Journal of Chromatography, 221 (1980) 367—370
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 679

Note

Simultaneous measurement of sterol and fatty acid composition in small samples

E.J.A. LEA* and S.P. JONES

The Membrane Laboratory, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ (Great Britain)

(First received April 15th, 1980; revised manuscript received July 17th, 1980)

A number of methods is commonly used to prepare fatty acid methyl esters for gas chromatography (GC): the sulphuric acid, toluene—benzene, methanol method [1]; the boron trifluoride method [2, 3]; and the sodium methoxide method [4]. The subject in general is reviewed by Christie [5]. This communication is concerned with the preparation and the hazards of analysis of fatty acid methyl esters and sterols from extracts of lipids and sterols, particularly where sample sizes are too small to permit separation of sterols prior to methylation. This is of particular importance in the analysis of lipids of erythrocyte and platelet membranes (which contain up to 50% sterol) when large blood samples are unobtainable (see e.g. Dyerberg and Bang [6]).

EXPERIMENTAL

In the experiments described here, lipid samples were refluxed with methanol—toluene—sulphuric acid (20:10:1, v/v) for 90 min at 90°C using up to a maximum of 10 mg of lipid sample for a 2-ml reaction mixture. The resulting fatty acid methyl esters and sterol products were extracted with hexane before concentrating for chromatography. Separation of methyl esters and sterol products was achieved using a Pye 104 gas chromatograph and flame ionisation detector fitted with a 5 ft. × 4 mm I.D. glass column packed with Silar 10C on 100—120 mesh Chromosorb Q (Supelco, Bellefonte, PA, U.S.A.). The output data were collected and processed using a Colombia Scientific

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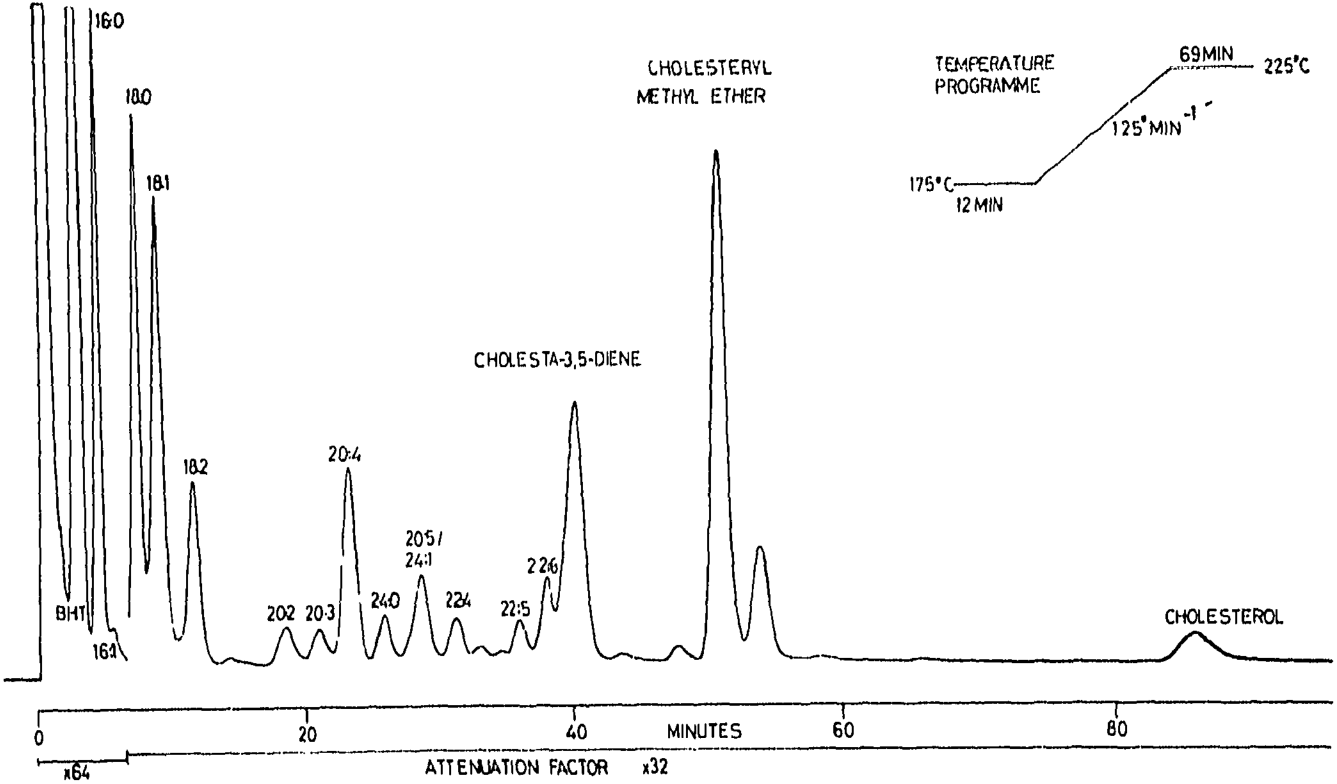

Fig. 1. Fatty acid methyl esters and sterol products of total human erythrocyte lipids from a 5-ml blood sample separated on a 5ft. × 4 mm I.D. glass column packed with Silar 10C on Chromosorb Q. The nitrogen carrier gas flow-rate was 40 ml/min. The temperature programme and attenuations used are indicated on the figure.

Instruments Supergrator 3 programmable computing integrator. The components thus separated were identified by the use of authentic standards [fatty acid methyl esters from Supelco; steroids from the Medical Research Council, Steroid Reference Collection (London, Great Britain)] and characterised by mass spectroscopy (Kratos DS-50SM mass spectrometry data system).

RESULTS AND DISCUSSION

Treatment of cholesterol alone under the above conditions results in the formation of two principal products, cholesta-3,5-diene and cholesteryl methyl ether. GC on Silar 10C shows that cholesterol and its products, including minor ones, all run after carbon No. 22:6, the first being cholesta-3,5-diene and the last, cholesterol, well separated from the others. The mass spectra for the three substances cholesta-3,5-diene, cholesterol methyl ether and cholesterol were characterized by the presence of their parent ions, *m/e* 368, 400 and 386 respectively and their expected major fragment ions, both in the case of the reaction mixture samples and in the reference samples run under the same conditions. Variation of the treatment time of sterol with methanol—toluene—sulphuric acid from 30 min to 4 h alters the relative yield of products as estimated from the gas chromatograms, but the sum of the products remains constant. This is exploited in the analysis of erythrocyte, platelet and serum samples where fatty acid composition and sterol content are determined from the same chromatogram, the latter as the sum of cholesterol and its components (Fig. 1).

Although the reports of shortcomings and artefacts of the different methods of preparation of fatty acid methyl esters number many, there are relatively few reports of these methods involving reactions with cholesterol producing cholesta-3,5-diene and cholesteryl methyl ether. Nonetheless, Morrison and Smith [3] reported the formation of these substances during treatment of sterol esters with boron trifluoride—methanol, and Kawamura and Taketomi [7] using a hydrochloric acid—methanol mixture. It seems not at all surprising therefore to find cholesta-3,5-diene and cholesteryl methyl ether produced from cholesterol using the sulphuric acid—methanol—toluene method. What may be a matter of some importance is that whilst the retention time of cholesterol on many polar GC columns is very long compared with those of the fatty acid methyl esters, and might thus be safely ignored in the analysis of fatty acids of lipid mixtures containing cholesterol, the retention time of cholesteryl methyl ether is shorter and that of cholesta-3,5-diene shorter still, raising the possibility of artefacts in measurements of fatty acid composition.

ACKNOWLEDGEMENTS

We are indebted to the Medical Research Council, Steroid Reference Collection Chemistry Department, Westfield College, London, Great Britain for the gift of steroid samples and to Mr. J. Eagles of ARC Food Research Institute, Norwich, Great Britain for the mass spectroscopy measurements.

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