Identification of Metabolites in a Human Plasma Standard Reference Material by Comprehensive two Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry.

Gauthier Eppe, Elizabeth A. McGaw, Nathan G. Dodder, Bruce A. Benner Jr, Karen W. Phinney, Michele M. Schantz

Novel aspect: Comprehensive identification of the metabolites. This is the first metabolomics Standard Reference Material from a national metrology institute.

Introduction

The human plasma metabolome is characterized by a large number of small molecules exhibiting a high diversity of chemical structures, physico-chemical properties and abundances. Complementary analytical platforms are necessary to reach its extensive coverage. Among them, comprehensive two dimensional gas chromatography (GC x GC) has been widely used in the separation of complex samples. Connected with time-of-flight mass spectrometry detector (TOFMS) and peak deconvolution software, it offers a major tool for non-targeted analysis in metabolomics. GC x GC-TOFMS was investigated to identify and characterize unknown polar and semi-polar low molecular weight compounds in a new Standard Reference Material (SRM) Metabolites in Human Plasma (SRM1950).

Methods

A Human plasma pool was obtained from a representative mix of healthy male and female donors. Extraction and protein precipitation was performed with methanol. The methanol extract was then evaporated to dryness prior to oximation and silylation derivatization. Two silylation agents *N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide (MTBSTFA) and *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) were evaluated. A standard solution of *n*-alkanes (SRM1494) was spiked into the vial before injection. Rtx-5 (40 m x 0.18 mm x 0.18 µm) /Rxi-17(1 m x 0.1 mm x 0.1 µm) columns were connected to a Pegasus IV TOFMS. Ions were generated by a 70 V electron beam at a current of 2.0 mA. Masses were acquired in full scan from 40 to 800 m/z at a rate of 200 spectra s^{-1}.

Preliminary results

The optimized GC x GC-TOFMS method was able to resolve more than 1000 peaks and therefore potential compounds. The data was first processed with ChromaTOF software for qualitative identification. Parameters included minimum similarity value with the NIST MS library, peak width, S/N ratio and retention time. Using these rules, the software automatically generated a list of approximately 250 compounds that fulfilled the criteria. In addition, the ChromaTOF software could provide linear retention indices (RI) in the first dimension even if the data were acquired in 2D mode. SRM1494, containing 18 *n*-alkanes (C_{10} to C_{34}), was used as RI markers. The silylation agents appear to be complimentary. For example, amino acids RI shifted when using MTBSTFA (1538-3201) compared to MSTFA (1106 to 2234) on the Rtx-5 phase, providing corroborative information. Experimental TOFMS fragmentation mass spectra were compared with

reference spectra available in the NIST MS library. Similarity and probability score values were used as qualitative indicators. Once confirmed by both silylation reagents, the metabolite was classified in a list. Currently, the compounds tentatively identified encompass several classes of metabolites such as amino acids (22 confirmed, 29 potential), organic acids (18 potential), fatty acids (14 confirmed, 22 potential), sugars, pharmaceutical residues such as analgesics and miscellaneous metabolites, providing approximately 100 metabolites. From this putative list, authentic compounds corresponding to most metabolites were analyzed to confirm identity. The peaks that remained unknowns required mass spectra interpretation as well as identification by other means such as LC/MS/MS and NMR to confirm presence.