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SHORT COMMUNICATION

The gas chromatographic determination of nicotinamide and thiamine* in vitamin preparations

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Methods available for the determination of thiamine and nicotinamide are generally slow and tedious. Gas chromatography alone offers the practical advantages of speed and convenience. It has already been shown (Senello & Argoudelis, 1969) that nicotinamide, ascorbic acid and pyridoxine can be converted to their BSA [N-O-bis (trimethylsilyl) acetamide] derivatives and quantitatively estimated in admixture using gas chromatography. The present communication outlines a method which avoids prior conversion to the silyl ether derivatives, thus eliminating an extra reaction stage and the need for careful monitoring of the conversion.

Experimental

A Varian Aerograph No. 204b gas chromatograph with a flame ionization detector was operated isothermally under the following conditions: injector 250°; oven 210° for nicotinamide and 190° for thiamine; detector 300°. The carrier gas was nitrogen flowing at 30 ml/min for nicotinamide and 9 ml/min for thiamine. The column was a 5 ft × $\frac{1}{8}$ in o.d. stainless steel coil packed with 4% silicone gum nitrile X.E.60 on acid-washed silanized chromosorb G (60/80 mesh).

Results and discussion

Calibration curves were constructed in the usual manner using nicotinamide over the weight ranges 1–8 mg/ml in methanol with 4 mg/ml phenacetin B.C.R.S. as an internal standard. For thiamine, the weight range included 1–10·6 mg/ml with acetyl-*o*-phenetidine (2·5 mg/ml) as internal standard. 1 μl injections of each solution were used throughout, and each estimation was made in triplicate. Peak areas were measured by the triangulation (peak height × $\frac{1}{2}$ height width) method. A plot of weight of vitamin against the ratio of peak areas (compound : internal standard) was linear over the range examined. Retention time for nicotinamide was 5 min at 210° and for thiamine 5 min at 190°. Resolution for nicotinamide/phenacetin was 3·4 and for thiamine/acetyl-*o*-phenetidine 3·6, as determined by the method of the British Pharmacopoeia 1968.

Pharmaceutical preparations were extracted as shown in Tables 1 and 2. The solutions (1 μl) were chromatographed in triplicate and the peak area ratios of the sample and internal standard determined. The vitamin content of the samples was

* With a note added in proof, on "the behaviour of thiamine under electron impact" by B. Blessington and D. W. Mathieson.

calculated by reference to the standard curves. Results are also shown in Tables 1 and 2.

Table 1. *Sample preparation and results for nicotinamide*

Sample and nicotinamide content	Amount of powder extracted with 25 ml methanol	Dilution	Nicotinamide found mg	B.P.C. method mg
Tablets of nicotinamide B.P.C. (50 mg)	≡ 5 tablets	5 ml of extract with internal standard* (40 mg) to 10 ml with methanol	52·1 53·2 54·1	52
Compound thiamine tablets B.P.C. (15 mg)	≡ 14 tablets	5 ml of extract with internal standard* (40 mg) to 10 ml with methanol	15·7 15·7 15·6	15·3
Strong compound thiamine tablets (20 mg)	≡ 10 tablets	5 ml of extract with internal standard* (40 mg) to 10 ml with methanol	20·1 20·0 19·8	20
Vitamins B and C injection B.P.C. 160 mg in 2 ml (ampoule 2)	—	1 ml of ampoule 2 with internal standard* (80 mg) to 20 ml with methanol	159·3 160·6 162·4	161

* Phenacetin British Chemical Reference Substance.

Table 2. *Sample preparation and results for thiamine*

Sample and thiamine content	Amount of powder extracted with 10 ml methanol	Dilution	Thiamine found mg	B.P.C. method mg
Compound thiamine tablets B.P.C. (1 mg)	≡20 tablets with internal standard* (12·5 mg)	—	0·95 0·97 0·99	0·99
Compound thiamine tablets strong B.P.C. (5 mg)	≡4 tablets with internal standard* (12·5 mg)	—	4·97 4·91 5·2	5·2
Vitamins B and C injection B.P.C. (strong for intravenous use) 250 mg in 5 ml (ampoule 1)	—	1 ml injection with internal standard* (30 mg) to 25 ml with methanol	257 253 249	252

* Acetyl-*o*-phenetidine.

To assay thiamine in the presence of nicotinamide (as in compound thiamine tablets B.P.C.) the two components must be determined separately at the requisite temperatures of 190° and 210° unless a temperature program is used. At the higher temperature, thiamine is only partially resolved from the solvent peak.

No interference with the method has been experienced from ascorbic acid, pyridoxine and riboflavine.

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REFERENCE

SENELLO, L. T. & ARGOUDELIS, C. J. (1969). *Analyt. Chem.*, **41**, 171–173.