**Introduction**

Accurate mass GC/MS methodologies have recently been gaining increased popularity in many applications, including metabolomics, by providing additional evidence of compound identity. While unit mass libraries and relevant screening approaches have been established for many years, accurate mass library creation and screening workflows represent significant challenges, since library curation for accurate mass spectra requires substantial effort. In addition, existing unit mass deconvolution and library search algorithms are not optimized for accurate mass data. To help improve reliability and increase throughput in metabolomics accurate mass applications, we created an accurate mass retention Time Locked (RTL) EI library of metabolites using accurate mass spectra acquired with high resolution 7200 GC/Q-TOF system. In addition, we also demonstrate metabolomics screening workflow optimized for accurate mass. The screening workflow involves feature detection (FD), accurate mass library search and statistical analysis, where feature detection uses a new profile-based algorithm that is capable to work with extremely complex matrices containing wide range of metabolite concentrations.

**Experimental**

Sample Preparation

Human breast cancer cell line MCF-7 and MDA-MB-468 cells were grown in DMEM with 10% FBS, 5% PenStrep, in 10% CO2, trypsinized, washed in PBS and flash frozen in liquid nitrogen. Prior to extraction, cells were quenched with methanol and dried using speed vacuum concentration system. Metabolites from approximately 1x10^6 cells were extracted in three replicates with acetonitrile:isopropanol:water (3:3:2). were dried under vacuum and derivatized by methoximation using a saturated solution of hydroxylamine HCl in pyridine.

Analytical Conditions

EI spectra have been acquired using an Agilent 7890B GC coupled to an Agilent 72000 accurate mass high resolution GC/Q-TOF (Figure 1). Standard metabolomics Fiehn method, retention time locked to d27-myxastic acid have been used. GC and MS conditions are described in Table 1.

**Data Processing Software Tools**

MassHunter Qualitative Analysis (B.07.00 SP1) software tools have been used to automatically convert accurate masses of fragment ions into theoretical masses for all the abundant fragments in the spectrum and to import the spectra into accurate mass metabolomics Personal Compound Database and Library (PCDL). The metabolite screening with the accurate mass metabolomics library was performed in Unknowns Analysis (UA) standalone tool of MassHunter Qualitative Analysis (B.06.00, pre-release) using new profile-based algorithm. Statistical analysis as well as pathway analysis were performed in Mass Profiler Professional (MPP), version 13.1.3.

**Results and Discussion**

The metabolite for the accurate mass metabolomics PCDL were selected to make sure that most of the GC/MS- amenable primary metabolites were included. The accurate mass metabolomics library was created by adding approximately 500 accurate mass EI spectra after correction of each fragment ion m/z to the theoretical m/z. The distribution of the PCDL entries into compound classes is shown on Figure 2.

To perform metabolomics screening we used the novel profile-based algorithm for feature detection (FD) optimized for accurate mass. First, to test the metabolomics PCDL we run the FD for one of the pathways (methionine salvage cycle) is shown on Figure 7b. The data processed in UA were imported as .CEF files into MPP for statistical as well as pathway analyses (Figures 7a and b). Pathway analysis revealed differences in a number of biochemical pathways for MCF-7 and MDA-MB-468. An example for one of the pathways (methionine salvage cycle) is shown on Figure 7b.

**Conclusions**

- A curated accurate mass metabolomics PCDL containing ~500 compound entries has been created using a GC/Q-TOF and MassHunter software tools.
- The accurate mass metabolomics PCDL was further tested using the MCF-7 and MDA-MB-468 cell extracts and metabolomics screening workflow using a novel profile based algorithm, followed by statistical analysis in MPP.