A new comprehensive GC×GC-HRTOFMS approach in metabolomics

N. Di Giovanni[a], M.-A. Meuwis[b], E. Louis[b], J-F. Focant[a]

[a] Organic and Biological Analytical Chemistry Group, MS Lab, Université de Liège

[b] Translationnal Gastroenterology Unit, GIGA-R, CHU (University Hospital), Liège

Using an optimized and validated GC×GC-HRTOFMS method we developed for the metabolic profiling of human serum, in combination with strict QA/QC system, we were able to highlight sets of biomarkers capable to discriminate between various inflammation phenotypes (high, low, remission, and control) representative of inflammatory bowel diseases.

During this proof-of-concept study, two main challenges of untargeted metabolomics were especially considered.

First, the issue of data handling –large datasets and low number of samples compared to variables- was solved by the definition of a workflow of data preprocessing and processing. It included the creation of a study template, the rigorous selection of good chromatographic quality features, the multiplication of statistical techniques submitted to re-sampling and test validation, and performance models designed to assess the ability of the candidate biomarkers to discriminate. In practice, 94 injections were made over 4 weeks, consisting of 70 study samples along with 16 QC samples and 8 reinjections due to QC system rejection. The all-chromatograms template included 524 verified features that were then reduced to less than two hundreds after selection of the ones having an analytical variation under 30%, based on the QC samples. This resulted in the finding of 36 robust biomarkers that positively discriminated between the different phenotypes of inflammation, including high and low inflammation, remission, and healthy statutes.

Second, the identification of unknown compounds was enhanced by using state-of-the-art high-resolution (HR) time-of-flight mass spectrometry and allowed to name and characterize putative biomarkers with higher degree of confidence. This is a mandatory step for further development of analytical chemistry in clinical applications, its use in routine laboratories and integration of the results obtained in biological pathways interpretation.

In conclusion, this study has shown the usefulness of optimized and fully controlled GC×GC in clinical research and the major role high resolution is to play in order to fully exploit the potential offered by state-of-the-art analytical techniques.