Robust and Versatile Workflow for Routine Lipidomics Analysis

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KEY FINDING

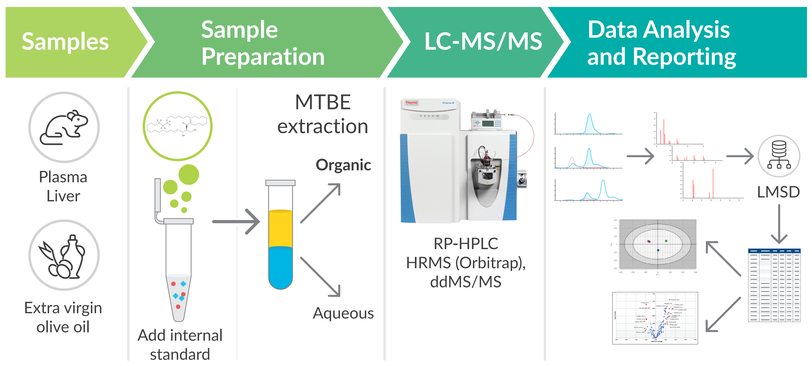
Optimization of data processing steps improves coverage and accuracy of lipidomics analysis.

INTRODUCTION

Identification and quantitation of lipids are the essential objectives of comprehensive lipidomics studies. The high number, diversity, and wide range of lipid abundances pose major challenges to the analysis of lipids by mass spectrometry and to the interpretation of complex data from biological samples. Such analyses typically require extensive and time-consuming manual curation to avoid reporting incorrect data and ensure accurate identification and quantitation.

The goal of this study was to establish a simple, robust, and versatile lipidomics LC-MS/MS workflow that increases the reliability of automated identification results and is applicable to a wide variety of biological samples. Herein, we compared lipid profiles in mouse plasma, liver tissue, and extra virgin olive oil with the workflow we developed.

WORKFLOW



Data processing steps (Lipostar 2.0)

Feature (*m/z*@rt) detection
- Peak picking, smoothing, and alignment
- Noise and artifact reduction

Lipid identification
- Lipid Maps Structural Database (LMSD)
- Mass accuracy filter and MS/MS fragment matching
- Assign lipid ID, scores, and confidence level

Quantitation
- Isotope correction
- Adduct clustering
- Normalization to the class-specific internal standard
- Normalization to amount of material (volume, weight)

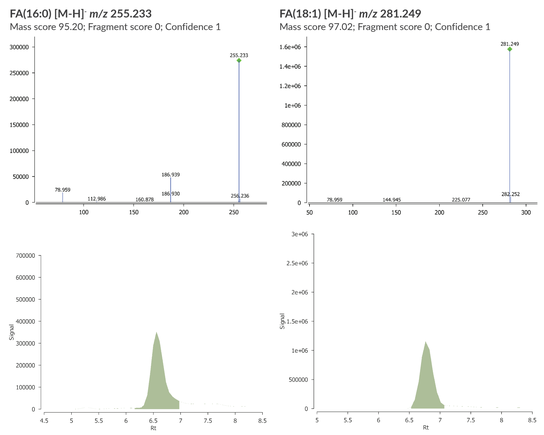
Manual peak review
- Manual review of results to verify identifications
- Report names at the correct structural information level

List of internal standards used

Internal Standard	Used to Normalize:
TG(16:0-$^2H_9$/16:0/16:0)	TG, other pos ions
CE(16:0-$^2H_9$)	CE
DG(16:0-$^2H_9$/16:0/0:0)	DG
SM(d18:1/16:0-$^2H_9$)	SM
Cer(d18:1-$^2H_7$/16:0)	Cer
CAR(17:0)-$^2H_3$	CAR
Cholesterol-$^2H_7$	Cho
PE(16:0-$^2H_9$/16:0)	PE, other neg ions
FA(16:0-$^2H_5$)	FA
PC(16:0-$^2H_9$/16:0)	PC
PI(16:0-$^2H_9$/16:0)	PI
PS(16:0-$^2H_9$/16:0)	PS
LPC(16:0-$^2H_9$)	LPC
FA(16:0/9-O-18:1-$^{13}C_4$)	FAHFA

WORKFLOW OPTIMIZATION

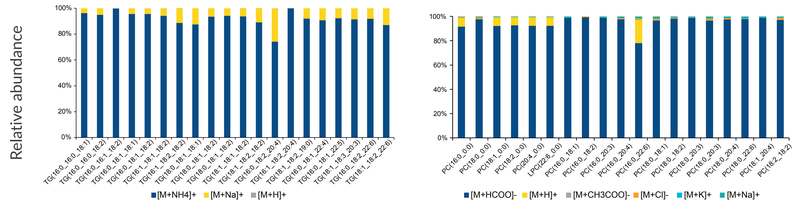
Approval Database to Automatically Identify Lipids Lacking Informative MS/MS Spectra



List of fatty acids in approval database

Lipid	Exact Mass	rt
FA(16:0-$^2H_5$)	260.264	6.52
FA(16:1)	253.217	4.79
FA(16:0)	255.232	6.60
FA(18:2)	279.232	5.06
FA(18:1)	281.248	6.79
FA(18:0)	283.264	8.69
FA(20:2)	307.264	7.28
FA(20:1)	309.279	8.73
FA(20:0)	311.295	10.66
FA(22:1)	337.311	10.55
FA(24:1)	365.349	12.31

Clustering of Signals from Different Adducts Helps Achieve Consistent Quantitation

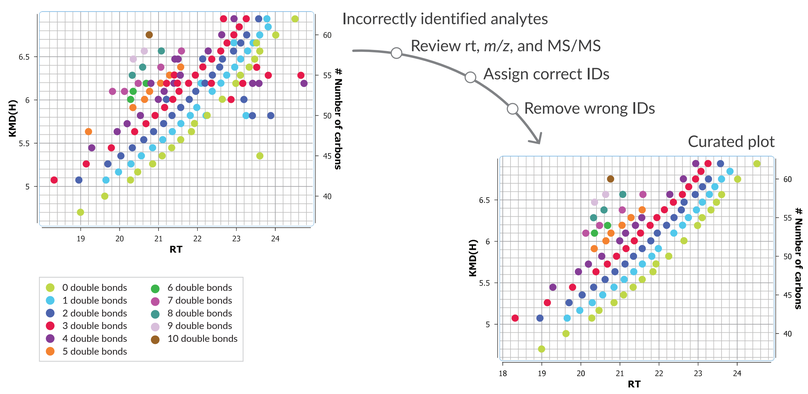


Quality Control Samples Help Monitor Instrument Performance and Precision

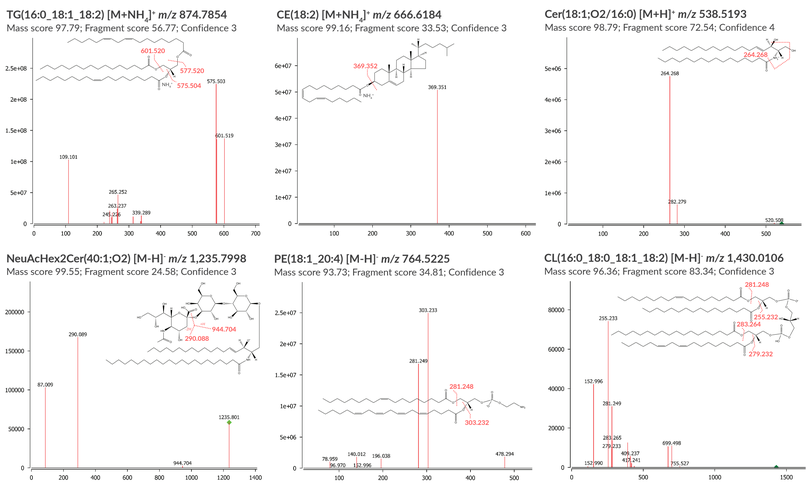
Average percent coefficient of variation of all identified molecular species by lipid class

Lipid Class	Plasma QC	Liver QC	Oil QC
Fatty acids	8.41	11.74	8.67
Diradylglycerols	5.26	9.28	8.54
Triradylglycerols	4.39	6.67	9.36
Glycerophosphocholines	4.27	5.91	11.47
Glycerophosphoethanolamines	7.48	7.37	8.36
Glycerophosphoglycerols	9.81	12.34	9.35
Cardiolipins	11.25	3.85	ND
Glycerophosphoinositols	5.99	7.82	10.08
Glycerophosphoserines	7.86	10.34	7.87
Ceramides	6.28	6.72	6.30
Sphingomyelins	3.70	4.81	6.03
Steryl esters	9.01	9.82	11.46

Kendrick Mass Defect Plots Help Reveal Incorrectly Identified Lipids

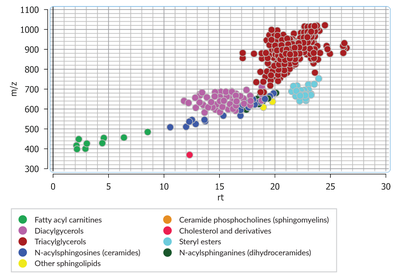


Lipids with Structurally Informative MS/MS Fragments are Identified Correctly with High Score

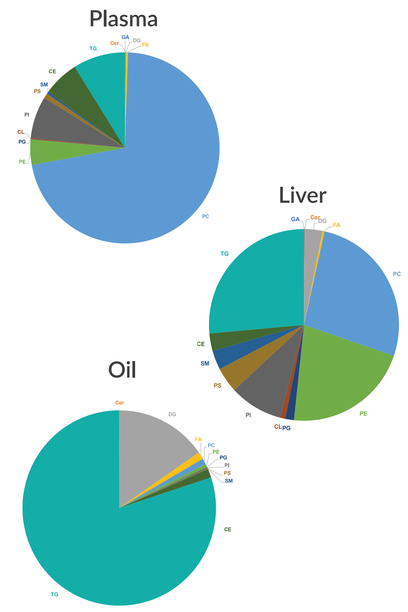


EXAMPLES OF QUALITATIVE AND SEMIQUANTITATIVE DATA REPORTING

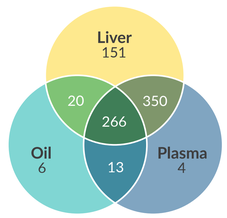
Lipid Separation by RP-HPLC (positive ions)



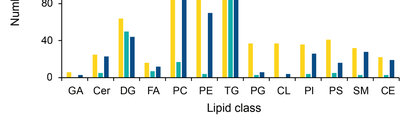
Relative Abundances of Lipid Classes



Matrix-specific Lipid Molecular Species Identified

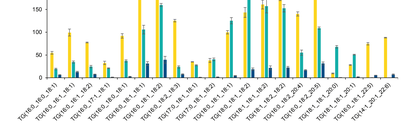


Lipids Identified by Class and Unidentified Features

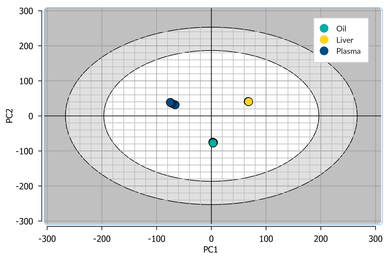


Matrix	Total number of features	
	Identified	Not identified
Plasma	633	4,358
Liver	787	5,321
Oil	305	3,847

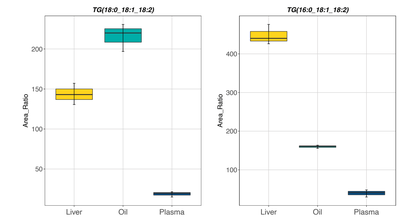
Levels of Selected TG Molecular Species



Principal Component Analysis



Comparison of the Levels of Two Triglycerides



CONCLUSIONS

- Well-established methods used for extraction, RP-HPLC and HRMS/ddMS/MS
- 14 class-specific deuterated internal standards used for signal normalization
- Matrix-specific quality control samples for monitoring of consistent performance
- Optimized data processing that reduces, but does not completely eliminate, manual intervention
  - Class-specific adduct clustering
  - Curated approval database for automated identification of lipids with uninformative MS/MS spectra
  - Kendrick plots to expedite review of incorrect identifications
  - HRMS and MS/MS used to report lipids at the appropriate structural level as recommended by current guidelines
- Further optimization is still required to achieve a fully (or mostly) automated lipidomics platform
  - Refining acquisition and filtering parameters to improve identifications
  - Expansion of the approval database to identify more non-fragmenting lipids
  - Complementing the LMSD with additional molecular species
  - Refinement and expansion of fragmentation rules

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