Lipid-class specific internal standard normalization of HILIC-MS/MS data embedded into untargeted data processing and interactive exploration

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Background

The field of lipidomics aims to study the diversity, abundance, and distribution of lipids within biological samples. Lipids are involved in important cellular functions such as the stabilization of cell membranes. Alterations of the lipidome have been shown to be correlated to changes in human health and disease.

In this context, the **complexity of lipid samples calls for appropriate normalization methods** which take into account both sample preparation and instrument-specific variations as well as the ionization efficiency of different lipid classes.

We present a **new software workflow that allows for accurate and precise normalization of lipid class concentrations** using stable isotope labelled internal standards (SIL-IS) that are representative for each lipid class.

LC Method

Lipid class separation was achieved on an iHILIC Fusion(+) column in 18 minutes, obtaining a **coelution of internal standards with their respective phospholipid class**.

Time [min]	0	0.2	8.2	8.5	11.5	12.0	18.0
% B	97	97	75	60	60	97	97

Tab. 1 Binary gradient for lipid class separation by HILIC using A: ammonium formate (35 mM; pH 3.5; 5 % acetonitrile) and B: Acetonitrile.

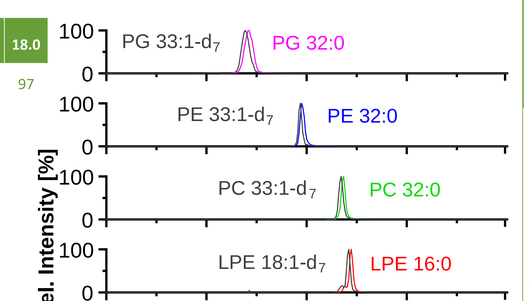

Fig. 1 Lipid class HILIC separation of PG, PE, PC, LPE, SM and LPC. A coelution of phospholipids and internal standard can be achieved by HILIC.

Data Processing

The data was processed and analyzed with **MetaboScape® 2023b** and the T-ReX® 4D workflow (Bruker).

Prior to untargeted feature finding, the **configured internal standards are detected** in a targeted fashion.

Internal standards may be **assigned to a lipid category**, lipid class or lipid subclass, according to the LIPID MAPS hierarchy.

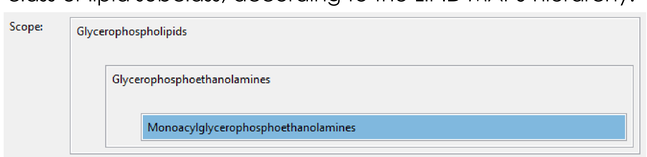

Fig. 2 Assignment levels of internal Standards, exemplified by 18:1(d7) Lyso PE.

After preprocessing, **rule-based lipid annotation** is applied, which also **classifies annotated features into the same hierarchy**.

Intensity normalization is then applied to all features which are covered by an IS according to their classification.

A **configurable dilution factor** allows to account for the dilution of the SPLASH® LIPIDOMIX®, if desired by the user.

This poster in a nutshell:

New software workflow that allows for accurate and precise normalization of lipid class concentration

Data were processed and analyzed with MetaboScape® 2023b and the T-ReX® 4D workflow

Normalization based on class-specific internal standards improved the mean relative standard deviation of the covered lipids from 14.3% to 8.7%.

Survey Plot

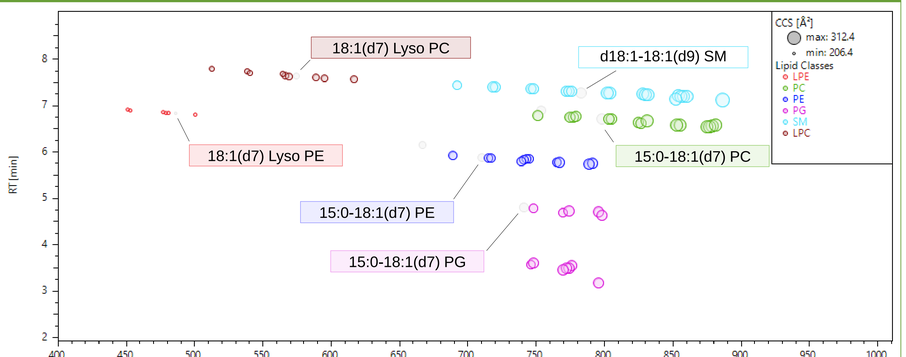

Fig. 3 Detected deuterated internal standards and lipids of the respective classes plotted on m/z vs retention time. The size of the markers represents the measured collisional cross section.
(* The lipids eluting between 3.2-3.6min were annotated as bis(monoacylglycero)phosphate (BMP). Lipid species of the regioisomeric classes phosphatidylglycerol (PG) and BMP were normalized by the same deuterated internal standard.)

Conclusions

Name	Neutral Formula	Ion	Conc. [µg/mL]	Standard for Compound Class	Detected
15:0-18:1(d7) PC	C41D7H73NO8P	$[M+HCOO]^-$	160.0	Diacylglycerophosphocholines	yes
15:0-18:1(d7) PE	C38H67D7NO8P	$[M-H]^-$	5.0	Diacylglycerophosphoethanolamines	yes
15:0-18:1(d7) PI	C42H75D7NO13P	$[M-H]^-$	10.0	Glycerophosphoinositols	no
18:1(d7) Lyso PC	C26H45D7NO7P	$[M+HCOO]^-$	25.0	Monoacylglycerophosphocholines	yes
18:1(d7) Lyso PE	C23H39D7NO7P	$[M-H]^-$	5.0	Monoacylglycerophosphoethanolamines	yes
18:1(d7) MAG	C21H33D7O4	$[M-H]^-$	2.0	Monoradylglycerols	no
15:0-18:1(d7) DAG	C36H61D7O5	$[M-H]^-$	10.0	Diradylglycerols	no
15:0-18:1(d7)-15:0 TAG	C51H89D7O6	$[M-H]^-$	55.0	Triradylglycerols	no
d18:1-18:1(d9) SM	C41H72D9N2O6P	$[M+HCOO]^-$	30.0	Phosphosphingolipids	yes
15:0-18:1(d7) PS	C39D7H67NO10P	$[M-H]^-$	5.0	Glycerophosphoserines	yes
15:0-18:1(d7) PG	C39D7H68O10P	$[M-H]^-$	5.0	Glycerophosphoglycerols	yes
15:0-18:1(d7) PA	C36D7H62O8P	$[M-H]^-$	10.0	Glycerophosphates	yes
18:1(d7) Chol Ester	C45D7H71O2	$[M-H]^-$	10.0	Cholesterol Ester	no
Cholesterol (d7)	C27H39OD7	$[M-H]^-$	100.0	Sterols	no

Tab. 2 T-ReX 4D data processing was configured to perform targeted feature detection for the deuterated lipids contained in SPLASH LIPIDOMIX. The targeted feature detection is configured via the neutral formula of the lipids and the expected ion, which allow determination of their theoretical isotopic patterns. The concentrations and compound classes are optional additions for the targeted feature detection but required to leverage the targets as internal standards. The configured compound classes arrange which of the later annotated lipid species will be normalized.

Normalization of 72 lipid species annotated by the rule-based lipid annotation (19 on species level, 53 on molecular species level).