Development of an analytical workflow for bronchoalveolar lavage fluids analysis using GCxGC-TOFMS

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Abstract (300 words)

Comprehensive two-dimensional gas chromatography (GC×GC) has become a method of choice for complex volatile mixture characterization. The two chromatographic dimensions and the possibility to hyphen high-speed high-resolution time-of-flight mass spectrometers (HRTOFMS) locates GC×GC as a method of choice for untargeted metabolomics. In this quest of the big picture, it is important to carefully apprehend every step of the analytical workflow from the sampling to the statistical process. The complexity of the approach cannot impact the analytical robustness.

In this project, bronchoalveolar lavage fluid (BALF) samples were analyzed by solid phase microextraction (SPME) coupled to GC×GC-TOFMS. A first study was set up to optimize the analytical conditions. Next, a larger sample set was used to demonstrate its applicability. Both sets were part of a discovery study for lung inflammation mechanisms characterization.

First, a pooled QC solution was used for optimization and daily system monitoring. Central composite design is a method of choice to establish optimal analytical conditions. For SPME, the peak intensity was used as a quality metric versus the fiber type, incubation time and temperature as variable parameters. For the GC×GC-TOFMS, normal and reversed column combinations were tested. Based on these optimal conditions, the samples were injected, and the optimization was performed for the pre-processing parameters. This analytical workflow allows us to identify asthma patients among other inflammatory diseases, such as COPD and sclerosis patients.

Next, the resulting workflow was then applied to study primary graft dysfunction (PGD) in 35 lung transplant patients. Primary graft dysfunction (PGD) is a major determinant of morbidity and mortality following lung transplantation. In this pilot study, severe PGD was differentiable from low grade PGD with an AUROC of 0.90 and an accuracy of 0.83 on test set samples.

In conclusion, SPME-GC×GC-TOFMS represents a go-to technique for the untargeted analysis BALF in a volatilomics context.