PURDUE UNIVERSITY Discovery Park

Bindley Bioscience Center

Metabolite Profiling Facility

Oxysterol Analysis

Brief Description:
Oxysterols can be detected and quantified in biological samples such as plasma, cells, or tissues. The samples may be saponified to look at total sterol content or extracted with organic solvent and the "free" sterol species evaluated. Picolinyl esters are made of each species prior to analysis to aid in ionization and selection. Quantitation of each analyte is based upon stable isotope dilution technique.* The prepared samples are separated on a Water's Xterra RP 18 phase HPLC column and detected using our Agilent 6460 triple quadrupole mass spectrometer in MRM mode. The run time is approximately 18 minutes per sample. Data are collected in positive electrospray ionization modes. Data are typically normalized to sample volume, weight, or protein content.

Normal Weight: plasma (200-1000 µL); tissue (50-500 mg); cells (~2E6)
Minimal Weight: plasma (200 µL); tissue (10 mg)

Special Handling: Samples should remain frozen at -80°C and in darkness prior to analysis if possible

References:

1. McDonald JG, Smith DD, Stiles AR, Russell DW. 2012. A comprehensive method for extraction and quantitative analysis of sterols and secosteroids from human plasma. J. Lipid Res. 53: 1399-1409.
2. Honda A, Yamashita K, Hara T, Ikegami T, Miyazaki T, Shirai M, Xu G, Numazawa M, and Matsuzaki Y. 2009. Highly sensitive quantification of key regulatory oxysterols in biological samples by LC-ESI-MS/MS. J. Lipid Res. 50: 350-357.

*Note: for absolute quantitation, it will require you purchase the stable labeled isotope for each analyte you wish to evaluate.

Table I: Analytes (3) reported. This list of analytes may be edited on request.

Compound Name	
lanosterol-d6	internal standard
lanosterol	analyte
25OH Cholesterol-d6	internal standard
25OH Cholesterol	analyte
Cholesterol-d7	internal standard
Cholesterol	analyte

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