Estimation of Thiamine by Inverse Isotope Dilution II

By WAYNE G. HARRIS*, WAYNE V. KESSLER, JOHN E. CHRISTIAN, and ADELBERT M. KNEVEL

In a continuation of a previous study, the cleavage of thiamine hydrochloride by sodium sulfite was proved to be a quantitative reaction. An inverse isotope dilution procedure based on this cleavage was shown to be both accurate and precise in the analysis of pure thiamine hydrochloride in quantities of 10, 50, and 100 mg. It was also used in the analysis of thiamine hydrochloride in 4 pharmaceutical products.

A previous paper (1) reported a preliminary study of a thiamine assay by inverse isotope dilution. Thiamine hydrochloride was cleaved with sodium sulfite-^{35}S to form ^{35}S labeled 4-amino-2-methylpyrimidyl-5-methanesulfonic acid as the derivative. Quantitative assays of thiamine in the presence of other vitamins were performed.

This assay procedure was studied further. The cleavage reaction was proved to be quantitative by direct isotope dilution. Samples containing 10, 50, and 100 mg. of thiamine hydrochloride and 4 commercial products were assayed. The results are reported in this paper.

EXPERIMENTAL

Preparation of Carrier.—The carrier was prepared in the manner reported previously. Purity was proved by paper chromatography with 2 solvent systems, propanol–ammonia (2:1) and butanol–acetic acid–water (4:2:1), and by potentiometric titration. The latter method gave an average purity of 100.3% for 3 determinations.

Measurement of Radioactivity.—The radioactivity of all samples was measured in the manner previously reported. Samples were counted for a period of time sufficient to maintain a counting error of 1.0% or less.

Proof That Cleavage Reaction Is Quantitative.—Williams *et al.* (2) reported that the cleavage reaction is quantitative. This report was based on the weight of product recovered in a cleavage reaction. The recovery was less than 100%.

In order to prove that the reaction is quantitative, direct isotope dilution was used. Labeled derivative was prepared by cleaving 5 Gm. of thiamine hydrochloride with sodium sulfite-^{35}S in the manner used for the preparation of the carrier. The purity of the product was confirmed by paper chromatography with the 2 solvent systems used for the carrier.

The direct isotope dilution procedure was performed in duplicate. Approximately 400 mg. of thiamine hydrochloride (U.S.P. reference standard), previously dried to constant weight over phosphorus pentoxide, was weighed accurately into a weighing bottle. The thiamine was dissolved in 3 ml. of water, 3.00 ml. of a 1 *M* sodium sulfite solution was added, the pH was adjusted to 4.9–5.0 with 2.6 *N* hydrochloric acid, and the reaction mixture was set aside for 12 hr.

A solution of the labeled derivative was prepared by dissolving 100.5 mg. in sufficient ammonia T.S. to make 25.00 ml. To the reaction mixture was added 5.00 ml. of this solution, the pH was again adjusted to 4.9–5.0, and the mixture was set aside for 12 hr. The crystals were collected with suction, washed with about 25 ml. of ice cold water, transferred to a 50-ml. beaker, and dissolved in the least amount of ammonia T.S. The pH was adjusted to 4.9–5.0 with glacial acetic acid, and the mixture was allowed to stand at room temperature for 12 hr. The crystals were collected, washed, and dissolved in about 5 ml. of water by boiling. The mixture was set aside at room temperature for 12 hr. The crystals were then collected, washed, and dried in an oven at 105° for 2 hr.

Approximately 50 mg. of the product was accurately weighed and dissolved in sufficient ammonia T.S. to make 50.00 ml. A sample was prepared for counting by adding 1.00 ml. of this solution to 15 ml. of scintillator. In a similar manner, a sample of the labeled derivative was prepared for counting in order to determine its initial specific activity.

The results are shown in Table I.

Table I.—Yield of Cleavage Reaction

Thiamine HCl Cleaved, mg.	Labeled Derivative Added, mg.[a]	Final Specific Activity, c.p.m./mg.	Thiamine HCl Recovered, mg.	Recovery, %
501.4	20.1	4378	499.7	99.7
342.2	20.1	6207	342.6	100.1

[a] Initial specific activity was 69,982 c.p.m./mg.

Table II.—Analysis of Thiamine Hydrochloride

Thiamine HCl Analyzed, mg.	Initial Sp. Act. of Derivative, c.p.m./mg.	Thiamine HCl Recovered, mg.
100.0	79,926[a]	100.3 ± 1.03[d]
50.0	81,654[b]	49.74 ± 0.53[e]
10.0	118,534[c]	10.07 ± 0.22[d]

[a] Average of 3 replicates. [b] Average of 4 replicates. [c] Average of 6 replicates. [d] Average of 12 replicates. [e] Average of 11 replicates.

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* Fellow of the National Defense Education Act. Present address: College of Pharmacy, South Dakota State University, Brookings.

TABLE III.—ANALYSIS OF THIAMINE HYDROCHLORIDE IN PHARMACEUTICAL PRODUCTS

Product	Thiamine HCl Content Labeled, mg.	Thiamine HCl Recovered, mg.		Internal Standard Recovered, %
		Isotope Dilution	Thiochrome Method	
A[a]	5	5.32 ± 0.04[c]		99.4[c]
B[b]	10	8.35 ± 0.16	11.4[c]	100.6
C[a]	50	50.25 ± 1.20		100.1
D[a]	100	105.8 ± 0.20	102	100.1

[a] Tablet. [b] Vitamin B complex capsule. [c] Average of 3 replicates.

Analyses of Thiamine Hydrochloride at 10, 50, and 100-mg. Levels.—These analyses were performed in the manner presented in the previous paper with the following exceptions. The thiamine hydrochloride was a manufacturing grade (Roche). The quantity and specific activity of the labeled sodium sulfite were adjusted to the amount of thiamine hydrochloride cleaved. For the 50 and 100-mg. levels, 6 mg. of carrier was used for each milligram of thiamine hydrochloride cleaved; for the 10-mg. level, the ratio was 30 to 1. In addition to recrystallization with glacial acetic acid, the carrier diluted derivative was recrystallized from water. The initial specific activity of the labeled derivative at each level was determined by performing the cleavage reaction on U.S.P. thiamine hydrochloride reference standard. The results are shown in Table II.

Analyses of Thiamine Hydrochloride in Pharmaceutical Products.—Four pharmaceutical products (Table III) with thiamine hydrochloride contents ranging from 5 to 100 mg. were selected for analysis. These analyses were performed in the manner used for pure thiamine hydrochloride with the following exceptions. For product *A*, the ratio of carrier added to thiamine hydrochloride cleaved was 60 to 1. The binders, diluents, and other insoluble materials present in the products were removed after the carrier was added by filtration with a Büchner funnel and a double thickness of Whatman No. 1 filter paper. Due to the large amounts of insoluble materials present, it was thought that constant stirring of the reaction mixture would be necessary for quantitative cleavage. It was shown that this was not necessary. For each product, 6 samples were cleaved. To 3 of these samples was added thiamine hydrochloride (Roche, for ampul use) as an internal standard in an amount equal to the labeled thiamine hydrochloride content of the product.

The average tablet weight and the average net capsule weight were determined for each product. For products *C* and *D*, 12 tablets were powdered in a mortar and an amount of the powder equivalent to 10 tablets was dissolved in water in a volumetric flask. An aliquot of this solution was used in the analysis. For product *A*, single tablets were used directly in the analysis. For product *B*, capsule fill equivalent to the average was used.

Products *B* and *D* were assayed by the thiochrome method by the supplier.

The results are shown in Table III.

DISCUSSION

The direct isotope dilution analysis proved that the cleavage reaction is quantitative. Thiamine hydrochloride, U.S.P. reference standard, was used to assure high purity.

The accuracy and precision of the inverse isotope dilution analysis were good for both pure thiamine hydrochloride and pharmaceutical products. For the pure thiamine hydrochloride the accuracy and precision were within 1 and 2.2%, respectively, for all 3 levels. For the pharmaceutical products, the precision and accuracy indicated by recovery of internal standard were comparable.

The reason for the poor agreement between the 2 methods of analysis for product *B* is not apparent. The recovery of internal standard was complete, and the analysis was run twice with consistent results.

SUMMARY

The inverse isotope dilution method was shown to be applicable for the analysis of pure thiamine hydrochloride in quantities of 10 mg. or greater. It was used for the analysis of thiamine hydrochloride in pharmaceutical products and was successful in three out of four.

REFERENCES

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