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Comparison of AOAC and high-performance liquid chromatographic methods for thiamin determination in foods

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Thiamin was determined in various foods by AOAC (manual) and high-performance liquid chromatographic (HPLC) methods. There was no statistical difference between the values obtained by the two procedures. HPLC assay was rapid, sensitive, accurate and economical, making it attractive for routine analysis of thiamin.

INTRODUCTION

Thiamin is one of the major water-soluble vitamins. The increased interest in the thiamin content of foods makes it highly desirable that we have a rapid and accurate method for determination of this vitamin which is widely applicable. While microbiological procedures are very accurate, they have the disadvantage of requiring several days to obtain results. Furthermore, many laboratories are not equipped to conduct microbiological determinations. There exists, therefore, a need for an accurate chemical method which can be used to analyse a large number and variety of samples in a short period of time.

Chemical methods officially recognized for determining thiamin in foods (AOAC, 1980) are lengthy, tedious and costly. These features make them undesirable for the task of analysing a large number of food samples as required for monitoring the nutritional quality of foods.

The use of high-performance liquid chromatography (HPLC) in food analysis is of increasing interest. HPLC methods have advantages over some chemical methods in their specific resolution characteristics which enable them to differentiate some closely related chemicals (Ang & Moseley, 1980). Also, the use of HPLC, decreases the time required for an analysis (Lumley & Wiggins, 1981). The use of HPLC methods for vitamin analysis is not new; however, these methods have not yet been widely tested. Few studies have been reported on the application of HPLC assay in the determination of vitamins in natural food products.

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There are more studies reported on the application of HPLC to fortified foods, however there is certainly a lack of studies directly comparing HPLC with other methods. This study was therefore undertaken to compare the HPLC method with the manual AOAC method for the determination of thiamin in several foods.

MATERIALS AND METHODS

Sample preparation

The foods selected represented some of the commodities most often consumed, including whole milk, skim milk powder, Cheddar cheese, raw peas, boiled potatoes, enriched wheat flour, enriched noodles, enriched rice, cornflakes and oranges. The cereal products were ground to a fine and uniformly mixed powder. All foods were homogenized by blending for a few minutes in a mechanical blender. All samples were stored in a freezer at −18°C until the analyses were carried out.

Procedure for the manual fluorometric method based on the AOAC procedure (AOAC, 1980)

Samples (2 g) were extracted with 0·2 N HCl (50 ml) by heating for 30 min in boiling water (stirring frequently), cooled, adjusted to pH 4·0–4·5, digested by mixing with 5 ml freshly prepared enzyme solution (suspension, 6 g takadiastase was shaken and diluted to 100 ml with 2·5 M sodium acetate solution), incubated overnight at 37°C, cooled, diluted to 100 ml with water and filtered (Whatman No. 541).

For purification, thiamin extract (25 ml) was eluted using an ion-exchange column (1 cm i.d., 20 cm length) with two successive 10 ml portions of boiling potassium chloride solution. The eluate was collected, cooled and diluted to 25 ml with acid potassium chloride.

For oxidation of thiamin, two 10 ml aliquots of samples eluate were put into two 100 ml separating funnels. To one funnel, alkaline potassium ferricyanide (5 ml) was added (test), and to the other funnel, 15% sodium hydroxide (5 ml) was added (blank). To each funnel, ethanol (5 ml) was added, mixed, water-saturated isobutanol (25 ml) was added and mixed for 2 min. The lower aqueous layer was removed, ethanol (2 ml) was added to the isobutanol layer and stirred carefully, ensuring that the aqueous layer was not disturbed.

Two 10 ml aliquots of thiamin working standard solution (0·05 μg ml^{-1}) were put into two 100 ml separating funnels and the same instructions were repeated.

The fluorescence of samples and blanks were measured on a Perkin-Elmer fluorometer (excitation 365 nm, emission 435 nm). One of the fluorometer cells was filled with 0·1 N sulphuric acid (to give zero deflection) and the other with a aliquot of the isobutanol layer from the standard determination (to give 100 deflection). The measurement of the fluorescence of the isobutanol should be made within 10 min and the isobutanol solution should not be exposed to bright light, to prevent the destruction of the thiochrome.

The thiamin content (mg per 100 g) of sample was calculated according to the formula:

$$\frac{(T_s - T_b) \times S_c \times V}{(S_s - S_b) \times W \times E}$$

where T_s is the sample reading, T_b is the blank reading, S_s is the standard reading, S_b is the standard blank reading, S_c is standard concentration (μg ml^{-1}), V is the volume of extract (ml), E is the aliquot (ml) taken for oxidation and W is the sample weight (g).

Procedure for the HPLC method

Thiamin was determined by HPLC, according to Van de Weerdohof *et al.* (1973) and Osborn and Voogt (1978).

Samples (2·0 g) were digested at 121°C for 30 min with 0·25 N sulphuric acid (10 ml) in a 50 ml centrifuge tube, cooled and mixed with 1·5 ml of buffer solution (equal volumes of sodium hydroxide, 160 g in 500 ml, and glacial acetic acid, 272 g in 500 ml) to give pH 4·6. The mixture was incubated in a shaking water bath at 40–45°C for 25 min with takadiastase (1 ml of a suspension in buffer containing 100 mg diatase ml^{-1}) and then for 2 h with papain (1 ml of suspension in buffer containing 100 mg papain ml^{-1}). The tubes were then heated with 45% trichloroacetic acid (2 ml) in a water-bath at 50–60°C for 5 min and centrifuged for 5 min at 30000 *g*.

The supernatants (100 ml) were injected onto the column (steel column, 250 × 4·5 mm i.d.; packing Merckosorb SI60, particle size 10 μm 'Applied Chromatography Systems') and eluted with eluting solution, pH 6·5 [disodium hydrogen phosphate dihydrate solution (11·88 g dissolved in 'HPLC' grade water (Rathburn) and made up to 1 litre) and potassium dihydrogen phosphate solutions (9·08 g dissolved in 'HPLC' grade water (Rathburn) and made up to 1 litre; the first solution (150 ml) and second solution (350 ml) were mixed and diluted to 1 litre, then 120 ml of ethanol were added] using a flow rate of 1 ml min^{-1} and alkaline ferricyanide solution [1 ml of potassium ferricyanide solution (1%) was combined with 24 ml of NaOH (15%) and 25 ml of water] was added at a flow rate of 0·3 ml min^{-1}. The pumping system comprised an Applied Chromatography System model 300 pump. The detector was a Perkin-Elmer spectrofluorimeter fitted with a liquid chromatography flow cell. The instrument was set on sensitivity ×10. The excitation wavelength was 366 nm and the emission wavelength was 464 nm.

Standards were prepared following the same procedure, replacing the sample with 2·0 ml of thiamin working standard solution (4 μg ml^{-1}).

The peak heights of samples and standards on the chromatograms were measured and the thiamin content was calculated as follows: The thiamin (mg 100 g^{-1}) in sample (expressed as thiamin chloride)

$$= \frac{0{\cdot}8 \times h}{W \times H}$$

where h is the height of the sample peak, H is the height of the standard peak and W is the weight (g) of the sample.

All determinations were carried out in glassware protected from light by aluminium foil.

RESULTS AND DISCUSSION

The AOAC (manual method) for thiamin determination was compared with the HPLC method using 10 different foods. The data obtained for thiamin analysis are presented in Tables 1–4. There appears to be close agreement in the value obtained by the two procedures. For example, the mean thiamin values determined for Cheddar cheese were 0·025 mg 100 g^{-1} by the AOAC method and 0·023 mg 100 g^{-1} by the HPLC method. However, the absolute mean values for the AOAC results may be very slightly higher than HPLC. This could be due to the presence of fluorescent impurities which could not be separated from the thiamin using the AOAC method (Fellman *et al.*, 1982). Table 5 shows that the *t*-statistic for the difference between the two methods did not exceed the tabulated value for the 0·05

Table 1. Comparison of thiamin contents (mg 100 g^{-1}) obtained by the two methods on whole milk, skim milk powder and Cheddar cheese

Sample No.	Whole milk		Skim milk powder		Cheddar cheese	
	AOAC method	HPLC method	AOAC method	HPLC method	AOAC method	HPLC method
1	0·031	0·033	0·429	0·373	0·024	0·020
2	0·031	0·030	0·453	0·378	0·026	0·027
3	0·030	0·031	0·484	0·401	0·022	0·021
4	0·035	0·032	0·430	0·414	0·024	0·025
5	0·034	0·028	0·464	0·434	0·023	0·020
6	0·037	0·024	0·430	0·447	0·027	0·025
7	0·038	0·032	0·481	0·470	0·026	0·020
8	0·036	0·038	0·485	0·475	0·028	0·026
Mean	0·034	0·031	0·457	0·424	0·025	0·023
$SD_{\sigma_{n-1}}$	0·003	0·004	0·025	0·039	0·002	0·003

Table 2. Comparison of thiamin contents (mg 100 g^{-1}) obtained by the two methods on raw peas, boiled potatoes and oranges

Sample No.	Raw peas		Boiled potatoes		Oranges	
	AOAC method	HPLC method	AOAC method	HPLC method	AOAC method	HPLC method
1	0·314	0·279	0·058	0·046	0·092	0·075
2	0·339	0·323	0·060	0·044	0·098	0·095
3	0·296	0·280	0·058	0·043	0·087	0·080
4	0·308	0·322	0·060	0·047	0·085	0·090
5	0·328	0·297	0·058	0·044	0·103	0·099
6	0·340	0·305	0·060	0·046	0·083	0·071
7	0·304	0·276	0·057	0·043	0·104	0·096
8	0·315	0·326	0·061	0·047	0·092	0·074
Mean	0·318	0·301	0·059	0·045	0·093	0·085
$SD_{\sigma_{n-1}}$	0·016	0·021	0·014	0·017	0·008	0·011

Table 3. Comparison of thiamin contents (mg 100 g^{-1}) obtained by the two methods on enriched wheat flour and enriched noodles

Sample No.	Enriched wheat flour		Enriched noodles	
	AOAC method	HPLC method	AOAC method	HPLC method
1	0·885	0·915	0·945	0·892
2	0·985	0·925	1·075	1·008
3	0·982	0·745	0·935	1·095
4	0·858	0·895	1·085	0·805
5	0·979	0·742	0·955	1·005
6	0·891	0·998	1·065	0·895
7	0·898	0·963	0·997	1·010
8	0·962	0·777	1·023	0·890
Mean	0·930	0·870	1·010	0·950
$SD_{\sigma_{n-1}}$	0·052	0·101	0·061	0·094

Table 4. Comparison of thiamin contents (mg 100 g^{-1}) obtained by the two methods on enriched rice and cornflakes

Sample No.	Enriched rice		Cornflakes	
	AOAC method	HPLC method	AOAC method	HPLC method
1	0·362	0·372	1·105	1·055
2	0·378	0·342	1·141	1·135
3	0·360	0·389	1·101	1·084
4	0·360	0·353	1·145	1·106
5	0·380	0·358	1·102	1·063
6	0·360	0·341	1·440	1·127
7	0·380	0·365	1·101	1·070
8	0·380	0·354	1·145	1·120
Mean	0·370	0·357	1·123	1·095
$SD_{\sigma_{n-1}}$	0·010	0·018	0·022	0·031

Table 5. *t*-Test results for comparison of AOAC and HPLC thiamin methods

Sample	*t*-value (calculated)	*t*-value (tabulated)[a]
Whole milk	1·71	2·13
Skim milk powder	2·0	2·13
Cheddar cheese	1·57	2·13
Raw peas	1·82	2·13
Boiled potatoes	1·78	2·13
Oranges	1·66	2·13
Enriched wheat flour	1·49	2·13
Enriched noodles	1·67	2·13
Enriched rice	1·79	2·13
Cornflakes	2·08	2·13

[a] Tabulated at the 0·05 probability level.

level of probability. Figure 1 shows the chromatographic separation of thiamin obtained in this study from the standard, enriched wheat flour and enriched noodles. Table 6 summarizes thiamin results obtained by both the AOAC and HPLC procedures and values reported in the literature. There is a good agreement between our values and other published values with all the foods examined.

The AOAC method is the manual fluorometric procedure in which thiamin is oxidised to thiochrome by ferricyanide. Many studies related to thiochrome formation showed that to obtain reproducible results with the manual procedure, it is important to control the pH, dissolved oxygen, temperature and concentration of potassium ferricyanide (Roy, 1979). Pelletier and Madere (1972) reported that a carefully controlled extraction of thiochrome in isobutanol is necessary to avoid excessive oxidation by the ferricyanide and to ensure uniform thiochrome extraction. However, when the manual thiochrome method has been used, many researchers have reported a great deal of conflicting data

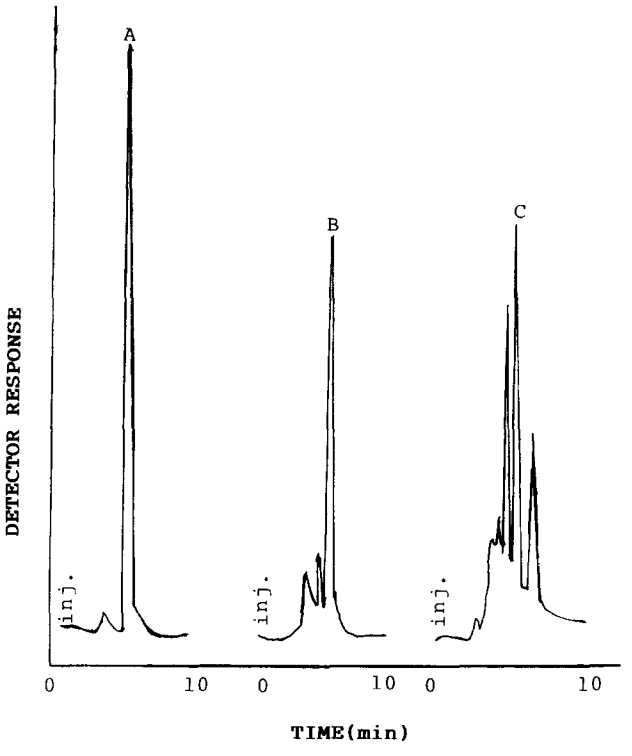

Fig. 1. Chromatograms of thiamin standard (A), enriched wheat flour (B), and enriched noodles (C).

for thiamin. Such differences could be attributed to the variations in the experimental conditions employed by analysts in different laboratories.

In the HPLC method, variables associated with the analysis can be controlled, thereby providing a thiochrome method which is highly reproducible and reliable for the routine analysis of thiamin from a wide variety of matrices. Pelletier and Madere (1975) compared the results of thiamin analysis by manual and automated procedures from a wide variety of food products.

Table 6. Comparison of AOAC and HPLC results (mean values) for thiamin in several foods with other published results

Food	Thiamin contents (mg 100 g^{-1})		
	AOAC method	HPLC method	Published data
Whole milk	0·034	0·031	0·035 (AOAC, 0·032 (HPLC)[a]
Skim milk powder	0·457	0·424	0·51 (AOAC), 0·41 (HPLC)[b]
Cheddar cheese	0·025	0·023	0·023 (AOAC), 0·028 (automated)[c]
Raw peas	0·318	0·301	0·310 (AOAC), 0·305 (HPLC)[a]
Boiled potatoes	0·059	0·045	0·041 (AOAC), 0·048 (automated)[c]
Oranges	0·093	0·085	0·080 (AOAC), 0·090 (automated)[c]
Enriched wheat flour	0·930	0·870	0·89 (HPLC)[d]
Enriched noodles	1·010	0·950	0·98 (HPLC)[d]
Enriched rice	0·370	0·357	0·362 (AOAC), 0·355 (HPLC)[e]
Cornflakes	1·123	1·095	0·080 (AOAC), 1·100 (HPLC)[d]

[a] Skurray, G. R. (1981).
[b] Fellman *et al.* (1982).
[c] Pelletier and Madere (1975).
[d] Kamman *et al.* (1980).
[e] Toma and Tabekhia (1979).

Their separation procedure was as follows: the samples were extracted according to the AOAC procedure, the extracts digested with clarase under toluene for 10 h at 40°C and interfering substances were eliminated from the extracts by using Decalso in an automated chromatographic column. Their results showed that the automated method was a suitable replacement for the manual AOAC procedure. They also reported that cleaning-up the sample with Decalso was essential to obtain valid results. Toma and Tabekhia (1979) determined thiamin in rice and rice products using the AOAC (manual method) and HPLC. Their statistical data showed no significant differences between the two methods. In 1981, Skurray reported a simplified HPLC procedure for analysis of thiamin in foods. He concluded that the HPLC procedure for thiamin assay is sufficiently accurate and precise and is applicable to almost any types of foods. He added that the combination of high-performance chromatography, ultraviolet and fluorescent detection has the advantage of speed, sensitivity and selectivity in determining thiamin in foods.

CONCLUSION

This study compared the values for thiamin obtained with either the AOAC method or the HPLC method for 10 common foods. There was no statistical difference found in the values obtained from the two procedures. Also, there was a good agreement between our values and other published values for thiamin. This work concluded that both the AOAC and HPLC assays for thiamin were sensitive, sufficiently accurate and completely reliable. Although the manual AOAC method is the most commonly used chemical procedure, it was lengthy, tedious and costly, which make it unsuitable for analysing the large numbers of food samples required for monitoring the nutritional quality of foods. The HPLC method offered many advantages: it is a specific and sensitive assay and is also faster and easier to perform compared with the manual AOAC method.

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