Data Acquisition Parameters Optimization of Quadrupole Orbitrap for Global Lipidomics on LC-MS/MS Time Frame

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Overview

Purpose: Comprehensive optimization of data-acquisition parameters of quadrupole Orbitrap mass spectrometers for global lipid profiling on a LC-MS timeframe.

Methods: Total lipid extract from liver was separated using reversed-phase chromatography and the eluting compounds were detected with a bench-top quadrupole Orbitrap mass spectrometer in data-dependant MS^2 acquisition mode under both positive and negative electrospray ionization conditions. The investigated acquisition parameters included Orbitrap mass resolution, using of exclusion mass list, maximum ion injection time, number of MS^2 experiments per detection cycle (Top-N parameter), normalized collision energy, and the use of stepped collision energies. Acquired data were processed and compared using the newly-released Thermo Scientific™ LipidSearch™ software.

Results: The presented data shows significant increase of identified lipids after optimization of several data acquisition parameters.

Introduction

Two different approaches of mass spectrometry based lipid profiling have been commonly used: shotgun (nano-infusion) and LCMS-based lipidomics. In contrast to nano-infusion experiments, LC-MS experiments require adjustment of MS parameters to meet the chromatographic behavior of investigated compounds. Appropriate adjustments of the acquisition parameters are also necessary to obtain the highest possible number of detected and subsequently identified lipid species. This work investigates crucial mass spectrometric parameters for LC-MS/MS data acquisition on Thermo Scientific ™ Q Exactive Plus™ mass spectrometer for global lipidomics.

Methods

Sample Preparation

Bovine Liver Total Lipid Extract (PN 181104C, 25 mg/mL) was purchased from Avanti Polar Lipids (Alabaster, AL). The sample was diluted 2:98 (v/v) in ACN / IPA / H_2O 47.5:47.5:5 (v/v/v).

Liquid Chromatography

Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system.

HPLC Column: Thermo Scientific Accucore C_{18} (2.1 x 150 mm, 2.6 μm) at 35 °C

Injection Volume: 2 μL
Flow Rate: 400 μL/min

Mobile Phase:

(A) 10 mM $HCOONH_4$ in 50% ACN (v) + 0.1% HCOOH (v);
(B) 2 mM $HCOONH_4$ in ACN / IPA / H_2O 10:88:2 (v/v/v) + 0.02% HCOOH (v)

HPLC Gradient:

Time	A%	B%	Time	A%	B%
0.00	65	35	24.00	0	100
4.00	40	60	24.10	65	35
12.00	15	85	28.00	65	35
21.00	0	100			

Mass Spectrometry

Q Exactive Plus mass spectrometer equipped with HESI-II probe. Polarity detection mode: Positive, Negative. Scan Mode: Data-dependant MS/MS. Mass Range: 250-1200 amu for Full-MS. Ion source settings: Spray Voltage = 3 kV (both ESI pos. and neg.), Vaporizer = 370 °C, Ion Transfer Tube = 285 °C, S-Lens = 45 %, Sheath Gas = 60, Auxiliary gas = 20, Sweep Gas = 1.

Data acquisition settings: AGC (MS) = 1e6, AGC (MS^2) = 1e5, Mass Range = 250 – 1200 Da, Fixed First Mass = 75 Da, Apex Trigger = N/A,

Initial (generic) Acquisition Settings (that were further optimized): Dynamic Exclusion = 6 s, Isolation Window = 1.0 Da, Top-4 experiments, Injection Time = 100 ms, Resolution MS = 70,000 (FWHM at *m/z* 200), Resolution MS^2 = 17,500 (FWHM at *m/z* 200) , Normalized Collision Energy (NCE) = 20 – 30 – 40

Data Analysis

Acquired data were automatically processed using LipidSearch software.

Search parameters: Precursor mass tolerance = 3 ppm, Product mass tolerance = 7 ppm, m-Score threshold = 3.

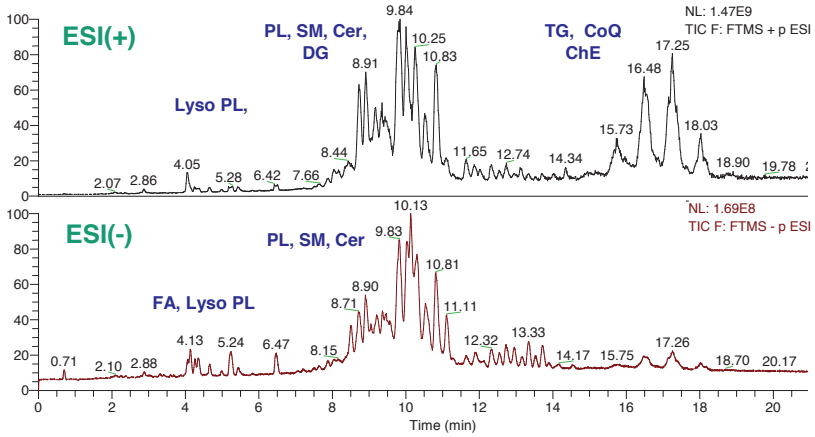
Results

LC-MS analysis and data evaluation

A bovine liver lipid extract (1 µg on column) was analyzed by LC-MS in dd-MS/MS mode under positive and negative electrospray ionization (Figure 1.).

Selected data acquisition parameters were optimized in a subsequent manner. Acquired data (duplicate injections) were processed in LipidSearch and the "numbers of identified lipid species" (with isomers considered to be the same) and the "numbers of identified lipid ions" were tabulated and compared. After each optimization step, the best particular setting (labeled with asterisk *) was used for optimization of the following parameter. The optimization was fully performed in positive ion mode. For negative ion mode, an Exclusion List (first step) was evaluated and optimized parameters from the positive ion mode were applied for optimizing Normalized Collision Energy (final step) .

FIGURE 1. Total ion Chromatograms (MS, R=70,000) of bovine liver lipid extract.



Optimization I. Exclusion Mass List

Exclusion Mass Lists were created from solvent injection runs, by averaging mass spectra (1 – 19 min), and exporting *m/z* values of the most intense ions (10 and 50 for positive, 100 for negative mode). When the Exclusion List was used a very significant increase in the number of identified lipid species was observed (Table 1).

TABLE 1. The effect of using an EXCLUSION LIST on the number of indentified lipids in positive and negative ion mode.

ESI (+)	Lipid Species (Ions)
No Excl. List	347 (659)
Excl. List 10	398 (788)
Excl. List 50 *	**448 (932)**

ESI (-)	Lipid Species (Ions)
No Excl. List	187 (329)
Excl. List 100 *	**216 (391)**

Optimization II. Dynamic Exclusion

Use of Dynamic Exclusion for dd-MS^2 acquisition effectively doubled the number of identified lipid groups (Table 2.). The highest number of identified lipid species was found at12 s exclusion time, whereas the number of identified "lipids ions" was virtually same at 8 s and 12 s. This result indicates that higher Dynamic Exclusion time allows recording MS^2 spectra of the lower intensity ions. However, shorter exclusion time permits obtaining MS^2 data of partially separated isomers as shown in Figure 2 for PC(36:3) isomers. For further optimization, Dynamic Exclusion was set to 8 s to favor acquisition of closely eluting isomers.

Optimization III. Quadrupole Isolation Window

As expected, number of identified lipids increases with a wider quadrupole isolation window (Table 3). However, it also gives a higher chance of false positive IDs. An isolation width of 1.2 Da appears to be a reasonable compromise for a good ion transmission and diminishing false positive lipid IDs.

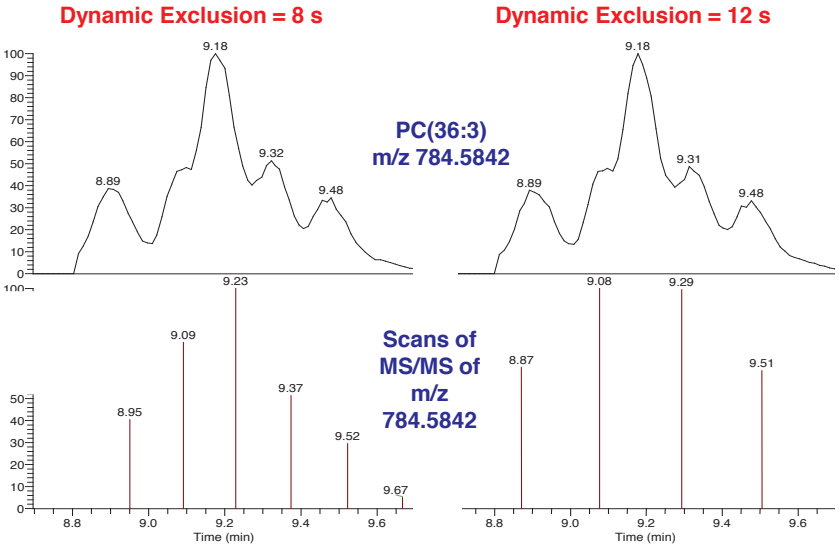
TABLE 2. DYNAMIC EXCLUSION and its effect on number of indentified lipids in positive ion mode.

DYNAMIC EXCLUSION	Lipid Groups (Ions)
No	222 (391)
4 s	452 (994)
6 s	448 (932)
8 s *	497 **(1080)**
12 s	**521** (1073)

TABLE 3. QUADRUPOLE ISOLATION Window and its effect on number of indentified lipids in positive ion mode.

QUADRUPOLE ISOLATION	Lipid Groups (Ions)
0.7 Da	488 (1029)
1.0 Da	497 (1080)
1.2 Da *	517 (1123)
1.5 Da	518 (1109)
2.0 Da	**523 (1172)**

FIGURE 2. DYNAMIC EXCLUSION settings of 8 s and 12 s and the number of MS/MS scans of PC(36:3) isomers, $[M+H]^+$ *m/z* 784.5842.



Optimization IV. FULL-MS RESOLUTION and TOP-N parameter

Physical characteristics the Orbitrap detector allows for 1) faster detection time and lower mass resolution with lower selectivity or 2) longer detection time and higher mass resolution with higher selectivity.

Mass spectrometric selectivity in full-MS data is critical for unambiguous determination of compounds in complex mixtures. Therefore, using very high mass resolution for MS scans is essential for correct identification of lipids in matrices as illustrated for coeluting isobaric lipids PE(18:1/20:5) and PC(16:1p/20:4) at RT = 8.5 min (Figure 4).

Based on the number lipid species identified from the acquired data (Table 4), the optimum combination of MS Resolution and Top-N experiments is observed at R = 70,000 and Top-10 MS^2. Using these parameters 10 MS scans were obtained for a typical chromatographic peak (12 s at the base) and was sufficient to provide MS^2 spectra of partially resolved isomers as demonstrated in Figure 3. More lipids may be identified with higher Top-N parameter, however this would result in less MS scans across a chromatographic peak and loss of information regarding coeluting isomers.

TABLE 4. The effect of MS RESOLUTION and TOP-N parameter on the number of indentified lipids in positive ion mode.

	Top 4	Top 5	Top 7	Top 10 *
MS RESOLUTION		Lipid Species (Ions)		
R = 35,000	-	503 (1216)	510 (1240)	516 (1249)
R = 70,000 *	517 (1123)	518 (1145)	**526 (1156)**	**527 (1148)**
R = 140,000	-	500 (1041)	512 (1073)	522 (1105)

FIGURE 3. XIC of PC(36:3) isomers, $[M+H]^+$ m/z 784.5842, with displayed scans recorded at R = 70,000 and Top-10 setting (Injection time = 75 ms).

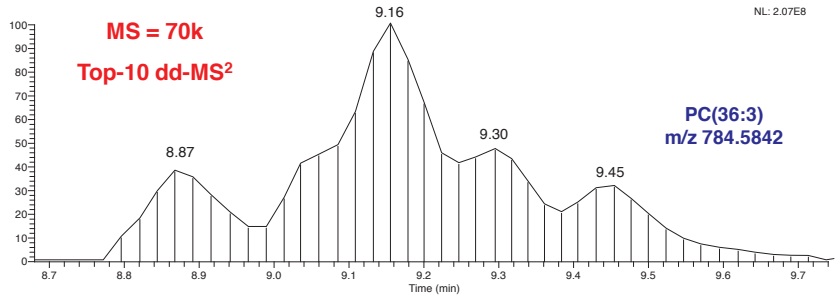


FIGURE 4. Mass spectra of PE(18:1/20:5) [$C_{43}H_{75}NO_8P$] and PC(16:1p/20:4) [$C_{44}H_{79}NO_7P$] at RT = 8.5 min recorded with different MS resolution

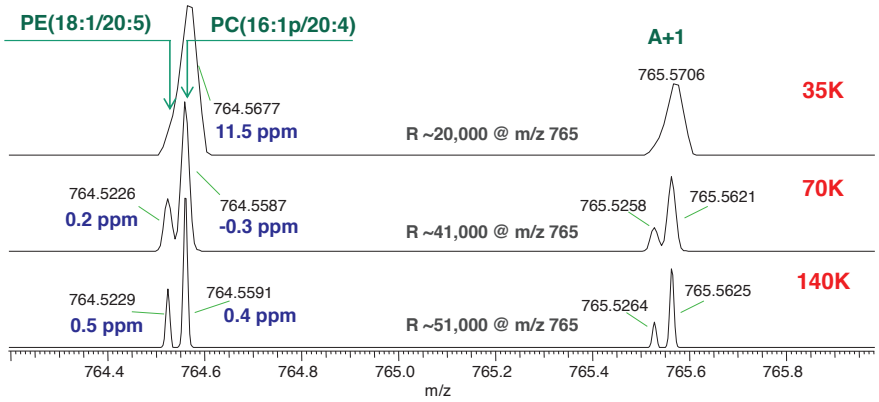


TABLE 5. INJECTION TIME and its effect on number of indentified lipids (ESI+)

INJ. TIME	Lipid Groups (Ions)	INJ. TIME	Lipid Groups (Ions)
50 ms	519 (1091)	100 ms	527 (1148)
75 ms *	**549 (1193)**	150 ms	520 (1086)

TABLE 6. NORMALIZED COLLISION ENERGY settings and its effect on a number of indentified lipids in positive and negative ion mode.

Col. Energy (+)	Lipid Groups (Ions)
20 - 30	552 (1209)
20 - 35	554 (1222)
20 - 40	546 (1221)
25 - 30	**563 (1257)**
25 - 35	**568 (1251)**
20 - 25 - 30	537 (1160)
20 - 25 - 35	545 (1190)
20 - 25 - 40	543 (1172)
20 - 30 - 40	549 (1193)

Col. Energy (-)	Lipid Groups (Ions)
20 - 30	247 (448)
20 - 35	247 (450)
20 - 40	247 (448)
20 - 45	236 (435)
25 - 40	**252** (455)
20 - 25 - 35	245 (446)
20 - 30 - 40	**247** (445)
20 - 30 - 45	240 (440)
20 - 30 - 50	245 (445)

Optimization V. INJECTION TIME

The optimum “Injection Time” was determined at setting of 75 ms as shown in Table 5.

Optimization VI. NORMALIZED COLLISION ENERGY

Normalized Collision Energy (NCE) conditions were evaluated individually for positive and negative ion mode. Optimization of NCE for global lipidomics is not as straight forward due to the different fragmentation patterns of various lipid classes and the necessity to generate particular fragment ions for proper identification.

This problem was solved using stepped collision energy that combines fragmentation at low and high energy within a single MS/MS event. Results of miscellaneous combination of two- and three-step NCE are summarized in Table 6. For positive ion mode, the highest number of identified lipids was found at 25 – 30 and 25 – 35 NCE, although other combinations exhibit similar results. For negative ion mode, the maximum number of identified lipids was achieved at 25 – 40 NCE, however, the results are very close to other combinations such as 20 – 30 – 40 NCE that has been previously used in the author's lab for LC-ddMS^2 analysis of lipids.

Overall comparison of results before and after the optimization

The numbers of lipids identified with generic (initial) and optimized settings are summarized in Table 7 (NCE 20 – 30 – 40 was used for negative mode and for positive ion mode NCE 25 – 35). In positive ion mode, the total number of identified lipids was increased almost by 50% when using an Exclusion List, and after optimization the overall increase was more than 100%. In the negative ion mode the increase of the number of identified lipids was 21% and 40%, respectively.

TABLE 7. Number of lipid species identified with generic and optimize settings

Lipid Class	Positive Ion Mode			Negative Ion Mode		
	Generic No Excl. List	Generic Excl. List	Optimized	Generic No Excl. List	Generic Excl. List	Optimized
Cer	22	29	35	0	0	1
CerG1	0	1	2	0	0	0
CerG2	0	0	1	0	0	0
CerG3	0	0	2	0	0	0
ChE	1	1	3	0	0	0
Co	1	2	2	0	0	0
DG	43	69	97	0	0	0
LPC	42	50	58	14	14	15
LPE	12	18	16	12	12	14
LPG	0	0	0	1	1	1
LPI	1	1	1	1	1	2
LPS	0	0	1	0	0	0
OAHFA	0	0	0	1	1	2
PC	214	279	358	98	117	141
PE	94	119	154	115	133	147
PG	2	5	7	9	13	18
PI	18	21	38	31	36	45
PS	0	1	1	34	52	54
SM	36	42	54	33	40	45
So	1	1	2	0	0	0
TG	216	395	637	0	0	0
dMePE	0	0	0	0	3	3
phSM	2	8	4	3	3	5
Total	**705**	**1042**	**1473**	**352**	**426**	**493**
%	***100***	***148***	***209***	***100***	***121***	***140***

Conclusion

For a global lipid profiling using Q Exactive Plus MS on LC-MS timeframe with run time of 28 min, optimums of following data-acquisition parameters were observed:

- Using of Exclusion List
- Dynamic Exclusion: 8 s
- Quadrupole Isolation Window: 1.2 Da
- Mass Resolution Setting: 70,000 for full-MS with Top-10 MS/MS experiment
- Injection Time: 75 ms
- Normalized Collision Energy – Positive: "25 – 30" or "25 – 35"
- Normalized Collision Energy – Negative: various stepped NCE such as "20–30–40"

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