4D SPECTRAL LIBRARY FOR METABOLITE ANALYSIS AND IDENTIFICATION OF HUMAN URINARY BIOMARKERS.

A. Koulouri[1,*], A. Lioupi[2], G. Theodoridis[2,3], C. Virgiliou[1,3]

[1]Department of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2]Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

[3]Biomic AUTh, Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thermi, Greece

*(*koulouriaspasia@outlook.com)*

ABSTRACT

Metabolomics is a powerful scientific approach for comprehensive study and analysis of small molecules (metabolites) in biological systems, offering insights into their functions, interactions, and roles in health and disease. The construction of a comprehensive spectral library is crucial in metabolomics, as it provides a valuable reference resource for the accurate identification and quantification of metabolites, enabling researchers to better understand complex biological processes and uncover potential biomarkers for various diseases. The main objective of this project is the development of an ion mobility-enhanced spectral “in-house” reference library for small molecule analysis. Accurate measurements of collision cross section (CCS), retention time (RT), and mass-to-charge ratio (*m/z*) values for individual small molecules significantly accelerate the process of curating and constructing a reference 4D library for efficient sample annotation in high throughput analysis. In the conduct of the procedure, reversed-phase liquid chromatography (RPLC) combined with tandem mass spectrometry, which are highly specific and efficient analytical techniques, were applied. The developed library was applied for the identification metabolites in human urine samples through RPLC-TIMS-TOF/MS analysis. Regarding the results of the study, among the 151 compounds subjected to analysis, 124 were detected by manual investigation using Data Analysis software. Of these, 71 were detected in both positive and negative ionization, while 37 were detected only in positive ionization and 16 only in negative ionization. CCS values were derived for 109 of these compounds, providing valuable information on the structure of the metabolites. Furthermore, of the 124 compounds that were detected, 83 of them were annotated in MetaboScape, which enabled their introduction into the “in-house” reference library. Then, the identification of the compounds in human urine took place using the “in-house” reference library constructed, an enhanced reference library that derives data from Human Metabolome Database (HMDB) and was provided by the instrument manufacturer, Bruker, and an analyte list.

KEYWORDS: 4D Metabolomics, mass spectral library, RP Chromatography, HRMS, Ion mobility