High pH Mobile Phase for LC-MS Global Lipidomics Profiling with Quadrupole Orbitrap Mass Spectrometer Detection

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ABSTRACT

Purpose: Compare high pH and low pH LC mobile phases for global lipidomics profiling.

Methods: Liver lipid extract was separated on C18 HPLC column in eluting compounds were detected using a Thermo Scientific™ Q Exactive™ HF mass spectrometer operated in data-dependent acquisition mode under positive and negative ESI conditions. Data were acquired using low pH and high pH mobile phases and processed by Thermo Scientific™ LipidSearch™ software.

Results: Insignificant or minor changes in retention times at low pH and high pH conditions were observed for most of the evaluated lipids. Significant increases of intensities of extracted chromatogram peaks of most of the investigated lipids were observed mostly in negative ion mode. The number of identified lipids in LipidSearch software was 16% higher for high pH conditions data.

INTRODUCTION

LC-MS techniques have been widely used for global lipidomics profiling. Most of the published LC-MS methods are performed using low pH mobile phases, with modifiers, typically consisting of acetic acid or formic acid and related ammonium salt. This work compares lipids identification/performance under low pH and high pH mobile phase LC-MS conditions.

MATERIALS AND 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 IPA / ACN 90:10 (v/v) and spiked with Avanti Polar Lipids SPLASH™ Lipidomix™ standard.

Liquid Chromatography

Thermo Scientific™ Vanquish™ Horizon binary UHPLC system.

HPLC Column: Thermo Scientific™ Accucore™ C18 (2.1 x 150 mm, 2.6 µm) at 35 °C

Injection Volume: 3 µL

Flow Rate: 400 µL/min

Mobile Phase 1 – Low pH:

(A) 10 mM HCOONH4 in 50% ACN (v) + 0.1% HCOOH (v);
(B) 10 mM HCOONH4 in IPA / ACN 90:10 (v/v) + 0.1% HCOOH (v)

Mobile Phase 2 – High pH:

(A) 10 mM HCOONH4 in 50% ACN (v) + 0.3% NH4OH (v) (NH4OH ~21% sol.); pH = 9.35
(B) 10 mM HCOONH4 in IPA / ACN 90:10 (v/v) + 0.3% NH4OH (v)

HPLC Gradient:

Time	A%	B%	Time	A%	B%
0.00	75	25	25.00	02	98
0.50	65	35	25.10	75	25
22.00	02	98	29.00	75	25

Mass Spectrometry

Q Exactive HF mass spectrometer equipped with HESI-II probe. Positive and negative detection mode. Data-dependent MS/MS scan mode. 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: Top-12 experiment, AGC (MS) = 1e6, AGC (MS2) = 1e5, Max Injection Time = 100 ms, Resolution MS = 120,000 (FWHM at *m/z* 200), Resolution MS2 = 30,00 (FWHM at m/z 200), Full-MS Mass Range = 250-1600 amu for ESI(+) and 200-1600 amu for ESI(-), Isolation Window = 1.0 Da, Fixed First Mass = 75 Da, Dynamic Exclusion = 6 s. Normalized Collision Energy (NCE) = 25 – 35 for positive and NCE = 20 – 30 – 40 for negative ion mode. Exclusion mass lists for both polarity modes were created from solvent injection runs by averaging mass spectra (1 – 23 min) and exporting m/z values of the 100 most intense ions .

Bovine Liver extract sample was injected in triplicate

Data Analysis

Acquired data were automatically processed using LipidSearch 4.2. software.

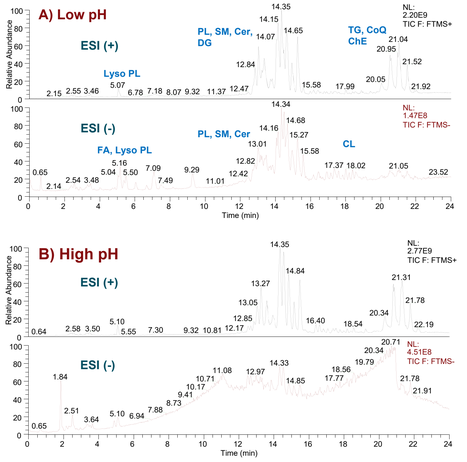
Search parameters: Precursor mass tolerance = 3 ppm, Product mass tolerance = 8 ppm, m-Score threshold = 3.

RESULTS

A bovine liver lipid extract (1.5 µg on column) spiked with Avanti SPLASH mixture was analyzed by LC-MS in dd-MS/MS mode under positive and negative electrospray ionization conditions. Data were acquired with A) low pH and B) high pH mobile phases (Figure 1).

Overall appearance of MS total ion chromatograms (TIC) in positive ion mode look very similar in both low pH and high pH conditions. In negative ion mode, abundant chemical noise background was observed at high pH conditions (Figure 1). The cause of that was not evaluated. Possibly, it can originate from solvent, column bleeding, or a combination of both.

Figure 1. Total ion chromatograms (MS) of bovine liver lipid extract recorded at A) low pH and B) high pH conditions.



Influence of mobile phase pH was visualized and evaluated on a set of lipids from various lipid classes. It consists of several deuterium labeled lipids (Avanti SPLASH mix) and selected natural lipids (Figures 2 and 3).

Retention Time

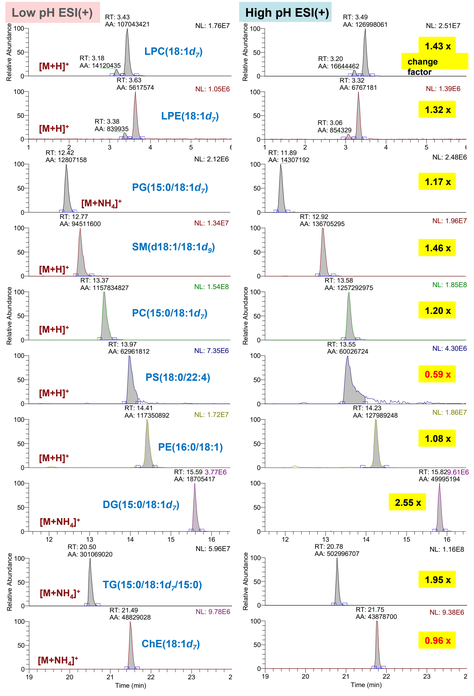
As apparent on the examples in Figures 2 and 3, most of the displayed lipids do not show any significant change in chromatographic retention time on pH of mobile phases. The subtle differences can be also attributed to the fact that low pH and high pH experiments were separately performed on two individual columns. The only dramatic difference was observed for fatty acids as can seen on FA(20:4) in Figure 3. This phenomenon is obviously caused by ionization of carboxylic group at high pH and its neutral form at low pH conditions.

Interestingly, LPC(18:1d7) and LPE(18:1d7) swap their relative retention times. While LPC(18:1d7) retention time does not really vary (permanently charged choline group), LPE(18:1d7) retention time is changing depending on protonated/neutral form of its amino group.

Chromatographic Peak Shape

Unchanged or a minor improvement of chromatographic peak shape was observed most of the displayed lipids in Figures 2 and 3. Noticeable improvement of chromatographic peak shape is observed on examples of PG, SM, PC, and CL (Figures 2 and 3). On the other hand, PS(18:0/22:4) showed significant decrease of peak quality (broader and tailing).

Figure 2. Positive ion mode extracted ion chromatograms for major ions of various lipids at low pH and high pH conditions. Signal intensity change factors are calculated for chromatographic peak apex at high-pH / low-pH.

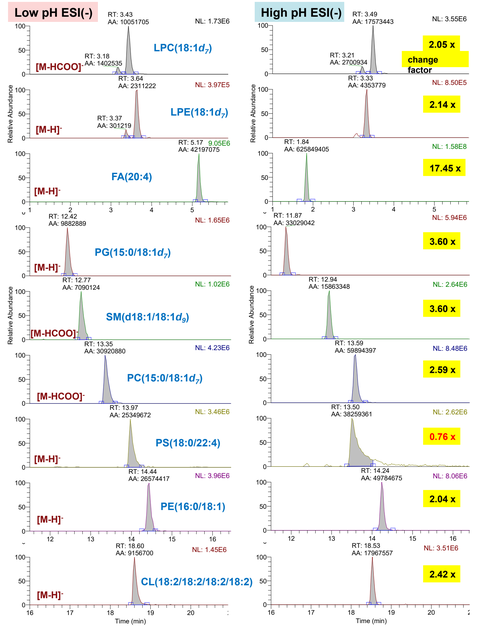


Chromatographic Peak Intensity

Changes in signal responses were compared on XIC peak intensities (not integrated areas) and their high-pH / low-pH "change factor" as it is displayed in Figures 2 and 3. In positive ion mode, the majority of the lipid species showed increase of peak intensities at high pH conditions. However, the changes were minor (< 1.5 x) for most of the compounds. Only PS(18:0/22:4) exhibited significant 0.59 x decrease of its peak intensity (though integrated peak area is very similar) and DG(15:0/18:1d7) peak intensity increased by 2.55 x change factor.

In negative ion mode, most of the example lipids (Figure 3) showed significant increase of their peak intensities at high pH conditions by change factor > 2.0 x. Only for PS(18:0/22:4), a decrease of peak intensity was observed (change factor of 0.76 x) at high pH.

Figure 3. Negative ion mode extracted ion chromatograms for major ions of various lipids at low pH and high pH conditions. Signal intensity change factors are calculated for chromatographic peak apex at high-pH / low-pH.



pH Influence on Type of "Main Ion"

The main ion (the most intense parent ion in both positive and negative mode) was mostly unchanged comparing low and high pH conditions. However, in several cases such as PG(15:0/18:1d7) it was observed that different main ions are found at low pH and high pH mobile phases. For PG(15:0/18:1d7) At low pH, [M+H]+ adduct is the main ion and its intensity is not significantly changing at high pH (Figure 2). However, in negative ion mode its [M-H]- ion is 3.6 x more intense at high pH than low pH conditions (Figure 3) making it the main ion for high pH conditions. This observation can be significant for data processing in software such as LipidSearch.

Table 1. Number of lipid species identified using LipidSearch software at low pH and high pH conditions.

	Filtered Count				Total Count		
Class	Low pH	High pH	*Difference*		Low pH	High pH	*Difference*
AcCa	11	15	4		11	15	4
CL	9	9	0		24	12	-12
Cer	81	103	22		101	139	38
ChE	9	9	0		9	10	1
Co	4	2	-2		4	3	-1
DG	24	59	35		95	165	70
DGMG	0	0	0		0	1	1
DLCL	0	0	0		3	2	-1
FA	1	5	4		1	6	5
GM3	0	0	0		1	0	-1
Hex1Cer	5	6	1		9	11	2
Hex2Cer	0	1	1		0	1	1
Hex3Cer	2	2	0		2	2	0
LPC	51	61	10		72	78	6
LPE	18	18	0		23	24	1
LPG	1	3	2		3	4	1
LPI	3	3	0		6	5	-1
LPS	7	3	-4		7	4	-3
MLCL	2	3	1		18	12	-6
PA	0	1	1		1	3	2
PC	284	298	14		451	469	18
PE	146	163	17		240	260	20
PG	14	20	6		25	25	0
PI	34	39	5		48	48	0
PS	26	36	10		45	63	18
SM	80	83	3		92	103	11
SPH	2	8	6		2	9	7
TG	150	175	25		253	391	138
WE	4	2	-2		4	2	-2
Total	**968**	**1127**	**+159**		**1550**	**1867**	**+317**
			+ 16%				***+ 20%***

Detected Lipid Species using LipidSearch

Acquired data were processed in LipidSearch software in an automated manner. Data results were filtered to show the most relevant lipid species. Number of identified lipid species is summarized in Table 1. LipidSearch software merged results (from both positive and negative ion mode) showed increased number of detected lipids at high pH than at low pH conditions. For filtered results, the number of lipids was increased by 16% (968 vs. 1127) and for unfiltered results the increase was 20% (1876 vs. 1550) . On a lipid class level, a significant increase of detected lipids in high pH solvent was observed, for example, for Diacylglycerols (146%, 59 vs. 24), Ceramides (27%, 103 vs. 81), and LPC lipids (20%, 61 vs. 51). Decrease of identified lipids was observed for low count occurring Co, LPS, and WE lipids (-2 or -4 counts).

High chemical noise background observed in negative ion mode at high pH conditions might result in ion suppression of low abundant lipid ions. Finding such high pH conditions that would solve that problem, can further increase number of detected and identified lipid.

CONCLUSIONS

Influence of high pH and low pH mobile phases on LC-MS global lipidomics profiling was performed using a Q Exactive mass spectrometer.

- High pH conditions increase signal intensity of various classes of lipids especially in negative ion mode.
- Number of identified lipids in LipidSearch software was increased by 16% in high pH solvents.
- Further investigation of high pH conditions that do not show elevated chemical background could result in increased number of identified lipids.

TRADEMARKS/LICENSING

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