Application Note

Lipidomics

Agilent

Trusted Answers

LC/MS Method for Comprehensive Analysis of Plasma Lipids

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Abstract

This Application Note describes the analysis of complex lipids in blood plasma. Lipids are extracted using a bi-phasic solvent system of methanol, methyl *tert*-butyl ether (MTBE), and water followed by separation and data acquisition of isolated lipids using reversed-phase liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (RPLC/Q-TOF MS). This workflow permits detection of over 270 unique lipid species covering the main plasma lipid classes.

Introduction

Many liquid chromatography (LC) modes have been used for the analysis of complex lipid mixtures. The three most important separation modes are reversed-phase LC (RPLC), normal-phase LC (NPLC), and hydrophilic interaction LC (HILIC)[1]. Among these methods, RPLC has been the most widely used method for analysis of complex lipids[2]. Typical methods in RPLC-based lipidomics use a short (50–150 mm) microbore column (1–2.1 mm id) with sub-2 µm particle size and C18 or C8-modified sorbent[2,3]. As a weak mobile phase, water or mixtures with organic solvent such as methanol or acetonitrile are used, while a strong mobile phase consists primarily of isopropanol mixed with other solvents (acetonitrile, methanol). Use of mobile phase modifiers is highly recommended to improve LC separation as well as ionization and detection of lipids. This Application Note presents a protocol for analysis of complex lipids in blood plasma using an Agilent ZORBAX EclipsePlus C18 column and the Agilent 1290 Infinity LC system coupled to the Agilent 6550 iFunnel Q-TOF MS using an Agilent Jet Stream (AJS) Technology ion source.

Experimental

Sample preparation

Extraction of plasma lipids was carried out using cold methanol, methyl *tert*-butyl ether (MTBE), and water[4,5]. Specifically, 225 µL of cold methanol containing a mixture of odd chain and deuterated lipid internal standards (LPE 17:1, LPC 17:0, PC 12:0/13:0, PE 17:0/17:0, d7-cholesterol, SM d18:1/17:0, Cer d18:1/17:0, d3-palmitic acid, DG 12:0/12:0/0:0, and d5-TG 17:0/17:1/17:0) was added to a 10 µL blood plasma aliquot in a 1.5 mL Eppendorf tube, then vortexed (10 seconds). Then, 750 µL of cold MTBE containing CE 22:1 (internal standard) was added, followed by vortexing (10 seconds) and shaking (six minutes) at 4 °C. Phase separation was induced by adding 188 µL of LC/MS-grade water followed by centrifugation at 14,000 rpm for two minutes. Two aliquots (each 200 µL) were collected and evaporated using a centrifugal evaporator. Dried lipid extracts were resuspended using of a methanol/toluene (9:1, v/v) mixture (150 µL for AJS(+) and 50 µL for AJS(−)) containing an internal standard CUDA (150 ng/mL), vortexed for (10 seconds) and centrifuged at 14,000 rpm for two minutes before LC/MS analysis.

Method

LC Separation	
Column	ZORBAX EclipsePlus C18, 100 × 2.1 mm, 1.8 µm
Mobile phase A, AJS(+)	60:40 (v/v) Acetonitrile:water with 10 mM ammonium formate and 0.1 % formic acid
Mobile phase B, AJS(+)	90:10 (v/v) Isopropanol:acetonitrile with 10 mM ammonium formate and 0.1 % formic acid*
Mobile phase A, AJS(−)	60:40 (v/v) Acetonitrile:water with 10 mM ammonium acetate
Mobile phase B, AJS(−)	90:10 (v/v) Isopropanol:acetonitrile with 10 mM ammonium acetate*
Flow rate	0.6 mL/min
Gradient	0 minutes 15 %B, 0–2 minutes 30 %B, 2–2.5 minutes 48 %B, 2.5–8.5 minutes 72 %B, 8.5–11.5 minutes 99 %B, 11.5–12 minutes 99 %B, 12–12.1 minutes 15 %B, 12.1–15 minutes 15 %B
Column temperature	60 °C
Injection	
Injection volume, AJS(+)	2 µL
Injection volume, AJS(−)	5 µL
Sample temperature	4 °C
Needle wash	10 seconds flush port (isopropanol)
Injector cleaning	Time 1: 0.1 minutes (bypass), Time 2: 11.6 minutes (mainpass/bypass), Time 3: 13.0 minutes (mainpass/bypass)
MS Detection	
Dual spray AJS ion source	Positive ionization or negative ionization
Drying gas temperature	200 °C
Drying gas flow	14 L/min
Nebulizer pressure	35 psi
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Capillary voltage	±3.5 kV
Nozzle voltage	±1 kV
Fragmentor	175 V
Acquisition speed	2 Spectra/s
Mass range	*m/z* 100–1,700
Extended dynamic range	2 GHz

* Ammonium salts should be dissolved first in a small aliquot of water, then added to the 90:10 isopropanol:acetonitrile.

Instrumentation

- Agilent 1290 Infinity LC system with a pump (G4220A), a column oven (G1316C), and an autosampler (G4226A)
- Agilent 6550 iFunnel Q-TOF MS system with an Agilent Jet Stream (AJS) ion source operated in positive or negative ion polarity

Software

- Agilent MassHunter for instrument control (revision B.05.00)
- Agilent MassHunter Qualitative Analysis software (revision B.05.00)
- Agilent MassHunter Quantitative Analysis software (revision B.05.01)

Results and discussion

For comprehensive screening, lipids need to be analyzed in both positive and negative electrospray mode. Optimal results for increased lipidome coverage are only obtained if two different mobile-phase modifier systems are used, as opposed to using a single common modifier for both ionization modes[6,7]. Based on our initial evaluation of standard lipid mixtures, we found that 10 mM ammonium formate with 0.1 % formic provided the best signal intensity for lipids in AJS(+), while 10 mM ammonium acetate should be preferred in AJS(–). Figure 1 shows examples of lipids detected in AJS(–). While a 2-fold increase in signal intensity was observed for internal standards such as LPC(17:0), PI(12:0/13:0), and PA(12:0/13:0), an over 300-fold increase of signal intensity was noted for d3-palmitic acid when using 10 mM ammonium acetate compared to 10 mM ammonium formate with 0.1 % formic.

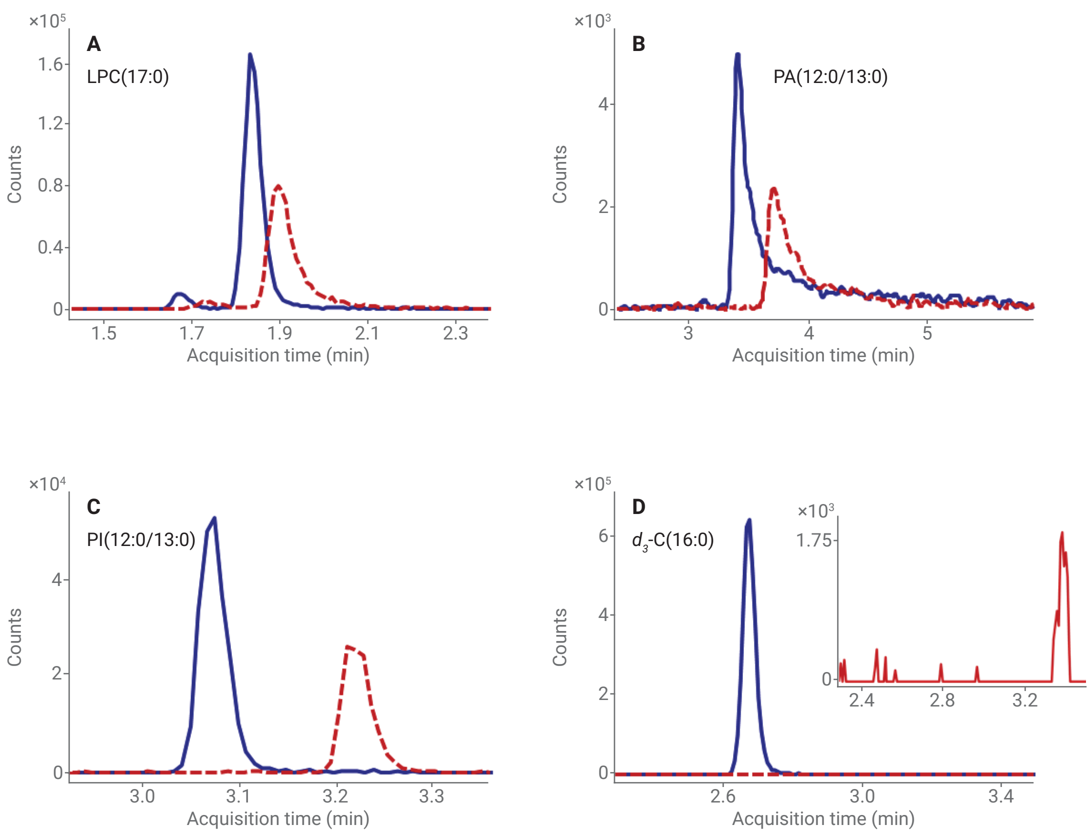

Figure 1. Impact of mobile phase modifiers on peak shape and signal intensity for selected lipids detected in AJS(–). 10 mM ammonium acetate (blue line), 10 mM ammonium formate with 0.1 % formic acid (red dashed line).

In the next step, we compared the lipidome coverage. We identified 350 lipids in total, more specifically, 192 lipids in AJS(+) and 158 lipids in AJS(−). Many lipids were detected as multiple molecular species (Table 1). For instance, DG, TG, and CE were detected as [M+NH$_4$]$^+$ and [M+Na]$^+$ species in AJS(+), or ceramides as [M+HAc−H]$^-$ and [M+Cl]$^-$ adducts in AJS(−). Plasma lipids were annotated based on their unique MS/MS fragmentation patterns using the open-access LipidBlast mass spectral library with NIST MS search software[8], or characterized as compound classes (nonesterified fatty acids).

Table 1. Identified blood plasma lipids. Grey cells indicate internal standards.

AJS(+)		
Lipid	**RT (min)**	***m/z***
CE (22:1) [M+NH$_4$]$^+$ ISTD	11.55	724.6966
CE (22:1) [M+Na]$^+$ ISTD	11.55	729.6520
Cer (d18:1/17:0) [M+H]$^+$ ISTD	6.84	552.5350
Cer (d18:1/17:0) [M+H-H$_2$O]$^+$ ISTD	6.84	534.5245
Cer (d18:1/17:0) [M+Na]$^+$ ISTD	6.84	574.5170
Cholesterol d7 [M-H$_2$O+H]$^+$ ISTD	5.39	376.3955
CUDA [M+H]$^+$ ISTD	0.76	341.2799
DG (12:0/12:0/0:0) [M+NH$_4$]$^+$ ISTD	4.79	474.4153
DG (12:0/12:0/0:0) [M+Na]$^+$ ISTD	4.79	479.3707
DG (18:1/2:0/0:0) [M+NH$_4$]$^+$ ISTD	3.37	416.3371
DG (18:1/2:0/0:0) [M+Na]$^+$ ISTD	3.37	421.2924
LPC (17:0) [M+H]$^+$ ISTD	1.90	510.3554
LPE (17:1) [M+H]$^+$ ISTD	1.35	466.2928
PC (12:0/13:0) [M+H]$^+$ ISTD	3.67	636.4599
PE (17:0/17:0) [M+H]$^+$ ISTD	6.95	720.5538
SM (d18:1/17:0) [M+H]$^+$ ISTD	5.49	717.5905
Sphingosine d17:1 [M+H]$^+$ ISTD	0.91	286.2741
TG d5 (17:0/17:1/17:0) [M+NH$_4$]$^+$ ISTD	11.11	869.8329
TG d5 (17:0/17:1/17:0) [M+Na]$^+$ ISTD	11.12	874.7882
CE (16:0) [M+NH$_4$]$^+$	11.19	642.6184
CE (16:0) [M+Na]$^+$	11.18	647.5738
CE (18:1) [M+NH$_4$]$^+$	11.19	668.6340
CE (18:1) [M+Na]$^+$	11.19	673.5894
CE (18:2) [M+NH$_4$]$^+$	10.99	666.6184
CE (18:2) [M+Na]$^+$	10.99	671.5738
CE (18:3) [M+NH$_4$]$^+$	10.78	664.6027
CE (18:3) [M+Na]$^+$	10.79	669.5581
CE (20:2) [M+NH$_4$]$^+$	11.01	694.6497

Lipid	RT (min)	*m/z*
CE (20:2) [M+Na]$^+$	11.02	699.6051
CE (20:3) [M+NH$_4$]$^+$	10.86	692.6340
CE (20:3) [M+Na]$^+$	10.86	697.5894
CE (20:4) [M+NH$_4$]$^+$	10.86	690.6184
CE (20:4) [M+Na]$^+$	10.86	695.5738
CE (22:2) [M+NH$_4$]$^+$	11.40	722.6810
CE (22:2) [M+Na]$^+$	11.40	727.6364
Cer (d40:1) [M+H]$^+$	8.75	622.6133
Cer (d40:1) [M+H-H$_2$O]$^+$	8.75	604.6027
Cer (d40:1) [M+Na]$^+$	8.75	644.5952
Cer (d41:1) [M+H]$^+$	9.08	636.6289
Cer (d41:1) [M+H-H$_2$O]$^+$	9.09	618.6184
Cer (d41:1) [M+Na]$^+$	9.09	658.6109
Cer (d42:1) [M+H]$^+$	9.40	650.6446
Cer (d42:1) [M+H-H$_2$O]$^+$	9.41	632.6340
Cer (d42:1) [M+Na]$^+$	9.40	672.6265
Cer (d42:2) [M+H]$^+$	8.75	648.6289
Cer (d42:2) [M+H-H$_2$O]$^+$	8.75	630.6184
Cer (d42:2) [M+Na]$^+$	8.75	670.6109
Cholesterol [M-H$_2$O+H]$^+$	5.42	369.3516
DG (34:1) [M+NH$_4$]$^+$	7.98	612.5562
DG (34:1) [M+Na]$^+$	7.97	617.5115
DG (34:2) [M+NH$_4$]$^+$	7.40	610.5405
DG (34:2) [M+Na]$^+$	7.42	615.4959
DG (36:2) [M+NH$_4$]$^+$	8.09	638.5718
DG (36:2) [M+Na]$^+$	8.13	643.5272
DG (36:3) [M+NH$_4$]$^+$	7.50	636.5562
DG (36:3) [M+Na]$^+$	7.51	641.5115
DG (36:4) A [M+NH$_4$]$^+$	6.91	634.5405
DG (36:4) A [M+Na]$^+$	6.91	639.4959
DG (36:4) B [M+NH$_4$]$^+$	6.97	634.5405
DG (36:4) B [M+Na]$^+$	6.98	639.4959

Lipid	RT (min)	*m/z*
DG (38:6) [M+NH$_4$]$^+$	6.80	658.5405
DG (38:6) [M+Na]$^+$	6.79	663.4959
GlcCer (d40:1) [M+H]$^+$	7.82	784.6661
GlcCer (d40:1) [M+Na]$^+$	7.82	806.6480
GlcCer (d42:1) [M+H]$^+$	8.53	812.6974
GlcCer (d42:1) [M+Na]$^+$	8.53	834.6793
GlcCer (d42:2) [M+H]$^+$	7.82	810.6817
GlcCer (d42:2) [M+Na]$^+$	7.82	832.6637
LPC (14:0) [M+H]$^+$	0.95	468.3085
LPC (15:0) [M+H]$^+$	1.18	482.3241
LPC (16:0) [M+H]$^+$	1.50	496.3398
LPC (16:1) [M+H]$^+$	1.07	494.3241
LPC (18:0) [M+H]$^+$	2.36	524.3711
LPC (18:1) [M+H]$^+$	1.68	522.3554
LPC (18:2) [M+H]$^+$	1.23	520.3398
LPC (18:3) [M+H]$^+$	0.97	518.3241
LPC (20:3) [M+H]$^+$	1.45	546.3554
LPC (20:4) [M+H]$^+$	1.20	544.3398
LPC (20:5) [M+H]$^+$	0.94	542.3241
LPC (22:5) [M+H]$^+$	1.31	570.3554
LPE (18:0) [M+H]$^+$	2.42	482.3241
LPE (18:2) [M+H]$^+$	1.26	478.2928
PC (28:0) [M+H]$^+$	4.56	678.5068
PC (30:0) [M+H]$^+$	5.24	706.5381
PC (31:0) [M+H]$^+$	5.59	720.5538
PC (32:0) [M+H]$^+$	5.97	734.5694
PC (32:1) [M+H]$^+$	5.38	732.5538
PC (32:2) [M+H]$^+$	4.83	730.5381
PC (33:1) [M+H]$^+$	5.72	746.5694
PC (33:2) [M+H]$^+$	5.17	744.5538
PC (34:0) [M+H]$^+$	6.72	762.6007
PC (34:1) [M+H]$^+$	6.08	760.5851

AJS(+) - continued

Lipid	RT (min)	*m/z*
PC (34:3) A $[M+H]^+$	4.95	756.5538
PC (34:3) B $[M+H]^+$	5.05	756.5538
PC (34:3) C $[M+H]^+$	5.15	756.5538
PC (34:4) $[M+H]^+$	4.75	754.5381
PC (35:1) $[M+H]^+$	6.46	774.6007
PC (35:2) A $[M+H]^+$	5.81	772.5851
PC (35:2) B $[M+H]^+$	5.90	772.5851
PC (35:3) $[M+H]^+$	5.38	770.5694
PC (35:4) $[M+H]^+$	5.08	768.5538
PC (36:1) $[M+H]^+$	6.83	788.6164
PC (36:2) $[M+H]^+$	6.27	786.6007
PC (36:3) A $[M+H]^+$	5.63	784.5851
PC (36:3) B $[M+H]^+$	5.73	784.5851
PC (36:4) A $[M+H]^+$	5.11	782.5694
PC (36:4) B $[M+H]^+$	5.43	782.5694
PC (36:5) A $[M+H]^+$	4.86	780.5538
PC (36:5) B $[M+H]^+$	4.98	780.5538
PC (38:2) $[M+H]^+$	6.95	814.6320
PC (38:3) $[M+H]^+$	6.48	812.6164
PC (38:4) A $[M+H]^+$	5.97	810.6007
PC (38:4) B $[M+H]^+$	6.17	810.6007
PC (38:5) A $[M+H]^+$	5.52	808.5851
PC (38:5) B $[M+H]^+$	5.72	808.5851
PC (38:6) A $[M+H]^+$	5.01	806.5694
PC (38:6) B $[M+H]^+$	5.27	806.5694
PC (38:7) $[M+H]^+$	5.11	804.5538
PC (40:4) $[M+H]^+$	6.71	838.6320
PC (40:5) A $[M+H]^+$	6.21	836.6164
PC (40:5) B $[M+H]^+$	6.47	836.6164
PC (40:6) A $[M+H]^+$	5.98	834.6007
PC (40:6) B $[M+H]^+$	6.48	834.6007
PC (40:7) $[M+H]^+$	5.37	832.5851
PC (o-32:0) $[M+H]^+$	6.46	720.5902
PC (o-34:0) $[M+H]^+$	7.24	748.6215
PC (p-34:1) or PC (o-34:2) $[M+H]^+$	6.01	744.5902
PC (p-34:2) or PC (o-34:3) $[M+H]^+$	5.88	742.5745
PC (p-36:2) or PC (o-36:3) $[M+H]^+$	6.64	770.6058
PC (p-36:4) or PC (o-36:5) $[M+H]^+$	5.76	766.5745
PC (p-38:3) or PC (o-38:4) $[M+H]^+$	6.66	796.6215
PC (p-38:4) or PC (o-38:5) $[M+H]^+$	5.98	794.6058
PC (p-42:4) or PC (o-42:5) $[M+H]^+$	7.44	850.6684

Lipid	RT (min)	*m/z*
PC (p-44:4) or PC (o-44:5) $[M+H]^+$	8.14	878.6997
PE (34:2) $[M+H]^+$	5.76	716.5225
PE (36:1) $[M+H]^+$	7.05	746.5694
PE (36:2) $[M+H]^+$	6.71	744.5538
PE (p-36:4) or PE (o-36:5) $[M+H]^+$	6.02	724.5276
PE (p-38:4) or PE (o-38:5) $[M+H]^+$	6.78	752.5589
SM (d30:1) $[M+H]^+$	3.81	647.5123
SM (d32:0) $[M+H]^+$	4.70	677.5592
SM (d32:1) $[M+H]^+$	4.43	675.5436
SM (d32:2) $[M+H]^+$	3.92	673.5279
SM (d33:1) $[M+H]^+$	4.78	689.5592
SM (d34:0) $[M+H]^+$	5.41	705.5905
SM (d34:1) $[M+H]^+$	5.12	703.5749
SM (d34:2) $[M+H]^+$	4.56	701.5592
SM (d36:0) $[M+H]^+$	6.17	733.6218
SM (d36:1) $[M+H]^+$	5.87	731.6062
SM (d36:2) $[M+H]^+$	5.27	729.5905
SM (d36:3) $[M+H]^+$	4.75	727.5749
SM (d38:1) $[M+H]^+$	6.68	759.6375
SM (d38:2) $[M+H]^+$	6.04	757.6218
SM (d39:1) $[M+H]^+$	7.06	773.6531
SM (d40:0) $[M+H]^+$	7.41	789.6844
SM (d40:1) $[M+H]^+$	7.42	787.6688
SM (d40:2) A $[M+H]^+$	6.70	785.6531
SM (d40:2) B $[M+H]^+$	6.81	785.6531
SM (d41:1) $[M+H]^+$	7.80	801.6844
SM (d41:2) A $[M+H]^+$	7.06	799.6688
SM (d41:2) B $[M+H]^+$	7.20	799.6688
SM (d42:1) $[M+H]^+$	8.16	815.7001
SM (d42:2) $[M+H]^+$	7.42	813.6844
SM (d42:3) $[M+H]^+$	6.82	811.6688
TG (40:0) $[M+NH_4]^+$	10.02	712.6450
TG (40:0) $[M+Na]^+$	10.02	717.6004
TG (40:1) $[M+NH_4]^+$	9.55	710.6293
TG (40:1) $[M+Na]^+$	9.51	715.5847
TG (42:0) $[M+NH_4]^+$	10.35	740.6763
TG (42:0) $[M+Na]^+$	10.34	745.6317
TG (44:0) $[M+NH_4]^+$	10.61	768.7076
TG (44:0) $[M+Na]^+$	10.61	773.6630
TG (44:1) $[M+NH_4]^+$	10.37	766.6919
TG (44:1) $[M+Na]^+$	10.37	771.6473

Lipid	RT (min)	*m/z*
TG (44:2) $[M+NH_4]^+$	10.12	764.6763
TG (44:2) $[M+Na]^+$	10.12	769.6317
TG (46:0) $[M+NH_4]^+$	10.84	796.7389
TG (46:0) $[M+Na]^+$	10.83	801.6943
TG (46:1) $[M+NH_4]^+$	10.63	794.7232
TG (46:1) $[M+Na]^+$	10.63	799.6786
TG (46:2) $[M+NH_4]^+$	10.42	792.7076
TG (46:2) $[M+Na]^+$	10.42	797.6630
TG (46:3) $[M+NH_4]^+$	10.17	790.6919
TG (46:3) $[M+Na]^+$	10.17	795.6473
TG (48:0) $[M+NH_4]^+$	11.02	824.7702
TG (48:0) $[M+Na]^+$	11.02	829.7256
TG (48:1) $[M+NH_4]^+$	10.86	822.7545
TG (48:1) $[M+Na]^+$	10.85	827.7099
TG (48:2) $[M+NH_4]^+$	10.66	820.7389
TG (48:2) $[M+Na]^+$	10.66	825.6943
TG (48:3) $[M+NH_4]^+$	10.45	818.7232
TG (48:3) $[M+Na]^+$	10.45	823.6786
TG (48:4) $[M+NH_4]^+$	10.22	816.7076
TG (48:4) $[M+Na]^+$	10.22	821.6630
TG (49:0) $[M+NH_4]^+$	11.11	838.7858
TG (49:0) $[M+Na]^+$	11.11	843.7412
TG (49:1) $[M+NH_4]^+$	10.96	836.7702
TG (49:1) $[M+Na]^+$	10.95	841.7256
TG (49:2) $[M+NH_4]^+$	10.77	834.7545
TG (49:2) $[M+Na]^+$	10.78	839.7099
TG (49:3) $[M+NH_4]^+$	10.58	832.7389
TG (49:3) $[M+Na]^+$	10.58	837.6943
TG (50:0) $[M+NH_4]^+$	11.20	852.8015
TG (50:0) $[M+Na]^+$	11.21	857.7569
TG (50:1) $[M+NH_4]^+$	11.04	850.7858
TG (50:1) $[M+Na]^+$	11.03	855.7412
TG (50:2) $[M+NH_4]^+$	10.87	848.7702
TG (50:2) $[M+Na]^+$	10.87	853.7256
TG (50:3) $[M+NH_4]^+$	10.69	846.7545
TG (50:3) $[M+Na]^+$	10.69	851.7099
TG (50:4) $[M+NH_4]^+$	10.49	844.7389
TG (50:4) $[M+Na]^+$	10.49	849.6943
TG (50:5) $[M+NH_4]^+$	10.30	842.7232
TG (50:5) $[M+Na]^+$	10.28	847.6786
TG (50:6) $[M+NH_4]^+$	10.12	840.7081

AJS(+) - continued

Lipid	RT (min)	*m/z*
TG (50:6) $[M+Na]^+$	10.13	845.6635
TG (51:2) $[M+NH_4]^+$	10.96	862.7858
TG (51:2) $[M+Na]^+$	10.96	867.7412
TG (51:3) $[M+NH_4]^+$	10.80	860.7702
TG (51:3) $[M+Na]^+$	10.80	865.7256
TG (51:4) $[M+NH_4]^+$	10.61	858.7545
TG (51:4) $[M+Na]^+$	10.61	863.7099
TG (52:0) $[M+NH_4]^+$	11.36	880.8328
TG (52:0) $[M+Na]^+$	11.36	885.7882
TG (52:1) $[M+NH_4]^+$	11.21	878.8171
TG (52:1) $[M+Na]^+$	11.21	883.7725
TG (52:2) $[M+NH_4]^+$	11.06	876.8015
TG (52:2) $[M+Na]^+$	11.06	881.7569
TG (52:3) $[M+NH_4]^+$	10.90	874.7858
TG (52:3) $[M+Na]^+$	10.90	879.7412
TG (52:4) $[M+NH_4]^+$	10.73	872.7702
TG (52:4) $[M+Na]^+$	10.72	877.7256
TG (52:5) $[M+NH_4]^+$	10.55	870.7545
TG (52:5) $[M+Na]^+$	10.55	875.7099
TG (52:6) $[M+NH_4]^+$	10.40	868.7389
TG (52:6) $[M+Na]^+$	10.40	873.6943
TG (53:0) $[M+NH_4]^+$	11.41	894.8484
TG (53:0) $[M+Na]^+$	11.41	899.8038
TG (53:1) $[M+NH_4]^+$	11.28	892.8328
TG (53:1) $[M+Na]^+$	11.28	897.7882
TG (53:2) $[M+NH_4]^+$	11.13	890.8171
TG (53:2) $[M+Na]^+$	11.14	895.7725
TG (53:3) $[M+NH_4]^+$	10.99	888.8015
TG (53:3) $[M+Na]^+$	11.00	893.7569
TG (53:4) $[M+NH_4]^+$	10.83	886.7858
TG (53:4) $[M+Na]^+$	10.82	891.7412
TG (54:0) $[M+NH_4]^+$	11.50	908.8641
TG (54:0) $[M+Na]^+$	11.49	913.8195
TG (54:1) $[M+NH_4]^+$	11.36	906.8484
TG (54:1) $[M+Na]^+$	11.36	911.8038
TG (54:2) $[M+NH_4]^+$	11.21	904.8328
TG (54:2) $[M+Na]^+$	11.22	909.7882
TG (54:3) $[M+NH_4]^+$	11.07	902.8171
TG (54:3) $[M+Na]^+$	11.08	907.7725
TG (54:4) $[M+NH_4]^+$	10.91	900.8015
TG (54:4) $[M+Na]^+$	10.92	905.7569

Lipid	RT (min)	*m/z*
TG (54:5) A $[M+NH_4]^+$	10.75	898.7858
TG (54:5) A $[M+Na]^+$	10.76	903.7412
TG (54:5) B $[M+NH_4]^+$	10.85	898.7858
TG (54:5) B $[M+Na]^+$	10.84	903.7412
TG (54:6) A $[M+NH_4]^+$	10.57	896.7702
TG (54:6) A $[M+Na]^+$	10.57	901.7256
TG (54:6) B $[M+NH_4]^+$	10.66	896.7702
TG (54:6) B $[M+Na]^+$	10.66	901.7256
TG (54:7) A $[M+NH_4]^+$	10.37	894.7545
TG (54:7) A $[M+Na]^+$	10.37	899.7099
TG (54:7) B $[M+NH_4]^+$	10.49	894.7545
TG (54:7) B $[M+Na]^+$	10.48	899.7099
TG (56:1) $[M+NH_4]^+$	11.51	934.8797
TG (56:1) $[M+Na]^+$	11.50	939.8351
TG (56:2) $[M+NH_4]^+$	11.37	932.8641
TG (56:2) $[M+Na]^+$	11.37	937.8195
TG (56:3) $[M+NH_4]^+$	11.23	930.8484
TG (56:3) $[M+Na]^+$	11.24	935.8038
TG (56:4) $[M+NH_4]^+$	11.09	928.8328
TG (56:4) $[M+Na]^+$	11.10	933.7882
TG (56:5) A $[M+NH_4]^+$	10.97	926.8171
TG (56:5) A $[M+Na]^+$	10.95	931.7725
TG (56:5) B $[M+NH_4]^+$	11.03	926.8171
TG (56:5) B $[M+Na]^+$	11.03	931.7725
TG (56:6) $[M+NH_4]^+$	10.86	924.8015
TG (56:6) $[M+Na]^+$	10.85	929.7569
TG (56:7) A $[M+NH_4]^+$	10.68	922.7858
TG (56:7) A $[M+Na]^+$	10.68	927.7412
TG (56:7) B $[M+NH_4]^+$	10.76	922.7858
TG (56:7) B $[M+Na]^+$	10.76	927.7412
TG (56:8) A $[M+NH_4]^+$	10.50	920.7702
TG (56:8) A $[M+Na]^+$	10.50	925.7256
TG (56:8) B $[M+NH_4]^+$	10.57	920.7702
TG (56:8) B $[M+Na]^+$	10.58	925.7256
TG (58:1) $[M+NH_4]^+$	11.61	962.9110
TG (58:1) $[M+Na]^+$	11.63	967.8664
TG (58:2) $[M+NH_4]^+$	11.51	960.8954
TG (58:2) $[M+Na]^+$	11.51	965.8508
TG (58:3) $[M+NH_4]^+$	11.39	958.8797
TG (58:3) $[M+Na]^+$	11.39	963.8351
TG (58:4) $[M+NH_4]^+$	11.66	956.8641

Lipid	RT (min)	*m/z*
TG (58:4) $[M+Na]^+$	11.64	961.8195
TG (58:5) $[M+NH_4]^+$	11.16	954.8484
TG (58:5) $[M+Na]^+$	11.15	959.8038
TG (58:6) $[M+NH_4]^+$	11.01	952.8328
TG (58:6) $[M+Na]^+$	11.02	957.7882
TG (58:8) $[M+NH_4]^+$	10.78	948.8015
TG (58:8) $[M+Na]^+$	10.78	953.7569
TG (58:9) $[M+NH_4]^+$	10.61	946.7858
TG (58:9) $[M+Na]^+$	10.61	951.7412
TG (60:2) $[M+NH_4]^+$	11.63	988.9267
TG (60:2) $[M+Na]^+$	11.63	993.8821
TG (60:3) $[M+NH_4]^+$	11.52	986.9110
TG (60:3) $[M+Na]^+$	11.51	991.8664

AJS(–)		
Cer (d18:1/17:0) [M+HAc-H]- ISTD	6.81	610.5416
Cer (d18:1/17:0) [M+Cl]- ISTD	6.82	586.4971
CUDA [M-H]- ISTD	0.56	339.2653
FA (16:0)-d3 [M-H]- ISTD	2.65	258.2518
LPC (17:0) [M+HAc-H]- ISTD	1.81	568.3620
LPE (17:1) [M-H]- ISTD	1.31	464.2783
PC (12:0/13:0) [M+HAc-H]- ISTD	3.63	694.4665
PE (17:0/17:0) [M-H]- ISTD	6.89	718.5392
PG (17:0/17:0) [M-H]- ISTD	5.72	749.5338
SM (d18:1/17:0) [M+HAc-H]- ISTD	5.45	775.5971
Cer (d33:1) [M+HAc-H]-	6.02	582.5103
Cer (d33:1) [M+Cl]-	6.03	558.4658
Cer (d34:1) [M+HAc-H]-	6.42	596.5259
Cer (d34:1) [M+Cl]-	6.42	572.4815
Cer (d34:2) [M+HAc-H]-	5.77	594.5103
Cer (d34:2) [M+Cl]-	5.78	570.4658
Cer (d36:1) [M+HAc-H]-	7.21	624.5572
Cer (d36:1) [M+Cl]-	7.22	600.5128
Cer (d38:1) [M+HAc-H]-	7.99	652.5885
Cer (d38:1) [M+Cl]-	7.98	628.5441
Cer (d39:1) [M+HAc-H]-	8.40	666.6042
Cer (d39:1) [M+Cl]-	8.40	642.5597
Cer (d40:0) [M+HAc-H]-	8.99	682.6355
Cer (d40:0) [M+Cl]-	8.99	658.5910
Cer (d40:1) [M+HAc-H]-	8.72	680.6198

AJS(–) - continued

Lipid	RT (min)	*m/z*
Cer (d40:1) [M+Cl]-	8.71	656.5754
Cer (d41:1) [M+HAc-H]-	9.08	694.6355
Cer (d41:1) [M+Cl]-	9.08	670.5910
Cer (d42:0) [M+HAc-H]-	9.59	710.6668
Cer (d42:0) [M+Cl]-	9.59	686.6223
Cer (d42:1) [M+HAc-H]-	9.39	708.6511
Cer (d42:1) [M+Cl]-	9.39	684.6067
Cer (d42:2) A [M+HAc-H]-	8.72	706.6355
Cer (d42:2) A [M+Cl]-	8.73	682.5910
Cer (d42:2) B [M+HAc-H]-	8.87	706.6355
Cer (d42:2) B [M+Cl]-	8.87	682.5910
Cer (d43:1) A [M+HAc-H]-	9.55	722.6668
Cer (d43:1) A [M+Cl]-	9.56	698.6223
Cer (d43:1) B [M+HAc-H]-	9.64	722.6668
Cer (d43:1) B [M+Cl]-	9.64	698.6223
FA (14:0) [M-H]-	1.69	227.2017
FA (15:0) A [M-H]-	2.04	241.2173
FA (15:0) B [M-H]-	2.14	241.2173
FA (16:0) [M-H]-	2.66	255.2330
FA (16:1) [M-H]-	1.92	253.2173
FA (17:0) A [M-H]-	2.97	269.2486
FA (17:0) B [M-H]-	3.06	269.2486
FA (17:1) [M-H]-	2.39	267.2330
FA (18:0) [M-H]-	3.38	283.2643
FA (18:1) A [M-H]-	2.89	281.2486
FA (18:1) B [M-H]-	2.96	281.2486
FA (18:2) [M-H]-	2.22	279.2330
FA (18:3) A [M-H]-	1.66	277.2173
FA (18:3) B [M-H]-	1.74	277.2173
FA (19:0) [M-H]-	3.72	297.2799
FA (20:0) [M-H]-	4.08	311.2956
FA (20:1) [M-H]-	3.49	309.2799
FA (20:2) [M-H]-	3.06	307.2643
FA (20:3) [M-H]-	2.57	305.2486
FA (20:4) [M-H]-	2.09	303.2330
FA (20:5) [M-H]-	1.59	301.2173
FA (20:6) [M-H]-	1.53	299.2017
FA (22:0) [M-H]-	4.90	339.3269
FA (22:1) [M-H]-	4.16	337.3112
FA (22:4) [M-H]-	2.90	331.2643
FA (22:5) A [M-H]-	2.32	329.2486
FA (22:5) B [M-H]-	2.41	329.2486

Lipid	RT (min)	*m/z*
FA (22:6) [M-H]-	1.86	327.2330
FA (24:0) [M-H]-	5.80	367.3582
FA (24:1) [M-H]-	4.94	365.3425
FA (26:0) A [M-H]-	6.25	395.3895
FA (26:0) B [M-H]-	6.73	395.3895
FA (28:0) [M-H]-	7.66	423.4208
GlcCer (d38:1) [M+HAc-H]-	7.06	814.6414
GlcCer (d40:1) [M+HAc-H]-	7.80	842.6727
GlcCer (d41:1) [M+HAc-H]-	8.15	856.6883
GlcCer (d42:1) [M+HAc-H]-	8.50	870.7040
GlcCer (d42:2) [M+HAc-H]-	7.79	868.6883
LPC (14:0) [M+HAc-H]-	0.81	526.3150
LPC (16:0) [M+HAc-H]-	1.43	554.3463
LPC (16:1) [M+HAc-H]-	0.81	552.3307
LPC (18:0) [M+HAc-H]-	2.27	582.3776
LPC (18:1) [M+HAc-H]-	1.61	580.3620
LPC (18:2) [M+HAc-H]-	1.19	578.3463
LPC (18:3) [M+HAc-H]-	0.81	576.3307
LPC (20:3) [M+HAc-H]-	1.40	604.3620
LPC (20:4) [M+HAc-H]-	1.17	602.3463
LPC (22:6) [M+HAc-H]-	1.10	626.3463
LPC (o-16:0) [M+HAc-H]-	1.71	540.3671
LPC (p-16:0) [M+HAc-H]-	1.67	538.3514
LPE (16:0) [M-H]-	1.48	452.2783
LPE (18:0) [M-H]-	2.36	480.3096
LPE (18:1) [M-H]-	1.67	478.2939
LPE (18:2) [M-H]-	1.23	476.2783
LPE (20:4) [M-H]-	1.19	500.2783
LPE (22:6) [M-H]-	1.16	524.2783
PC (32:0) [M+HAc-H]-	5.92	792.5760
PC (32:1) [M+HAc-H]-	5.32	790.5604
PC (32:2) [M+HAc-H]-	4.79	788.5447
PC (33:1) [M+HAc-H]-	6.03	804.5760
PC (33:2) [M+HAc-H]-	5.49	802.5604
PC (34:0) [M+HAc-H]-	6.68	820.6073
PC (34:1) [M+HAc-H]-	6.04	818.5917
PC (34:2) [M+HAc-H]-	5.48	816.5760
PC (34:3) A [M+HAc-H]-	4.90	814.5604
PC (34:3) B [M+HAc-H]-	5.00	814.5604
PC (34:3) C [M+HAc-H]-	5.14	814.5604
PC (34:4) [M+HAc-H]-	4.72	812.5447
PC (36:1) [M+HAc-H]-	6.79	846.6230

Lipid	RT (min)	*m/z*
PC (36:2) [M+HAc-H]-	6.23	844.6073
PC (36:3) A [M+HAc-H]-	5.59	842.5917
PC (36:3) B [M+HAc-H]-	5.68	842.5917
PC (36:4) A [M+HAc-H]-	5.06	840.5760
PC (36:4) B [M+HAc-H]-	5.39	840.5760
PC (36:5) A [M+HAc-H]-	4.83	838.5604
PC (36:5) B [M+HAc-H]-	4.94	838.5604
PC (38:2) [M+HAc-H]-	6.90	872.6386
PC (38:3) [M+HAc-H]-	6.44	870.6230
PC (38:4) [M+HAc-H]-	6.12	868.6073
PC (38:5) A [M+HAc-H]-	5.44	866.5917
PC (38:5) B [M+HAc-H]-	5.65	866.5917
PC (38:6) [M+HAc-H]-	5.23	864.5760
PC (40:4) [M+HAc-H]-	6.67	896.6386
PC (40:5) A [M+HAc-H]-	6.17	894.6230
PC (40:5) B [M+HAc-H]-	6.43	894.6230
PC (40:6) [M+HAc-H]-	5.94	892.6073
PC (p-34:0) or PC (o-34:1) [M+HAc-H]-	6.03	804.5760
PC (p-34:1) or PC (o-34:2) [M+HAc-H]-	5.97	802.5967
PC (p-34:2) or PC (o-34:3) [M+HAc-H]-	5.83	800.5811
PC (p-36:3) or PC (o-36:4) [M+HAc-H]-	5.84	826.5967
PC (p-36:4) or PC (o-36:5) [M+HAc-H]-	5.71	824.5811
PC (p-38:4) or PC (o-38:5) A [M+HAc-H]-	5.93	852.6124
PC (p-38:4) or PC (o-38:5) B [M+HAc-H]-	6.46	852.6124
PC (p-38:5) or PC (o-38:6) [M+HAc-H]-	5.81	850.5967
PC (p-44:4) or PC (o-44:5) [M+HAc-H]-	8.08	936.7063
PE (34:1) [M-H]-	6.25	716.5236
PE (34:2) [M-H]-	5.69	714.5079
PE (36:1) [M-H]-	7.01	744.5549
PE (36:2) [M-H]-	6.45	742.5392
PE (36:3) [M-H]-	5.81	740.5236
PE (36:4) [M-H]-	5.59	738.5079
PE (38:4) [M-H]-	6.36	766.5392
PE (38:6) [M-H]-	5.40	762.5079
PE (p-34:2) or PE (o-34:3) [M-H]-	6.07	698.5130
PE (p-36:2) or PE (o-36:3) [M-H]-	6.85	726.5443

AJS(–) - continued

Lipid	RT (min)	*m/z*
PE (p-36:4) or PE (o-36:5) [M-H]-	5.96	722.5130
PE (p-36:5) or PE (o-36:6) [M-H]-	5.48	720.4974
PE (p-38:3) or PE (o-38:4) [M-H]-	6.86	752.5600
PE (p-38:4) or PE (o-38:5) [M-H]-	6.72	750.5443
PE (p-38:5) or PE (o-38:6) [M-H]-	6.06	748.5287
PE (p-38:6) or PE (o-38:7) [M-H]-	5.76	746.5130
PE (p-40:6) or PE (o-40:7) [M-H]-	6.52	774.5443
PE (p-40:7) or PE (o-40:8) [M-H]-	5.86	772.5287
PI (32:1) [M-H]-	5.73	807.5029
PI (34:1) [M-H]-	4.94	835.5342
PI (34:2) [M-H]-	4.49	833.5186
PI (36:2) [M-H]-	5.11	861.5499
PI (36:4) [M-H]-	4.44	857.5186
PI (38:3) [M-H]-	5.30	887.5655
PI (38:4) [M-H]-	5.04	885.5499
PI (40:6) [M-H]-	4.90	909.5499
SM (d32:0) [M+HAc-H]-	4.65	735.5658
SM (d32:1) [M+HAc-H]-	4.39	733.5501
SM (d32:2) [M+HAc-H]-	3.89	731.5345
SM (d33:1) [M+HAc-H]-	4.73	747.5658
SM (d34:0) [M+HAc-H]-	5.36	763.5971
SM (d34:1) [M+HAc-H]-	5.08	761.5814
SM (d34:2) [M+HAc-H]-	4.52	759.5658
SM (d36:0) [M+HAc-H]-	6.17	791.6284
SM (d36:1) [M+HAc-H]-	5.84	789.6127
SM (d36:2) [M+HAc-H]-	5.23	787.5971
SM (d36:3) [M+HAc-H]-	4.72	785.5814
SM (d38:1) [M+HAc-H]-	6.62	817.6440
SM (d38:2) [M+HAc-H]-	6.00	815.6284
SM (d39:1) [M+HAc-H]-	7.03	831.6597
SM (d40:1) [M+HAc-H]-	7.38	845.6753
SM (d40:2) A [M+HAc-H]-	6.67	843.6597
SM (d40:2) B [M+HAc-H]-	6.78	843.6597
SM (d40:3) [M+HAc-H]-	6.03	841.6440
SM (d41:2) [M+HAc-H]-	7.16	857.6753
SM (d42:2) A [M+HAc-H]-	7.38	871.6910
SM (d42:2) B [M+HAc-H]-	7.55	871.6910
SM (d42:3) [M+HAc-H]-	6.78	869.6753

Lipid species were either detected in only one ionization mode or in both ionization modes. There were 276 unique lipid species identified in total. These unique lipid species belong to 12 lipid classes from polar lipids such as lysophosphatidylcholine over nonesterified fatty acids to very lipophilic compounds such as triacylglycerols and cholesteryl esters. Figure 2 shows the lipid classes and the number of uniquely identified lipids; AJS(+) exclusively detected cholesteryl esters (CE), cholesterol, diacylglycerol (DG), and triacylglycerol (TG) species, while AJS(–) permitted detection of nonesterified fatty acids and phosphatidylinositols (PI). Overlap among specific lipids was observed for lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), phosphatidylcholines (PC), phosphatidylethanolamines (PE), sphingomyelins (SM), and ceramides detectable in both ionization modes.

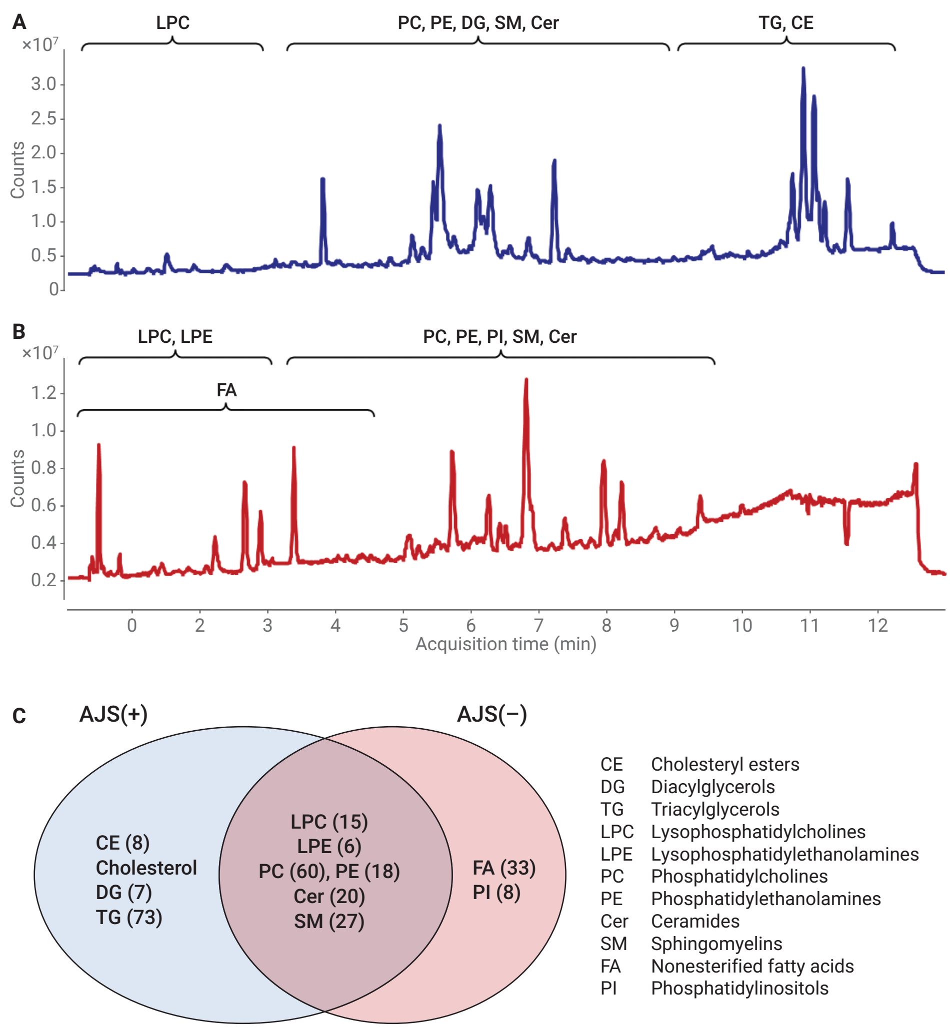

Figure 2. Total ion chromatogram of plasma lipids acquired using (A) LC–AJS(+)-Q-TOF MS and (B) LC–AJS(-)-Q-TOF MS. Elution of particular lipid classes for each ionization mode is highlighted by retention time ranges. The Venn diagram (C) illustrates the number of shared (shown in pink) and unique (shown in blue, red) lipids detected in plasma extracts.

Conclusions

The 1290 Infinity LC system with a ZORBAX EclipsePlus C18 column and optimized LC gradient was used for fast separation of blood plasma lipids with 15 minutes cycle time injection-to-injection. Use of different mobile-phase modifiers for each ionization mode allowed for increased lipidome coverage. Detection with the 6550 accurate-mass Q-TOF system facilitated identification of over 270 unique lipid species combining data from positive and negative ion modes. Semiquantification of lipids was achieved using 11 internal standards for the major lipid classes in plasma.

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