Tissue/Cell Lipid G.C. Analysis

Lipid G.C. analysis for tissues/cells

Expand

Tissue/Cell Lipid G.C. Analysis

Lipids are extracted using the method of Folch-Lees (1). The extracts are filtered and lipids recovered in the chloroform phase. Individual lipid classes are separated by thin layer chromatography using Silica Gel 60 A plates developed in petroleum ether, ethyl ether, acetic acid (80:20:1) and visualized by rhodamine 6G. Phospholipids, diglycerides, triglycerides and cholesteryl esters are scraped from the plates and methylated using BF_3 /methanol as described by Morrison and Smith (2). The methylated fatty acids are extracted and analyzed by gas chromatography. Gas chromatographic analyses are carried out on an Agilent 7890A gas chromatograph equipped with flame ionization detectors and a capillary column (SP2380, 0.25 mm x 30 m, 0.20 µm film, Supelco, Bellefonte, PA). Helium is used as the carrier gas. The oven temperature is programmed from 160 °C to 230 °C at 4 °C/min. Fatty acid methyl esters are identified by comparing the retention times to those of known standards. Inclusion of lipid standards with odd chain fatty acids permits quantitation of the amount of lipid in the sample. Dipentadecanoyl phosphatidylcholine (C15:0), diheptadecanoin (C17:0), trieicosenoin (C20:1), and cholesteryl eicosenoate (C20:1) are used as standards.

References

1. J. Folch, M. Lees, and G.H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226: 497-509.
2. R. Morrison and L.M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5: 600-608.

Tissue/Cell Cholesterol G.C. Analysis

Unesterified Cholesterol

Internal standard is added to a portion of the lipid extract, concentrated under nitrogen and then solubilized in carbon disulfide to inject onto the gas chromatograph.

Total Cholesterol

Internal standard (5-a-cholestane) is added to a portion of the lipid extract and then saponified at 80 C in 1 N KOH in 90% methanol for 1 hour. The nonsaponifiable sterol is extracted into hexane, concentrated under nitrogen, and then solubilized in carbon disulfide to inject onto the gas chromatograph.

Samples are analyzed on an Agilent 7890A gas chromatograph equipped with an HP-50+ column (0.53 mm i.d x 30 m, Agilent) and a flame ionization detector. The oven temperature is 260° C and nitrogen is used as the carrier gas.

Adapted from:

Rudel, L.L., K. Kelley, J.K. Sawyer, R. Shah, and M.D. Wilson. 1998. Dietary monounsaturated fatty acids promote aortic atherosclerosis in LDL receptor-null, human apoB100-overexpressing transgenic mice. Aterioscler.Thromb. Vasc. Biol. 18: 1818-1827.