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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.

Cyrielle Clément^{1,2}, Julia Soullier^{1,2}, Roselyne Gautier^{1,3}, Carla Orlandi^{1,3}, Emilien Jamin^{1,3}, H el ene Duplan⁴, Carine Jacques⁴, Nuria Pell Vidal⁵, Nathalie Jonca⁵, Pauline Le Faouder^{1,2}, Justine Bertrand-Michel^{1,2}
¹MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, Toulouse, 31077, France, ²I2MC, Universit e de Toulouse, Inserm, Universit e Toulouse III – Paul Sabatier (UPS), Toulouse, France., ³Toxalim (Research Center in Food Toxicology), Universit e de Toulouse, INRAE, ENVT, INP-Purpan, Toulouse, France, ⁴Centre de R&D Pierre Fabre - Centre de Recherche Pierre Fabre, ⁵INSERM, Infinity - Institut Toulousain des Maladies Infectieuses et Inflammatoires

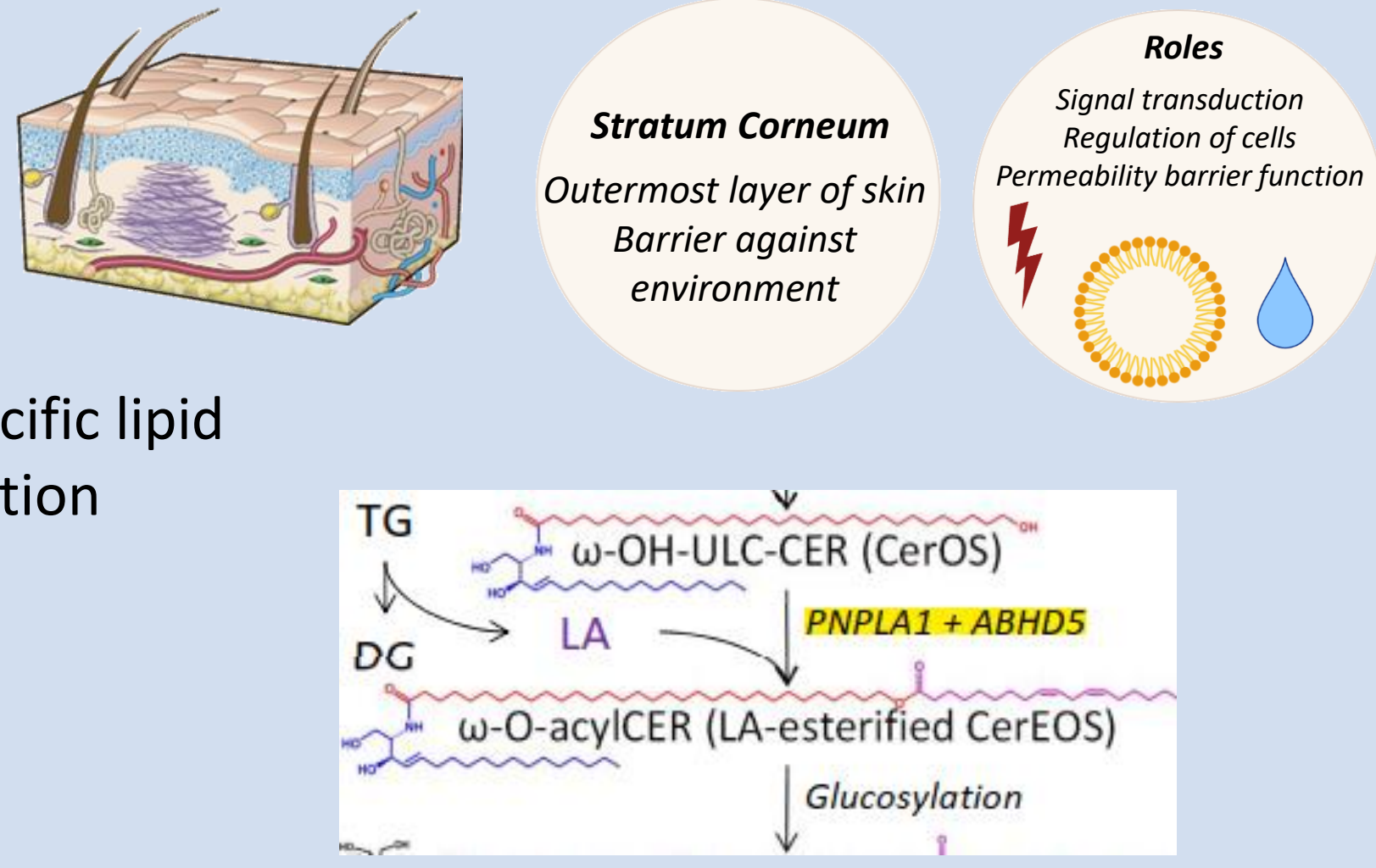
Biological context

✓ Skin ceramide are key partners of epidermal barrier

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

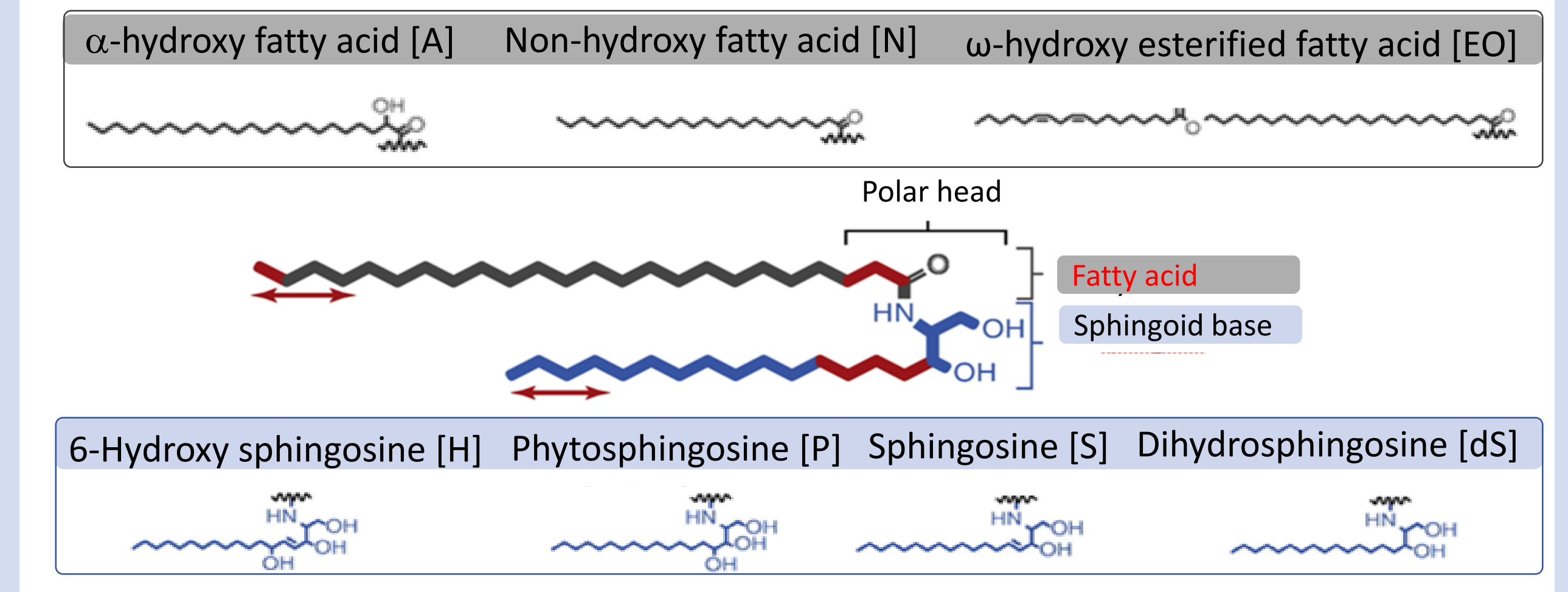
✓ Diseases such as ichthyosis are impacted by ceramide quality

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

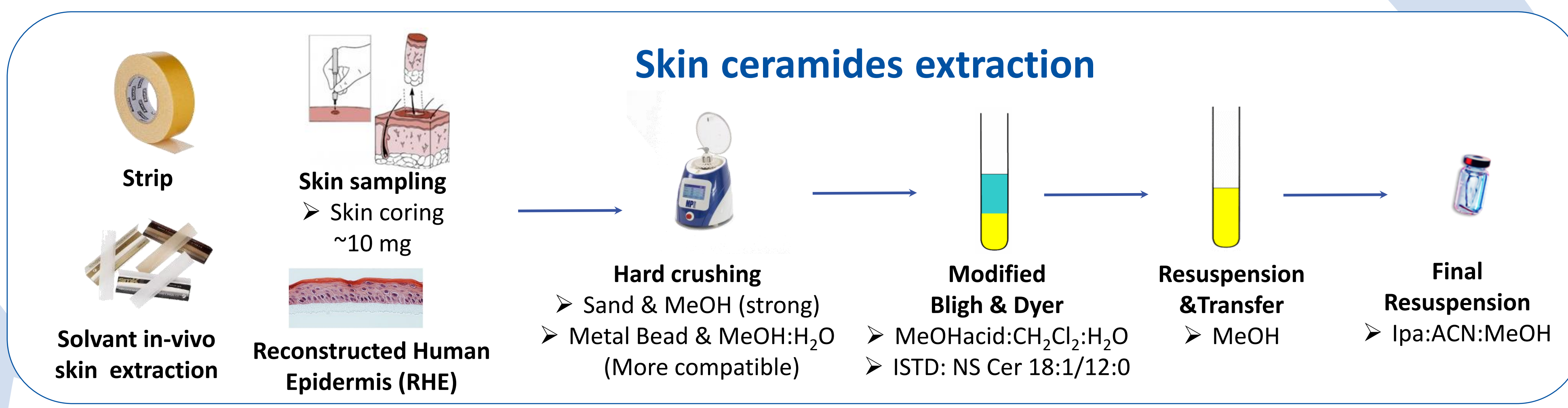


➢ PNPLA1 mutated patients present a default in CerEOS synthesis

Ceramides structures

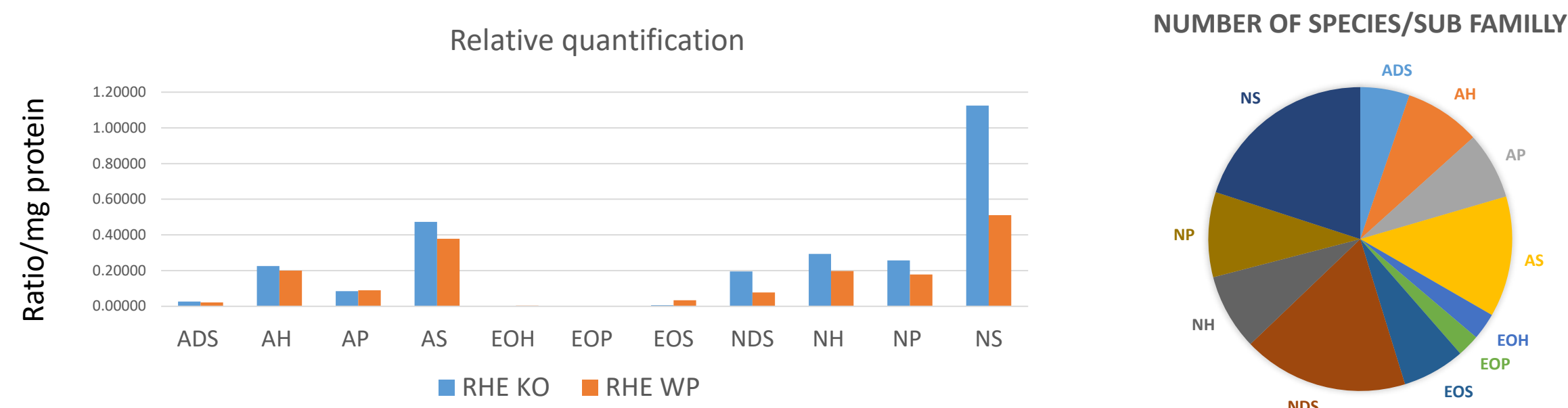


✓ Huge and complex diversity of epidermal ceramides

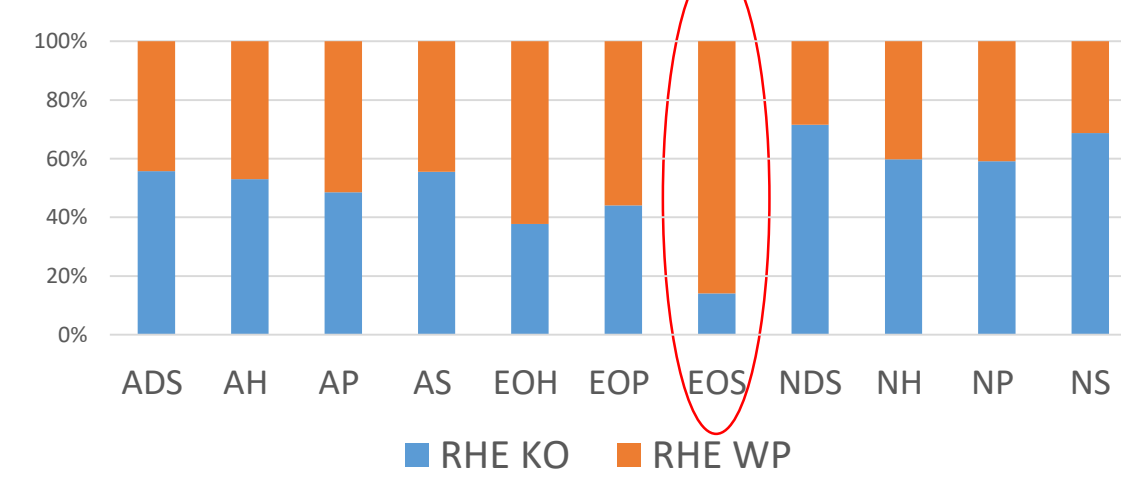


RHE analysis

✓ Analysis of 209 ceramides species in 11 subclasses



✓ Decrease of CerEOS in PNPLA1 KO



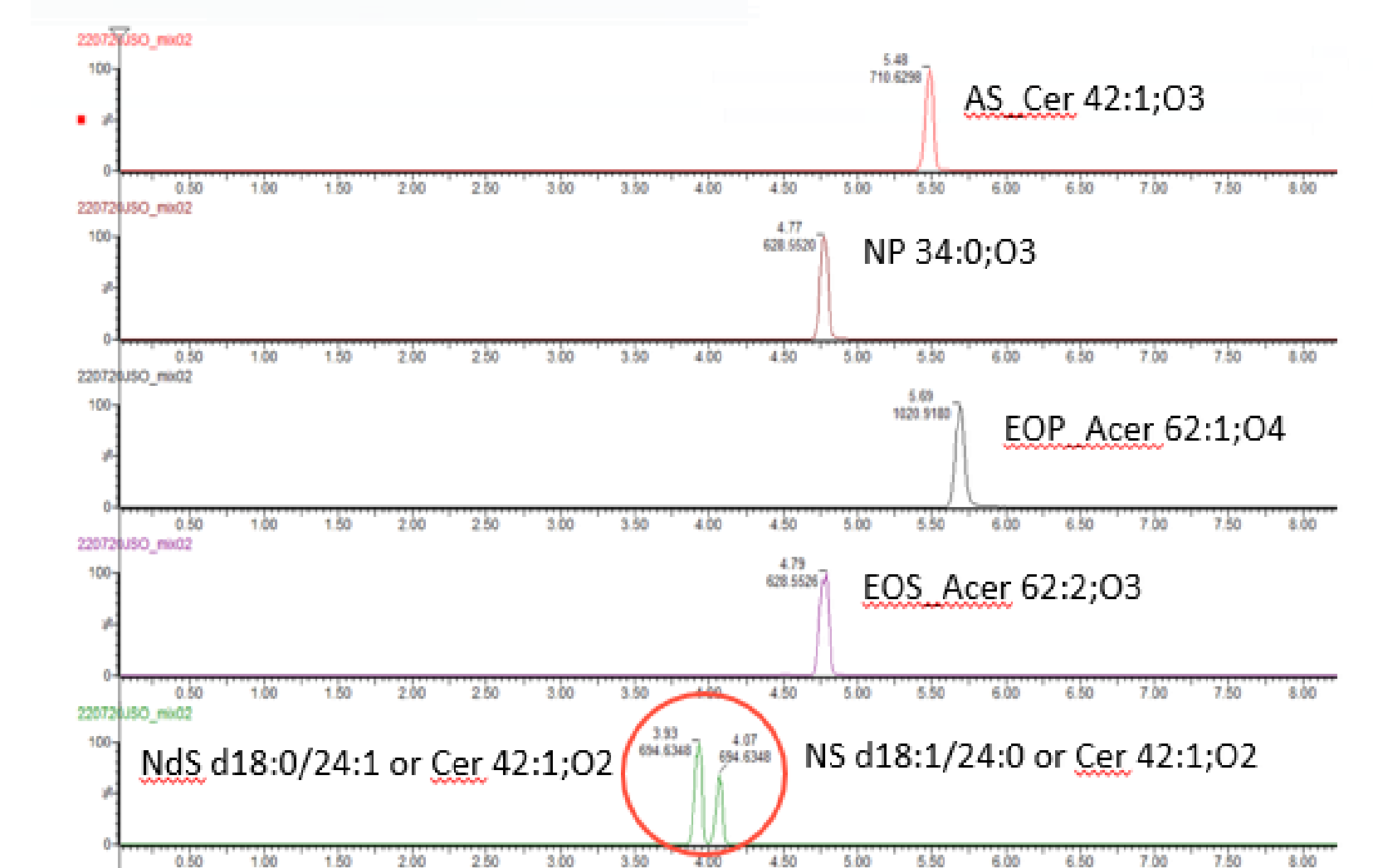
SFC-QTOF analysis

✓ 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

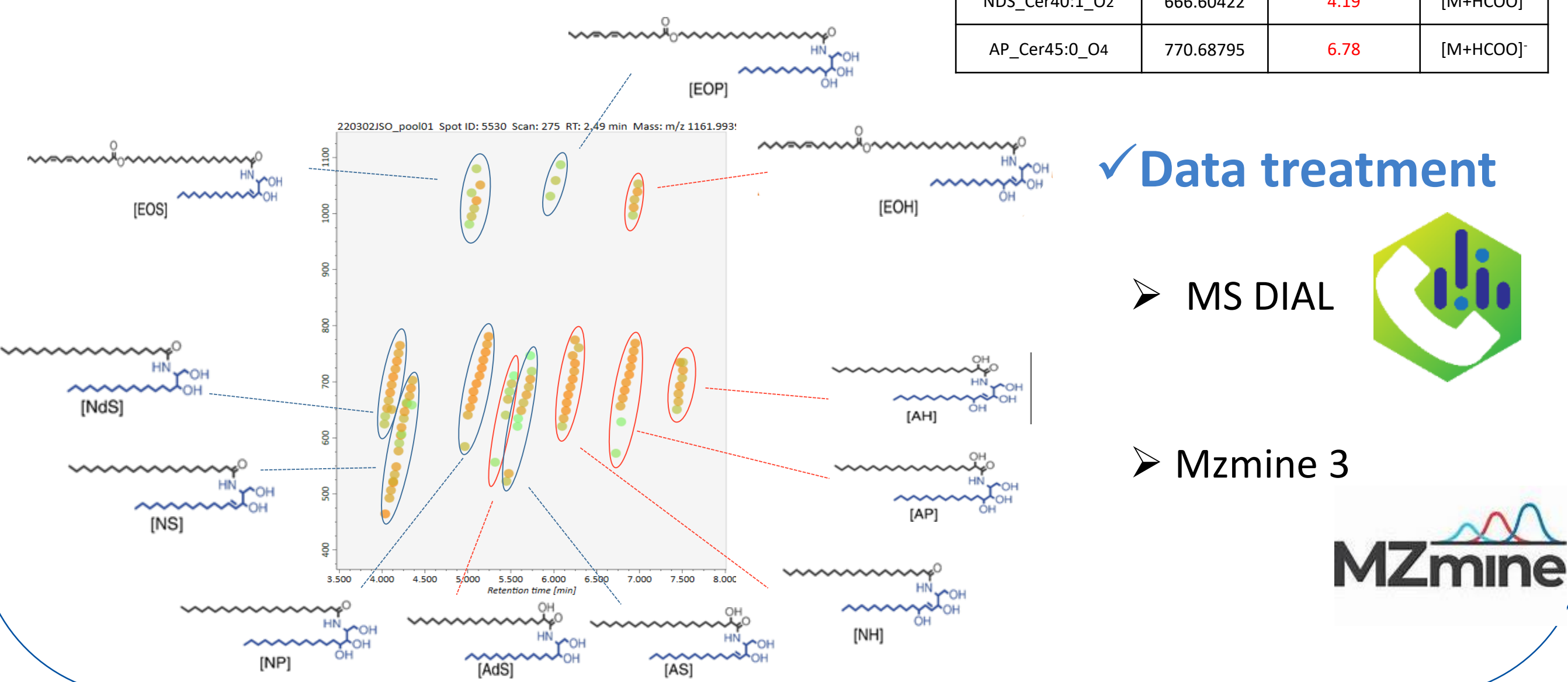


Data Processing

✓ 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

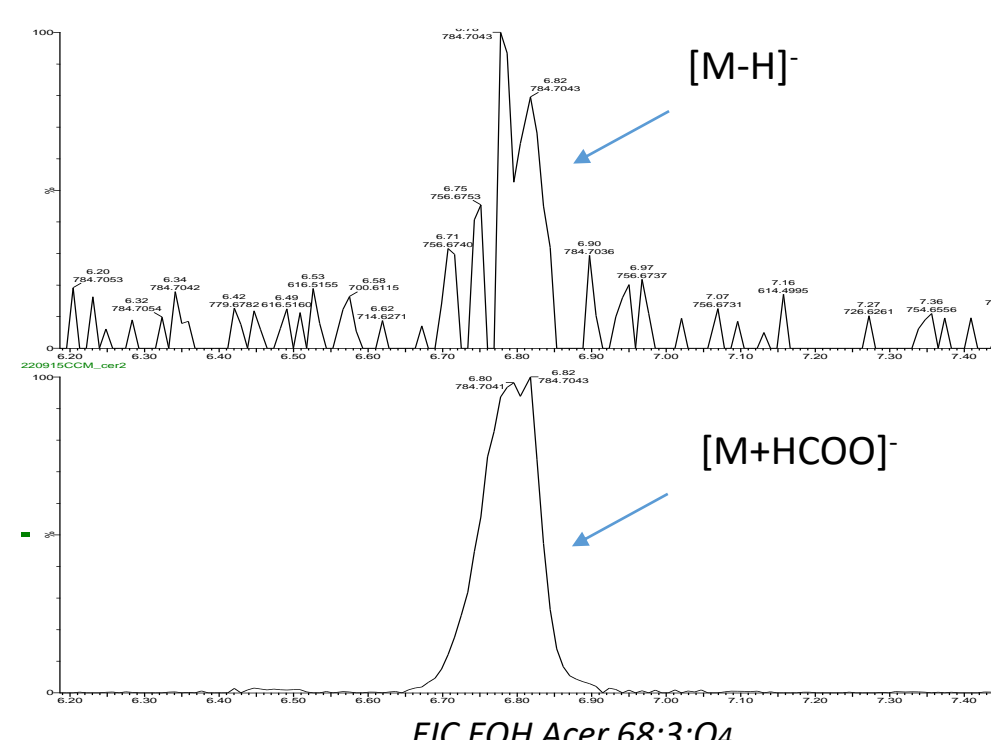
➢ MS DIAL

➢ Mzmine 3

Ceramides characterization by MS/MS acquisition

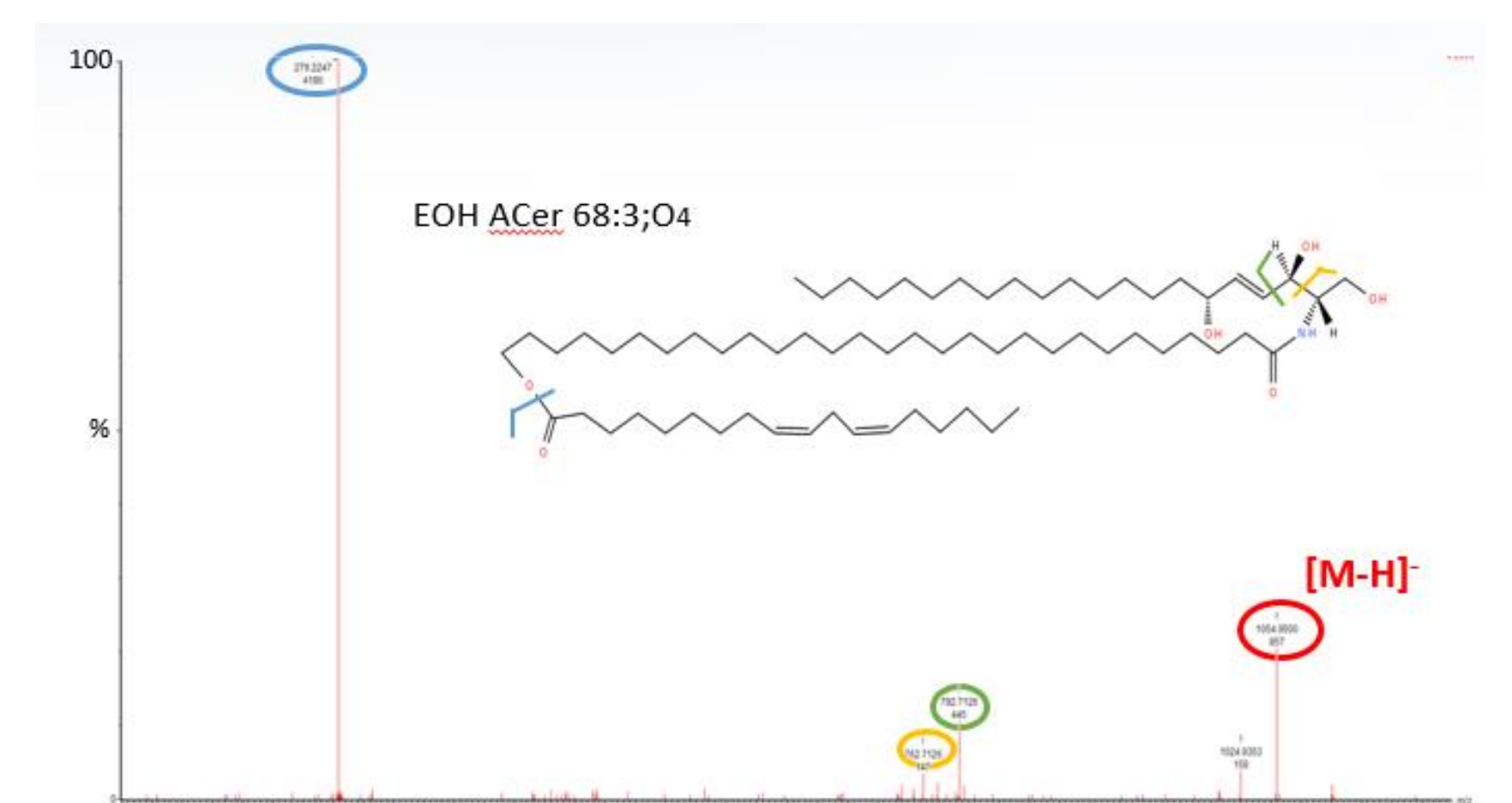
✓ Mass detection

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

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