Evaluation of the Quantification Capabilities of Untargeted Lipidomics Approaches

Paul D. Kennedy, Dilrukshika S.W. Palagama, Matthew J. Kwiatkowski, Samantha K. Goodwin, Miguel A. Gijón

Cayman Chemical, Ann Arbor, MI, USA

KEY FINDING

Untargeted lipidomics analysis provides useful quantitation of hundreds of molecular species in human plasma using single-point or multipoint calibration, the latter being more accurate across all lipid classes.

INTRODUCTION

- Untargeted lipidomics uses MS to analyze hundreds of molecular species in biological samples.
- Single-point calibration based on the known amounts of internal standards is used in many studies, especially those using shotgun (i.e., without chromatography) MS analysis.
- Questions remain about the accuracy of single-point calibration compared to the traditional interpolation in multipoint calibration curves, as well as its suitability in LC-MS studies, where internal standards may not coelute with most of the endogenous analytes.
- The objective of this study is to assess the accuracy of quantitation of lipids in human plasma by LC-MS using either single-point or multipoint calibration curves with authentic or surrogate lipid standards, using a new preparation of human reference plasma and methods validated by comparison with published values from the NIST SRM 1950 Metabolites in Human Plasma material.

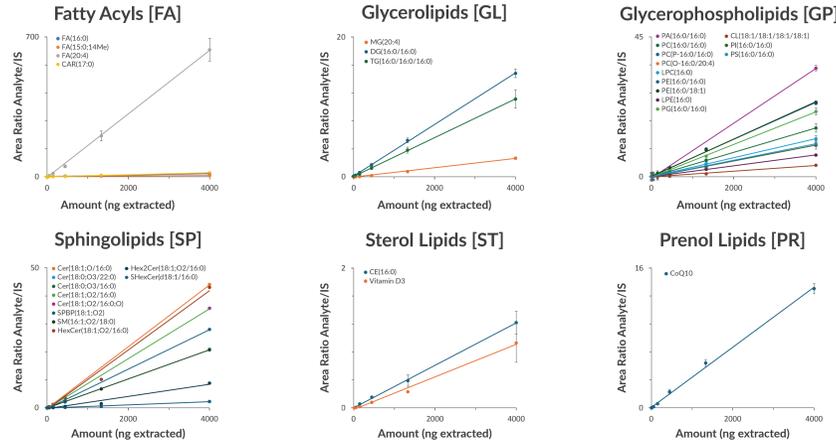*(The authors declare no competing financial interest.)*

WORKFLOW
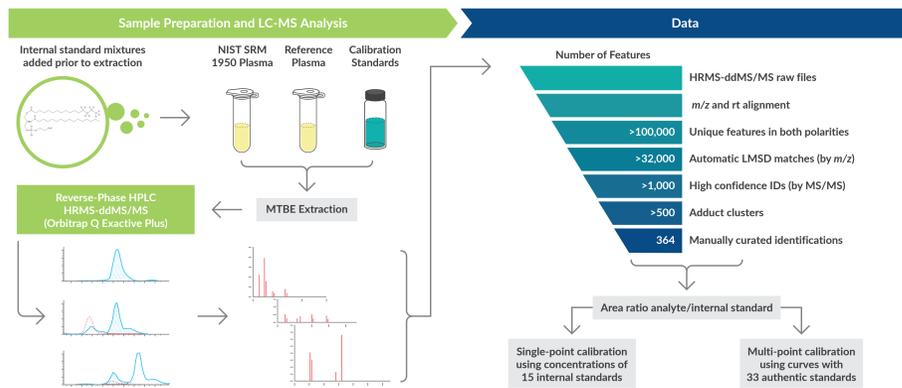
Calibration Standard	Internal Standard Used	LLOQ (ng)	ULOQ (ng)
FA(16:0)	FA(16:0-d₃)	49.4	4000
FA(15:0;14Me)	FA(16:0-d₃)	49.4	4000
FA(20:4(5Z,8Z,11Z,14Z))	FA(16:0-d₃)	1.82	4000
CAR(17:0)	CAR(17:0)-d₃	0.2	4000
MG(20:4)	TG(16:0-d₅/16:0/16:0)	5.4	4000
DG(16:0/16:0)	DG(16:0-d₅/16:0/0:0)	0.6	4000
TG(16:0/16:0/16:0)	TG(16:0-d₅/16:0/16:0)	0.2	4000
PA(16:0/16:0)	PE(16:0-d₅/16:0)	5.4	4000
PC(16:0/16:0)	PC(16:0-d₅/16:0)	0.6	4000
PC(P-16:0/16:0)	PC(16:0-d₅/16:0)	1.8	4000
PC(O-16:0/20:4)	PC(16:0-d₅/16:0)	5.4	4000
LPC(16:0)	LPC(16:0-d₅)	0.6	4000
PE(16:0/16:0)	PE(16:0-d₅/16:0)	0.6	4000
PE(16:0/18:1(9Z))	PE(16:0-d₅/16:0)	0.6	4000
LPE(16:0)	LPE(16:0-d₄)	0.2	4000
PG(16:0/16:0)	PG(16:0-d₅/16:0)	0.2	4000
LPG(18:0)	PE(16:0-d₅/16:0)	0.6	4000
CL(18:1(9Z)/18:1(9Z)/18:1(9Z)/18:1(9Z))	PE(16:0-d₅/16:0)	5.4	4000
PI(16:0/16:0)	PI(16:0-d₅/16:0)	0.6	4000
PS(16:0/16:0)	PS(16:0-d₅/16:0)	5.4	4000
Cer(18:1;O/16:0)	Cer(d18:1-d₇/16:0)	1.8	4000
Cer(18:0;O3/22:0)	Cer(d18:1-d₇/16:0)	1.8	4000
Cer(18:0;O3/16:0)	Cer(d18:1-d₇/16:0)	1.8	4000
Cer(18:1;O2/16:0)	Cer(d18:1-d₇/16:0)	0.6	4000
Cer(18:1;O2/16:0;O)	Cer(d18:1-d₇/16:0)	0.6	4000
SPBP(18:1;O2)	Cer(d18:1-d₇/16:0)	50	4000
SM(18:1;O2/18:0)	SM(d18:1/16:0-d₃)	1.8	4000
HexCer(18:1;O2/16:0)	Cer(d18:1-d₇/16:0)	0.6	4000
Hex2Cer(18:1;O2/16:0)	Cer(d18:1-d₇/16:0)	1.8	4000
SHexCer(d18:1/16:0)	Cer(d18:1-d₇/16:0)	0.6	4000
CE(16:0)	CE(16:0-d₇)	49.4	4000
Vitamin D3	TG(16:0-d₅/16:0/16:0)	49.4	4000
CoQ10	TG(16:0-d₅/16:0/16:0)	0.6	4000

Internal Standard	Amount Extracted (ng)
FA(16:0-d₃)	400
CAR(17:0)-d₃	50
DG(16:0-d₅/16:0/0:0)	50
TG(16:0-d₅/16:0/16:0)	100
PC(16:0-d₅/16:0)	100
LPC(16:0-d₅)	100
PE(16:0-d₅/16:0)	50
LPE(16:0-d₄/0:0)	100
PG(16:0-d₅/16:0)	100
PI(16:0-d₅/16:0)	100
PS(16:0-d₅/16:0)	100
Cer(d18:1-d₇/16:0)	100
SM(d18:1/16:0-d₃)	100
Cholesterol-d₇	1000
CE(16:0-d₇)	1000

RESULTS

Calibration curves used for multipoint calculations

Effect of volume extracted in the number of curated molecular species quantitated

Lipid Class	Volume of Plasma Extracted (µL)				
	60	20	6.7	2.2	0.74
FA	30	30	27	24	6
CAR	6	6	2	0	0
MG	2	2	2	2	0
DG	28	26	26	27	14
TG	82	82	82	77	36
PA	3	3	3	1	0
PC	63	61	56	49	27
PE	38	38	36	26	11
PG	6	5	5	3	1
PI	21	21	20	18	9
PS	3	3	2	0	0
Cer	22	22	21	18	4
S1P	1	0	0	0	0
SM	23	23	23	20	8
Gangliosides	6	6	6	6	0
SHexCer	3	3	3	2	0
HexCer	10	9	8	8	3
Sterols	21	19	15	15	6
CoQ10	1	1	1	0	0
Total	**369**	**360**	**338**	**296**	**125**

Number of curated molecular species indentified by class

Validation of the calculated concentrations using single-point or multipoint calibration, as compared with the published median of the means (MEDM) for NIST SRM 1950 plasma
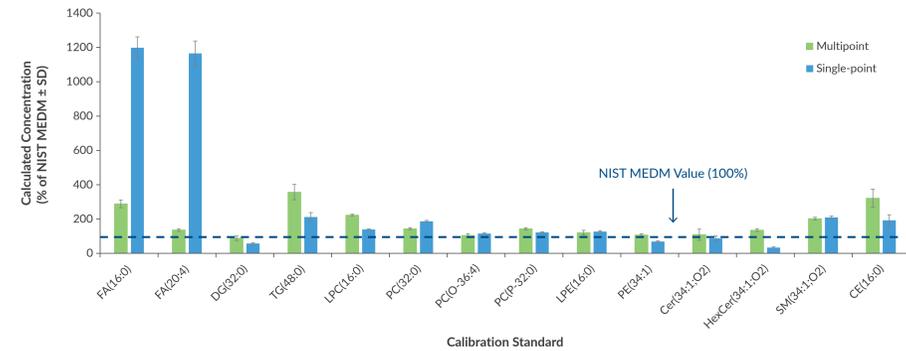
Comparison of total concentrations of each lipid class, as the sum of all molecular species identified, using single-point or multipoint calibration
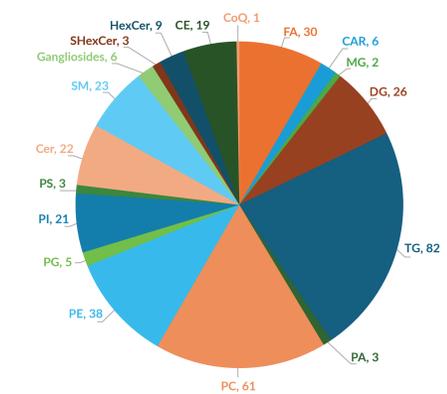
Total lipid concentration (ng/µl), by class

Lipid Class (Abbreviation)	Multipoint Calibration	
	Concentration	SD
Fatty Acids and Conjugates (FA)	380.4	43.2
Monoradylglycerols (MG)	0.5	0.0
Diradylglycerols (DG)	110.5	10.6
Triradylglycerols (TG)	647.0	107.0
Glycerophosphates (PA)	2.2	0.2
Glycerophosphocholines (PC)	804.9	20.2
Glycerophosphoethanolamines (PE)	24.2	2.1
Glycerophosphoglycerols (PG)	15.7	1.1
Glycerophosphoinositols (PI)	46.5	0.2
Glycerophosphoserines (PS)	1.0	0.0
Ceramides (Cer)	14.6	1.2
Phosphosphingolipids (SM)	420.5	20.5
Gangliosides	0.2	0.0
Sulfatides (SHexCer)	0.1	0.0
Neutral glycosphingolipids (HexCer)	6.4	1.0
Sterols (CE)	5399.0	175.6
Quinones (CoQ)	0.8	0.1
Total	**7874.4**	**381.0**

Calculated concentrations (ng/µl) of the sums of glycerophosphocholines using single-point calibration or multipoint calibrations using the curves for three different PC standards

PC Subclass (number of features)	Published MEDM (ng/µl)	Curve Used for Multipoint Calibration			Single-Point Calibration
		PC(16:0/16:0)	PC(O-16:0/20:4)	PC(P-16:0/16:0)	
Diacylglycerophosphocholines (18)	502.9	502.9	502.9	502.9	686.0
1-Alkyl,2-acylglycerophosphocholines (6)	31.6	502.9	502.9	502.9	33.8
1-(1Z-Alkenyl),2-acylglycerophosphocholines (6)	20.7	502.9	502.9	502.9	18.0
Relative Error (%)	-	17.3	157.1	107.1	32.9

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CONCLUSIONS

- Untargeted lipidomics analysis by LC-MS provides excellent linearity of the signals for 33 lipid standards across several orders of magnitude.
- Calculated plasma concentrations using either single-point or multipoint calibration are in reasonable agreement with published data, validating the workflow.
- Although there are differences dependent on the lipid classes monitored, multipoint calibration results in improved accuracy across all the classes.
- Better results are obtained when using the isotopically labeled internal standard corresponding to the calibration standard, and a calibration curve from a structurally similar standard.
- The method presented here allows for the confident identification and quantitation of hundreds of lipid molecular species from sub-microliter volumes of human plasma.