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Note

Gas chromatography of thiamine and derivatives*

Thiamine (T*HCl), or vitamin B_1, is a heterocyclic nitrogenous base widely distributed in plants and animals. The coenzyme, thiamine pyrophosphate (TPP), is involved in α-keto acid metabolism. Assays for T*HCl and TPP have been made by photometric, fluorometric, polarographic and microbiological methods[1]. These methods generally lack sensitivity, specificity and are time-consuming.

Analyses of whole blood or serum have not proved to be satisfactory for determining thiamine status in man and animals. Methods for blood thiamine relying on the formation of thiochrome[2–4] require acid and enzymic hydrolysis of thiamine phosphates.

Since gas chromatography (GC) offers not only speed, selectivity but also sensitivity, the development of a GC method for the analysis of thiamine and its derivatives would be of great value in nutritional status studies, analysis of thiamine in foods and also in studies of the biochemical function of thiamine. Since the polar nature and low volatility of T*HCl prohibits its direct analysis at temperatures at which it is stable, derivatization of thiamine prior to GC analysis has been tried. AMOS AND NEAL[5] used gas chromatography–mass spectrometry for the separation and determination of trimethylsilyl derivatives of thiamine metabolites. However, silylation and GC analysis of both the pyrimidine and thiazole moieties of thiamine were unsuccessful and the method was useful only in conjunction with established methods of ion-exchange and thin-layer chromatography. The GC method for the analysis of thiamine in food reported by DWIVEDI AND ARNOLD[6] required the splitting of the thiamine into methyl-4-amino-5-hydroxymethyl-pyrimidine and 4-methyl-5-hydroxyethyl-thiazole and GC analysis using a flame ionization detector. JANECKE AND VOEGE[7] achieved volatility and separation of seven B-complex vitamins with the exception of thiamine.

The development of the "double derivatization" technique[8,9] combined with a GC nitrogen detection system provided a convenient method for the amino acid analysis in plants[10] and blood serum[11]. This report concerns the development of a GLC analysis of trifluoroacetyl T*HCl and TPP derivatives with an ordinary polar column, ethylene glycol adipate (EGA).

Materials and methods

Solutions (5 m*M*) of T*HCl, oxythiamine (Nutritional Biochemicals), TPP (Cal-Biochem) and 2-hydroxy-4-methyl-pyridine (Aldrich Chem. Co.) were prepared in 0.1 *N* HCl. Thiochrome was prepared by oxidizing thiamine with alkaline ferricyamine and extracting with isobutanol. A 10-μl aliquot of the solution or 20 μl of rat plasma were introduced into a cone-shaped micro-vial (0.5-ml volume) for acylation without prior cleanup. The preparation of the acyl derivatives included:

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(1) drying the samples at 70° under a stream of dry nitrogen, (2) adding 100 μl of dichloromethane–trifluoroacetic anhydride (3:1), (3) ultrasonic mixing for 1 min, and (4) acylation at 150° for 5 min.

A Micro Tek Model MT-220 gas chromatograph and a four-column oven equipped with a Coulson electrolytic conductivity detector were used in this study. The column was a 6 × ¼ in. I.D. U-glass packed with 0.325 w/w% EGA on 80–100 mesh AW HT Chromosorb W (preheated at 140° for 12 h). Various parameters used in the GLC analysis for thiamine are outlined in Table I.

TABLE I

GAS CHROMATOGRAPHIC CONDITIONS FOR THE SEPARATION OF THIAMINE AND DERIVATIVES

Column	0.325 % (w/w) EGA on 80–100 mesh AW HT Chromosorb W
Column temp., °C	75
Helium flow-rate, ml/in.	60
Hydrogen flow-rate, ml/in.	50
Pyrolyzer temp., °C	820
Inlet temp., °C.	200
Chart speed, in./min.	0.5

TABLE II

RELATIVE RETENTION TIME FOR THIAMINE AND DERIVATIVES

Thiamine derivatives	*Retention time (min)*
Injection	0
T*HCl (thiamine HCl)	7.4
TPP (thiamine pyrophosphate)	3.6
OTCl (oxythiamine HCl)	5.3
2-Hydroxy-4-methylpyridine	5.8
Thiochrome[a]	7.4

[a] A chromogenic three-ring compound of thiamine after oxidation.

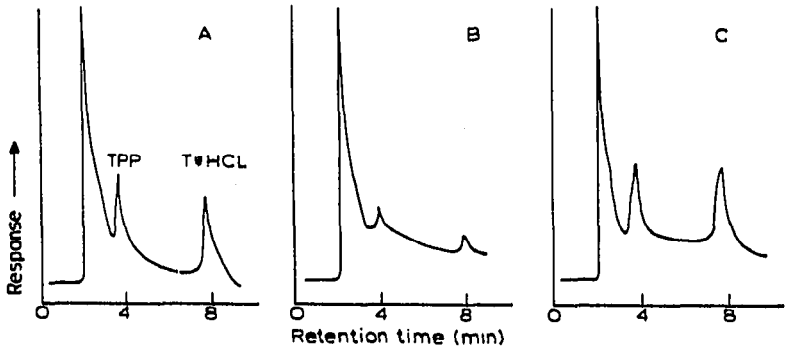

Fig. 1. Gas chromatograph curves obtained from a 6 × ¼ in. column containing 0.325 % (w/w) EGA on 80–100 mesh AW HT Chromosorb W(preheated at 140° for 12 h). Curves: (A) 0.5 μg TPP and 0.5 μg T*HCl; (B) 0.6 μg rat plasma; (C) 0.6 μg rat plasma, 0.5 μg TPP and 0.5 μg T*HCl.

Table II shows the relative retention data for thiamine and its derivatives. Thiochrome, an oxidative product of thiamine, was acylated and eluted at a retention time equivalent to the parent T*HCl. The good separation of T*HCl and TPP is of particular interest. Fig. 1 illustrates the representative chromatograph curves of T*HCl and TPP with and without rat plasma. The rat plasma showed measurable amounts of both compounds. An attempt was made to determine the optimal acylation time and temperature from 25° for 1–20 h to 150° for 5 to 30 min. The results showed that the best acylation occurred at 150° for 5 min, which gave one sharp peak for T*HCl or TPP.

In quantitative work, a stable internal standard will be required for necessary calibration. A series of volatile nitrogenous compounds including heterocyclic amines are being investigated.

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