Journal of Chromatography, 193 (1980) 470–475
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CHROM 12,696

Note

Modified procedure for determining vitamin B_1 by gas chromatography

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(Received January 10th, 1980)

Thiamine, a water soluable vitamin, has been analyzed by a variety of methods–chemical, physical, animal assay and microbiological[1,2]. The more recent physical methods include gas chromatography (GC)[3], and liquid chromatography[4]. But the recommended standard chemical assay procedure is still based on the thiochrome reactions[5], first reported in 1936.

Hilker and Mee[6] chromatographed thiamine and various derivatives. Dwivedi and Arnold[7] cleaved thiamine into 4-methyl-5-(2-hydroxyethyl) thiazole, and a relatively volatile compound (b.p. 145°/2 mm), which they chromatographed. The cleavage reaction is quantitative, but the chromatographing procedure used by Dwivedi and Arnold suffers from having to be carried out at low temperatures to prevent decomposition of the thiazole derivatives. They also experienced excessive tailing caused by the hydroxy group in this compound.

This paper reports on modifications of the procedure of Dwivedi and Arnold. These modifications, made possible by the recent advances in GC instrumentation, were carried out in an effort to make GC determination of thiamine more competitive with the standard spectrophotometric procedure. The usefulness of this modified procedure has been demonstrated by using it to quantitate the thiamine content of various formulations of commercial vitamins and standard thiamine solutions. The procedure has been carried out with both internal and external standards, using a flame-ionization detector (FID) and a nitrogen–phosphorus detector (NPD) for the internal and the external standard method, respectively.

EXPERIMENTAL

Apparatus and settings

A Perkin-Elmer Sigma 1 gas chromatograph equipped with a FID and a NPD was used for all of the analyses. The column was preconditioned 6 ft. × 2 mm I.D. coiled glass packed with 5% OV-17 on Chromosorb W AW DMCS (80–100 mesh) (TexLab, Baton Rouge, LA, U.S.A.). The injector and detector of the instrument were all-glass lined.

The set-up parameters for the FID runs were: injector temperature, 175 °C; detector, 200 °C; and the initial oven temperature, 130 °C. This initial oven temperature was held for 2 min into the run, after which it was increased at a rate of 30 °C/min up to 190 °C. The flow-rate was set at 18 ml/min.

For any given analysis, the amplifier range setting remained fixed throughout the run; the console attenuation, however, was changed at various times during the run, as specified in the program, to give a neat chromatogram. At 2.5 min the instrument was zeroed and the integrator activated. In all FID determinations, cyclooctanol was used as the internal standard. For the NPD runs the injection and detector temperature was 200 °C. Each run was carried out isothermally at an oven temperature of 180 °C.

Procedure

Over-the-counter commercial vitamin preparations and laboratory prepared standard solutions were used. Enough vitamin tablets were dissolved in 50 or 100 ml of water to give a thiamine concentration of 20–300 ppm (depending on tablet concentration). A 10-ml volume of this solution was pipetted into a 125-ml erlenmeyer flask containing 1.5 g of sodium bisulfite. (Larger concentrations of up to 5 g of sodium bisulfite were used with some of the standard solutions, described below, containing higher concentrations of thiamine.) After adding enough water to the flask to bring the contents up to 25 ml, the flask was placed in a boiling water bath for 30 min. A 5-g amount of sodium chloride was then added, and the solution was allowed to cool.

Following cooling, the pH was adjusted to 10–11 (hydrion paper) with 2 *N* sodium hydroxide. The contents were transferred to a separatory funnel and extracted with three 42-ml portions of chloroform. The extract (a total volume of 126 ml) was evaporated at room temperature. The residue was taken up in diethyl ether and filtered through 2 g of anhydrous sodium sulfate into a 10-ml volumetric flask containing 1 ml of the internal standard. Additional ether was poured into the pear-shaped flask and filtered through the anhydrous sodium sulfate into the volumetric flask until the total volume reached the 10-ml mark. One-microliter samples of this solution were used for the GC analysis. When the procedure was carried out without an internal standard (NPD runs), the injections were made with a Hamilton constant-rate syringe.

Thiamine hydrochloride standard solutions

Thiamine hydrochloride (U.S. Biochemical, Cleveland, OH, U.S.A.) was dried at 100 °C for 4 h under vacuum. A 4000-ppm solution was prepared from the pure thiamine hydrochloride. Concentrations lower than 4000 ppm were prepared by dilution. Higher concentrations were prepared by using multiples of 10 ml of the 4000-ppm solution. Analyses of these solutions were carried out by the above procedure.

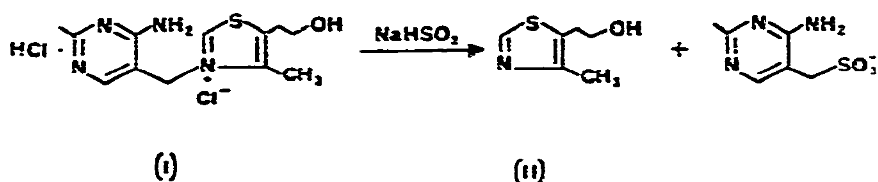
Preparation of internal and external standards and standardization of the instrument

Separate solutions of 1000 ppm of 4-methyl-5-(2-hydroxyethyl)thiazole and 800 ppm of cyclooctanol were prepared in benzene. One milliliter each of the 1000-ppm solution and 800-ppm solution was pipetted into a 10-ml volumetric flask and diluted to the mark with ether to give a calibration standard of 80–100 ppm of 4-methyl-5-(2-hydroxyethyl) thiazole to cyclooctanol. Response factors for these two compounds were calculated by a method inherent in the Sigma 1 data system. The 800-ppm cyclooctanol solution was used as the source of the internal standard

for all analyses. The 80–100 ppm calibration standard was run at the beginning and the end of each analysis. The external standard was prepared from purified 4-methyl-5-(2-hydroxyethyl)thiazole to a concentration of 5 μg/ml. Printouts from the Sigma 1 reported all analyses in ppm of 4-methyl-5-(2-hydroxethyl)thiazole. By using the proper conversion factors, the result of each analysis was converted to μg/ml or mg/tablet of thiamine.

RESULTS AND DISCUSSION

The vitamin determination is based on the method of Dwivedi and Arnold[7] with modifications in the instrumentation, chromatographing procedure and procedure for cleaving thiamine into 4-methyl-5-(2-hydroxyethyl)thiazole (II).



Thiamine (I) was cleaved[8] into 4-methyl-5-(2-hydroxyethyl)thiazole and purified (b.p. 145 °C/2 mm). The purified thiazole derivative was used as an external standard and as a reagent for standardizing the instrument for the internal standard analyses. By raising the reaction temperature to 100 °C, the cleavage time was decreased from overnight at room temperature (or 3 h at 40–50 °C) to less than 30 min without any loss in reaction yield. The reported tailing of peaks was eliminated by using silylated supports.

Dwivedi and Arnold used standard metal columns, which limited the column temperature to 100 °C or less. Their retention times were on the order of 12 min with a peak width of 2–3 min. In the present work, it was found that decomposition occurred with metal columns at 150 °C, with complete degradation of the 4-methyl-5-(2-hydroxyethyl)thiazole occurring at temperatures above 180 °C. No degradation at all was found to occur with an all-glass system up to temperatures of 225 °C. No runs were ever carried out at temperatures above 225 °C.

Although OV-17 was used as the liquid phase in the reported analyses, various other phases such as OV-1, OV-210 and OV-225 can be used.

Column bleeding, not FID sensitivity, is the limiting factor in an FID analysis.

Results of the analyses of standard solutions and multivitamin preparations are given in Tables I, II and III. Each of the observed values in these tables is reported on the basis of ten trials at the 99.9% confidence level. The data in Table I shows the analyses of standard solutions with the NPD. This table shows that thiamine can be detected at concentrations below 0.8 μg/ml and quantitated with acceptable accuracy at 2 μg/ml. The NPD is intensitive to materials that bleed off the column. It is usually necessary, however, to change response factors in the method from day to day when performing NPD analyses.

Tables II and III give the FID results. The data in Table II show that the procedure can be used to determine concentrations from 10 μg/ml to more than

TABLE I

SUMMARY OF NPD ANALYSES OF STANDARD SOLUTIONS

Observed concentration	*Calculated concentration*
0.8 ± 0.2	0.8
1.9 ± 0.1	2
5.0 ± 0.2	5
6.8 ± 0.3	7
10.1 ± 0.4	10
30.2 ± 1.0	30
40.0 ± 1.2	40

TABLE II

SUMMARY OF FID ANALYSES OF STANDARD SOLUTIONS

Observed concentration (μg/ml)	*Calculated concentration (μg/ml)*	*Observed concentration (μg/ml)*	*Calculated concentration (μg/ml)*
9.5 ± 1.5	10	800 ± 18	800
19.0 ± 1.4	20	2006 ± 24	2000
29.0 ± 1.1	30	3942 ± 64	4000
98.0 ± 2.1	100	8281 ± 107	8000
194 ± 6	200	9757 ± 133	10,000
489 ± 16	500	19,811 ± 300	20,000

20,000 μg/ml. A typical FID chromatogram is shown in Fig. 1 for a 100 ppm standard solution. Fig. 2 shows a typical NPD chromatogram for a 2 ppm standard solution.

Although the GC method is not as sensitive as the standard spectrophotometric method (0.1–12 μg/ml), the fact that the GC method requires only 1 μl of sample for analysis enables it to be used to determine overall sample concentrations that are much smaller than the lower limits of sample concentration that can be determined spectrophotometrically. In order words, even a solution whose thiamine concentration is very small can be concentrated down to the 2 μg/ml for which accurate quantitation can be obtained by NPD analysis.

The results of Table II show a relatively large percent deviation at low concentrations, but at higher concentrations the standard solutions analyze to within 2–4% of their calculated concentrations.

Application of the GC method to the analyses of various commercial vitamin preparations (Table III) gives results that agree quite well with reported values. The data in Table III are particularly good, in light of the fact that actual concentrations of vitamin tablets often are not exactly the same as the values reported on bottle labels. Park[9], using colorimetric and fluorimetric techniques, reported that the potency of various vitamin preparations ranged from 94–103% of the reported amounts.

CONCLUSION

The GC method for determining thiamine has been modified and updated for quantitation with modern equipment. The concentration range of detection with

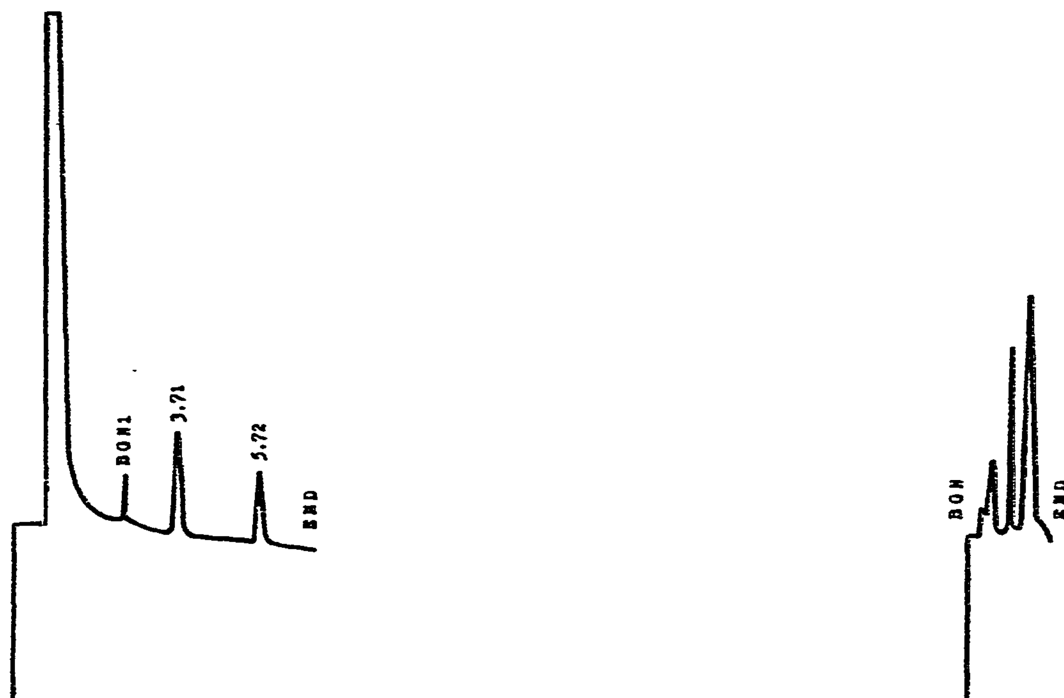


Fig. 1. FID chromatogram for a 100-ppm standard solution of thiamine with an 80-ppm internal standard of cyclobutanol. The peaks appearing at 3.71 and 5.72 min are due to cyclooctanol and the thiazole derivative, respectively.

Fig. 2. NPD chromatogram for a 2-ppm standard solution of thiamine. The first and third peaks are due to the solvent and the thiazole derivative, respectively. The middle peak arises from a change in the attenuation and integration parameters, as specified in the program.

TABLE III

SUMMARY OF FID ANALYSES OF COMMERCIAL VITAMIN PREPARATIONS

Name	*Observed mg/tablet*	*Reported mg/tablet*
Thermgram-M	10.4 ± 0.3	10
Becotin-T	15.3 ± 0.3	15
Allbees with C	14.8 ± 0.2	15
Family formula multivitamin	3.0 ± 0.1	3.0
Treat	1.8 ± 0.1	1.9
Wheat Vim	13.1 ± 0.2	12.5
Stresstab-600	16.2 ± 0.4	15

FID is 10–20,000 μg/ml. The NPD can be used to detect thiamine concentrations as low as 0.8 μg/ml. The advantages of this modified GC procedure lie in its increased speed, easier analytical calculations, and fewer manipulations. GC eliminates interferences arising from colored substances that are inherent problems associated with colorimetric methods. At the same time the sensitivity of GC, with NPD, is comparable to that of colorimetry.

ACKNOWLEDGEMENT

This research was supported by the National Institutes of Health, Grant No. RR08025-07.

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