

An automated workflow for rapid alignment and identification of lipid biomarkers obtained from chip-based direct infusion nanoelectrospray tandem mass spectrometry

Jens Hoefkens¹; TOBIAS KIND²; Kent Pinkerton³; Oliver Fiehn²

¹*Genedata Inc, Waltham, MA*; ²*UC Davis - Metabolomics, Davis, CA*; ³*UC Davis Center for Health and the Environment, Davis, CA*

Keywords: Biomarkers; Ionization, Chip-based Nanospray; Lipid; Mass Spectrometry, Fourier Transform; MS/MS, Direct Mixture Analysis;

Novel Aspect: Flexible workflow environment for automated alignment and identification of lipid biomarkers from infusion and tandem mass spectra

Introduction

Polar lipids (glycerophospholipids, saccharolipids) are routinely analyzed with chromatographic and mass spectrometric techniques. Chip based direct infusion nanoelectrospray tandem mass spectrometry can provide lipid profiles or lipid fingerprints with short infusion times (two minutes). Using high resolution FT-ICR-MS or low resolution linear ion trap mass spectra in positive and negative mode together with data dependent tandem mass spectrometry polar lipids can be semi-quantified and identified. The major challenge is however not the analysis itself but automated data handling and data evaluation of the obtained results. We present efforts which include a reusable workflow environment for rapid alignment and identification of lipids from infusion tandem mass spectra. Results from an environmental tobacco smoke study and plasma and lung tissue are shown.

Methods

Lipid extracts were infused into a linear ion trap mass spectrometer coupled to an FT-ICR-MS using a chip based infusion system. Mass spectra were acquired from 400-1000 m/z with positive ionization and negative ionization and 100,000 resolving power and 0V CID voltage. Data dependent MS2 scans were performed on the 40 highest peaks in the full scan spectrum and scanned in low resolution mode with 30V CID voltage. The data alignment of the high resolution and low resolution mass spectra was performed using Genedata Expressionist. The statistical data evaluation was performed using univariate and multivariate methods. Unique lipid species were identified by matching tandem mass spectra against a database of known fragment ions.

Preliminary results

Lipid extracts from heart, plasma and lungs from rats which were exposed to environmental tobacco smoke and clean air were analyzed. Extraction and infusion solvent were optimized to obtain stable spray conditions which are important for good ion statistics. Raw files from a LTQ-FT mass spectrometer were directly imported into the workflow system. An alignment workflow for high-resolution and low-resolution mass spectra was built using Refiner MS. It included a spectrum grid alignment, spectrum averaging, intensity thresholding, peak detection, peak shaping and alignment export. The post-processing time for a sample setup of 50 infusion experiments took around 15 seconds. Additionally MS/MS spectra were extracted from data dependent scans. A matrix containing all samples and all aligned ions above a certain signal noise threshold was obtained as a result file. Data was analyzed performing unsupervised statistical tests, cluster analysis, principal component analysis (PCA) and supervised multivariate partial least square analysis (PLS). Identification of lipid species was performed by matching specific sn1 and sn2 fatty acid chain fragmentation with the experimentally obtained MS/MS product ions. Specific neutral loss fragments were used for identification. In case of overlapping saturated and un-saturated species even FT-ICR-MS can not resolve those species. Differences in tandem mass spectra obtained at 40V internal collision voltage (CID) can be used to identify isobaric species. Furthermore isotopic pattern are used to cluster certain peak groups together. MS1 peaks which show similar tandem mass spectra are additionally used to identify true lipid compounds. Although tandem mass spectra fragments can be queried easily online from the LipidMaps database, it is favorable to perform such calculations directly inside the workflow. Therefore in-silico fragmentation of certain lipid species was performed using MassFrontier and the obtained fragment database was used to identify unique lipid biomarkers. Different lipid patterns revealed alterations during exposure to tobacco smoke.