions of H_2O, and the washings were filtered into the same volumetric flask. The filtrate was diluted with water to 20 ml, and the solution was subjected to colorimetry.

Preparation of Standard——Potassium iodide, previously dried at 105°C for 4 h, was used as the standard material. The standard solution was prepared by dissolving about 75 mg of potassium iodide in 200 ml of H_2O, followed by stepwise dilution with 12.5% potassium carbonate to 0.5—4 μg of potassium iodide per ml.

Analytical Procedure——Five ml of the sample solution was placed in a test tube, and 3 ml of 6 N H_2SO_4 and 2 ml of 0.1 N potassium permanganate were added. The mixture was heated on a boiling water bath (*ca.* 95°C) for 15 min, then cooled. One ml of 1% sodium nitrite was added to the solution. The mixture was shaken until the color of potassium permanganate disappeared, then 1 ml of 10% ammonium sulfamate was added, and the whole was allowed to stand for 10 min at room temperature with occasional shaking. Then 1 ml of potato starch reagent and 1 ml of 0.4% potassium iodide were added in this order to the solution, and the mixture was transferred to a 20-ml volumetric flask. The tube was washed with water, the washings were added to the same volumetric flask, and the solution was diluted with water to 20 ml. The solution was allowed to stand for 10 min, then the absorbance was measured at about 600 nm against the blank solution.

One mg of potassium iodide is equivalent to 1.3513 mg of sodium liothyronine.

Results

Ignition Method

The ignition technique was an important factor in the analysis of sodium liothyronine. In the preliminary studies, good results were obtained when the sample was mixed with potassium carbonate in the mortar, transferred to the crucible, and ignited. However, the sample prepared merely by hand-mixing in the crucible according to the USP method did not give reproducible results. Therefore, the former method was chosen for the analysis of sodium liothyronine, and an agate mortar was used, as the mixture did not adhere to it. A sample containing 0.05 mg of sodium liothyronine was used for ignition, to which 2.5 g of anhydrous potassium carbonate was added. The amount of potassium carbonate used is twice that used in the USP method, as a ratio to the amount of sodium liothyronine.

Analytical Conditions

Various conditions were tested. Table I shows the effect of ignition time. The ignition temperature was fixed between 675 and 700°C, which is the same as that of the USP method. To examine the effect of igniting as much excipient materials as possible, the tablet containing

Table I. Effect of Ignition Time in the Present Method[a]

Found (%)[b] Ignition time (min)					
10	15	20	30	45	60
77.0	91.2	95.9	96.7	96.0	97.1

a) Ignition sample; tablet containing 5 μg of sodium liothyronine.
b) Mean value of duplicate determinations. Conditions other than those given above were held constant according to the standard procedure.

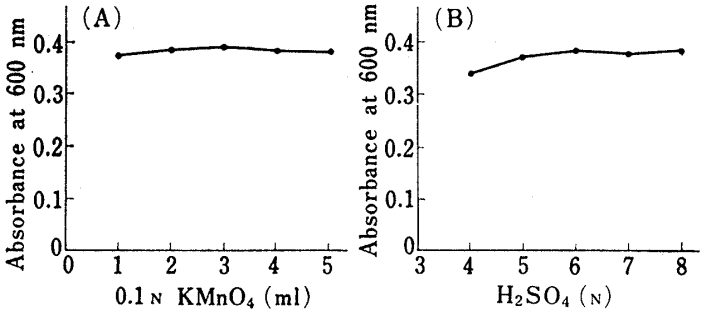

Fig. 1. Effect of Oxidation of Iodide to Iodate with Potassium Permanganate in the Presence of Sulfuric Acid on Color Development

Conditions were studied using 5 ml of potassium iodide solution (1.85 μg/ml). Conditions other than that to be tested were held constant according to the standard procedure.
(A): 1% sodium nitrite was added until the color of potassium permanganate disappeared.
(B): Amount of H_2SO_4 of each concentration; 3 ml.

5 μg of sodium liothyronine was used. The results indicate that the time required for ignition is between 20 and 60 min. The ignition time was set at 30 min. As shown in Fig. 1, the oxidation reaction of iodide to iodate with permanganate in the presence of acid was studied. A little more sodium nitrite was used than was needed for reducing excess permanganate. The optimum amount of color development was observed between 1 to 5 ml of 0.1 N potassium permanganate and at 3 ml of sulfuric acid of 5 to 8 N concentration. Accordingly, it was decided to use 2 ml of 0.1 N potassium permanganate, 3 ml of 6 N sulfuric acid, and 1 ml of

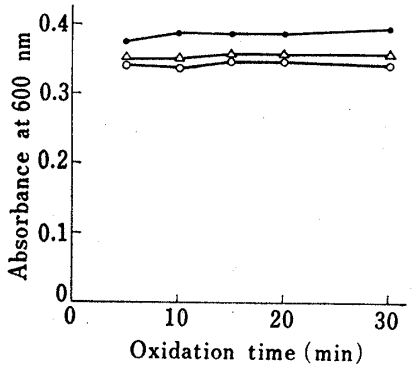

Fig. 2. Effect of Oxidation Time and Temperature with Potassium Permanganate in the Presence of Sulfuric Acid on Color Development

—●—: heating in a boiling water bath (*ca.* 95°C).
—○—: room temperature.
—△—: 40°C.
Other conditions were as given in Fig. 1.

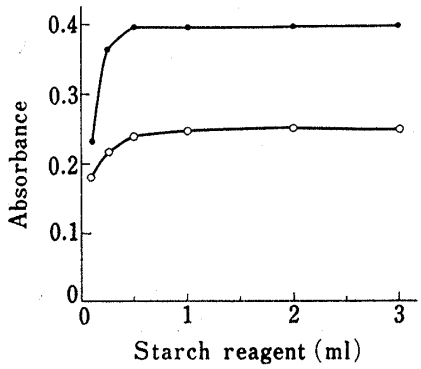

Fig. 3. Effect of Amount of Potato Starch and Soluble Starch Reagents on Color Development

—●—: potato starch reagent (at 600 nm).
—○—: soluble starch reagent (at 560 nm).
Other conditions were as given in Fig. 1.

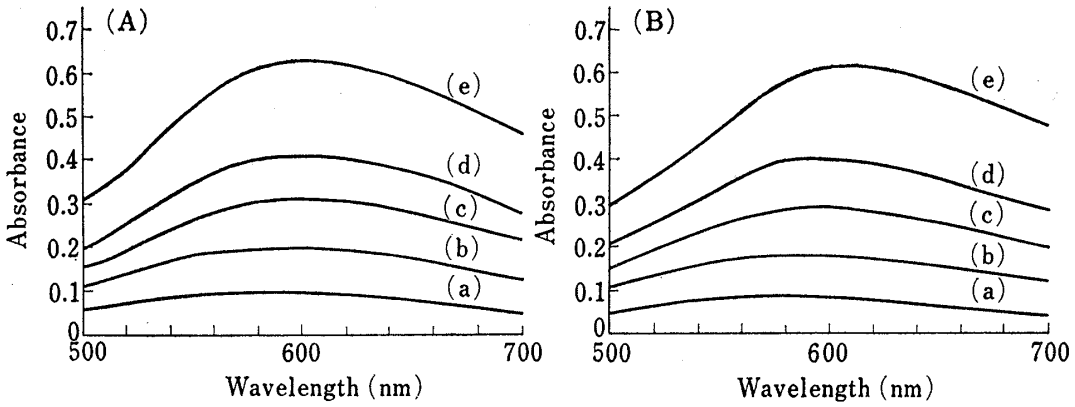

Fig. 4. Absorption Spectra of Iodine–Starch Reaction by the Present Method

(A): Curves were obtained using 1 ml of 0.4% potassium iodide, which was added in order to liberate iodine to be analyzed.
(B): Curves were obtained using 1 ml of 0.1% potassium iodide, which was added in order to liberate iodine to be analyzed.
Amounts of potassium iodide used for measurement of the absorbance: (a) 2.54, (b) 5.08, (c) 7.62, (d) 10.16, and (e) 15.24 μg/20 ml.
Conditions other than those given above were held constant according to the standard procedure.

1% sodium nitrite. Fig. 2 shows the effect of the oxidation time and temperature. The color intensity reached a plateau on heating in a boiling water bath during 5 to 30 min. In contrast the colors were of somewhat lower intensity when solutions were allowed to stand at room temperature and at 40°C, although they each reached a plateau at about the same time. Heating in a boiling water bath for 15 min was chosen as the standard condition. The effect of ammonium sulfamate for destroying the excess nitrite was investigated in connection with the effect of the standing time for the reaction. It was found that increase of the amount of ammonium sulfamate had the effect of suppressing the development of blue color in the blank. When 1 ml of ammonium sulfamate at a concentration of 2% or less was used, except during the absorbance measurement, the sample had to be kept in the dark. On the other hand, there was no significant difference between 5 and 30 min standing time with occasional shaking at room temperature after addition of the reagent. Therefore, 1 ml of 10% ammonium sulfamate and a standing time of 10 min were selected for this procedure. A comparison of the effect of potato starch and soluble starch reagents on the color development is shown in Fig. 3. The color intensity with potato starch reagent was about 1.6 times that with soluble starch reagent, on comparing the absorbance at the maximum wavelengths of about 600 nm for the former and of about 560 nm for the latter. Use of 1 ml of the potato starch reagent was suitable. The absorption spectra are shown in Fig. 4, which also shows a comparison of the effect of 0.1 and 0.4% potassium iodide added in order to liberate iodine. The results indicate that the lower the concentration of potassium iodide used, the more gradually the red shift occurred in relation to the increase of the absorbance. This shift, however, could be repressed by adding potassium iodide at a concentration of 1% or more, but the rate of formation of blue color in the blank, increased on increasing the amount of the iodide. No color developed in the blank for up to 2 h in the range of 0.1 to 0.7% potassium iodide and for up to 30 min in the range of 0.8 to 1%. Fig. 5 shows the effect of amount of potassium iodide.

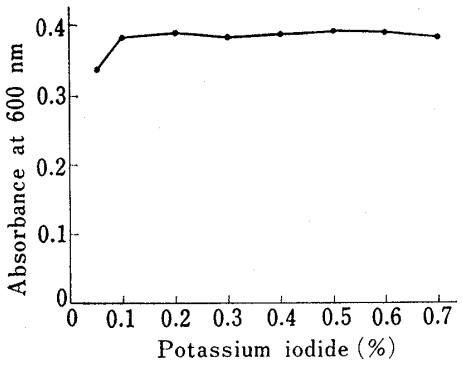

Fig. 5. Effect of Concentration of Potassium Iodide added in order to liberate Iodine for Analysis by Color Development

Amount of potassium iodide of each concentration: 1 ml. Other conditions were as given in Fig. 1.

The optimum color intensity was obtained in the range of 0.1 to 0.7% of the reagent. Although a slight red shift was still observed in this concentration range, it was possible to obtain a useful standard curve by using 1 ml of 0.4% potassium iodide, since the absorption spectrum of the iodine-starch reaction usually had such a broad peak that the slight shift had little effect on the curve. On the basis of the above results, measurement of the absorbance was carried out within 2 h at about 600 nm. The regression equation is as follows: $Y=0.0426X-0.012$ ($\gamma=0.999$; Y=absorbance; X=concentration of potassium iodide, μg/20 ml; Fig. 4A.)

The results of analyses of sodium liothyronine in tablets and in synthetic samples are summarized in Table II.

Table II. Assay Results and Recovery Tests of Sodium Liothyronine in Tablets by the Present Method

Labelled amount μg/tablet	Pharmaceutical sample No.	Found (%)						Mean value ($\bar{X}_6$, %)	C.V. (%)
25	1	98.5	96.1	99.4	96.8	97.2	99.0	97.8	1.35
	2	97.2	98.8	96.5	99.0	95.8	98.5	97.6	1.36
	3	95.9	97.9	98.0	99.5	96.8	97.3	97.5	1.47
5	1	91.5	94.0	92.7	91.0	92.1	94.6	92.7	1.52
	2	97.2	98.7	96.0	95.1	97.5	96.6	96.9	1.29
	3	96.2	99.1	98.5	99.0	96.8	95.8	97.6	1.51
	4	99.6	100.1	97.9	95.4	95.9	98.5	97.9	2.00

Labelled amount μg/tablet	Synthetic sample [a] No.	Found (%)						Mean value ($\bar{X}_6$, %)	C.V. (%)
25	1	96.5	100.9	98.0	99.5	97.2	97.5	98.3	1.67
5	1	95.8	97.9	98.0	99.0	98.6	96.0	97.6	1.38

a) Synthetic sample was prepared as follows: A solution of 0.4% (w/v) sodium liothyronine in methanol (0.25 ml for 5 μg/tablet and 1.25 ml for 25 μg/tablet) was added to the mixture of the excipient materials except sodium liothyronine, corresponding to 200 tablets of each labelled tablet, and was mixed well. The resulting mixture was spread thinly on a sheet of paper, allowed to stand overnight at room temperature, and used as the synthetic sample.

Discussion

Conversion of iodide to iodate and release of iodine with potassium iodide gives a six-fold increase of iodine atoms with respect to the original iodide. This iodine-starch reaction has been widely used for the determination of iodide or iodate,[19] though there are considerable differences in their color intensities. The use of linear starch (amylose), which only produces the deep blue color with iodine, for colorimetric iodometry has been reported and its use as a stable reagent was discussed by Lambert and his co-workers.[19b,20] The sensitivity of the present method is rather high compared with those of the Lambert method[19b,20d] and other methods.[19a,c,d] From the regression equation, the molar absorption coefficient is 137000 on the basis of the concentration of the original potassium iodide, and the threshold value (the potassium iodide content for zero absorbance) is 0.28 μg/20 ml. The threshold does not become a critical factor in measurement of the absorbance by the present method. Several workers have reported that the calibration curve of the iodine-starch complex does not pass through the origin.[19c,d,21] It is believed that the threshold is caused by hydrolysis of iodine to hypoiodous acid (HOI), and that the existence of a micro amount of hypoiodous acid is necessary for the formation of iodine-starch complex.[22] It was found that addition of potato starch reagent and 0.4% potassium iodide, in this order, was necessary for constant color development.

If this order of addition of the two reagents is reversed, the sensitivity tends to decrease and the threshold value becomes greater. Therefore, starch should be added before iodide is added in the solution. There is no need to allow the solution to stand in the dark for color development.

The ignition method is recommended for pharmaceutical analysis as an effective method to overcome the effect of a high ratio of excipient materials to sodium liothyronine. A synthetic sample was prepared by addition of methanol solution of sodium liothyronine to excipient materials, and recovery tests gave reproducible results.

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