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High Throughput Lipid Identification and Quantification Using a Directed HRAM LC-MS-MS approach on a Modified Quadrupole-Orbitrap Mass Spectrometer

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ABSTRACT

Purpose: Develop a robust and reproducible HPLC MS-MS method which enables untargeted lipid identification and targeted screening of thousands major lipid molecule for getting estimated concentrations of identified lipid species in a single HPLC MS-MS run on a Thermo Scientific™ Q Exactive™ HF-X hybrid quadrupole-Orbitrap™ mass spectrometer.

Methods: Analysis of lipid extracts from bovine heart and human plasma. An isotopically labeled lipid standard mixture (SPLASH™ Lipidomix® Mass Spec Standard, Avanti Polar Lipids) was spiked into each sample prior to lipid extraction. A large inclusion list (positive and negative ions) covering 4074 lipid molecular species from 14 major lipid classes was generated in-silico using Thermo Scientific™ LipidSearch™ software and used to direct the LC MS-MS data acquisition. LipidSearch pre-release software was used for lipid identification and quantitation. The estimated concentrations of identified lipid species from 14 major lipid classes were calculated relative to the known concentration of internal standards included in the SPLASH mixture.

Results: A short synopsis of the extensive results obtained in this single experiment.

INTRODUCTION

Lipids play a key role in cell, tissue and organ physiology. Diseases such as cancer and diabetes involve disruption of metabolic enzyme pathways. Lipidomics studies aim to identify and quantify thousands of cellular lipid species in order to provide a more detailed understanding of the biological function of lipids and subsequently to identify unique lipid biomarkers for early disease detection.

Traditionally, two different lipidomics approaches are employed using MS-based platforms:

1) Untargeted approaches are designed to analyze all detectable lipids in total lipid extracts including unknowns without prior knowledge. This unbiased approach is preferred for discovering novel biomarkers and unique lipid species that play a significant biological role in systems biology.
2) Targeted approaches are used to analyze pre-defined groups of lipids based on prior knowledge. This approach is biased and is preferred for putative biomarker confirmation.

However, it remains challenging to detect low abundance lipid species efficiently and carry out absolute quantitation for the identified lipid molecular species through the untargeted approach. Since only the targeted lipid species are measured the main limitation of a targeted approach is that all other lipid information is lost requiring re-analysis if the expected biomarkers are not confirmed.

Our goal for this study is to merge the benefits of the untargeted and targeted approaches into a single LC-MS/MS workflow to enable identification of more lipid species and quantification over major lipid classes in a high-throughput fashion. The results presented here demonstrate that thousands of individual lipid species can be identified and quantified from complex biological samples. The newly-developed workflow implements a very large pre-defined lipid precursor ion inclusion list for directed MS/MS data acquisition on a Q Exactive HF-X mass spectrometer. This comprehensive approach allows simultaneous unbiased novel lipid identification and determination of estimated concentrations for more than 1000 lipid species while targeting more than 4000 targeted lipid species across 14 lipid classes in a single HPLC MS-MS run.

MATERIALS AND METHODS

Sample Preparation

Total lipid extract (25 mg/mL) from bovine heart was purchased from Avanti Polar Lipids, Inc. An organic solvent mixture of IPA/MeOH (1:1) was used to dilute the bovine lipid extract to a final concentration of 0.5 mg/mL containing the SPLASH standard mixture which was spiked in with 1:10 dilution factor. Two human EDTA plasma samples were purchased from BioServe Biotechnologies which were recovered from whole blood of healthy volunteers and diabetic patients, respectively. Each plasma sample aliquot (60 µL) was spiked with 10 µL of SPLASH standard before lipid extraction. Human plasma lipids were extracted using chloroform, methanol and water[1], dried down and reconstituted into 100 µL of IPA/MeOH (1:1) giving a 1:10 dilution of the internal standards.

HPLC Conditions

A Thermo Scientific™ Vanquish™ UHPLC system performed separations using the gradient conditions shown in Table 1. Mobile phase A was 60:40 Acetonitrile / Water and mobile phase B was 90:10 IPA / Acetonitrile; both A and B contained 10mM ammonium formate and 0.1% formic acid. The column was a Thermo Scientific™ Accucore™ C30 column (2.1 x 150mm, 2.6µm) operated at 45 ℃, flow rate of 260 µL/min. The injection volume was 2 µL for bovine heart and 3 µL for human plasma injected in triplicate.

MS Conditions

A Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap™ mass spectrometer and a Q Exactive HF-X mass spectrometer were used. Table 2 shows the MS instrument setup and Table 3 shows the number and fatty acid group range of targeted lipid species from the inclusion lists (positive and negative ion).

Table 1. HPLC Gradient

Time	%A	%B
-3	70	30
0	70	30
2	57	43
2.1	45	55
12	35	65
18	15	85
20	0	100
25	0	100
25.1	70	30
28	70	30

Table 2. MS Set-Up

HESI Source	Q Exactive HF/HFX
Sheath gas 40; Aux gas 10	MS, R = 120K FWHM at m/z 200 Pos: 250 - 1200 amu; Neg: 250 -1000 amu
Spray voltage 3200	MS/MS, inclusion list enabled; Top 20 dd MS-MS, 35 ms max.; R=15K FWHM at m/z 200; Top 15 dd MS-MS, 65 ms max.; R=30K FWHM at m/z 200
S-Lens 50 (QE HF)	MS/MS Isolation Width 1.0 Da
RF lens 40 (QE HFX)	Stepped NCE; Pos.: 25, 30; Neg.: 20, 30, 40
Cap. Temp. 300	MS AGC target, 1E+6
Heater Temp 325	MS/MS AGC target, 1E+5

Table 3. Number of Lipid Species covered by the inclusion lists (positive and negative ion). The precursor ions of targeted lipid species are generated *in-silico* using LipidSearch software. The lipid class selection corresponds to the isotopically-labeled lipid counterparts of the SPLASH standard mixture. The range of fatty acid sum composition per lipid class was selected based on previous discovery experimental data.

Splash Mixture	Lipid Classes	Number of Lipid Species	Fatty Acid Sum Composition
15:0-18:1(d7) PC	PC	492	17:0 - 51:1
15:0-18:1(d7) PE	PE	511	16:0 - 48:2
15:0-18:1(d7) PS(sodium salt)	PS	398	27:1 - 48:6
15:0-18:1(d7) PG(sodium salt)	PG	332	32:1 - 49:6
15:0-18:1(d7) PI(ammonium salt)	PI	188	32:1 - 42:6
15:0-18:1(d7) PA(sodium salt)	PA	302	33:1 - 49:8
18:1(d7) LPC	LPC	129	14:0 - 33:3
18:1(d7) LPE	LPE	54	14:0 - 22:0
18:1(d7) Chol Ester	Chol Ester	57	16:2 - 26:5
18:1(d7) MG	MG	164	15:2 - 38:0
15:0-18:1(d7) DG	DG	509	28:4 - 57:4
15:0-18:1(d7)-15:0 TG	TG	759	30:0 - 72:5
18:1(d9) SM	SM	178	30:2 - 53:6
Cholesterol (d7)	Cholesterol		

Data Processing

LipidSearch 4.1 SP2 software was used for lipid identification and quantitation. The estimated concentration of identified lipid species across 14 major lipid classes were calculated relative to the isotopically-labeled internal lipid standards included in the spiked-in SPLASH standard.

RESULTS

Directed HPLC MS-MS method development using bovine heart

There are two main goals while developing this workflow. First, we want to get the lipid identification coverage as high as possible. Second, we would like to get estimated concentrations of identified lipid species across major lipid classes. To accomplish these goals, the developed workflow needs to be able to acquire MS-MS data on the eluting lipid species over a wide concentration range. In addition, the workflow needs to provide reproducible quantitative results with low LOD. The new Q Exactive HF-X mass spectrometer implements a brighter ion source and allows more ions into the Orbitrap analyzer compared to the current Q Exactive HF mass spectrometer. With the improved ion transfer efficiency of the Q Exactive HF-X platform, it takes less time to acquire each MS/MS spectrum and thus provides higher lipid identification coverage (Figure 1).

Figure 1. The comparison of the number of identified lipid molecular species from 1µg bovine lipid extract on the Q Exactive HF and Q Exactive HF-X platforms using 2 different MS/MS acquisition conditions. The Q Exactive HF-X MS obtained an average of 18% more lipid species identified using the brighter ion source even while using a short ion injection time (35ms).

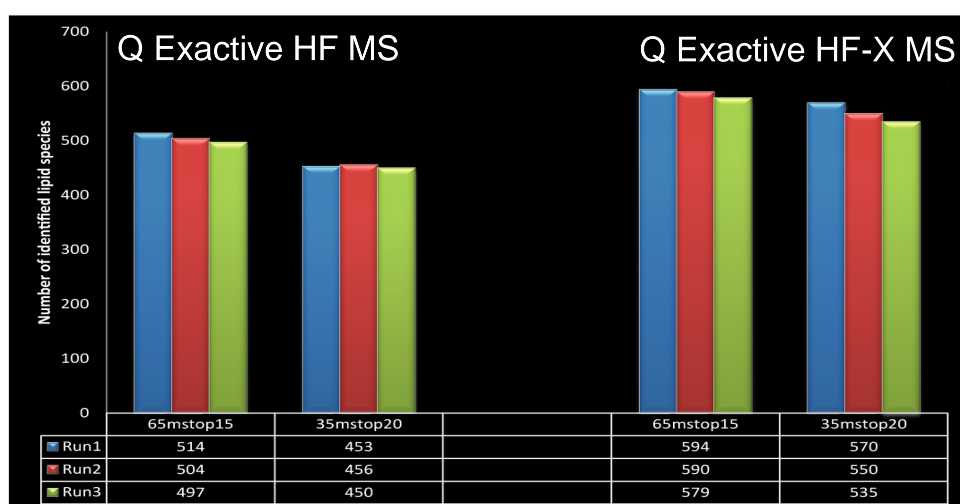


Figure 2. Flow Chart for the Directed HPLC MS-MS Workflow o the Q Exactive HF-X MS

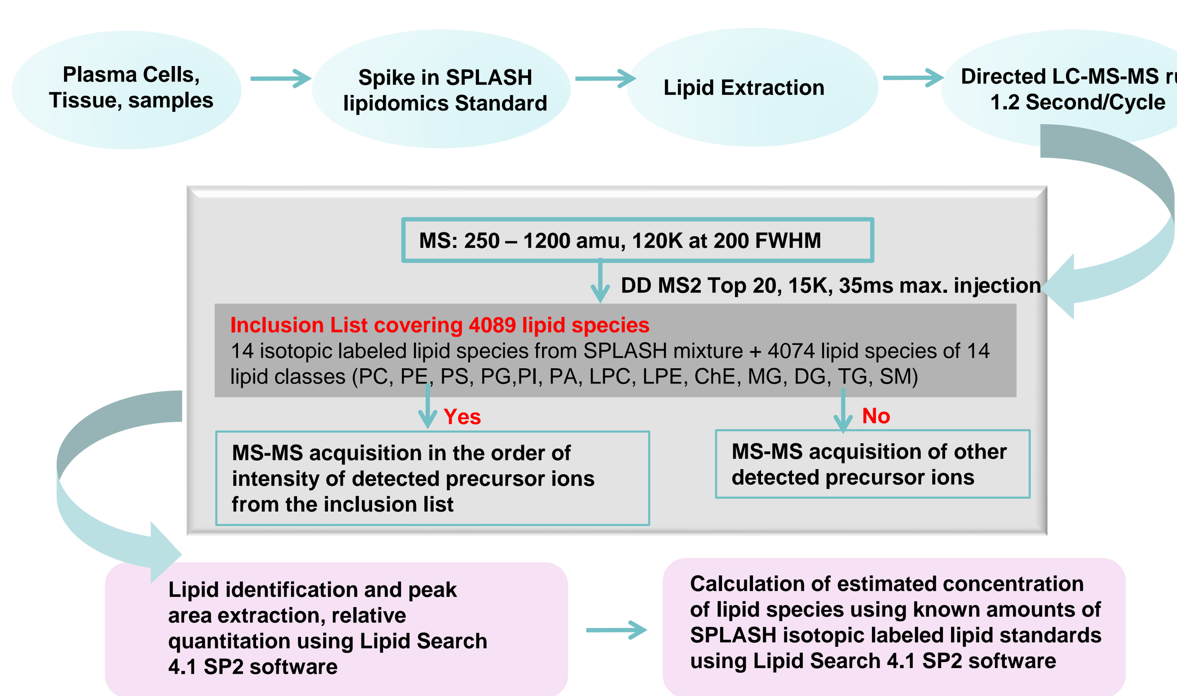
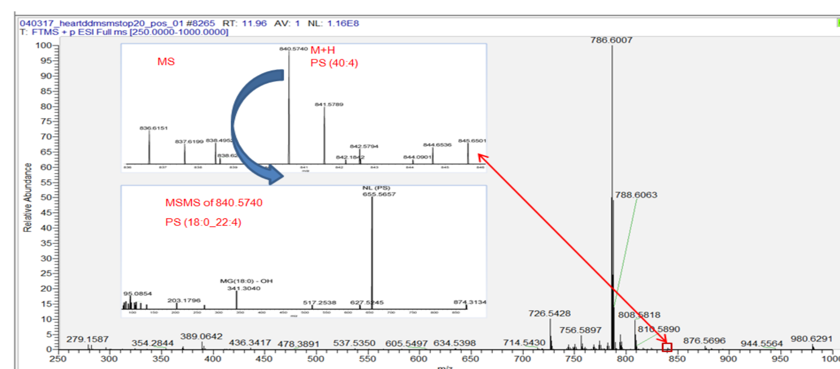


Figure 2 shows the flow chart of the directed LC MS-MS workflow. The workflow is designed to identify and simultaneously estimate the concentration of identified lipid species across major 14 lipid classes. A large inclusion list covering 4089 lipid species including internal standards (Table 3) is used to give priority to the MS/MS acquisition of these known lipids. The directed MS-MS acquisition strategy allows the instrument to efficiently collect MS-MS data for very low abundance lipid species while still obtaining MS/MS of unknown lipid species which are not included. Figure 3 shows the MS[2] spectrum of a very low abundance precursor ion (m/z 840.5740) included in the positive inclusion list that was triggered for MS-MS using the re-directed LC MS-MS workflow. The high quality MS-MS data provided significant fragment ion information to give a confident identification of PS (18:0_22:4).

Figure 3. Identification of a Low Abundance PS Species using the Directed LC MS-MS Workflow



Applying the directed LC MS-MS workflow to human plasma samples for high throughput lipid identification and quantitation

The directed LC MS-MS workflow was used to analyze two human plasma samples (control & diabetic). The lipid identification, relative quantitation and calculation of estimated concentration of identified lipid species using the spiked-in SPLASH standard mixture were carried out using Lipid Search 4.1 Sp2 software. With the directed MS-MS approach, very low abundance lipid ions were triggered for MS-MS, yielding deep lipidome identification coverage over the complex human plasma samples. The majority of the identified lipid species showed reproducible peak areas, yielding precise quantitation results. Figure 4 shows the CV (coefficient of variation) of spiked-in d7-PE (833 ng/mL) was less than 5% in *both* positive and negative ion modes. In this study, 1244 lipid species were quantified with less than 30% CVs. Most lipids were identified from the targeted lipid classes and provided estimated concentrations by using the spiked-in isotopic labeled lipid standards. Table 4 shows partial quantitation results for 125 PE species down to 2 ng/mL range of LOD/LOQ. Overall, concentrations were estimated for more than 1100 lipid species across 13 lipid classes (Table 5).

Figure 4. Observed coefficient of variation for spiked 15:0-18:1(d7) PE (830ng/ml)

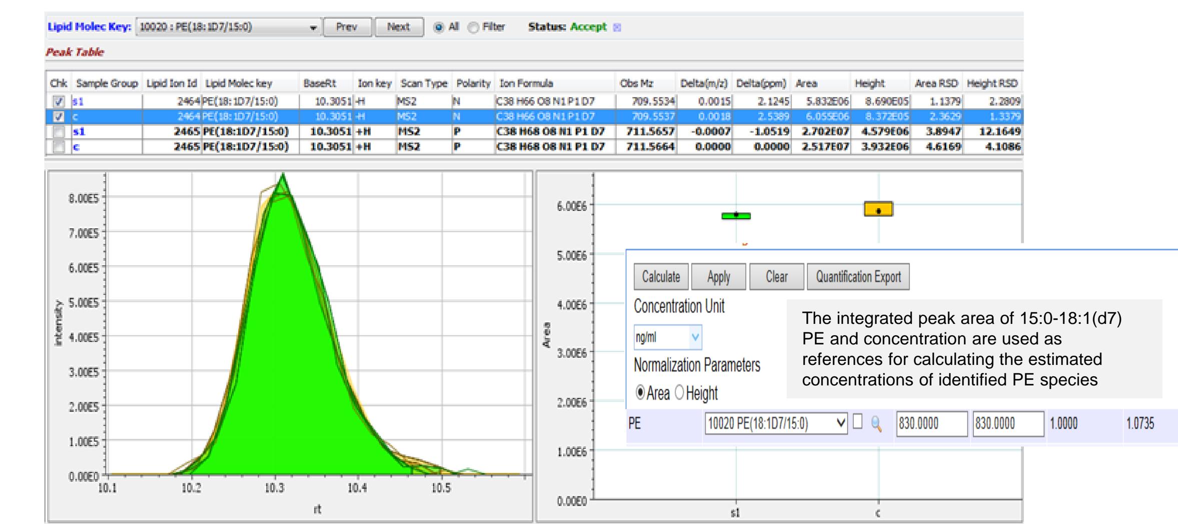


Table 4. Partial list of estimated concentrations of identified 125 PE species and fold changes between the control and diabetic human plasma samples

*c: control *s1: Diabetic

LipidMolec	Calc Mass	Formula	BaseRt	MainArea[c*]	MainArea[s1*]	AreaRSD[c]	AreaRSD[s1]	Estimated concentration (ng/ml). [c]	Estimated concentration (ng/ml). [s1]	Fold change (s1/c)
PE(18:0/16:1)	717.5309	C39 H76 O8 N1 P1	8.945	5.37E+06	4.74E+07	2.1	1.7	177	1455	8.21
PE(16:0/20:5)	737.4996	C41 H72 O8 N1 P1	8.477	2.01E+06	1.49E+07	17.6	3.3	66	458	6.91
PE(18:0/18:3)	741.5309	C41 H76 O8 N1 P1	8.208	1.33E+06	8.11E+06	14.3	1.9	44	249	5.69
PE(18:1/18:2)	741.5309	C41 H76 O8 N1 P1	7.831	1.62E+06	8.98E+06	11.3	7.3	54	276	5.15
PE(18:0/18:3)	741.5309	C41 H76 O8 N1 P1	8.467	7.95E+05	4.22E+06	7.4	2.6	26	130	4.95
PE(18:1/18:2)	741.5309	C41 H76 O8 N1 P1	10.041	1.98E+07	1.04E+08	2.3	1.0	654	3180	4.86
PE(16:0/22:4)	767.5465	C43 H78 O8 N1 P1	11.069	5.50E+05	2.73E+06	9.9	10.1	18	84	4.62
PE(18:1/18:1)	743.5465	C41 H78 O8 N1 P1	11.639	7.24E+06	3.55E+07	17.4	9.6	239	1089	4.57
PE(16:0/18:2)	715.5152	C39 H74 O8 N1 P1	7.636	1.22E+06	5.47E+06	26.8	2.2	40	168	4.17
PE(18:0/20:5)	765.5309	C43 H76 O8 N1 P1	10.443	1.04E+07	4.38E+07	10.9	7.5	343	1345	3.92
PE(16:0/18:2)	715.5152	C39 H74 O8 N1 P1	10.171	7.09E+05	2.92E+06	6.3	6.3	23	90	3.84
PE(18:0/22:4)	795.5778	C45 H82 O8 N1 P1	13.416	3.74E+05	1.51E+06	9.7	6.5	12	47	3.78
PE(18:0/20:3)	769.5622	C43 H80 O8 N1 P1	10.512	6.08E+05	2.45E+06	9.6	1.3	20	75	3.75
PE(20:1/20:3)	795.5778	C45 H82 O8 N1 P1	10.048	1.41E+06	5.60E+06	5.1	2.2	47	172	3.69
PE(16:0/18:2)	715.5152	C39 H74 O8 N1 P1	7.342	2.27E+05	8.65E+05	9.1	4.6	7	27	3.55
PE(18:2/18:2)	739.5152	C41 H74 O8 N1 P1	6.546	6.18E+04	2.34E+05	28.2	5.9	2	7	3.53
PE(18:1/20:5)	763.5152	C43 H74 O8 N1 P1	8.377	3.92E+05	1.44E+06	10.7	5.1	13	44	3.43
PE(18:0/20:3)	769.5622	C43 H80 O8 N1 P1	12.716	2.57E+06	9.21E+06	4.8	5.2	85	283	3.33
PE(38:4)	767.5465	C43 H78 O8 N1 P1	8.168	5.45E+06	1.91E+07	1.1	3.3	180	586	3.26
PE(20:4e)	501.2855	C25 H44 O7 N1 P1	19.878	8.07E+05	2.81E+06	18.9	46.0	27	86	3.24
PE(18:0/18:1)	745.5622	C41 H80 O8 N1 P1	13.877	1.18E+07	3.90E+07	5.5	3.2	388	1199	3.09
PE(34:1e)	703.5516	C39 H78 O7 N1 P1	13.052	2.18E+05	7.03E+05	8.2	4.1	7	22	3.01
PE(34:0p)	703.5516	C39 H78 O7 N1 P1	12.926	2.18E+05	6.92E+05	8.2	3.3	7	21	2.96
PE(18:0/22:4)	795.5778	C45 H82 O8 N1 P1	10.486	1.76E+06	5.47E+06	1.5	2.5	58	168	2.89
PE(18:1/18:2)	741.5309	C41 H76 O8 N1 P1	10.406	5.04E+06	1.54E+07	23.3	2.3	166	473	2.85
PE(18:0p/20:3)	753.5672	C43 H80 O7 N1 P1	13.943	3.50E+06	1.03E+07	0.8	9.8	115	315	2.74
PE(20:2/20:4)	791.5465	C45 H78 O8 N1 P1	7.966	1.40E+06	4.03E+06	1.0	1.3	46	124	2.69
PE(18:1/20:4)	765.5309	C43 H76 O8 N1 P1	9.76	2.62E+07	7.44E+07	5.4	6.0	863	2284	2.65
PE(18:0/20:5)	765.5309	C43 H76 O8 N1 P1	8.046	4.88E+06	1.38E+07	1.9	1.8	161	423	2.63
PE(54:4)	991.7969	C59 H110 O8 N1 P1	19.112	4.15E+05	1.17E+06	5.3	12.2	14	36	2.62
PE(16:0/18:1)	717.5309	C39 H76 O8 N1 P1	11.473	2.37E+07	6.65E+07	4.3	3.7	780	2042	2.62
PE(38:6)	763.5152	C43 H74 O8 N1 P1	6.761	2.23E+05	6.09E+05	5.1	2.2	7	19	2.54
PE(18:0/20:3)	769.5622	C43 H80 O8 N1 P1	13.005	1.91E+05	5.18E+05	6.7	6.4	6	16	2.53
PE(50:2)	939.7656	C55 H106 O8 N1 P1	18.735	4.29E+05	1.13E+06	11.3	17.2	14	35	2.46
PE(18:0/20:3)	769.5622	C43 H80 O8 N1 P1	9.87	2.39E+07	6.20E+07	6.4	3.6	789	1904	2.41
PE(40:2)	799.6091	C45 H86 O8 N1 P1	13.943	2.29E+05	5.86E+05	15.7	4.0	8	18	2.39
PE(18:0/18:2)	743.5465	C41 H78 O8 N1 P1	12.108	2.91E+07	7.25E+07	2.2	2.2	961	2227	2.32
PE(54:3)	993.8126	C59 H112 O8 N1 P1	19.67	3.39E+05	8.34E+05	8.3	13.9	11	26	2.29
PE(36:2e)	729.5672	C41 H80 O7 N1 P1	13.745	5.25E+05	1.27E+06	17.8	16.9	17	39	2.26
PE(16:0/20:4)	739.5152	C41 H74 O8 N1 P1	9.622	8.64E+07	2.06E+08	4.6	8.6	2850	6325	2.22
PE(38:5)	765.5309	C43 H76 O8 N1 P1	12.108	8.30E+06	1.97E+07	4.1	6.2	274	605	2.21
PE(20:1/18:2)	769.5622	C43 H80 O8 N1 P1	9.523	1.38E+07	3.22E+07	2.5	3.0	455	989	2.17
PE(20:0/18:1)	773.5935	C43 H84 O8 N1 P1	13.209	1.23E+07	2.86E+07	3.2	2.9	405	877	2.17
PE(16:0/18:2)	715.5152	C39 H74 O8 N1 P1	9.884	1.52E+07	3.48E+07	1.9	1.9	501	1070	2.14
PE(18:1p/18:1)	727.5516	C41 H78 O7 N1 P1	12.817	3.28E+06	7.17E+06	8.1	9.9	108	220	2.04
PE(20:0/20:3)	797.5935	C45 H84 O8 N1 P1	12.079	9.26E+06	2.00E+07	11.8	5.4	305	615	2.02
PE(40:7p)	773.5359	C45 H76 O7 N1 P1	10.609	8.83E+05	1.86E+06	15.6	6.0	29	57	1.97
PE(18:0/18:2)	743.5465	C41 H78 O8 N1 P1	9.633	6.18E+06	1.29E+07	10.4	3.4	204	395	1.94
PE(52:3)	965.7813	C57 H108 O8 N1 P1	18.937	9.18E+05	1.87E+06	12.9	12.9	30	57	1.90
PE(18:1p/20:4)	749.5359	C43 H76 O7 N1 P1	11.126	1.94E+06	3.91E+06	10.5	4.1	64	120	1.88
PE(39:2)	785.5935	C44 H84 O8 N1 P1	12.669	2.08E+05	4.12E+05	18.8	12.2	7	13	1.84
PE(18:0/18:1)	745.5622	C41 H80 O8 N1 P1	10.858	8.63E+07	1.69E+08	0.2	1.1	2847	5176	1.82
PE(18:1/20:4)	765.5309	C43 H76 O8 N1 P1	10.088	2.29E+06	4.44E+06	1.1	3.0	76	136	1.81
PE(16:0p/22:4)	751.5516	C43 H78 O7 N1 P1	12.127	5.79E+06	1.10E+07	6.9	14.0	191	337	1.76
PE(18:0/22:5)	793.5622	C45 H80 O8 N1 P1	9.231	6.10E+06	1.15E+07	6.7	4.8	201	352	1.75
PE(43:6)	833.5935	C48 H84 O8 N1 P1	9.549	1.99E+08	3.74E+08	2.8	4.5	6576	11489	1.75
PE(40:2)	799.6091	C45 H86 O8 N1 P1	13.537	1.00E+06	1.88E+06	13.1	1.7	33	58	1.74
PE(20:0/20:4)	795.5778	C45 H82 O8 N1 P1	11.203	2.63E+07	2.84E+07	4.2	3.8	867	872	1.01
PE(18:1D7/15:0), IS	710.5591	C38 H67 O8 N1 P1 D7	10.305	2.52E+07	2.70E+07	4.6	3.9	830	830	1.00
PE(18:0p/20:4)	751.5516	C43 H78 O7 N1 P1	12.962	9.90E+07	1.05E+08	1.5	2.6	3265	3220	0.99
PE(37:1)	759.5778	C42 H82 O8 N1 P1	11.994	1.25E+06	1.25E+06	5.0	3.0	41	38	0.93
PE(37:1)	759.5778	C42 H82 O8 N1 P1	11.591	3.68E+05	3.66E+05	13.5	30.4	12	11	0.93
PE(37:2)	757.5622	C42 H80 O8 N1 P1	10.023	1.88E+06	1.79E+06	5.6	5.2	62	55	0.89
PE(19:0/18:2)	757.5622	C42 H80 O8 N1 P1	10.374	3.06E+06	2.91E+06	5.4	8.5	101	89	0.89
PE(18:0e)	481.3168	C23 H48 O7 N1 P1	4.362	3.47E+07	3.23E+07	15.6	8.7	1145	991	0.87
PE(20:0p/20:4)	779.5829	C45 H82 O7 N1 P1	15.305	7.96E+06	7.38E+06	10.4	1.3	262	227	0.86
PE(18:0e/20:4)	753.5672	C43 H80 O7 N1 P1	10.35	5.06E+06	4.70E+06	2.5	4.8	167	144	0.86
PE(20:1p/18:2)	753.5672	C43 H80 O7 N1 P1	10.352	5.09E+06	4.69E+06	0.8	4.4	168	144	0.86
PE(18:0p/20:5)	749.5359	C43 H76 O7 N1 P1	11.513	1.54E+07	1.40E+07	7.1	38.8	506	429	0.85
PE(36:5p)	721.5046	C41 H72 O7 N1 P1	10.575	1.37E+09	1.22E+09	3.7	1.6	45266	37346	0.83
PE(34:0p)	703.5516	C39 H78 O7 N1 P1	14.897	5.81E+05	5.04E+05	19.9	25.9	19	15	0.81
PE(18:0p/22:6)	775.5516	C45 H78 O7 N1 P1	12.377	4.68E+07	4.02E+07	1.4	4.0	1544	1233	0.80
PE(16:0p/20:5)	721.5046	C41 H72 O7 N1 P1	9.346	6.64E+05	5.70E+05	18.9	10.7	22	18	0.80
PE(20:0p/20:4)	779.5829	C45 H82 O7 N1 P1	10.453	2.71E+06	2.28E+06	5.8	0.4	89	70	0.78
PE(18:0e/22:4)	781.5985	C45 H84 O7 N1 P1	11.889	5.93E+05	4.78E+05	11.0	11.3	20	15	0.75
PE(39:4)	781.5622	C44 H80 O8 N1 P1	10.121	7.89E+05	6.35E+05	9.8	9.1	26	19	0.75
PE(18:0p/16:0)	703.5516	C39 H78 O7 N1 P1	11.762	1.58E+06	1.27E+06	4.1	5.5	52	39	0.75
PE(20:0p/20:4)	779.5829	C45 H82 O7 N1 P1	12.247	7.97E+05	5.93E+05	17.8	14.0	26	18	0.69
PE(18:0p/20:4)	751.5516	C43 H78 O7 N1 P1	9.989	5.65E+06	4.00E+06	11.8	8.0	186	123	0.66
PE(20:0e/20:4)	781.5985	C45 H84 O7 N1 P1	12.692	2.15E+06	1.46E+06	9.9	18.2	71	45	0.63
PE(20:0/22:6)	819.5778	C47 H82 O8 N1 P1	10.706	1.19E+07	7.95E+06	7.2	4.6	392	244	0.62
PE(39:6)	777.5309	C44 H76 O8 N1 P1	13.221	5.26E+07	3.11E+07	5.1	11.1	1735	955	0.55
PE(20:0p/22:6)	803.5829	C47 H82 O7 N1 P1	14.834	3.79E+06	2.22E+06	5.3	16.6	125	68	0.55
PE(42:7p)	801.5672	C47 H80 O7 N1 P1	12.31	3.43E+05	1.77E+05	27.4	27.8	11	5	0.48
PE(49:0)	929.7813	C54 H108 O8 N1 P1	21.192	1.02E+06	3.17E+05	10.8	10.2	34	10	0.29
PE(37:5)	751.5152	C42 H74 O8 N1 P1	13.226	2.16E+07	5.18E+06	7.2	39.7	712	159	0.22

Another advantage of the directed HPLC MS-MS workflow is the capability to discover unknown lipid species which are not included in the targeted inclusion list. For untargeted lipids, the workflow still provides lipid identification and fold changes based on the integrated peak area information (Figure 5). The identification and quantitation summary is shown in the Table 5. 1244 lipid species were identified after filtering with main ions per lipid class and CVs. Among them, the estimated concentration results were obtained for 1202 lipid species across lipid classes including ChE, DG, LPC, LPE, MG, PA, PC, PE, PG, PI, PS, SM and TG using the spiked SPLASH internal standard.

Figure 5. Identification and relative quantitation of d18:1/24:1 ceramide

d18:1/24:1 Cer [M+H]+ m/z 648.6278, rt = 17.06min

CV = 5.5%

Ratio = 0.377
p-value = 0.0003

Table 5. Identification and quantitation summary for the two human plasma samples

Lipid Sub-class	Filtered	Estimated concentration
AcCa	7	
Cer	64	
CerG1-G3	29	
ChE	20	√
DG	38	√
LPC	47	√
LPE	9	√
MG	8	√
PA	14	√
PC	188	√
PE	125	√
PG	11	√
PI	59	√
PS	15	√
SM	205	√
TG	405	√
Total Species	1244	1144

CONCLUSIONS

- A robust and reproducible directed LC MS-MS workflow enables untargeted lipid identification and targeted screening of thousands of major lipid species for obtaining estimated concentrations of identified lipid species in a single HPLC MS-MS run on the Q Exactive HF-X mass spectrometer.
- Deep human plasma lipidome coverage was observed using the directed LC MS-MS workflow; 1244 lipid species were detected with CVs less than 30%.
- The concentrations of 1144 lipid species across the major lipid classes were estimated using the spiked SPLASH internal standard mixture. The LOD/LOQ for identified and quantified PE lipid species was 2 ng/mL.
- By using the commercially available SPLASH Lipidomix Mass Spec Standard for estimating the concentration of lipid species, the directed LC MS-MS workflow enables more reliable quantitative comparisons between or among different laboratories and studies.
- Directed LC MS-MS workflow can be applied to any complex biological samples including plasma, serum, tissues, cells and foods.

TRADEMARKS/LICENSING

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