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Global analysis of lipids by SFC-HRMS (Q-Tof) : an analytical and data processing challenge

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Analytical challenge

Objectives

- Have a complete lipidomic profiling
- Perform relative quantification of lipid subspecies

Optimal lipid extraction

- Polar and unpolar lipids
- Only one internal standard per sub-classes

Optimal analytical method

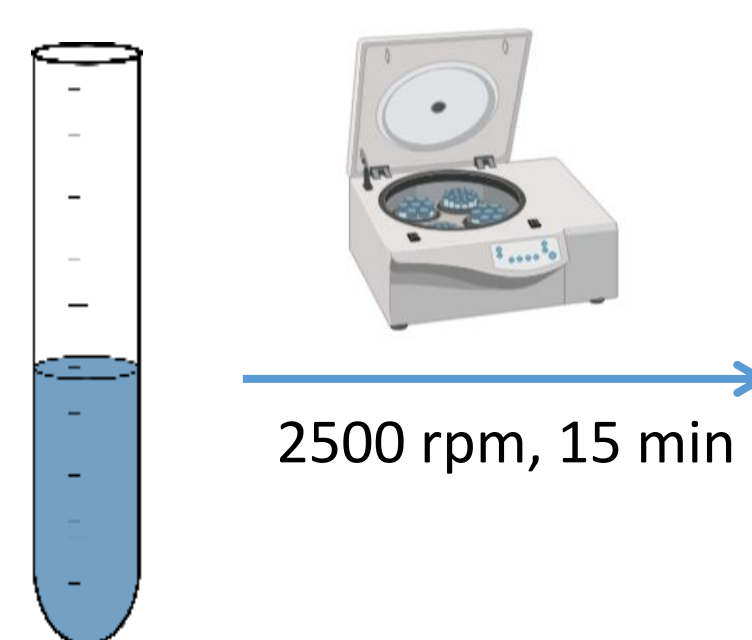
- Large scale of concentration
- Adducts diversity
- Analysis in positive and negative ionisation mode

Global processing method

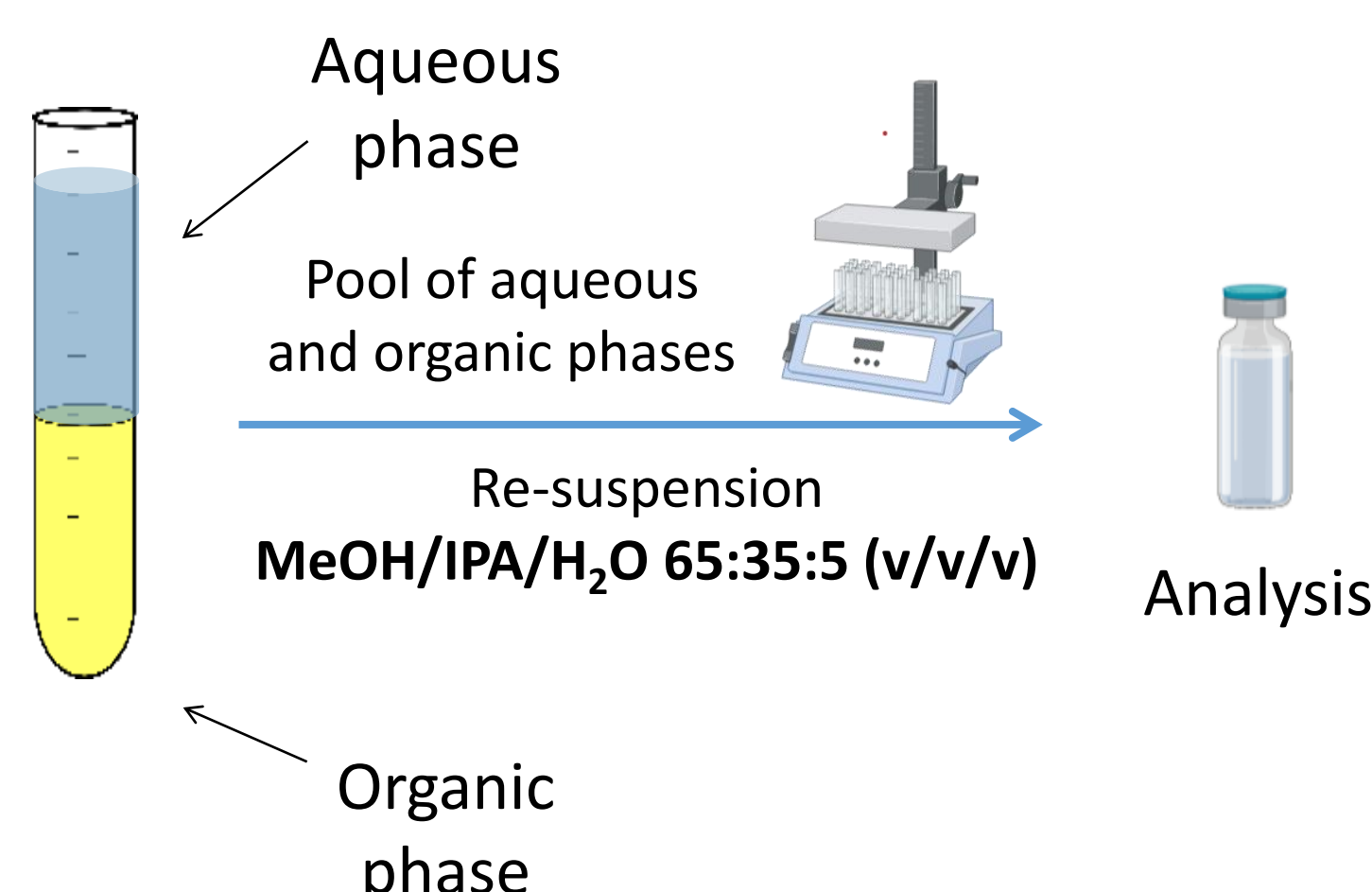
- Presence of isomeric and isobaric species
- High diversity of peak width and retention time

Global extraction [1]

- ▶ 10 µL of mix Internal Standard
- ▶ 10 µL of plasma



- ▶ QSP 100 µL of H₂O
- ▶ 490 µL of CHCl₃/MeOH 1:1 (v/v)
- ▶ Vortex 1 hour and add 75 µL of H₂O



Processing method

× Tools of data processing for semi-targeted analysis

Tools	PROS	CONS
Progenesis QI	Improved matching, peak picking and normalisation, user friendly	Need three adducts per lipid to annotate
UNIFI, Waters	Outperforms MassLynx as custom software	Need continuum data, Deisotopisation not compatible with polar head separation and QTOF resolution
MassLynx, Waters	Custom software => high reliability of peak detection	Very time-consuming
MSDial	Free software compatible with all analysis, and all technology, easy to use	Data loss, alignment problems (mass shift), no deisotopisation

Necessity to find a more robust and less time-consuming solution

✓ MZmine3 [3] : a software for optimising all parameters of each step of an integrative workflow

1. Import data

- MS detector
- Noise level
- MS Convert => mzml

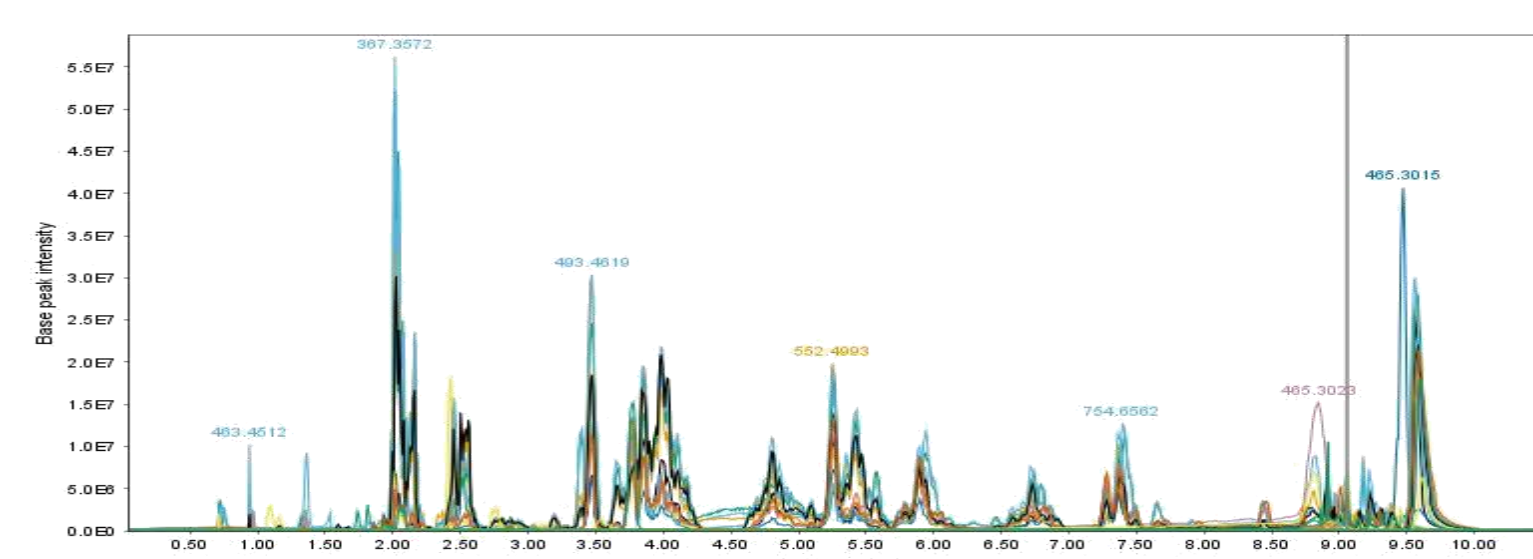
2. Raw data filtering : Crop filter

- Retention time range
- m/z range

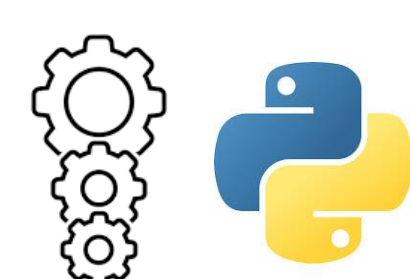
3. ADAP chromatogram builder

- Min consecutive scan
- Intensity
- Absolute height

MZmine 3



Automated processing



4. Local minimum resolver

- Peak duration
- Minimum scans
- Threshold

5. Alignment

- m/z tolerance (sample to sample)
- Rt tolerance
- m/z and rt weight

6. Annotation Local database (CSV)

- Database file
- Column
- m/z and rt tolerance

Conclusion

MZmine 3

- Meets our data reprocessing criteria
- Adaptable to different methods (targeted, semi-targeted, untargeted methods)
- Fast processing speed
- Not user friendly : takes a lot of time to learn how to use it
- Needs help of an IT

Analytical method [2]

✓ Optimised SFC conditions

Column : Torus DEA 130Å, 1.7µm, 3 x 100 mm; Waters

A solvent : SCCO₂

B solvent (=Modifier) : MeOH:H₂O 98:2 (v/v) 20 mM Amm.Acetate

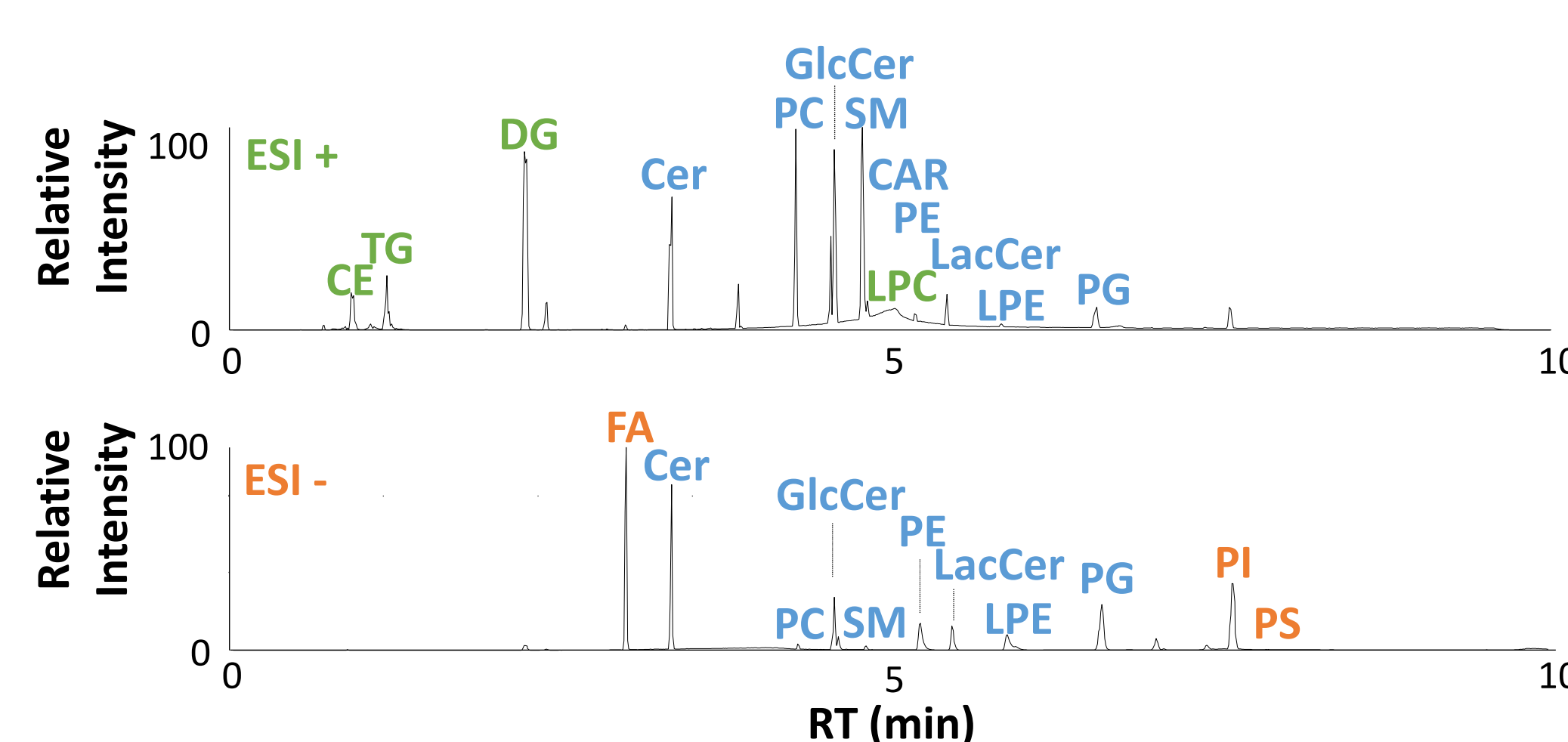
Flow rate : 1.2 mL/min

ISM : MeOH:H₂O 95/5 (v/v) at 0.1 mL/min



UPC²-QTOF, XEVO G2XS, Waters

✓ Separation of 16 sub-classes by polar head in 9 min



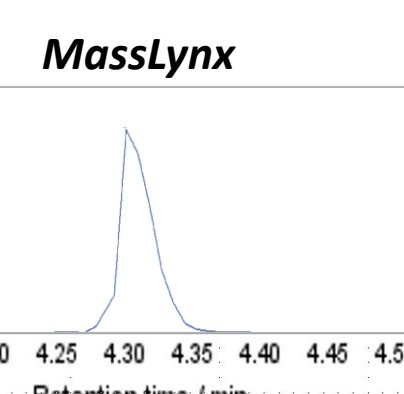
✓ Development of homemade Database containing

=> 550 lipids described with Lipid Maps Abbrev name, Formula, Adduct, RT, CHEBI ID

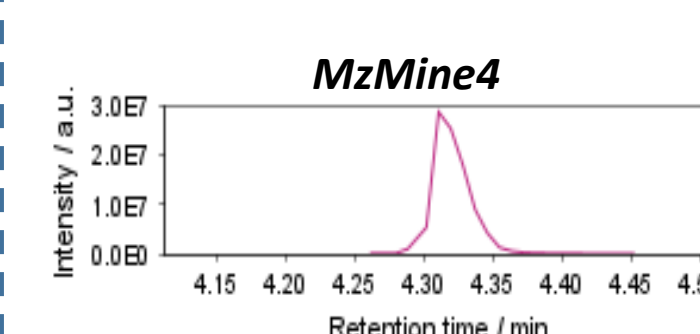
Validation on human plasma

Feature detection

Chromatogram builder



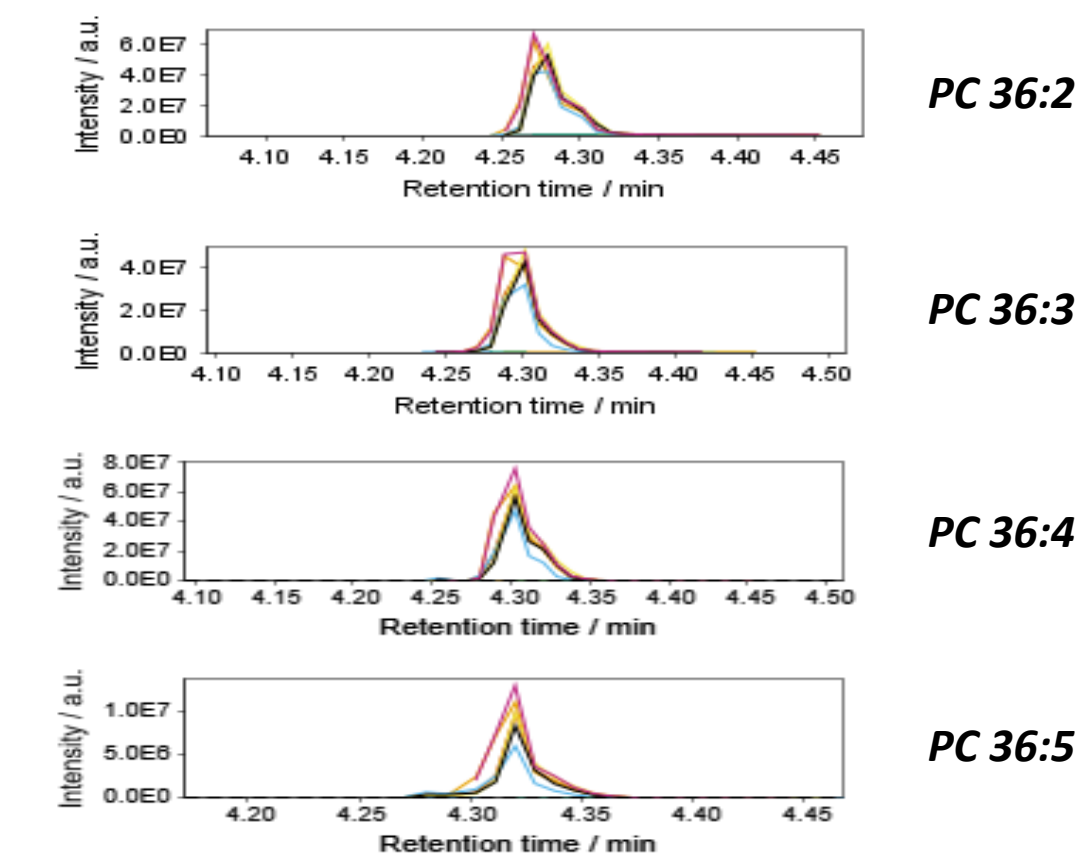
m/z	808.583
RT	4.31
Max intensity	2.88E7



m/z	808.5827
RT	4.31
Max intensity	2.9E7

Same EIC between MassLynx and MzMine

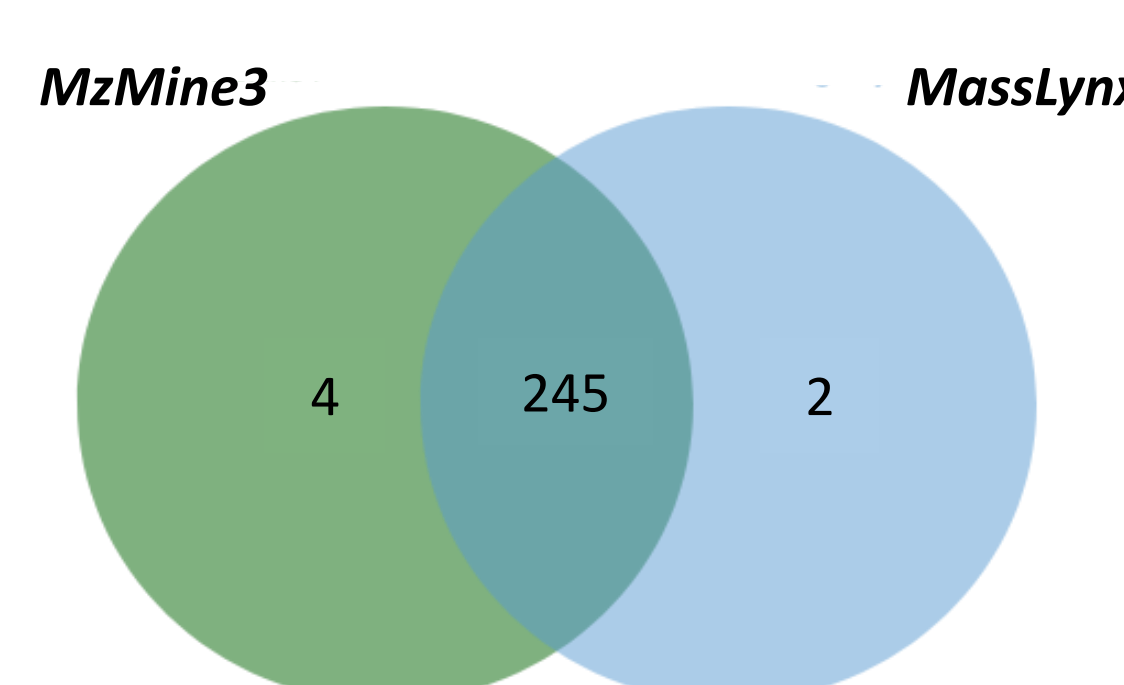
Alignment



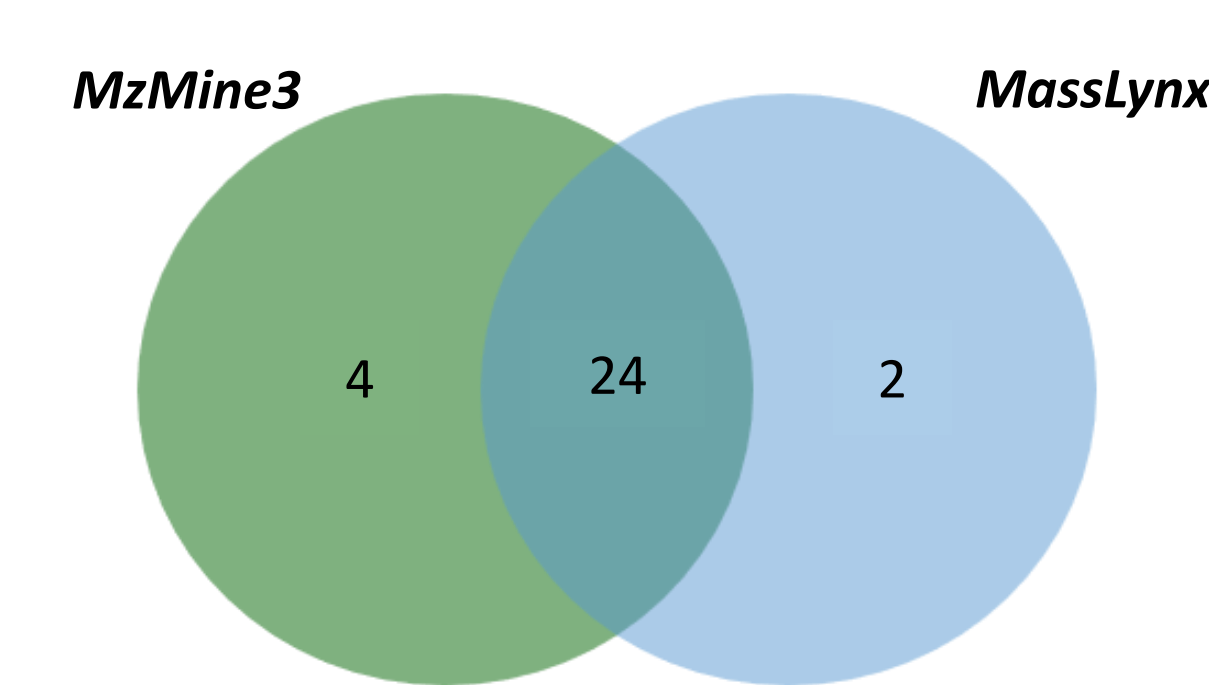
Reliable process, no loss of compound inter samples

Annotation

ESI Positive analysis



ESI Negative analysis



Exact match
Differences with minor species that are difficult to integrate

Perspectives

- Use of Data Connect (Waters) instead of MSConvert
- More precise separation of isotopic masses
- Use of Kendrick's diagram to implement DB
- Work in untargeted method

References

[1] Annotation of the human cerebrospinal fluid lipidome using high resolution mass spectrometry and a dedicated data processing workflow, Seyer *et al.*, *Metabolomics* 2016

[2] Untargeted Lipidomic Profiling of Dry Blood Spots Using SFC-HRMS, Le Faouder *et al.*, *Metabolites* 2021

[3] Integrative analysis of multimodal mass spectrometry data in MZmine 3, Schmid *et al.*, *Nature Biotechnology* 2023

* Pictures obtained from Biorender

