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

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NIST is developing a standard reference material, Metabolites in Human Plasma (SRM1950), to serve as a common material to help researchers evaluate new techniques or platforms in this field. Both qualitative and quantitative information will be provided to the users of this material.
Comprehensive two dimensional gas chromatography (GCxGC) connected with time-of-flight mass spectrometry detector (TOFMS) and peak deconvolution software offers a major tool for non-targeted analysis in metabolomics. GCxGC-TOFMS was first investigated to identify and characterize unknown polar and semi-polar low molecular weight compounds in SRM1950. Prior to GC analysis, a derivatization step is necessary to increase the volatility of the metabolites. The optimized GCxGC-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 (cut-off value of 600), peak width, S/N ratio of 10 and retention time. Using these rules, the software automatically generated a list of approximately 250 compounds that fulfilled the criteria. In addition, linear retention indices (RI) in the first dimension were calculated using SRM1494, containing 18 *n*-alkanes (C_{10} to C_{34}). Currently, more than 100 metabolites were confirmed by triplicate analyses and authentic compounds standards. The metabolites list encompassed several classes of metabolites such as amino acids, hydroxyl/carboxylic acids, fatty acids, carbohydrates, alcohols, miscellaneous organics including some exogenous compounds. Among this list, seventeen amino acids were quantified by GC-TOFMS and GCxGC-TOFMS. Two different derivatization agents were investigated: tertbutyl silyl (MTBSTFA) and propyl chloroformate (PCF). In GC-TOFMS, terbutyl silyl and propyl chloroformate derivatives provided comparable concentrations. However, some amino acids are not able to be quantified under the conditions used with PCF. GCxGC TOFMS provided comparable data with GC-TOFMS. The added sensitivity of GCxGC did not seem to improve the precision.