4D-Lipidomics workflow utilizing PASEF acquisition mode for increased throughput

ASMS 2021 – FP 499

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Introduction

Lipids represent a biological compound class with a broad variety of chemical structures. Their essential biological roles make them interesting biomarkers in clinical research [1]. However, confident lipid annotation can be time consuming, and it requires an unambiguous differentiation of co-elution isobaric and isomeric compounds. The presented **4D-Lipidomics workflow for increased throughput** simplifies and streamlines the annotation and validation process using mobility enhanced MS data.

Methods

Lipids from NIST SRM 1950 reference plasma (Sigma-Aldrich, Germany) were extracted using methyl-tert-butyl ether (MTBE) and the residues were dissolved in Methanol : Dichloromethane (9:1) [2]. The C18 reversed phase chromatography was performed using an Elute UHPLC system (Bruker). Run times were 5, 10 and 20 minutes, respectively. MS- and MS/MS data were acquired in positive and negative ESI mode using the **timsTOF Pro** (Bruker) instrument in Parallel Accumulation Serial Fragmentation (**PASEF**) MS/MS mode [3,4]. The 4D data (*m/z*, RT, mobility, and intensity) was processed using a preliminary version of **MetaboScape 2022** (Bruker).

MS	timsTof Pro
Source	Apollo II ESI source
Ioniz. mode	ESI (+ and -)
Acquis. mode	PASEF MS/MS, 100 ms ramp time

LC	Elute UHPLC
Column	YMC Triart C18 column (100x2.1 mm, 1.9 µm)
Column oven temp.	55°C (20 min grad.), 60°C (10 min grad.), 65°C (5 min grad.)
Mobile phase	A: MeCN/H2O (60:40 10 mM ammonium formate, 0.1% FA) B: IPA/MeCN/H2O (90:8:2, 10 mM ammonium formate, 0.1% FA)

	20 min	10 min	5 min
Flow rate	0.4 mL/min	0.5 mL/min	0.55 mL/min
Gradient	0 min: 40% B 2 min: 43% B 2.1 min: 50% B 12 min: 54% B 12.1 min: 70% B 18.0 min: 99% B 18.1 min: 40% B 20 min: 40% B	0 min: 50% B 0.6 min: 50% B 2.0 min: 55% B 6.5 min: 80% B 6.6 min: 95% B 8.6 min: 99% B 8.7 min: 50% B 10 min: 50% B	0 min: 50% B 0.3 min: 50% B 0.75 min: 60% B 0.85 min: 85% B 3.0 min: 80% B 3.05 min: 95% B 4.33 min: 99% B 4.35 min: 50% B 5.0 min: 50% B

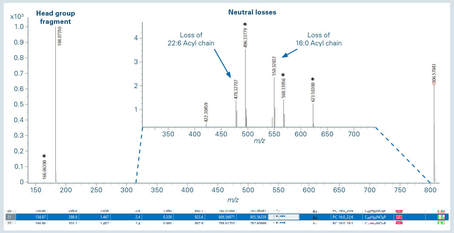
References

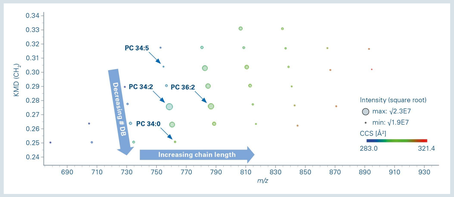
[1] Züllig et al. (2020), Mass Spectrom. Rev., early view.
[2] Matyash (2008), V. et al., J. Lipid Res., 49, 1137-1146
[3] Application Note LCMS-158, Using Parallel Accumulation Serial Fragmentation (PASEF) to speed up untargeted 4D lipidomics LC-MS/MS workflows.
[4] Application Note LCMS-175, Investigating the increased lifespan in C. elegans daf-2 mutants by 4D-Lipidomics.
[5] Tsugawa et al. (2020), Nature Biotechnology, 38, 1159–1163.
[6] Hayen et al. (2018), Rapid Commun. Mass Spectrom., 32, 981-991.
[7] Liebisch et al., The Journal of Lipid Research, 61, 1539-1555.

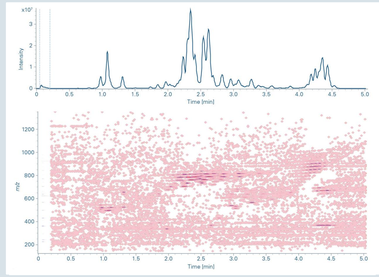
Workflow

1. 4D-PASEF acquisition. PASEF delivers high-quality MS/MS data with a large precursor coverage (red squares) of typically >65% in single acquisitions even at short run times.

2. 4D-Processing. The T-ReX 4D algorithm combined common adducts and isotopes belonging to the same compound into features.







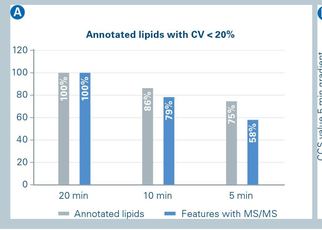
3a. Rule-based annotation. The rule-based lipid features fragmentation rules for a wide range (> 40) of common lipid sub classes. It annotates based on the precursor *m/z,* isotopic pattern, and characteristic fragments in the MS/MS spectra. Predicted CCS values serve as additional qualifier.

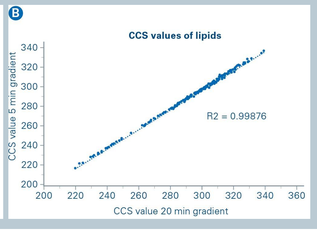
3b. CCS-aware LipidBlast. Additionally, the CCS containing open-source MS/MS library LipidBlast can be used. It was compiled by Tsugawa and Fiehn et al. [5] and contains > 1/2 million of CCS values for lipids, together with their in-silico generated MS/MS spectra.

4. Deep Analysis 4D-KMD plots. 4D Kendrick mass defect (**KMD**) plot filtered to display unique PC lipids. This plot shows *m/z* on the x-axis vs. KMD (CH_2) on the y-axis. Trend lines can be used to assess potential wrong annotations or missing ones [6].

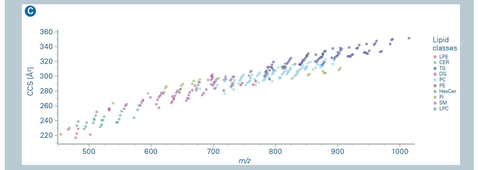
Results

A. The maximum number of unique identified lipids were observed with 20 min gradient time. **Increasing the chromatographic speed four-fold, the presented workflow still yielded 75%** of the maximum observed lipids annotations.





B. Comparison of observed lipid **CCS values** from 5- and 20-min gradient run presents the power of CCS values as a **qualifier**.



C. Distribution of the detected lipids in the *m/z* and **CCS** space (5 min runs, ESI-(+)). For different lipid classes, **clear trends** can be observed that enable **validation of the annotations**.

Overview

- **PASEF** enables **higher throughput** lipid profiling.
- The **lipid annotation** tool in MetaboScape provides **rule-based annotation** for >40 sub-classes and reports according to most recent guidelines [7].
- Increasing the chromatographic speed four-fold, the presented workflow still yielded 75% of the maximum annotations observed.
- The comparison of the measured CCS values for the 20- and 5-min gradients demonstrated the **independence of CCS values from the chromatography**.

4D-Lipidomics