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Letter to the Editor

Rapid gas chromatographic determination of phytanic acid from serum of a patient suffering from Refsum's disease

Sir,

In a preceding paper [1] a new rapid technique was described for measuring free phytanic acid added to serum. Now, a small amount of serum of a patient suffering from Refsum's disease, in which phytanic acid is bonded to various components, such as cholesterol, phospholipids, triglycerides, has been assayed, to control the transesterification of these lipids. Addition of trimethylphenyl ammonium hydroxide (TMPAH) seems to improve the precision of the method. The sample was assayed by both the new and the traditional methods [2]. Further research has been carried out to confirm the effectiveness of the method.

EXPERIMENTAL

The instruments and reagents were as described in ref. 1.

Extraction procedure

Aliquots of 0.5 ml of patient's serum were added to a series of 13-ml glass tubes, and 0.1 ml of 12 *M* hydrochloric acid solution was then added. The samples were left for 20 min in a water-bath at 30°C. After addition of 8 ml of diethyl ether and mechanical agitation for 30 min, the tubes were centrifuged for 2 min at 2000 *g* and the upper layer was transferred to a second series of test-tubes containing 0.05 ml of the internal standard (methylpentadecanoate) solution; 0.3 ml of 0.1 *M* TMPAH was then added to each tube. After 30 min the samples were evaporated to dryness under nitrogen. The residue was redissolved in 0.1 ml of 0.1 *M* TMPAH, and 2 μl of the resulting solution were injected into the gas chromatograph.

To determine the calibration curve, various amounts of phytanic acid (0.05, 0.4 or 0.6 ml of a 0.1 mg/ml solution) were added to blank serum, which was treated as described above. A second series of 0.5 ml of patient's serum and cali-

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TABLE I

LEVEL OF PHYTANIC ACID EXTRACTED FROM SERUM OF A PATIENT AFFECTED BY REFSUM'S DISEASE DETERMINED BY RAPID METHYLATION AND BY A TRADITIONAL METHOD

Method	*n*	Concentration found (mean ± S.D.) (μg/ml)
Rapid methylation	4	33.3 ± 1.22
Traditional	4	29.4 ± 3.1

bration standards was prepared simultaneously [2] and assayed by the same gas chromatographic procedure.

Recovery

Analytical recoveries were estimated as follows. Known amounts of the substance were added to pooled phytanic acid-free serum. Aliquots (0.5 ml) of the serum were taken through the extraction procedure without an internal standard, which was added to the ether layer (0.05 ml of the stock solution). A second series of standards was prepared by extracting 0.5 ml of blank serum and adding the substance and the marker simultaneously in the ether layer when TMPAH was added. The peak-area ratios of the extracted standards were compared with the ratios obtained from the standards to which the marker and the substance were added after extraction. The analytical recoveries so measured were corrected to absolute recoveries by the calculated ratio between the volume of diethyl ether added and the volume of the diethyl ether withdrawn and evaporated during the extraction procedure.

Reproducibility

The reproducibility was estimated from duplicate analyses of known amounts (10, 80 and 120 μg/ml) of the substance added to pooled phytanic acid-free serum. Samples were stored at −25°C until the analysis and assayed once every eight days.

RESULTS AND DISCUSSION

The reproducibility of the assay was tested by analysing serum samples containing standard phytanic acid at concentrations of 10, 80 and 120 μg/ml. The average coefficients of variation of eight determinations at each concentration were 3.36, 1.82 and 2.19%, respectively. An increase in the amount of TMPAH added to the ether layer seems to improve the methylation of the fatty acid. The phytanic acid levels measured in patient's serum by the traditional and by the new method are shown in Table I. The results are based on four analyses for each procedure. The small amount of sample available ruled out more accurate investigations, but the transesterification of triglycerides and other lipids containing ester bonds with phytanic acid seems to occur rapidly and completely. The reduc-

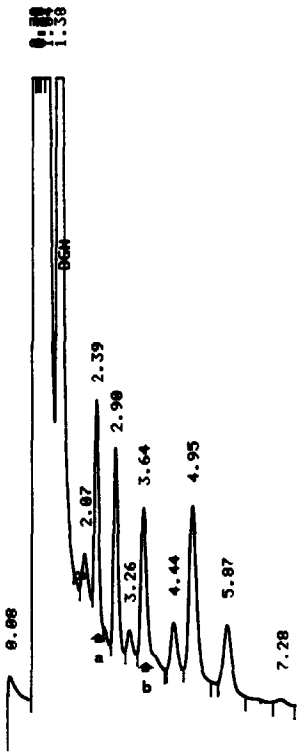

Fig. 1. Chromatogram obtained from rapid methylation of extracted serum of the patient. Peaks: a = methylpentadecanoate; b = methylated phytanic acid.

tion in the number of manipulative steps seems to improve the extraction efficiency of the new method in comparison with the traditional procedure.

A chromatogram obtained from the injection of extracted serum from the patient is shown in Fig. 1. Table II shows that the recovery from serum is high and constant in the range examined.

These results confirm the advantages of high specificity, sensitivity and rap-

TABLE II

RECOVERY OF PHYTANIC ACID FROM HUMAN SERUM

Concentration added (μg/ml)	Concentration found (mean ± S.D., $n = 4$) (μg/ml)	Recovery (mean ± S.D.) (%)
10	9.6 ± 0.46	96.0 ± 0.92
40	39.2 ± 0.66	98.0 ± 1.65
80	78.6 ± 1.01	98.2 ± 1.26
120	118.0 ± 1.71	98.3 ± 1.42

idity of the method, which appears to be suitable for the routine monitoring of phytanic acid in subjects suffering from Refsum's disease.

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1 L. Cingolani and D. Croci, J. Chromatogr., 339 (1985) 375.
2 W. Stoffel, F. Chu and A.E.H. Ahrens, Anal. Chem., 31 (1959) 307.

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