High speed untargeted 4D-lipidomics LC-MS/MS workflows with Parallel Accumulation Serial Fragmentation (PASEF)

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Introduction

The search for new and validated biomarkers is of particular interest in clinical areas like oncology[1,2] or neurology[3]. As lipids play an important role in many diseases, the area of lipidomics has become central for clinical research.
While there is a more in-depth oriented approach to ID as many lipids as possible, clinically-oriented projects often demand a high-throughput for large sample cohorts. Therefore, a short cycle time per sample is necessary to realize research projects with hundreds or even thousands of samples in a reasonable time frame. In order to keep up with this, the analytical instrumentation needs to deliver a high data quality at high acquisition speeds. This is realized by the PASEF (Parallel Accumulation Serial Fragmentation) acquisition mode on the timsTOF Pro system.[4]

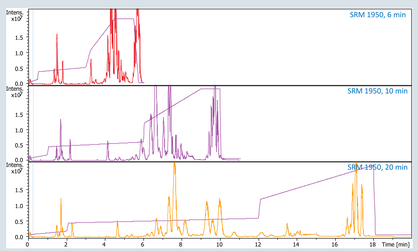

Fig. 1 Different gradient lengths applied to SRM 1950 lipid extract

Methods

Lipids from NIST SRM1950 reference plasma were extracted based on a protocol from Shevchenko *et al.*[5] The reversed phase based LC separation was performed using an Elute UHPLC system (*Bruker Daltonics*) and a YMC Triart C18 column (100 x 2.1mm, 1.9µm). Run times were 6, 10 and 20 minutes, respectively (**Fig. 1**). The MS data was acquired in positive ESI mode using a timsTOF Pro instrument (*Bruker Daltonics*) in PASEF MSMS mode. The transfer parameters were optimized for 100-1500 m/z, precursors were fragmented from 300-1500 m/z.

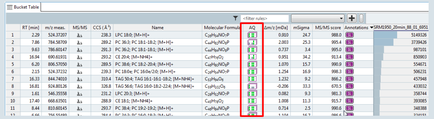

Fig. 2 Screenshot of MetaboScape 5.0 showing the bucket table of the 20 minute LC-PASEF analyses. The AQ score gives feedback on the quality of IDs

Run time [min]	Buckets	Buckets with MS/MS	MS/MS coverage [%]	IDs
20	2591	1624	62	392
10	2260	1338	59	283
5	2123	1166	55	213

Table 1 Lipids identified in SRM 1950 at different LC run times

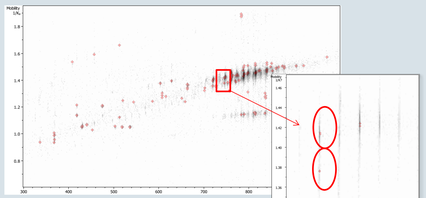

Fig. 3 In the time range of 4.6 to 4.7 minutes, PASEF picked 102 precursors, some of them several times. Multiply charged ions were excluded from fragmentation. The cutout shows two co-eluting isobaric lipids that were fragmented separately by PASEF (see also **Fig. 4**)

The resulting data were processed considering all four dimensions: m/z, RT, mobility and intensity using MetaboScape 5.0 (*Bruker Daltonics*). The T-ReX 4D algorithm combines all specified adducts and isotopes in the specified range of 300-1200 m/z into the bucket table. Features occurring in blank samples were subtracted. The remaining buckets were matched against the open source MS/MS library LipidBlast[6], and identified based on the fitting of m/z, isotopic pattern and MS/MS spectra (**Fig. 2**). In order to compare the number of assigned lipid classes on sum composition level, only the most abundant species of each class was considered (**Fig. 5**).

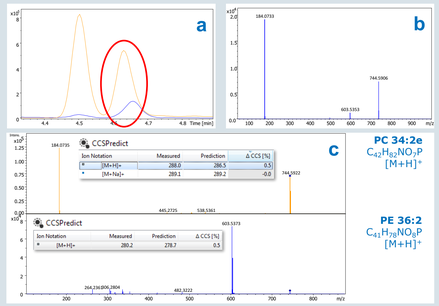

Fig. 4a Extracted ion chromatograms traces of an isobaric PC (744.5911, orange) and PE (744.5575, blue). **b** mixed MS/MS spectrum without ion mobility separation. **c** clean PASEF MS/MS spectra separated by TIMS and predicted CCS values

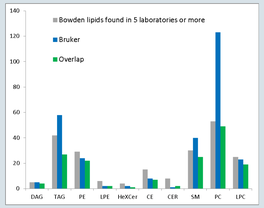

Fig. 5 Comparing the identified lipid classes with data from an interlaboratory study.

Especially for the Phosphatidylcholines (PCs), the presented approach shows improved lipid identifications

Results

- The high speed of PASEF allowed to reduce the LC run times to 5 minutes while still identifying a very large number of lipids (**Fig. 1** and **Table 1**)
- Around 390 lipids of SRM 1950 were identified in 20 min LC-PASEF analyses (**Table 1**)
- PASEF fragmented up to 9 precursors per 100 ms ramp (**Fig. 3**)
- The trapped ion mobility technology (TIMS) allowed the separation of isobars or isomers. This was demonstrated on a pair of co-eluting isobaric PE and PC species (**Fig. 4**)
- **CCSPredict** implemented in MetaboScape was used to predict the CCS values of identified lipids. As shown in **Fig. 4c**, this increased the confidence in the identification of two co-eluting isobaric PC and PE species with <1% deviation! (also see **Poster THP 395**)
- 286 lipid classes were identified at sum composition level, in comparison to 217 classes described in an interlaboratory comparison[7]. The overlap was 158 classes (**Fig. 5**)

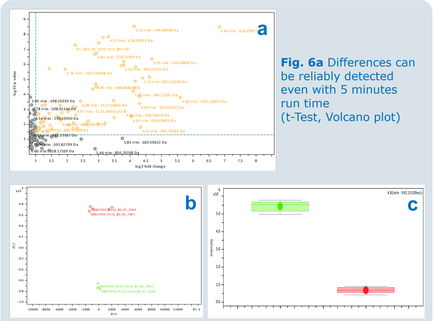

Fig. 6a Differences can be reliably detected even with 5 minutes run time
(T-Test, Volcano plot)

Fig. 6b PCA plot showing the grouping of spiked vs. non-spiked samples. c box plot of one of the spiked compounds

- A spike-in experiment with 5 and 20 min run times showed that even with reduced gradient times, the spiked "biomarkers" were reliably detected (**Fig. 6**)

Summary

The potential of the PASEF acquisition mode to increase the sample throughput was demonstrated. The crucial ability to separate co-eluting isobaric compounds and to identify differences between sample groups was maintained. With this, PASEF is demonstrated to be an optimal acquisition mode for deep profiling applying longer LC gradient times as well as for projects with high turnover needs, e.g. in clinical metabolomics studies.

References

(1) Röhrig, F., Schulze, A., *Nat. Rev. Cancer 16*, 732–749 (**2016**)
(2) Vriens, K. *et al.*, *Nature 566*, 403–406 (**2019**)
(3) Yang, Q., Vijayakumar, A. & Kahn, B. B., *Nat. Rev. Mol. Cell Biol. 19*, 654–672 (**2018**)
(4) Meier, F. *et al.*, *J. Proteome Res. 14*, 5378–5387 (**2015**)
(5) Shevchenko, A. *et al.*, *J. Lipid Res.*, *49*, 1137-1146 (**2008**)
(6) https://fiehnlab.ucdavis.edu/projects/LipidBlast
(7) Bowden, J. A. *et al.*, *J. Lipid Res. 58*, 2275–2288 (**2017**)

Conclusions

- The PASEF technology enables to increase the sample throughput using 4D lipid profiling by a factor of almost four
- Even at reduced LC run times, the ion mobility separates co-eluting isobaric or isomeric compounds and provides accurate and reproducible CCS values for high confident lipid ID
- Complementary to an in-depth "ID as many as possible" approach, PASEF enables a very fast lipid profiling based on MS/MS spectra

4D-Lipidomics