Applying Trapped Ion Mobility separation (TIMS) in combination with Parallel Accumulation Serial Fragmentation (PASEF) for analysis of lipidomics samples

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Introduction

Aiming at a deeper understanding of biochemistry, the analysis of lipid species is an important area. Untargeted lipidomics workflows target the profiling of changes in the lipidome in order to discover relevant lipids as potential biomarkers. This approach relies on robustly identifying a large number of lipids and statistically evaluating their relative abundances. We present an improved lipidomics identification workflow that is based a combination of LC-MS and TIMS separation together with a very fast data dependent MS/MS fragmentation (PASEF)[1]. The comprehensive data sets are processed and statistically evaluated with MetaboScape software in combination with SimLipid software for identification of the lipids. Serum samples of 4 different species were evaluated (human, pig, chicken, bovine).

Methods

Serum samples were extracted using a liquid-liquid extraction method (methanol/methyl tert-butyl ether). LC separation was performed using a Bruker Elute UHPLC system (32 min gradient program)[2]. MS data acquisition was performed in Parallel Accumulation Serial Fragmentation mode (PASEF) in combination with TIMS separation on a Bruker timsTOF PRO instrument (see Figure 1).

The instrument was set to cover a broad mobility range in TIMS mode while acquiring MS/MS fragmentation spectra in Parallel Accumulation Serial Fragmentation mode (PASEF, see Figure 2) during the LC gradient. The resulting data sets were processed in a novel MetaboScape 4.0 software (Bruker) version using a 4-dimensional feature finder designed to include the mobility separation dimension. Features were annotated using SimLipid 6.04 software (PREMIER Biosoft).

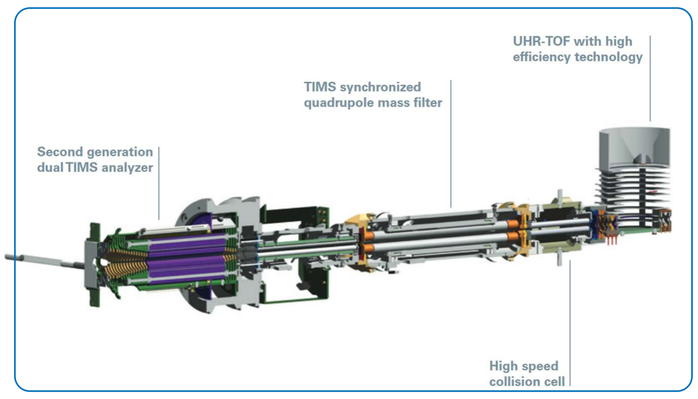

Figure 1. Ion optics of the timsTOF Pro instrument including a dual TIMS analyzer and QTOF mass spectrometer.

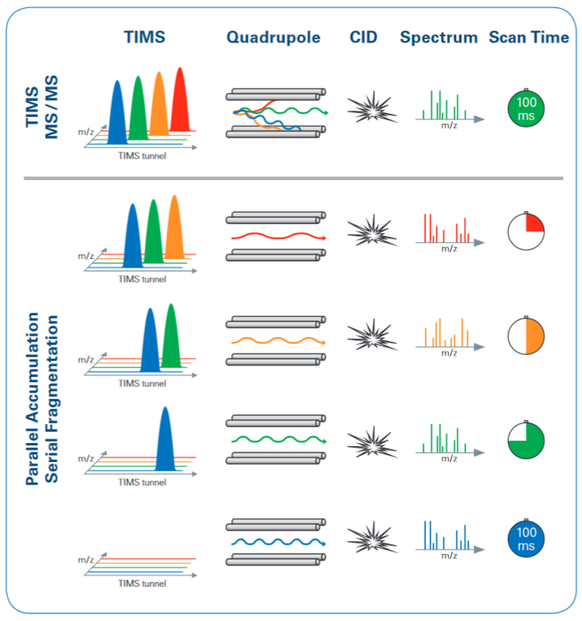

Figure 2. Illustration of the PASEF method in comparison with the standard TIMS MS/MS

Results

Feasibility of the TIMS system was first checked using a targeted infusion method with highest mobility separation settings. Two close isobaric lipids (18:0-18:1 PE and C16-18:1 PC) were mixed and both the protonated and sodiated ions could be well separated in the mobility dimension with TIMS resolution up to 180 (Figures 3a) and 1b)).

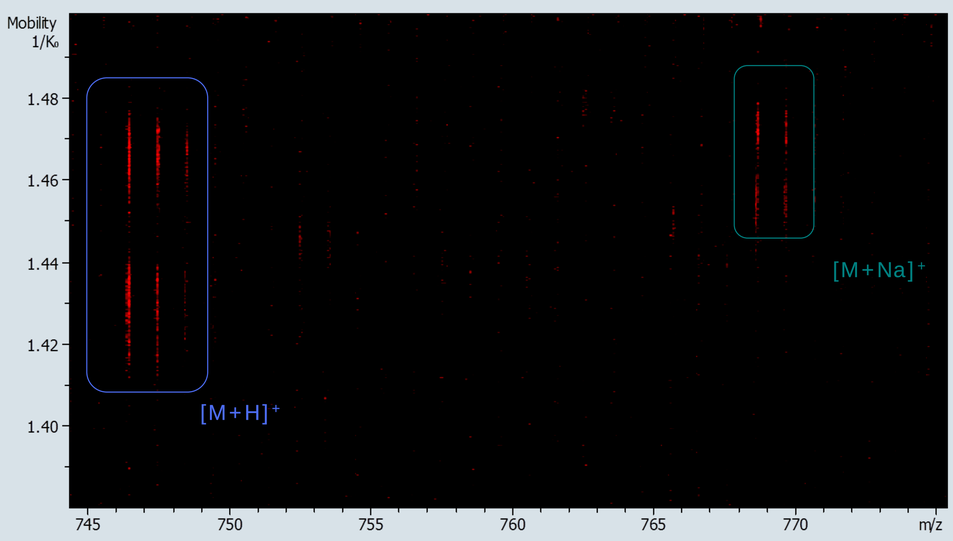

Figure 3a) Heatmap of close isobaric PC/PE lipid pair separated in mobility dimension (targeted infusion method)

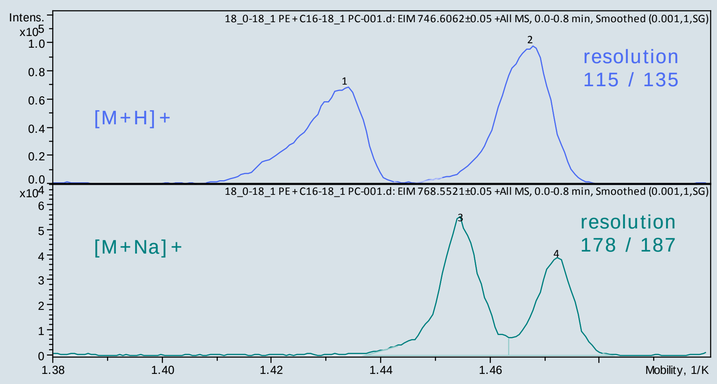

Figure 3b) Mobiliograms for protonated and sodiated ions of PC/PE lipid pair (targeted infusion method)

A broader untargeted TIMS-MS method was then developed and applied to a 32 minute LC gradient separation[2]. Extracted serum samples of the different species were analyzed using this method.

To accommodate for the additional dimension of information in LC-TIMS-MS data a dedicated 4-dimensionsal feature finder algorithm was developed and implemented into MetaboScape software. The algorithm (T-ReX 4D) enables robust detection of features including recursive extraction and automatically assigns available PASEF MSMS spectra to the found MS1 features.

In this evaluation only features were accepted that could be found in all 3 replicates of an analyzed serum sample. All features with associated MSMS spectra were then exported to SimLipid software for lipid identification.
SimLipid allows for the identification of multiple lipids in a single MSMS spectrum. These chimeric spectra were still occasionally observed, but only the single best lipid ID per MSMS spectrum was used for the presented results. Although SimLipid implements a proprietary algorithm that allows for identification of fatty acid chain length due to fragmentation patterns, this list was further filtered and condensed to avoid duplicates and over-specification by using short names (e.g. PC(32:1) instead of e.g. PC(16:0_16:1)). Table 1 shows number of identified lipids for the different evaluation methods. Most abundant main classes of identified lipids were glycerophosphocholines (PC), triacylglycerols (TAG), phosphosphingolipids and glycerophosphoethanolamines (PE). Relative abundances of these classes per analyzed species are shown in Figure 4.

Figure 5 shows MetaboScape results after processing all species sample replicates in a single feature list (bucket table). The software allows for running multiple statistical analyses (Principal Component Analysis used here). The scores and loadings plots show a clear separation of the groups with a high degree of reproducibility regarding the replicates. Automatically generated collisional cross section values (CCS) are available for each feature facilitating alternative library search workflows with increased identification confidence.

Table 1) Number of identified lipids in different species

	Human	Pig	Chicken	Bovine
Lipid IDs (common in all replicates)	442	234	330	270
Unique lipid IDs (short name)	340	208	255	232

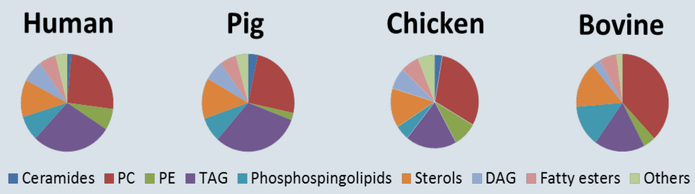

Figure 4) Relative abundance of lipid main classes per species

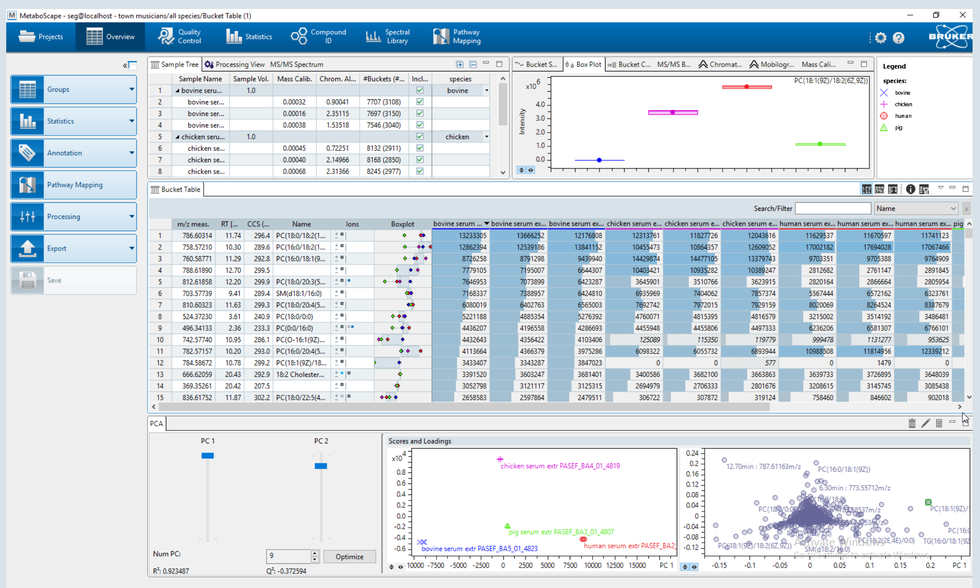

Figure 5) Serum samples of 4 different species processed in MetaboScape software

Conclusions

- Newly developed 4-dimensional feature finder (T-ReX 4D) in MetaboScape enables thorough and reproducible extraction of all relevant features including collisional cross sections and corresponding MSMS Information
- Targeted approach allows TIMS separation of (close) isobaric lipids with resolution of 180
- SimLipid and MetaboScape were integrated in a streamlined workflow for enabling LC-TIMS-PASEF based lipidomics research)

timsTOF / PASEF

References

(1) Meier et al.; J. Proteome Res. 2015, 14:5378-5387
(2) Wörmer et al.; Org Geochem 2013, 59:10-21