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Note

Improved method for gas chromatographic determination of phytanic acid

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Refsum's disease (Heredopathia Atactica Polyneuritiformis) is characterised by the inability of the patient to break down or excrete ingested phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) resulting in abnormally high levels in the blood plasma[1]. Symptoms include retinitis pigmentosa, chronic polyneuropathy, and ichthyosis leading to eventual death when blood plasma levels remain elevated[1,2]. Despite the low occurrence in the population ($< 1:10^6$) extensive work has been done on dietary treatment[1,3–6].

The research was originally undertaken to determine the levels of phytanic acid in New Zealand foodstuffs. The method of Masters-Thomas *et al.*[7] was followed. In order to ensure complete separation of the fatty acids they must first be hydrogenated using Adams' platinum oxide catalyst. The laboratory preparation of the catalyst[8] and the frequent regeneration necessary are both time-consuming and expensive. This coupled with the time required for the hydrogenation of food samples results in a most time-inefficient method. This paper describes the alternative procedure developed to avoid the hydrogenation step.

Methyl phytanate cannot be separated from other naturally occurring fatty acid methyl esters on either polar or non-polar gas chromatographic (GC) phases[7] (see also Table I). On the polar stationary phase, polyethyleneglycol adipate, methyl phytanate has a similar logarithmic retention volume to methyl heptadecanoate which is present in many foodstuffs. On the non-polar stationary phase, Apiezon L, methyl phytanate elutes with the methyl esters of unsaturated C_{18} fatty acids. A comparison of the relative logarithmic retention volumes for the methyl esters (see Tables I and II) suggested that a combination of the two phases could result in effective separation of methyl phytanate from other fatty acid methyl esters.

MATERIALS AND METHODS

Preparation of GC column

A 2 m × 3 mm I.D. glass column was packed using a 1:1 ratio of 10% diethyleneglycol succinate (DEGS-PS) on 80–100 mesh Supelcoport and 3% methyl

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TABLE I

LOG RETENTION VOLUMES OF FATTY ACIDS RELATIVE TO METHYL PENTADECANOATE ON POLAR AND NON-POLAR PHASES*

Fatty acid methyl ester	*Polar stationary phase (10% PEGA)*	*Non-polar stationary phase (10% APL)*
Phytanic ($C_{16:0}$, tetramethyl)	0.269	0.439
Heptadecanoic ($C_{17:0}$)	0.272	0.355
Stearic ($C_{18:0}$)	0.415	0.527
Oleic ($C_{18:1}$)	0.460	0.465
Linoleic ($C_{18:2}$)	0.545	0.445
Linoleic ($C_{18:3}$)	0.654	0.445

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silicone gum (SE-30) on 100–120 mesh Gas-Chrom Q: the polar DEGS-PS at the injector end, and the non-polar SE-30 at the detector end.

Preparation of methyl phytanate and methyl heptadecanoate

The procedure outlined by Patton and Benson[9] was used to synthesise phytanic acid from phytol. Methylation of the acids was as outlined by Masters-Thomas *et al.*[7].

Preparation of test samples

A standard fatty acid methyl ester mixture was used (GLC-10 Supelco, Bellefonte, PA, U.S.A.). GLC-10 consists of 10 mg each of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$. To the GLC-10 mixture was added 10 mg each of methyl phytanate and methyl heptadecanoate.

GC conditions

Aliquots of 0.5 ml were injected onto a Shimadzu GC-5A gas chromatograph with flame ionization detector (190°C; nitrogen flow-rate, 25 ml/min).

TABLE II

ADDITIVE LOG RETENTION VOLUMES OF FATTY ACIDS RELATIVE TO METHYL PENTADECANOATE ON A THEORETICAL POLAR/NON-POLAR PHASE SYSTEM (CALCULATED FROM TABLE I)

Fatty acid methyl ester	*Additive log retention volume*
Phytanic ($C_{16:0}$, tetramethyl)	0.708
Heptadecanoic ($C_{17:0}$)	0.627
Stearic ($C_{18:0}$)	0.942
Oleic ($C_{18:1}$)	0.925
Linoleic ($C_{18:2}$)	0.990
Linoleic ($C_{18:3}$)	1.099

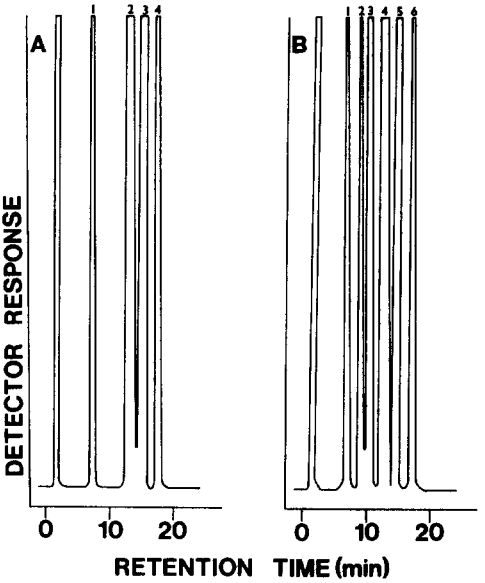

Fig. 1. Gas–liquid chromatogram of fatty acid methyl esters. (A) Peaks: 1 = $C_{16:0}$; 2 = $C_{18:0}$ and $C_{18:1}$; 3 = $C_{18:2}$; 4 = $C_{18:3}$. (B) Peaks: 1 = $C_{16:0}$; 2 = $C_{17:0}$; 3 = $C_{16:0}$ (tetramethyl); 4 = $C_{18:0}$ and $C_{18:1}$; 5 = $C_{18:2}$; 6 = $C_{18:3}$.

RESULTS AND DISCUSSION

Typical examples of the traces obtained after injection of GLC-10 (Fig. 1A) and GLC-10 plus methyl phytanate and methyl heptadecanoate (Fig. 1B) are given. (The positions of methyl phytanate and methyl heptadecanoate were confirmed by injecting them onto the column separately.) It can be seen in Table III that the column has successfully separated the phytanic acid from the remaining fatty acids and corrobated the elution order indicated by Table II.

The slightly lower column temperature of 190°C increases the total elution time by approximately 10 min (as compared to Masters-Thomas *et al.*'s[7] 200°C). However, this is arguably well compensated for by the exclusion of the hydrogenation step. A considerable saving in time (2.5 to 3.5 h per sample) and cost (of platinum catalyst)

TABLE III

ELUTION TIME FOR FATTY ACID METHYL ESTERS

Fatty acid methyl ester	*Fig. 1A*	*Fig. 1B*
Palmitic ($C_{16:0}$)	6 min 30 sec	6 min 30 sec
Heptadecanoic ($C_{17:0}$)	–	9 min 10 sec
Phytanic ($C_{16:0}$, tetramethyl)	–	10 min 35 sec
Stearic ($C_{18:0}$) / Oleic ($C_{18:1}$)	12 min 50 sec	12 min 50 sec
Linoleic ($C_{18:2}$)	14 min 50 sec	14 min 50 sec
Linoleic ($C_{18:3}$)	17 min 10 sec	17 min 10 sec

is now feasible using this two-phase column. The routine analysis of food and/or blood plasma for phytanic acid can therefore be carried out more efficiently.

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