Chromatographic techniques for separation and quantification of lipids, lipoproteins, and related metabolites

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INTRODUCTION

The Lipidomics Core Facility is a pay-per-use facility developed to assist researchers at the University of Alberta and other institutions in measuring lipid-related compounds from a variety of sources. The Core is a project initiated by the **Group on the Molecular and Cell Biology of Lipids**, the members of which have strong backgrounds and experience in lipid biochemistry. Financial and administrative supports for this facility have been provided since 2008 from the **Women and Children's Health Research Institute (WCHRI)**. Currently, the **Faculty of Medicine and Dentistry** is the major supporter of the core, with ongoing financial contributions from WCHRI. This allows the core to provide its services at rates that are affordable to most basic and clinical researchers. This is often preferable to the expense and technical complexity of individual labs purchasing and operating their own major equipment similar to what is available in this facility.

In recent years, there has been considerable emphasis placed on lipid-related research fields including obesity, diabetes, nutrition, neuroscience, and cardiovascular health by public health groups as well as research funding agencies. **The Lipidomics Core Facility provides an invaluable service to scientists in these and other fields of research for many reasons including the following:**

1. Alterations in lipid and lipoprotein levels in the blood, cells, or sub-cellular compartments of experimental subjects can be indicative of changes at the regulatory, cellular, and/or genetic levels.

2. When working with cultured cells, measurement of lipids either within these cells or secreted into the culture medium can demonstrate experimental differences between treatments.

3. Accumulation of certain lipids in the blood, tissues, or organs like the liver can often be indicative of disease; changes in lipid levels in response to dietary factors, drug administration, genetic manipulation, or any other treatment can be measured in our facility.

In this poster, we demonstrate a few examples of typical results from experimental samples, using various chromatographic techniques.

SAMPLE CHROMATOGRAMS

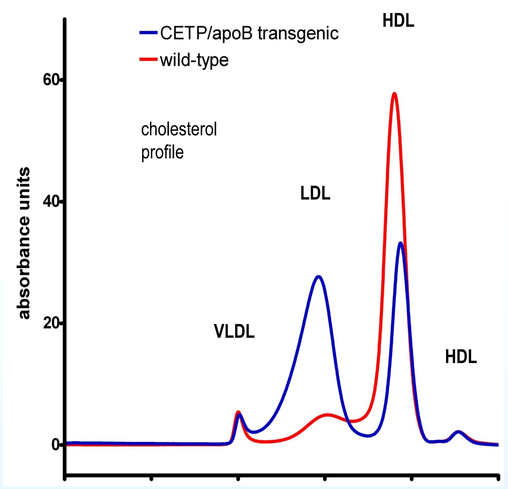

Figure 1: CETP/apoB transgenic mice show redistribution of cholesterol out of HDL particles and into LDL particles. Isolated mouse plasma (12 µL) was directly injected into an Agilent 1200 HPLC equipped with a Superose 6 gel filtration FPLC column. Cholesterol was visualized using in-line infusion of Infinity cholesterol reagent (Thermo) and detection at 500 nm.

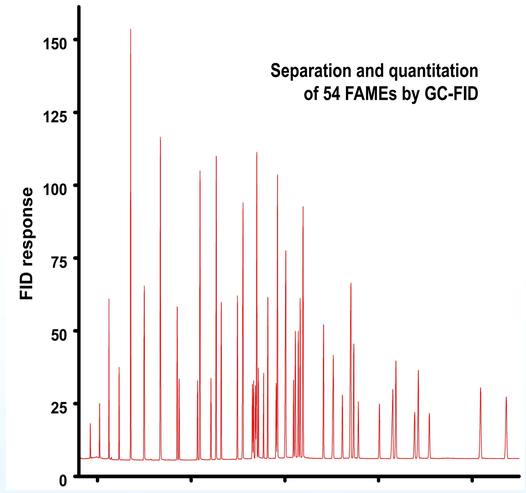

Figure 3: Separation of fatty acid methyl ester commercial standards by gas chromatography. For a typical analysis, lipids are extracted using chloroform:methanol (2:1); lipid classes may be pre-separated if desired. Lipids are hydrolysed, and fatty acids chemically modified to their methyl ester derivatives; these "FAMEs" are separated with an Agilent CPSil-88 column on an Agilent 6890 GC equipped with a flame-ionization detector.

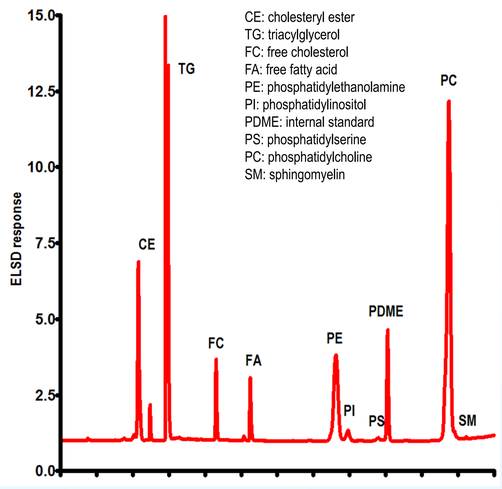

Figure 5: Separation of mouse liver lipids by HPLC. For a typical analysis, lipids are extracted using chloroform:methanol (2:1), dried under nitrogen, and resuspended in chloroform:isooctane (1:1); 5 µL of this extract is injected directly into an Agilent 1100 HPLC equipped with an Onyx monolithic silica normal phase column. Detection is performed with an Alltech evaporative light scattering detector or a Corona Ultra RS charged-aerosol detector.

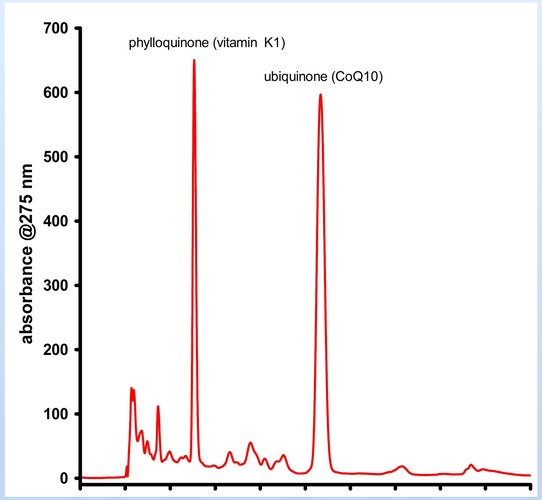

Figure 2: Separation and quantitation of coenzyme Q10 and vitamin K1 from a supercritical carbon dioxide extract. CoQ10 was enriched by extraction with supercritical carbon dioxide (F. Temelli lab, AFNS), then quantified by reversed-phase HPLC* on an Agilent Poroshell 120 column with vitamin K1 as an internal standard. Detection was by UV @275 nm.

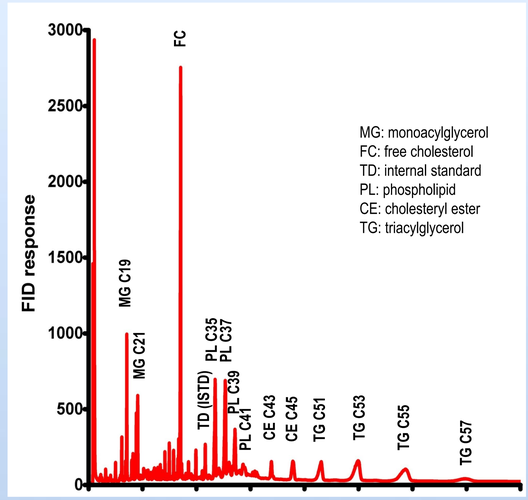

Figure 4: Separation of total lipids from mouse liver by GC. Lipids were extracted using chloroform:methanol (2:1) and phospholipids were digested with phospholipase-C to remove polar head groups. Lipids were then chemically modified to their trimethylsilyl ester derivatives and injected into an Agilent 6890 GC equipped with a Zebron ZB-5 column and flame-ionization detector. Numbers in peak labels represent the number of carbon atoms.

***FPLC=fast-protein liquid chromatography; GC=gas chromatography; HPLC=high-performance liquid chromatography**

Special thanks to Rineke Steenbergen and Norman Kneteman (Department of Surgery) for the CETP/apoB transgenic mouse FPLC data, to David Villanueva Bermejo and Feral Temelli (Department of Agricultural, Food, and Nutritional Science) for the CoQ10 data, and to Nancy Ling and Dennis Vance (Department of Biochemistry) for the total lipid by GC data.

While we are constantly evolving to add new methods as needed, current services include:

- **lipoprotein profiling** from plasma, cultured cell medium, and intracellular compartments by FPLC*
- Non-destructive **fractionation and collection of lipoproteins (VLDL, LDL, HDL)** by FPLC
- measurement of **fatty alcohols**, **fatty acids, and total lipid classes** by GC*
- measurement of **bile acids, phospholipids, total lipid classes, resveratrol, and sulforaphane** by HPLC*
- measurement of **fat-soluble vitamins** by HPLC
- Measurement of **inositol phosphates and phytate** by HPLC

We have substantial mass spectrometry expertise in the lab, and investigators are welcome to discuss potential LC-MS analysis as needed. We also offer customized chromatographic analyses based on the needs of individual users. **Rates start at $15/sample**. Please feel free to email audric.moses@ualberta.ca if there is a technique you would like to see included in our services, or if you have any other questions.