Shotgun Lipidomics with High Analytical Confidence

Julio L. Sampaio[1]; Kai Schuhmann[2]; Madalina Oppermann[3]; Kai Simons[1]; Markus Kellmann[3]; Andrej Shevchenko[2]

[1]Lipotype GmbH, Dresden, Germany; [2]MPI-CBG, Dresden, Germany; [3]Thermo Fisher Scientific, Bremen, Germany

Aim

Ceramides can be identified in **high resolution** instruments, by **top-down** (from the intact precursor mass) or bottom-up (from MSMS) experiments in both positive and negative ion mode. We propose that **the correlation of quantitative profiles** from these 4 orthogonal and complementary identification strategies **increases the identification confidence** by **decreasing the false positive identifications.**

Introduction

- Lipidomics **lacks consensual criteria** for reliable lipid identification and quantification. This contributes to **poor inter-laboratory concordance** of lipid profiles.
- **Ceramides are prone to false positives** due to the relative simplicity of their atomic composition.
- **Ceramide structural variability** impacts on the fragmentation pathways making the analyses of MS/MS spectra cumbersome in complex lipid extracts.
- In **high resolution tandem mass spectrometers**, lipid species of the same class can be identified and quantified in orthogonal and complementary ways.

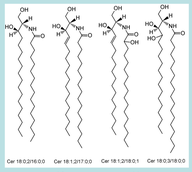
Methodology

Automated Shotgun Lipidomic platform

- **Robotic sample preparation and infusion** for high-throughput analysis (**200 samples/day**).
- MS analyses in the **QExactive** allows:
  - acquisitions in positive and negative ion mode with **fast polarity switch**
  - fragmentation of all masses (DIA) from both polarities in **less than 5 minutes**.

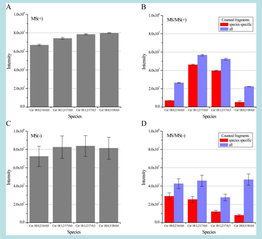
Approach

Ceramide structure variability in mammalian cells



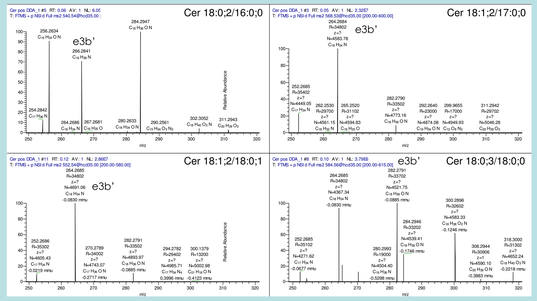
Ceramides in mammalian cells can be composed of sphinganine (18:0;2), sphingosine (18:1;2) or phytosphingosine (18:0;3) amide linked to fatty acids or alpha-hydroxylated fatty acids.

Ceramide ionization and fragmentation efficiency



Although the **ionization of ceramides** is **comparable in both polarities**, the **fragmentation is structure dependent** so **correction factors** need to be calculated for accurate quantification.

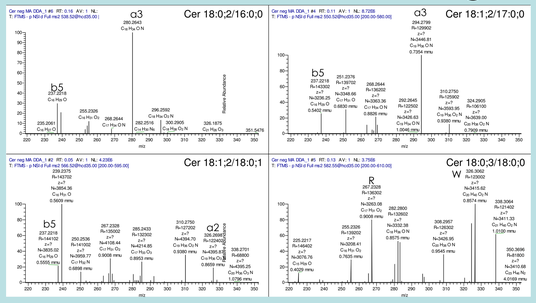
Ceramide fragmentation in positive ion mode…



Positive ion mode	18:0;2/16:0;0	18:1;2/17:0;0	18:1;2/17:0;1	18:0;3/18:0;0
Fragments	e4b' (284.2947) **e3b' (266.2841)** (256.2634) e4b'' (254.2842)	e4b' (282.2791) **e3b' (264.2685)** e4b'' (252.2686)	e4b' (282.2790) **e3b' (264.2684)** e4b'' (252.2685)	C18H40O3N (318.3000) C18H38O2N (300.2896) **C18H36ON (282.2791)** C18H34N (264.2685)
Reference	Hsu et al. JASMS 2002	Hsu et al. JASMS 2002	Hsu et al. JASMS 2002	

Fragmentation of ceramides **in positive ion mode only provides structural information relating to the LCB**. Moreover, the different standards show **redundant fragmentation** (*e. g.* m/z = 264.264) making it impossible to reliably identify and quantify the different ceramide backbones if they were to co-exist in the same sample.

… and in negative ion mode

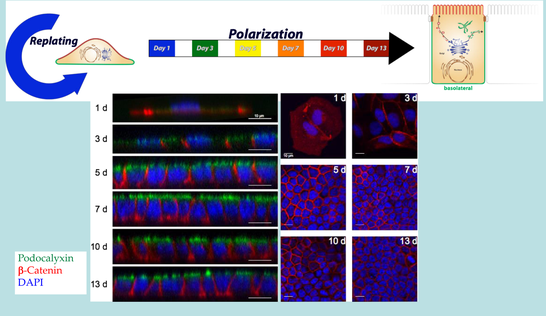


Negative Ion Mode	18:0;2/16:0;0	18:1;2/17:0;0	18:1;2/17:0;1	18:0;3/18:0;0
Fragments	a1 (296.2584) **a3 (280.2635)** a4 (255.2319) b4 (239.2369) **b5 (237.2213)**	a1 (310.2752) **a3 (294.2802)** a4 (268.2646) b4 (251.2380) **b5 (237.2213)**	**a2 (326.3062)** a3 (310.2750) a4 (285.2433) a6 (267.2328) a8 (239.2375) **b5 (237.2213)**	X (338.3065) **W (326.3065)** T (308.2959) U (282.2802) **R (267.2330)** Q (255.2330) P (225.2224)
Reference	Hsu et al. JASMS 2002	Hsu et al. JASMS 2002	Hsu et al. JASMS 2002	Lee et al. RCM, 2002

Fragmentation **in negative ion mode** is richer than in positive ion mode providing **complementary structural information relating to both LCB** (*e. g.* b5) and **amide linked fatty acid** (*e. g.* a3) .

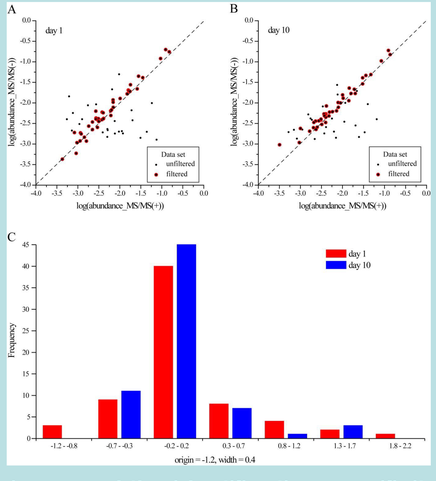
Application

Polarization of epithelial cells



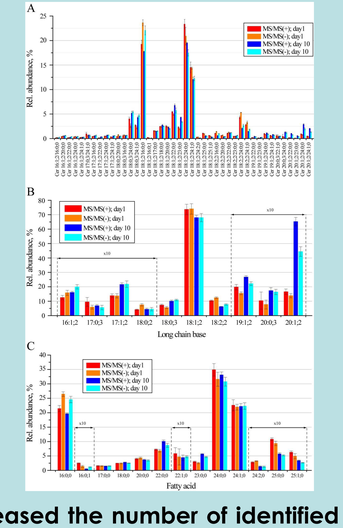
MDCK cells are a model for epithelial formation. **Ceramide remodelling** is required in this process since the newly formed apical membrane is enriched in sphingolipids.
It has been shown previously (Sampaio, et. al., PNAS, 2011) that, with the progression of polarization of MDCK cells, **ceramides get longer and more hydroxylated** but it remained unclear if these changes occurred at the LCB or fatty acid level.

Correlation of positive and negative ion mode data



We **increased the identification specificity** by correlating independent profiles and observed that aprox. **30%** of tentatively matched lipids showed **poor correlation** and were discarded as **false positives**, leaving **31 consistently determined** individual molecular species.

Detailed ceramide profile



We **increased the number of identified species by 20%** providing **full structural characterization** of the ceramide molecules.
Ceramide remodelling occurred mainly at the **long chain base.**
This **approach is generic** and can be **extended to other lipid classes.**