Title: High Performance GC- and GCxGC-TOFMS Metabolomics-Based Approach for the Discovery of Potential Cancer Biomarkers in Plasma

Authors: Tomas Kovalczuk1, David E. Alonso2 and Joe Binkley2

1 LECO EATC Berlin, Max-Dohrn-Str. 8–10, Germany
2 LECO Corp., LSCA Centre, St. Joseph, MI, USA

Abstract

An elevated death rate exists for individuals with hepatocellular carcinoma (HCC). There is a critical need for the discovery of early stage HCC biomarkers to trigger rapid intervention and more effective medical treatment. Metabolomics is ideal for the investigation of cancer physiology and discovery of markers for disease diagnosis. The main objective of this study was to implement an untargeted analytical methodology for annotation of candidate cancer biomarkers in humans using GC-TOFMS and GCxGC-TOFMS. In general, GC-TOFMS techniques resulted in excellent chromatographic resolution and expeditious sample characterization. GCxGC-high resolution (HR)TOFMS was implemented for the identification of unknowns and confirmation of statistically significant metabolites. Plasma samples were treated with acetonitrile/isopropanol/water, centrifuged and supernatant materials were placed under vacuum to remove solvents. Dry samples were derivatized in two-steps: 1) Methoximation and 2) silylation. Derivatized samples were injected into the chromatograph and separation performed using two columns with varying polarities. Mass spectra were collected using an ion source temperature of 250 °C, mass range of 30 to 510 and an acquisition rate of 10 spectra per second (200 sps for GCxGC). Data were processed using untargeted Peak Find and compounds were characterized through retention index filtering, similarity searches and formula determinations using accurate mass ions (HRTOFMS). GCxGC-TOFMS processing resulted in composition maps (Contour plots) displaying wide variety of metabolites including acids, diacids, amino acids, fatty acids, bases, monosaccharides, disaccharides, sugar phosphates, sterols, nucleosides and others. Differentiation of the control and disease samples was performed through statistical processing of the data (e.g., XCMS). This work resulted in the identification of several candidate markers for HCC.