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New method to characterize epidermal Ceramides by Supercritical Fluid Chromatography (SFC) coupled to High Resolution Mass (Q-ToF) : Application to various samples with skin barrier defect. Conclusion & outlooks Skin ceramides extraction SFC-QTOF analysis Data Processing Ceramides characterization by MS/MS acquisition

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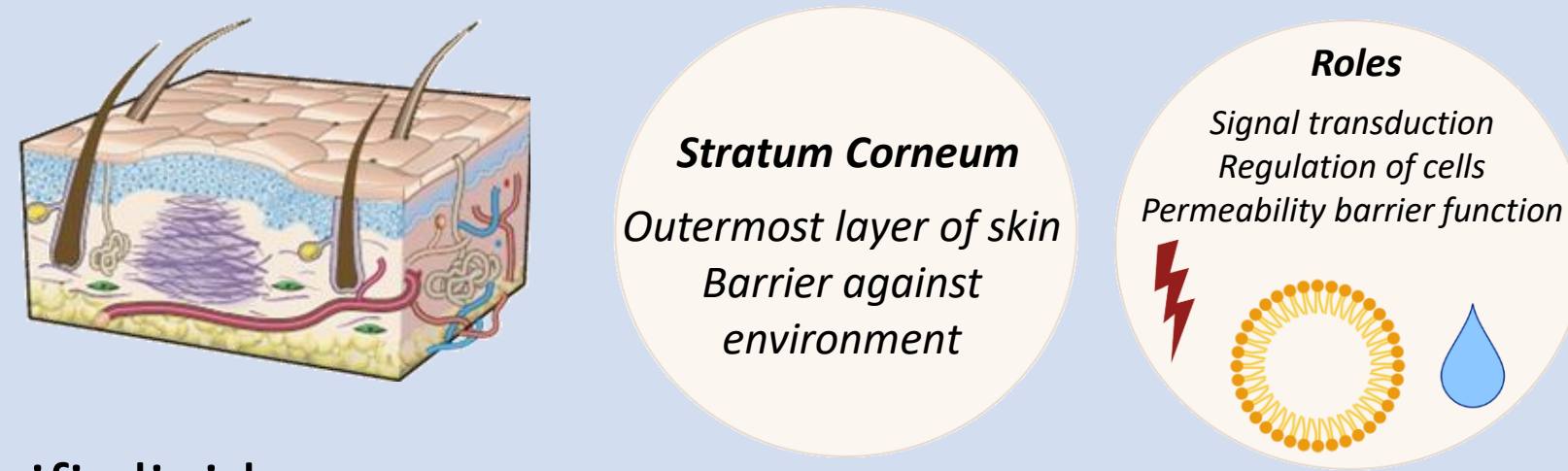
Cyrielle Clement, Julia Soullier, Roselyne Gautier, Carla Orlandi, Emilien Jamin, et al.. New method to characterize epidermal Ceramides by Supercritical Fluid Chromatography (SFC) coupled to High Resolution Mass (Q-Tof): Application to various samples with skin barrier defect. Conclusion & outlooks Skin ceramides extraction SFC-QTOF analysis Data Processing Ceramides characterization by MS/MS acquisition. ILS (International Lipidomics Society) Conference, Aug 2023, Vienne, Austria. hal-04338851

New method to characterize epidermal Ceramides by Supercritical Fluid Chromatography (SFC) coupled to High Resolution Mass (Q-ToF) : Application to various samples with skin barrier defect.

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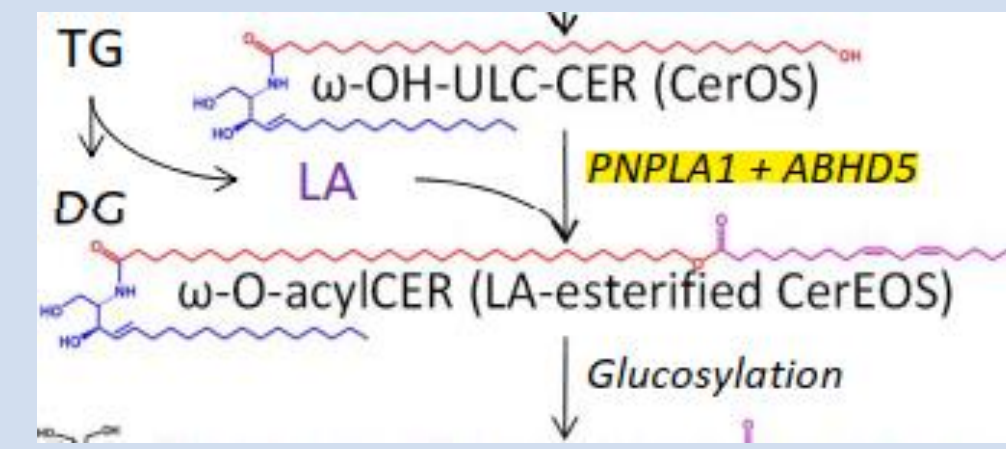
Biological context

✓ Skin ceramide are key partners of epidermal barrier



➢ ω-O-Acylceramide is an epidermis specific lipid with a crucial role for skin barrier function

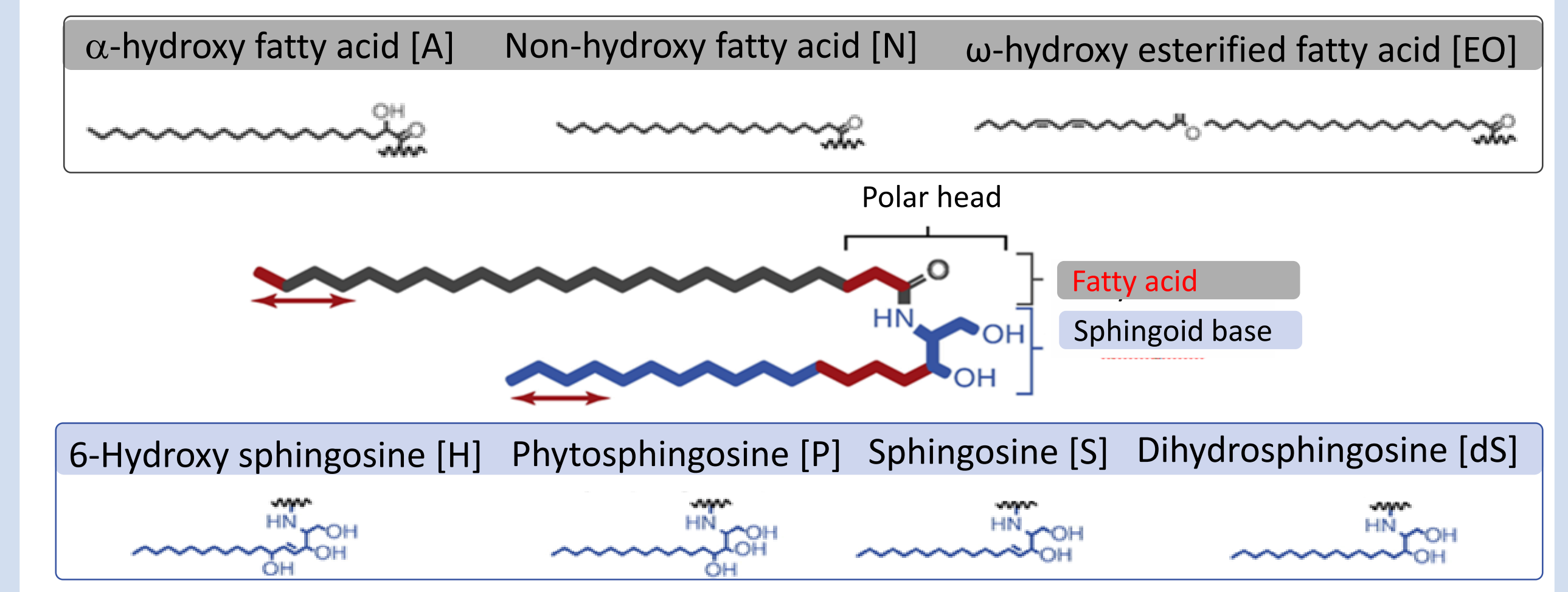
✓ Diseases such as ichthyosis are impacted by ceramide quality



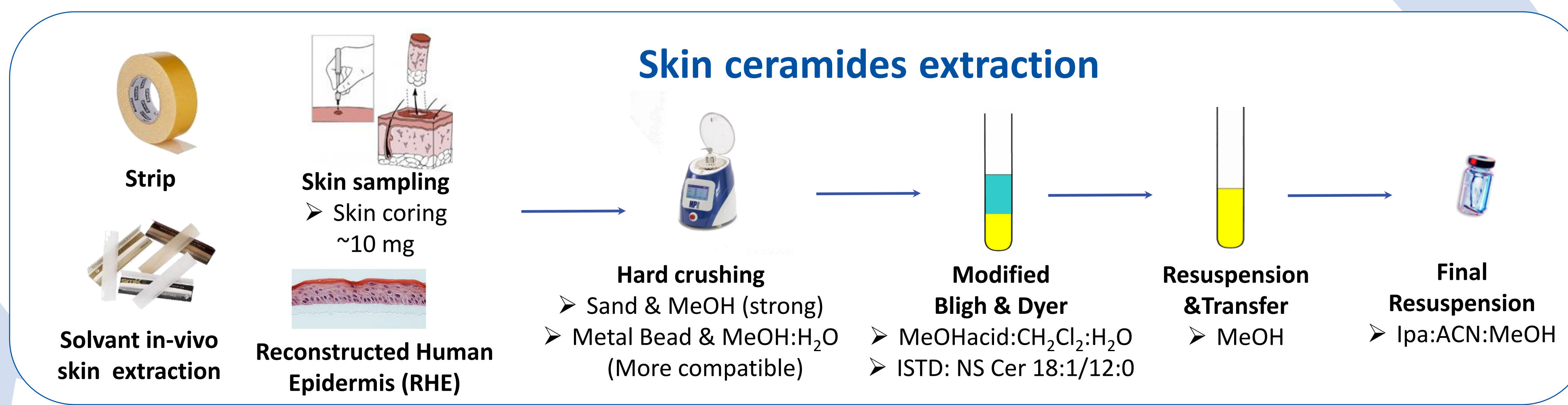
➢ Mutation in patatin-like phospholipase domain-containing 1 (PNPLA1) causes autosomal recessive congenital ichthyosis

➢ PNPLA1 mutated patients present a default in CerEOS synthesis

Ceramides structures

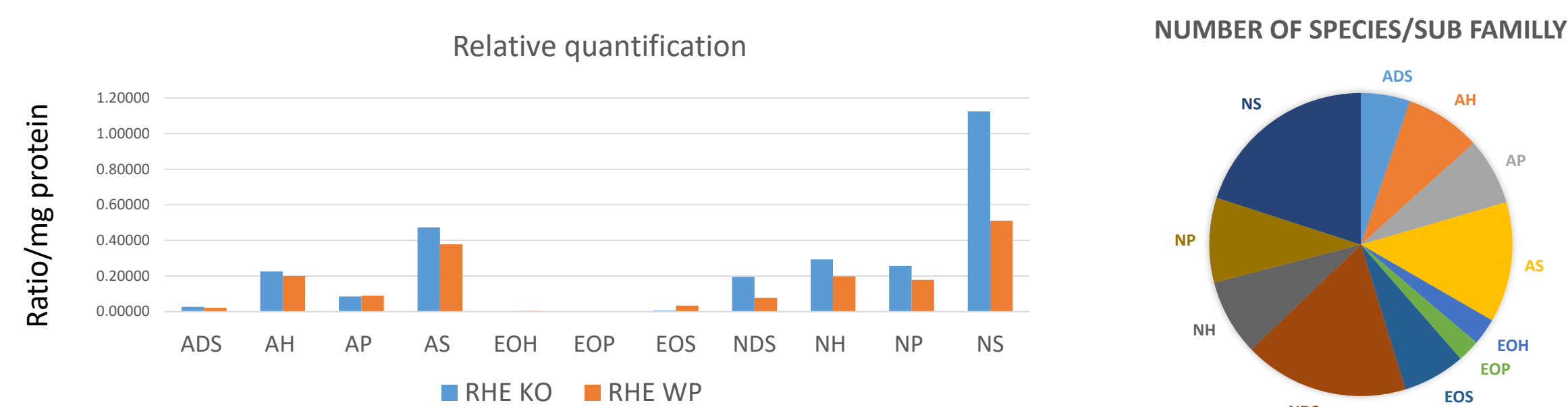


✓ Huge and complex diversity of epidermal ceramides

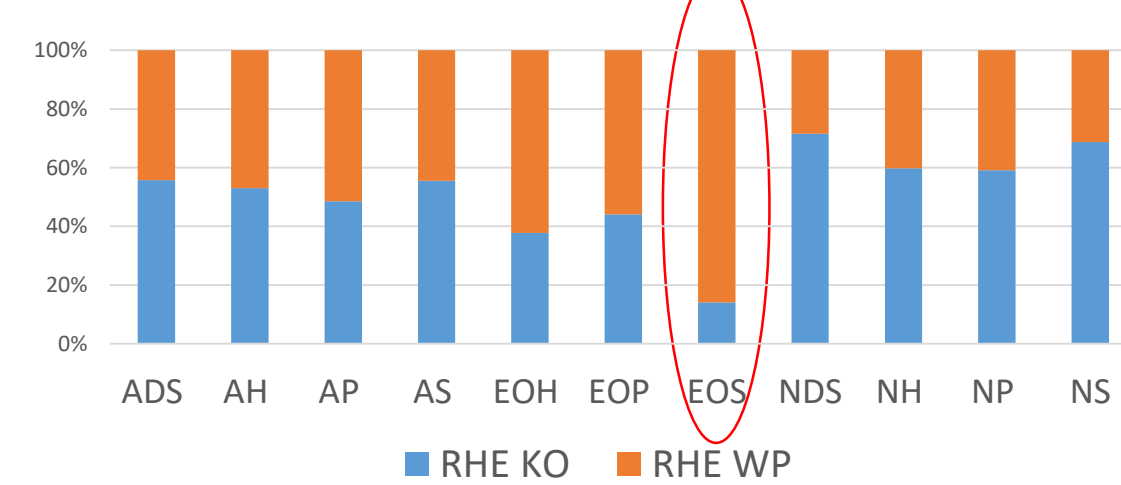


RHE analysis

✓ Analysis of 209 ceramides species in 11 subclasses



✓ Decrease of CerEOS in PNPLA1 KO



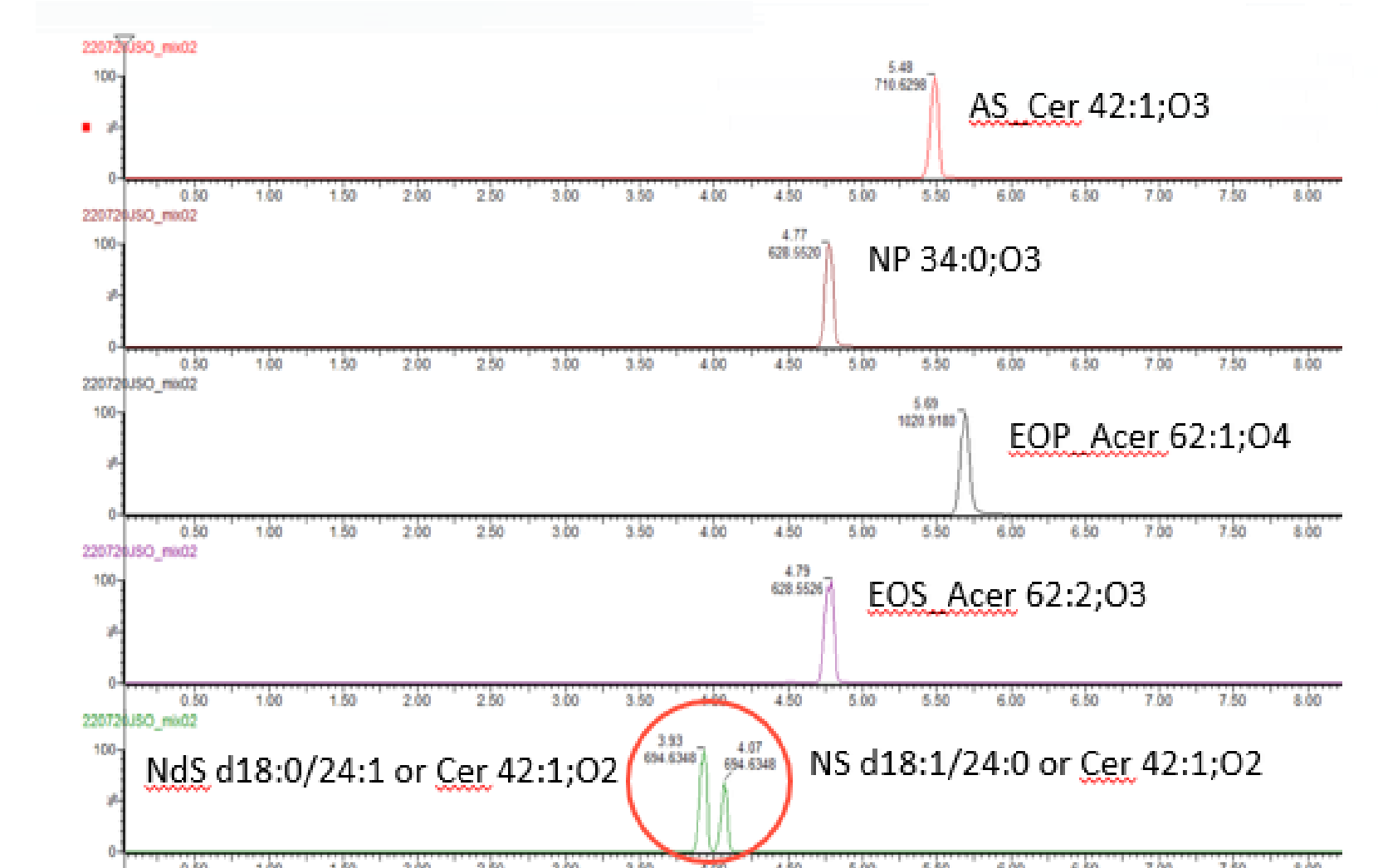
SFC-QTOF analysis

✓ Optimized SFC conditions

Column : Torus DIOL 130Å, 1.7µm, 3 x 100 mm; Waters
A solvent : SCCO₂
B solvent (=Modifier) : IPA:ACN:MeOH 30:30:40 (v/v/v)
 0.1% acid formic
Flow rate : 1.1 mL/min
ISM : Methanol at 0.1 mL/min



✓ Separation by polar heads in 8min

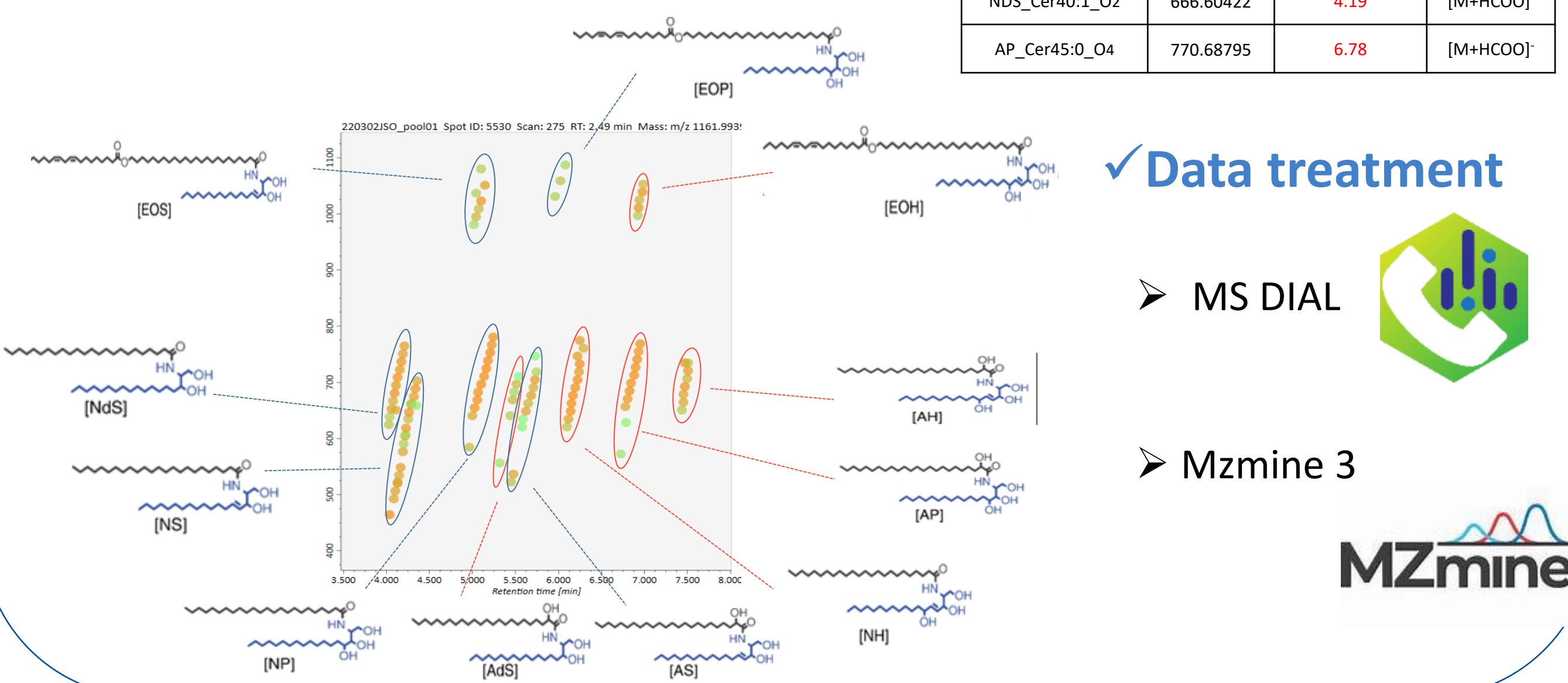


Data Processing

✓ Home made data base:

➢ 280 species for 11 sub-classes

Metabolite	m/z	RT (min)	Adduct
ADS_Cer34:0_O3	600.52088	5.09	[M+HCOO] ⁻
NDS_Cer40:1_O2	666.60422	4.19	[M+HCOO] ⁻
AP_Cer45:0_O4	770.68795	6.78	[M+HCOO] ⁻



✓ Data treatment

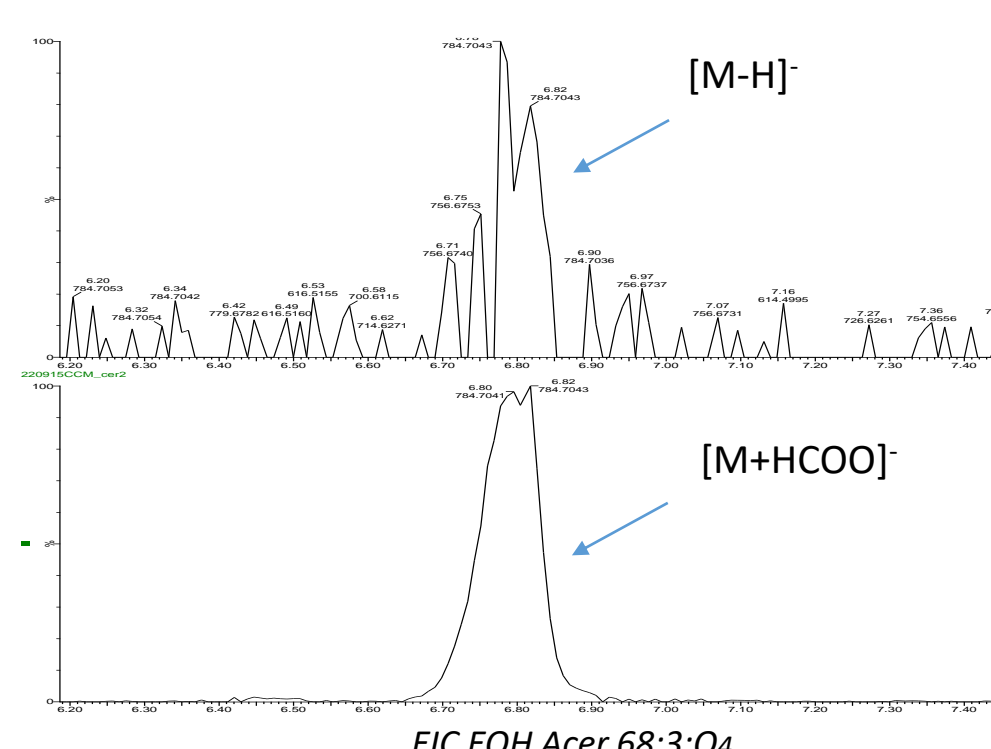
➢ MS DIAL

➢ Mzmine 3

Ceramides characterization by MS/MS acquisition

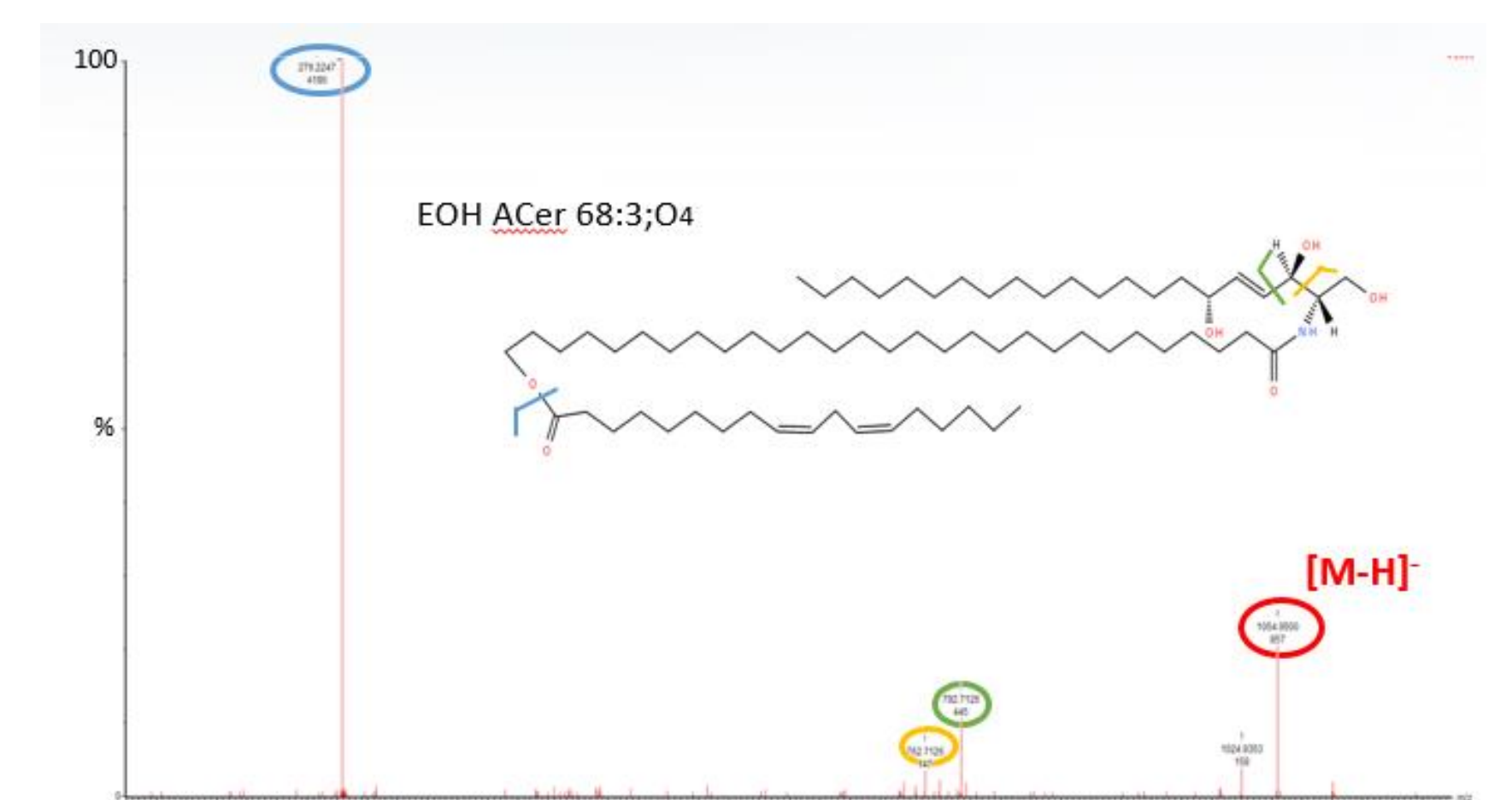
✓ Mass detection

➢ ESI negative mode
 ➢ [M+HCOO]⁻ adduct



✓ MS/MS fragmentation

➢ Validation of MS/MS for all on [M+HCOO]⁻



Conclusion & outlooks

- ✓ Development of a specific and robust method for skin ceramides quantitative profiling using SFC-QTOF
- ✓ Application to RHE sample in the context of ichthyosis diseases
- ✓ Need to develop the analysis of Cer EOS precursor: CerEOS
- ✓ Continue the optimization of extraction on various sample (i.e Strip)

References

- [1] Smeden et al, Biochimica et Biophysica Acta, 2014 (<https://doi.org/10.1016/j.bbalip.2013.10.002>)
- [2] Smeden et al, Biochimica et Biophysica Acta, 2014 (<http://dx.doi.org/10.1016/j.bbalip.2013.11.006>)
- [3] Jamin et al, EJMS, 2018 (DOI: 10.1177/1469066718815380) Forum lipidomic meeting, ILS, Vienna, 2023

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