Poster Reprint

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Targeted Lipidomic Analysis of Pediatric Leukemia Cells Using LC-MS/MS Triple Quadrupole

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Introduction

Various research has shown that lipids play important roles during cancer development, progression, and treatment. Leukemia is the most frequent childhood cancer. A challenge in treating leukemia is eradicating leukemia stem cells (LSCs), which are inherently resistant to chemotherapeutics. Although there is great interest in designing therapeutics to target LSCs, clinical translation has been hampered by limited characterization of the biological properties of these heterogeneous cells. While intensive research efforts have been devoted to characterizing the genetics of these tumors, the comprehensive study of lipids has been relatively unexplored. In this study, an easy and fast lipidomics sample preparation method and a rapid (16-min run), sensitive LC-QQQ based targeted lipidomics workflow was developed to quantitatively study the alternation of lipid profiles in bone marrow leukemia cells acquired at initial diagnosis and at relapse diagnosis and at relapse. The targeted lipidomic approach was applied for identification and quantification of over 1200 lipids from about 50 classes in progenitor cells from leukemia bone marrow acquired at diagnosis. A representative subject cell sample was spiked with the internal standard mix containing 97 isotope labelled compounds at different levels with at least 3 replicates. The method was validated in terms of identification, accuracy, precision, matrix effect and linearity of calibration curves. The quantitation was performed using extracted matrix calibration curve. This validated method allowed the detailed lipid profiling by LC-QQQ to identify predictive lipid biomarkers for pediatric leukemia development and progress.

Agilent 1290 Infinity II LC with 6495 Triple Quadrupole LC/MS System.

Experimental

Chromatographic Conditions-Agilent 1290 Infinity II Bio LC

- ✓ Agilent targeted lipidomics chromatographic method as described previously[1]
- ✓ 16-minute RP method designed for comprehensive coverage of major lipid classes
- ✓ Combination of Agilent Deactivator Additive and the Agilent Bio LC improves peak shape and detection for metal-sensitive lipids

[1]Huynh, K, et al. A Comprehensive, Curated, High-Throughput Method for the Detailed Analysis of the Plasma Lipidome. Agilent Application Note 5994-3747EN, 2021

MS Conditions-Agilent 6495 Triple-Quadrupole

Parameters	
MS acquisition	Dynamic MRM
Ion source	Agilent Jet Stream electrospray ionization (AJS ESI positive/negative)
Drying gas temperature	150 °C
Drying gas flow	17 L/min
Nebulizer	20 psi
Sheath gas heater	200 °C
Sheath gas flow	10 L/min
Capillary	3500 V ESI+ / 3000 V ESI-
Nozzle voltage	1000 V ESI+ / 1500 V ESI-
High pressure RF voltage	150 V ESI+ / 200 V ESI-
Low pressure RF voltage	60 V ESI+ / 110 V ESI-

Sample Preparation

- ✓ Prepare 1M subject cell pellet
- ✓ Add 75% ice cold methanol spiked with IS
- ✓ Vortex, sonicate
- ✓ Homogenize with ceramic beads
- ✓ Extract with 3 volumes of 1:1:1 acetonitrile : isopropanol : acetone
- ✓ Vortex, centrifuge and collect the supernatant

Experimental

Lipid Classes (52 Sub-Classes)

Lipid Class	Lipid Subclass	Full Name
AC	AC	Acylcarnitine
AC	AC-OH	Hydroxylated acylcarnitine
BA	BA	Bile acid
CE	CE	Cholesteryl ester
CE	dimethyl-CE	Dimethyl-cholesteryl ester
CE	methyl-CE	Methyl-cholesteryl ester
Cer	Cer(d)	Ceramide
Cer	Cer(m)	Deoxyceramide
Cer	Cer1P	Ceramide-1-phosphate
Cer	dhCer	Dihydroceramide
Cer	dhCer1P	Dihydroceramide-1-phosphate
Cer	dhHex2Cer	Dihydrodihexosylceramide
Cer	dhHexCer	Dihydromonohexosylceramide
Cer	dhS1P and dhSph	Dihydrosphingosine-1-phosphate
Cer	Hex2Cer	Dihexosylceramide
Cer	Hex3Cer	Trihexosylcermide
Cer	HexCer	Monohexosylceramide
Cer	S1P	Sphingosine-1-phosphate
Cer	SHexCer	Sulfatide
Cer	SM	Sphingomyelin
Cer	Sph	Sphingosine
CL	CL	Cardiolipin
COH	COH	Free Cholesterol
DE	DE	Dehydrocholesterol ester
DE	methyl-DE	Methyl-dehydrocholesteryl ester
DG	DG	Diacylglycerol
FFA	FFA	Free fatty acid
Glycerophospholipids	LPC	Lysophosphatidylcholine
Glycerophospholipids	LPC(O)	Lysoalkylphosphatidylcholine (lysoplatelet activating factor)
Glycerophospholipids	LPC(P)	Lysoalkenylphosphatidylcholine (plasmalogen)
Glycerophospholipids	LPE	Lysophosphatidylethanolamine
Glycerophospholipids	LPE(P)	Lysoalkenylphosphatidylethanolamine (plasmalogen)
Glycerophospholipids	LPG	Lysophosphatidylglycerol
Glycerophospholipids	LPI	Lysophosphatidylinositol
Glycerophospholipids	LPS	Lysophosphatidylserine
Glycerophospholipids	PA	Phosphatidic acid
Glycerophospholipids	PC	Phosphatidylcholine
Glycerophospholipids	PC(O)	Alkylphosphatidylcholine
Glycerophospholipids	PC(P)	Alkenylphosphatidylcholine (plasmalogen)
Glycerophospholipids	PE	Phosphatidylethanolamine
Glycerophospholipids	PE(O)	Alkylphosphatidylethanolamine
Glycerophospholipids	PE(P)	Alkenylphosphatidylethanolamine (plasmalogen)
Glycerophospholipids	PG	Phosphatidylglycerol
Glycerophospholipids	PI	Phosphatidylinositol
Glycerophospholipids	PIP1	Phosphatidylinositol monophosphate
Glycerophospholipids	PS	Phosphatidylserine
GM3	GM3	GM3 ganglioside
MAG	MAG	Monoacylglycerols
OxSpecies	OxSpecies	Oxidised lipids
TG	TG [NL]	Triacylglycerol
TG	TG(O) [NL]	Alkyldiacylglycerol
Ubiquinone	Ubiquinone	Ubiquinone

Subject Cell Collection

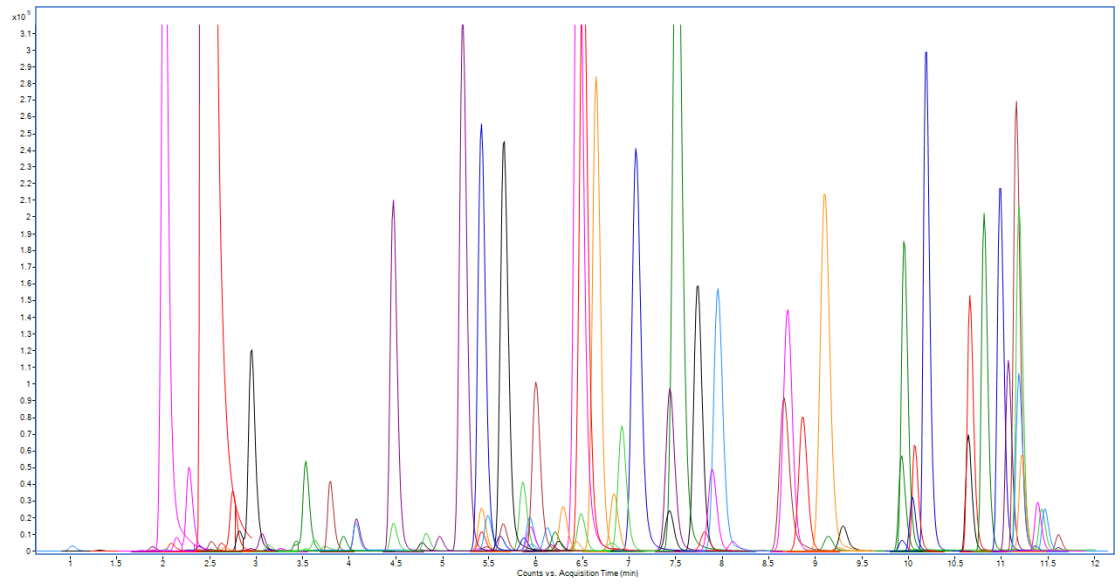
- ✓ Cells were collected from pediatric subjects in accordance with an approved Stanford Review Board protocol
- ✓ The control samples are progenitor cells from healthy young adult bone marrow
- ✓ Leukemia bone marrow cells were acquired at diagnosis and at relapse

Results and Discussion

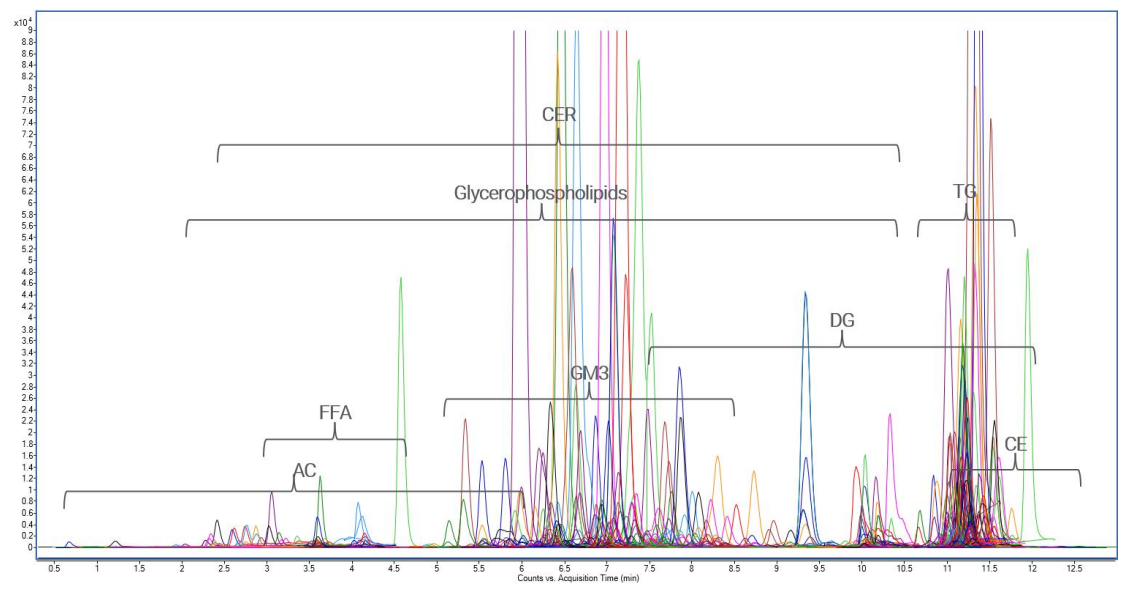
Method Validation Procedure

- ✓ 97 internal standards that represent majority of the included lipid classes were selected to evaluate the method performance
- ✓ Three sets of standards (extracted matrix-matched standards, post-extraction matrix-matched standards and standards in solvent) were prepared at 0.01, 0.02, 0.05, 0.1, and 0.5 µg/mL with 3 or 4 replicates to test the linearity, limit of quantitation (LOQ), accuracy, reproducibility, and matrix effect (ME)

Profile of Internal Standards Spiked in Subject Cell



Profile of Lipids in Subject Cells



Method Validation Results

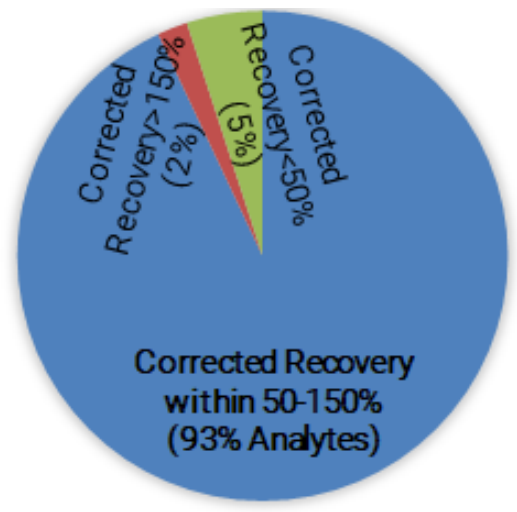
- ✓ Extracted matrix-matched standards provide accurate results by compensating for both matrix effects and potential recovery losses
- ✓ Over 90% of analyte corrected recoveries (CR, within the 50-150% range), CVs (≤30%) and matrix effect (±50%) were obtained at and above the LOQs
- ✓ The coefficient of determination (r^2) values of matrix extracted calibration curves were >0.95 for the majority of analytes ranging from 0.01 (or 0.02) to 0.5 µg/mL

Results and Discussion

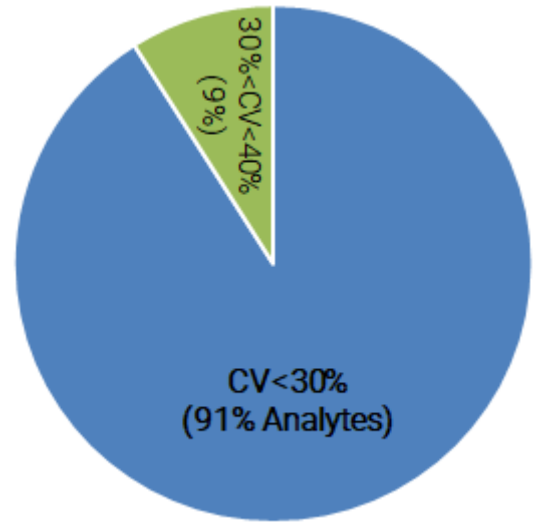
Internal Standard Specific Conditions and LOQ in Cell

Internal Standard	Transition *m/z*	RT min	LOQ in Cell µg/mL	Internal Standard	Transition m/z	RT min	LOQ in Cell µg/mL
AC(16:0)-d3	403.4 > 85.1	2.45	0.01	PC (17:0_18:1)-d5	779.6 > 184.1	7.51	0.01
CE(14:1)-d7	619.6 > 376.5	11.12	0.02	PC (17:0_20:3)-d5	803.6 > 184.1	7.08	0.01
CE (16:1)-d7	647.6 > 376.5	11.36	0.02	PC (17:0_22:4)-d5	829.6 > 184.1	7.42	0.01
CE (18:0)-d6	676.7 > 376.5	11.60	0.02	PC (P-18:0_18:1)-d9	781.6 > 184.1	8.66	0.01
CE (18:1)-d7	675.7 > 376.5	11.61	0.01	PE (15:0_18:1)-d7	711.6 > 570.5	6.81	0.01
CE (20:3)-d7	699.6 > 376.5	11.42	0.01	PE (17:0_14:1)-d5	681.5 > 540.5	5.92	0.01
CE (22:4)-d7	725.7 > 376.5	11.46	0.01	PE (17:0_16:1)-d5	709.5 > 568.5	6.83	0.01
Cer (d18:1_16:1)-d7	543.5 > 271.4	6.63	0.01	PE (17:0_18:1)-d5	737.5 > 596.5	7.88	0.01
Cer (d18:1_18:0)-d7	573.6 > 271.4	8.69	0.01	PE (17:0_20:3)-d5	761.5 > 620.5	7.43	0.01
Cer (d18:1_18:1)-d7	571.5 > 271.4	7.73	0.01	PE (17:0_22:4)-d5	787.6 > 646.6	7.80	0.01
Cer (d18:1_20:1)-d7	599.6 > 271.4	8.85	0.01	PE (P-18:0_18:1)-d9	739.5 > 348.3	9.13	0.01
Cer (d18:1_22:1)-d7	627.7 > 271.4	9.95	0.01	PG (15:0_18:1)-d7	759.6 > 570.6	5.59	0.01
Cer (d18:1_24:1)-d7	655.6 > 271.4	10.18	0.01	PG (17:0_14:1)-d5	729.5 > 540.5	4.96	0.01
Cer1P (d18:1/12:0)	562.4 > 264.3	4.06	0.01	PG (17:0_16:1)-d5	757.5 > 568.5	5.63	0.01
Cholesterol-d7	411.5 > 411.5	4.46	0.01	PG (17:0_18:1)-d5	785.5 > 596.5	6.47	0.01
Cholic Acid-d4	430.3 > 359.3	1.03	0.01	PG (17:0_20:3)-d5	809.5 > 620.5	6.11	0.01
DG (15:0_18:1)-d7	605.5 > 299.5	9.23	0.01	PG (17:0_22:4)-d5	835.5 > 646.5	6.40	0.01
DG (17:0_14:1)-d5	575.6 > 332.3	8.11	0.01	PI (15:0_18:1)-d7	847.6 > 570.6	5.38	0.01
DG (17:0_16:1)-d5	603.6 > 332.3	9.29	0.01	PI (17:0_14:1)-d5	817.6 > 540.6	4.76	0.01
DG (17:0_18:1)-d5	631.6 > 332.3	10.06	0.01	PI (17:0_16:1)-d5	845.6 > 568.6	5.40	0.01
DG (17:0_20:3)-d5	655.6 > 332.3	9.92	0.01	PI (17:0_18:1)-d5	873.5 > 596.5	6.20	0.01
DG (17:0_22:4)-d5	681.6 > 332.3	10.04	0.01	PI (17:0_20:3)-d5	897.5 > 620.5	5.85	0.01
dhCer (d18:0_8:0)	428.4 > 266.4	4.47	0.01	PI (17:0_22:4)-d5	923.6 > 646.6	6.15	0.01
FFA (18:1)-d9	290.3 > 290.2	3.79	0.01	PS (15:0_18:1)-d7	755.5 > 570.5	5.45	0.01
GlcCer (d18:1_15:0)-d7	693.6 > 271.3	5.84	0.01	PS (17:0_14:1)-d5	725.5 > 540.5	4.82	0.01
Hex3Cer (d18:1_17:0)	1038.7 > 264.3	5.95	0.01	PS (17:0_16:1)-d5	753.5 > 568.5	5.47	0.01
LacCer (d18:1_15:0)-d7	855.6 > 271.3	5.41	0.01	PS (17:0_18:1)-d5	781.5 > 596.5	6.28	0.01
LPC (15:0)-d5	487.3 > 184.1	2.28	0.01	PS (17:0_20:3)-d5	805.5 > 620.5	5.93	0.01
LPC (17:0)-d5	515.4 > 184.1	2.94	0.01	PS (17:0_22:4)-d5	831.5 > 646.5	6.23	0.01
LPC (18:1)-d7	529.4 > 184.1	2.74	0.01	S1P (d18:1) d7	387.2 > 271.3	2.08	0.02
LPC (19:0)-d5	543.4 > 184.1	3.53	0.01	SHexCer (d18:1_12:0)	724.8 > 264.3	3.92	0.01
LPE (15:0)-d5	445.3 > 304.3	2.38	0.01	SM (d18:1_15:0)-d9	698.6 > 193.1	5.41	0.01
LPE (17:0)-d5	473.3 > 332.3	3.06	0.01	SM (d18:1_16:1)-d9	710.6 > 193.1	5.21	0.01
LPE (18:1)-d7	487.3 > 346.3	2.85	0.01	SM (d18:1_18:1)-d9	738.7 > 184.1	5.98	0.01
LPE (19:0)-d5	501.3 > 360.3	3.63	0.01	SM (d18:1_20:1)-d9	766.6 > 193.1	6.91	0.01
LPG (15:0)-d5	493.3 > 304.3	2.02	0.01	SM (d18:1_22:1)-d9	794.7 > 193.1	7.94	0.01
LPG (17:0)-d5	521.3 > 332.3	2.65	0.01	SM (d18:1_24:1)-d9	822.7 > 193.1	9.08	0.01
LPG (19:0)-d5	549.3 > 360.3	3.24	0.01	Sph (d17:1)	286.3 > 268.3	2.01	0.01
LPI (13:0)	548.3 > 271.3	1.30	0.01	TG (41:0) [NL-13:0]-d5	731.6 > 500.4	10.64	0.01
LPI (15:0)-d5	581.3 > 304.3	1.87	0.01	TG (43:1) [NL-15:1]-d5	757.7 > 500.4	10.66	0.01
LPI (17:0)-d5	609.3 > 332.3	2.51	0.01	TG (45:1) [NL-17:1]-d5	785.7 > 500.4	10.81	0.01
LPI (19:0)-d5	637.3 > 360.3	3.11	0.01	TG (47:1) [NL-15:1]-d5	813.7 > 556.4	10.98	0.01
LPS (15:0)-d5	489.3 > 304.3	1.88	0.01	TG (48:1) [NL-18:1] d7	829.8 > 523.5	11.07	0.01
LPS (17:0)-d5	517.3 > 332.3	2.51	0.01	TG (49:1) [NL-17:1]-d5	841.8 > 556.5	11.16	0.01
LPS (19:0)-d5	545.3 > 360.3	3.13	0.01	TG (51:2) [NL-19:2]-d5	867.8 > 556.5	11.18	0.01
PA (15:0_18:1)-d7	685.6 > 570.6	5.97	0.02	TG (53:3) [NL-17:1]-d5	893.8 > 608.5	11.18	0.01
PC (15:0_18:1)-d7	753.6 > 184.1	6.45	0.01	TG (55:4) [NL-19:2]-d5	919.8 > 608.5	11.21	0.01
PC (17:0_14:1)-d5	723.6 > 184.1	5.65	0.01	TG (57:4) [NL-21:2]-d5	947.9 > 608.5	11.39	0.01
PC (17:0_16:1)-d5	751.6 > 184.1	6.50	0.01				

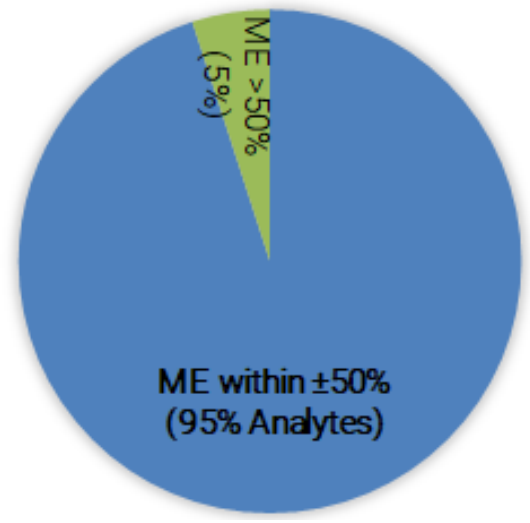
Corrected Accuracy



Reproducibility



Matrix Effect



Statistical results for quantitative analysis based on five spiked levels at 3 or 4 replicates

Conclusions

- ✓ A lipid profiling workflow in cells targeting over 1200 lipids in 54 sub-classes was developed and validated
- ✓ This workflow is ready to be used to accurately quantitate the change of lipidomic profiles in the development and treatment of pediatric leukemia

The Future Plan

- ✓ The lipid profile of 200 samples taken from leukemia bone marrow in relapsed to non-relapsed cases will be compared to identify lipid predictors of relapse, which could guide the development of biomarkers for the prediction of relapse

https://www.agilent.com/en/promotions/asms

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