# IDENTIFICATION OF METABOLITES IN A HUMAN PLASMA STANDARD REFERENCE MATERIAL BY MULTIPLE GC/MS APPROACHES

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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 often necessary to manage its complexity. Among them, gas chromatography coupled to mass spectrometry (GC/MS) and mass spectral library searching is one of the most versatile and widely applied technology platforms involved in metabolite identification. In recent years, the standardization of qualitative and quantitative information as well as reporting of metadata has been discussed. According to the recommendations of the Metabolomics Society, the highest level of unambiguous non-novel metabolite identification requires a minimum of two independent and orthogonal parameters, such as 'retention index' (RI) and 'mass spectrum' with authentic compounds, in order to be compliant with the minimum standards for reporting metabolomic data. NIST is developing a Standard Reference Material, Metabolites in Human Plasma (SRM 1950), to serve as a common material to help researchers evaluate qualitative and quantitative analytical methods.

The study presented here investigates the potential of identification and characterization of endogenous metabolites in the biofluid SRM 1950 by means of different GC/MS approaches. Split/splitless one dimensional (1D) GC coupled to time-of-flight mass spectrometry (TOFMS), split/splitless comprehensive two dimensional (2D) gas chromatography (GC x GC)-TOFMS and on-column GC-quadupole MS methods are evaluated. The comparison focuses on the RI of silylated metabolites calculated in the first dimension of the three GC/MS methods; on the potential of mass spectral matching to identify metabolites with NIST library (e.g. mass spectral similarity and probability scores) using automated mass spectral deconvolution softwares (Leco ChromaTOF® and NIST AMDIS); and, the confirmation of numerous potential non-novel metabolites in SRM 1950 based upon the co-characterization with authentic compounds (RI and authentic mass spectra libraries for GC-TOFMS and GC x GC-TOFMS only).