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Analysis of Individual Thyroid Tablets

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Abstract □ A procedure was developed for the assay of individual thyroid tablets or composite samples equivalent to as little as 0.033 g (0.5 gr) of thyroid. The sample is ignited in a closed atmosphere of oxygen and, after a series of redox reactions, the iodine is determined spectrophotometrically as the triiodide ion. The results agree well with those obtained by the USP thyroid tablet assay.

Keyphrases □ Thyroid—analysis of individual tablets, spectrophotometric method measuring triiodide ion □ Iodine—analysis of individual thyroid tablets □ Spectrophotometry—analysis, individual thyroid tablets, measurement of triiodide ion

The USP thyroid tablet monograph assay (1) is not suitable for individual tablet analysis because the level of iodine in an individual tablet is usually too low to produce an acceptable titration volume. In an effort to provide an individual tablet analysis, the following method was developed. It also provides a satisfactory alternative to the compendial assay method (1).

The proposed method utilizes essentially the same chemical transformations that form the basis of the compendial monograph assay (Scheme I).

$$\text{thyroid} + Na_2CO_3 \longrightarrow I^- + \text{fusion products}$$

$$I^- + 3Br_2 + 3H_2O \xrightarrow{\text{boil}} IO_3^- + 6Br^- + 6H^+$$

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

Scheme I

In contrast to the compendial assay, which requires alkaline fusion of the equivalent of 1 g of thyroid and a final thiosulfate titration, the proposed procedure requires oxygen flask ignition of the equivalent of 30 mg of thyroid and a final spectrophotometric determination. The procedure is applicable to coated or uncoated tablets ranging from 0.033 to 0.4 g (0.5 to 6 gr) of thyroid. Potassium iodate is used as the standard, and the measurement is made on aliquots of the iodate solutions equivalent to 5–35 μg of iodine.

EXPERIMENTAL

Reagents—The standard potassium iodate solution is prepared by quantitatively diluting 0.05 *M* potassium iodate (2) stepwise to 1 in 2500 to obtain a solution equivalent to 2.538 μg I_2/ml.

The bromine–sodium acetate test solution is prepared as directed in Ref. 3.

Acetate buffer, pH 3.9, consists of 1.0 *M* sodium acetate in 5.5 *M* acetic acid.

Buffered potassium iodide is prepared just prior to use by mixing 1.5% (w/v) potassium iodide in pH 3.9 acetate buffer. The solution should remain colorless for several hours.

Assay Preparation—Oxygen ignition[1] was performed using 1-liter conical ignition flasks fitted with all-glass stopper sample holders.

Individual Tablets—Securely fasten one thyroid tablet in a suitable platinum basket in contact with a small strip of black Schöniger ignition paper. Suspend the basket from the sample

[1] Thomas-Ogg oxygen flask ignitor.

holder into the ignition flask containing 15 ml of water. (For a large coated tablet, use 15 ml of 60% acetic acid.) Flush the flask thoroughly with oxygen, remove the oxygen inlet, and quickly inject 1–2 ml of bromine–sodium acetate test solution into the flask with a medicine dropper. Clamp the stopper securely and ignite the sample.

After the flask has cooled sufficiently for handling, swirl the contents for 4–5 min, allowing the solution to wet *all* internal surfaces repeatedly. Then chill the flask in cold water. In an efficient hood, carefully release the stopper and rinse it and the walls of the flask with water. Add a few silicon carbide boiling chips, and gently boil the solution until its vapors are negative to a moistened potassium iodide–starch test paper and the volume of the solution is conveniently reduced to facilitate its transfer. Do not reduce the volume below 10 ml.

Quantitatively transfer the cooled solution with the aid of water through a funnel, containing a small, tightly packed pledget of fine glass wool, into a volumetric flask of suitable size to obtain a solution concentration in the range equivalent to 0.5–3.5 μg of I_2/ml when diluted to volume.

Sample Composites—Weigh into a No. 0 or 00 gelatin capsule an amount of powdered sample composite equivalent to 65–130 mg of thyroid. Proceed as already described, transferring the solution to a 100-ml volumetric flask, and dilute to volume with water.

Procedure—Pipet 10-ml aliquots of sample, standard, and water to serve as a blank into separate 25-ml volumetric flasks. Add 10 ml buffered potassium iodide solution to each flask, and dilute to volume with water. Mix well and let stand for at least 5 min.

With a suitable spectrophotometer, record the spectrum relative to the blank in 1-cm cells from 460 to 340 nm. Correct the maximum absorbance at about 350 nm by subtraction of the absorbance at 450 nm.

Calculate the percent of iodine per grain of thyroid per tablet by the formula:

$$\%\,I_2 = \frac{(A_u350 - A_u450) \times C \times V_u \times 0.1}{(A_s350 - A_s450) \times D \times 64.8} \qquad \text{(Eq. 1)}$$

where C = concentration of standard (micrograms of I_2 per milliliter), V_u = volume of sample preparation (milliliters), D = declared content of thyroid (grains), and 64.8 = milligrams per grain. When assaying a sample composite, the formula is multiplied by average weight/weight, where average weight = average weight per tablet (milligrams), and weight = sample weight (milligrams).

RESULTS

Various thyroid tablets, coated and uncoated, ranging from 0.033 to 0.4 g (0.5 to 6 gr) in declared thyroid content, were assayed by the developed procedure. However, to emphasize the more significant stages in the evolution of the method, the analytical results obtained at these developmental stages are included in Table I with results using the proposed procedure. The validity of the results was established by comparison with results obtained by the USP thyroid method (1).

DISCUSSION

The primary concerns in the development of this assay of individual thyroid tablets were: *(a)* the suitability and ease of sample preparation and *(b)* the sensitivity and accuracy of the determinative step. This latter concern was adequately dealt with by Graham *et al.* (4) in the development of a procedure for the determination of levothyroxine sodium or liothyronine sodium in tablets. The first concern required a considerable amount of experimentation until a set of conditions was established that gave acceptable results with the various thyroid tablets studied.

In contrast to the free iodoamino acids (4), thyroid-bound iodine cannot be conveniently oxidized directly to iodate with bromine–sodium acetate test solution. No iodate appeared to have been produced by this process in several attempts, thus eliminating this most direct approach to sample preparation.

The remaining immediate options were: *(a)* scale down the USP ignition technique (1) and *(b)* adapt the oxygen flask procedure of Moody *et al.* (5). The first was rejected in favor of the second when the number of operations required to obtain the test solution was considered to be too large.

The results obtained under the conditions used by Moody *et al.* (5) for ignition—*viz.*, 1 liter of oxygen and 30 ml of 0.5 *M* sodium hydroxide as the absorbing solution, are shown in Table I. When the averaged individual tablet analyses were compared with the compendial assay, only Samples B, C, and H gave good agreement. Other anomalies were notable when comparisons were made among the averaged individual tablet assays, composite assays, and compendial assays:

1. Composite and individual tablet assays did not agree (Samples C, D, and F).
2. Composite and compendial assays agreed but individual tablet assays did not (Sample F).
3. Composite assay did not agree with the compendial assay but the individual tablet assay did (Sample C).
4. Composite and individual tablet assays agreed but did not agree with the compendial assay (Samples A and G).

At this point, the observation that some of the large coated tablets burned with a white incandescence while others did not suggested that there may be an insufficiency of oxygen for the combustion of some samples. It was also evident that the degree of subdivision of the sample affected the combustion.

When composite samples were ignited using 2 liters of oxygen with the same absorbing solution, significant changes were noted in Samples C, D, and G (Table I). This modification appeared to be adequate until attention was focused on Sample A. The change in assay was hardly significant, yet there must have been sufficient oxygen for complete combustion of the sample, since ignition of twofold amounts of the sample did not result in decreased assay values.

It was recalled that difficulty was encountered during an attempted determination of the iodine content of iopanoic acid by a similar ignition procedure[2], and it appeared that the same or an analogous situation was occurring with this thyroid sample. When applied to iopanoic acid, the oxygen flask combustion technique produces elemental iodine. The sodium hydroxide solution appeared to have absorbed the iodine during the scrubbing stage; but on the subsequent acidification preliminary to bromine oxidation, iodine vapor was released from solution. This loss was completely circumvented by substituting an aqueous solution of the acidic bromine–sodium acetate reagent in place of the sodium hydroxide solution. Although no iodine was visible during the ignition of the thyroid sample, this scrubbing technique was applied to a composite of Sample A (2 liters of oxygen) with complete success, giving an assay of 0.195%, which agreed well with the compendial assay. The procedure was repeated using a 1-liter flask, which gave an assay of 0.196%.

Since the ignition in 2-liter flasks was cumbersome and reduced the number of samples that could be conveniently handled, all samples were reassayed in 1-liter flasks, using the bromine oxidizing reagent as the scrubbing solution (Table I).

One further modification was made in view of the behavior of coated Samples E–H. When these samples were ignited in 2-liter flasks, the white ash remaining appeared inert when it came in contact with the acidic scrubbing solution. The ash remaining when these same samples were ignited in a 1-liter flask effervesced vigorously when it came in contact with the absorbing solution and the color of the solution was greatly diminished. Since it is desirable to maintain a low pH and an excess of free bromine for the oxidation to iodate, the recommended vehicle for the scrubbing reagent is 60% acetic acid.

The discrepancy remaining in the individual tablet and composite assays of Sample G is attributed to the sampling of this product.

SUMMARY

The developed procedure provides the same information as the USP thyroid and thyroid tablet monograph assays. Neither the compendial procedure nor this proposed procedure provides the highly desirable detailed information concerning the thyroxine and liothyronine content of thyroid samples.

This procedure is recommended for adoption by the USP because of its convenience and comparable accuracy to the compen-

[2] J. H. Graham, unpublished data.

Table I—Comparison of Results of Analysis of Thyroid Tablets by Individual Tablet Assay and Composite Assay (5), Proposed Method, and USP Method (1)

Sample	Individual[a] Tablet Assay, %	Composite[a], %	Composite[b], %	Composite[c], %	Individual[c] Tablet Assay, %	USP Method, %
A						
0.5 gr	0.183, 0.177, 0.182	0.183, 0.180, 0.182	0.183, 0.179, 0.187	0.194, 0.197	0.195, 0.194, 0.190	0.193, 0.193, 0.193,
63.7 mg/tablet	0.181, 0.174, 0.183	0.182, 0.182	0.190		0.195, 0.194, 0.234	0.193, 0.192, 0.193,
					0.189, 0.190, 0.191	0.192, 0.194, 0.196
Average	0.180	0.182	0.185	0.196	0.197	0.193
B						
1 gr	0.224, 0.218, 0.229	0.226, 0.213, 0.224	0.222, 0.220	0.237, 0.232	0.246, 0.246, 0.228	0.223, 0.228
195.8 mg/tablet	0.259, 0.226, 0.228				0.252, 0.266, 0.231	
					0.240, 0.249, 0.233	
					0.236	
Average	0.231	0.221	0.221	0.235	0.243	0.226
C						
2 gr	0.208, 0.202, 0.211	0.173, 0.174, 0.154	0.213	0.212, 0.213	0.217, 0.215, 0.211	0.197, 0.212
358.3 mg/tablet	0.210, 0.210, 0.210	0.155, 0.144, 0.179			0.211, 0.200, 0.201	0.205, 0.206
					0.208, 0.198, 0.195	
					0.207	
Average	0.209	0.163	0.213	0.213	0.206	0.205
D						
2 gr	0.194, 0.193, 0.199	0.164, 0.172, 0.145	0.196	0.200, 0.201	0.202, 0.201, 0.202	0.201, 0.200
	0.196, 0.192, 0.185	0.150, 0.151			0.202, 0.200, 0.197	
					0.193	
Average	0.193	0.156	0.196	0.201	0.200	0.201
E						
5 gr, coated				0.203, 0.207	0.207, 0.166, 0.208	0.203, 0.200
748.2 mg/tablet					0.172, 0.200, 0.196	
					0.202, 0.204, 0.238	
					0.188	
Average				0.205	0.198	0.202
F						
6 gr, coated	0.160, 0.137, 0.119	0.200, 0.194		0.209, 0.203, 0.204	0.168, 0.208, 0.158	0.202, 0.200
781.9 mg/tablet	0.178, 0.194, 0.180			0.201, 0.197	0.181, 0.171, 0.208	
	0.227, 0.159, 0.174				0.207, 0.220, 0.211	
	0.189					
Average	0.172	0.197		0.203	0.192	0.201
G						
6 gr, coated	0.122, 0.125, 0.111	0.120, 0.120, 0.124	0.195, 0.194	0.202, 0.198, 0.195	0.190, 0.183, 0.173	0.200, 0.201
853.0 mg/tablet	0.119, 0.105, 0.127	0.129, 0.121, 0.126		0.194, 0.195	0.213, 0.179, 0.190	
	0.121, 0.175, 0.164				0.185, 0.203, 0.187	
	0.120				0.169, 0.175, 0.200	
					0.181, 0.181	
Average	0.129	0.123	0.195	0.197	0.186	0.201
H						
6 gr, coated	0.187, 0.173, 0.162	0.186, 0.172		0.179, 0.182	0.161, 0.193, 0.152	0.173, 0.177
696.2 mg/tablet	0.182, 0.211, 0.161	0.177			0.160, 0.170, 0.165	
	0.200, 0.167, 0.167				0.188, 0.210, 0.209	
	0.143, 0.188				0.186	
Average	0.177	0.178		0.181	0.179	0.175

[a] One liter of oxygen, sodium hydroxide scrubbing solution. [b] Two liters of oxygen, sodium hydroxide scrubbing solution. [c] Proposed method. One liter of oxygen, bromine scrubbing solution.

dial procedure [the two methods agreed within 2.0% (average of eight sample composites)] in handling small samples, as is needed for individual tablet analysis or when the quantity of sample is too small for assay by the compendial procedure.

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NMR Spectroscopic Analysis of 2-Mercapto-5-methyl-1,3,4-thiadiazole in Cefazolin

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Abstract □ A rapid, sensitive, and accurate method for the quantitative analysis of 2-mercapto-5-methyl-1,3,4-thiadiazole in cefazolin is presented. The method utilizes NMR spectroscopy and is based on the difference in the chemical shift of the methyl protons on free thiadiazole and the thiadiazole moiety of cefazolin.

Keyphrases □ Cefazolin and cefazolin sodium—NMR analysis of free 2-mercapto-5-methyl-1,3,4-thiadiazole □ 2-Mercapto-5-methyl-1,3,4-thiadiazole—NMR analysis in cefazolin and cefazolin sodium □ NMR spectroscopy—analysis, 2-mercapto-5-methyl-1,3,4-thiadiazole in cefazolin and cefazolin sodium

Free thiadiazole may be found in cefazolin (sodium salt and free acid) as a decomposition product or as unreacted starting material. The contaminant may be determined by high-pressure liquid chromatography[1], but the procedure is tedious and time consuming. The NMR method presented in this paper is specific for free thiadiazole and provides a rapid, sensitive, and accurate quantitative analysis. The method takes advantage of the difference in the chemical shift of the methyl protons on free thiadiazole and the thiadiazole moiety of cefazolin; the NMR signals appear at 2.54 and 2.84 ppm, respectively (Fig. 1).

Quantitative analysis of pharmaceuticals and chemical mixtures by NMR spectroscopy has generally been accomplished with the use of an internal standard such as hexamethylcyclotrisiloxane (1, 2), succinic acid (3), fumaric acid (4), *tert*-butanol (5), and 2,3,5-tribromothiophene (6). However, for the quantitation of free thiadiazole in cefazolin, a calibration curve was derived from spectral data on known mixtures prepared from pure thiadiazole and pure cefazolin (sodium salt or free acid). Considerable time is saved, and potential weighing errors are

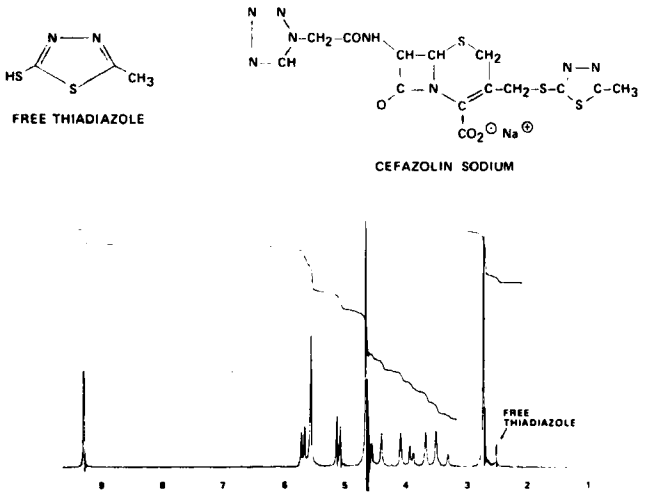

Figure 1—*NMR spectrum of cefazolin sodium containing free thiadiazole.*

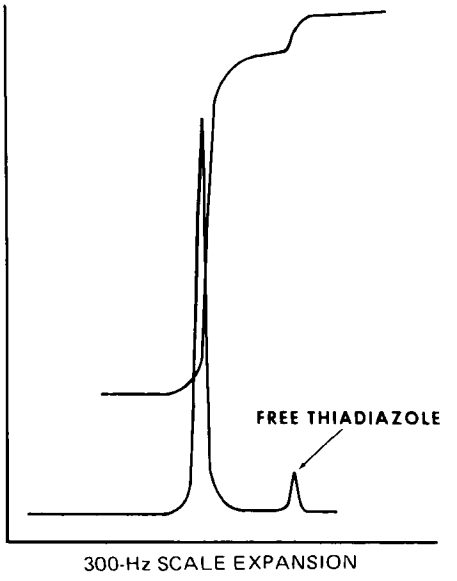

Figure 2—*Quantitative NMR spectrum of free thiadiazole in cefazolin sodium.*

[1] Unpublished data.