Application Note

Clinical Research

Lipidomics Analysis of Human Plasma Using Agilent Bond Elut Lipid Extraction 96-Well Plates

By liquid chromatography/mass spectrometry

Authors

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Abstract

This application note presents a novel SPE method using Agilent Bond Elut Lipid Extraction 96-well plates for high-throughput human plasma lipidomics sample preparation. The plasma sample was first protein precipitated. The entire homogenate was loaded into the Bond Elut Lipid Extraction plate for gravity elution. After the washing and elution steps, the extracted sample was run on LC/Q-TOF for lipid profiling using Agilent MassHunter Lipid Annotator software. In addition, the isotopic lipid internal standards were used to study recovery of the major lipid classes using LC/TQ. Both quantitative recovery and qualitative profiling studies demonstrate equivalent method performance between the SPE high-throughput method on the 96-well plate and the previous method based on the 1 mL cartridge. Compared to the 1 mL cartridge SPE method, the high-throughput SPE method on a 96-well plate saves time and effort, increases sample processing productivity, and improves automation compatibility.

Introduction

Lipidomics is a branch of analytical biochemistry that deals with the large-scale study of lipid molecules in biological systems. Lipidomics research has gained more attention over the past decade, which was driven by new insights into functional lipid-lipid and lipid-protein interactions in biochemical systems. Lipidomics research has developed rapidly facilitated by advances in analytical technology, including liquid chromatography, mass spectrometry, and informatics. In return, the new research motivations and advanced analytical capabilities have led to the increased demand for a fast and convenient, accurate and precise, and high throughput lipidomics sample preparation workflow to allow for the larger cohorts in biological research.

One of the primary bottlenecks in any lipidomics workflow is sample preparation. Traditional common sample preparation workflows for lipidomics analyses have used liquid-liquid extraction (LLE) methods[1-4], using various extraction solvent or solvent mixture. However, these methods are time consuming, labor intensive, difficult to automate, and have poor reproducibility.

Bond Elut Lipid Extraction products were developed using EMR-Lipid sorbent, which was demonstrated to provide slightly better or equivalent performance to the traditional LLE methods,[5] but offered significant time and labor saving, improved ease-of-use, and better reproducibility. The workflow using Bond Elut Lipid Extraction 1 mL cartridges for human plasma lipidomics analysis was previously demonstrated,[6] providing a convenient method for small number of samples preparation. The work in this application note will demonstrate a high-throughput workflow on Bond Elut Lipid Extraction 96-well plates.

Experimental

Chemicals and reagents

LC/MS grade acetonitrile (ACN), methanol (MeOH), dichloromethane (DCM), and isopropanol (IPA) were purchased from Honeywell (Muskegon, MI). HPLC grade butanol (*n*-BuOH), ammonium acetate, and medronic acid were purchased from Sigma-Aldrich (St. Louis, MO). NIST SRM 1950 metabolites in human plasma standard was obtained from Sigma-Aldrich (St. Louis, MO). UltimateSPLASH One and EquiSPLASH internal standards were purchased from Avanti Polar Lipids Inc. (Alabaster, AL).

Solutions and standards

The protein precipitation (PPT) extraction solvent, ACN/MeOH (95/5, v/v), was prepared by mixing 95 mL of ACN with 5 mL of MeOH. This solvent was freshly made and stored in a freezer for minimal 10 to 15 minutes before using. The washing solvent, ACN:water (9:1, v/v), was prepared by mixing 90 mL of ACN with 10 mL of water. The elution solvent, DCM: MeOH (1:2, v/v), was prepared by mixing 25 mL of DCM with 50 mL of MeOH. The reconstitution solvent, n-BuOH: MeOH (1:1, v/v), was prepared by mixing 25 mL of n-BuOH with 25 mL of MeOH.

Equipment and materials

Detection for the lipid profiling study was accomplished with an Agilent 6545 LC/Q-TOF equipped with an ESI source with Agilent Jet Stream ionization technology. The chromatographic separation was performed with an Agilent 1290 Infinity II LC consisting of a high-speed binary pump, thermostatted multisampler, and multicolumn thermostat.

Detection for the lipid recovery study was accomplished with an Agilent 6490 LC/TQ equipped with an ESI source with Jet Stream ionization technology. The chromatographic separation was performed with a 1290 Infinity II LC consisting of a high-speed binary pump, thermostatted multisampler, and multicolumn thermostat.

Sample preparation equipment included:

- Agilent Bond Elut Lipid Extraction 96-well plate, 2 mL (part number 5610-2042)
- Agilent square-well collection plate, 2 mL (part number 5133009)
- Agilent square 96-well plate sealing mat (part number 5133005)
- Agilent PlateLoc thermal microplate sealer (part number G5402A/G)
- Agilent PlateLoc microplate heat seals (part number 16985-001)
- Glass-coated 2 mL collection plates or 2 mL collection plate with glass insert
- Agilent positive pressure manifold 96 processor (PPM-96) (part number 5191-4116)
- Pipettes and repeater (Eppendorf, USA)
- Multiprobe plate vortexer (VWR, USA)
- Sonicator (VWR, USA)
- 96-well plate evaporator
- ViaFlo 96 liquid handler (Integra Biosciences, USA)

Instrument conditions

The LC/Q-TOF method conditions are listed in Table 1, and LC/TQ method conditions are listed in Table 2 and Appendix Table A1.

Table 1. Analytical instrument method conditions for the LC/Q-TOF used for lipidomics analysis.

Parameter	Value
Agilent 6545 LC/Q-TOF with Dual Agilent Jet Stream ESI Source	
Instrument Mode	2 GHz, extended dynamic range, *m/z* 1,700
Polarity	Positive and negative
Gas Temperature	210 °C
Drying Gas (Nitrogen)	13 L/min
Nebulizer Gas	45 psi
Sheath Gas	250 °C, 12 L/min
Capillary Voltage	3,500 V (+), 3,000 V (-)
Nozzle Voltage	0 V
Fragmentor	160 V
Oct 1 Rf Vpp	750 V
Acquisition Speed	**MS-only:** 3 spectra/second (MS) **Auto MS/MS:** 3 spectra/second (MS), 4 spectra/second (MS/MS)
Auto MS/MS Parameters	**Isolation width:** narrow (~1.3 amu) **Collision energy:** 20, 35 eV
Reference Correction	Two points at *m/z* 121.050873 (+), 922.009798 (+) Two points at *m/z* 119.036320 (-), 980.016375 (-)
Agilent 1290 Infinity II LC	
Analytical Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 x 100 mm, 1.9 µm, narrow bore LC column (part number 695675-902)
Column Temperature	60 °C
Injection Volume	1 µL
Autosampler Temperature	5 °C
Needle Wash	15 sec in wash port, MeOH: IPA (1:1, v/v)
Mobile Phase	A) water: MeOH (9:1, v/v) with 10 mM ammonium acetate and 10 µM medronic acid B) ACN: MeOH: IPA (3:2:5, v/v/v) with 10 mM ammonium acetate
Flow Rate	0.3 mL/min
Gradient Program	**Time (min) % B** 0.0 60 0.5 60 3.0 84 9.5 87 10.5 95 14.0 96 14.5 100 20.0 100 20.1 60
Stop Time	22 minutes
Post Time	5 minutes

Table 2. Analytical instrument method conditions for the LC/TQ.

Parameter	Value
Agilent 6490 LC/TQ with Dual Agilent Jet Stream ESI Source	
Instrument Mode	2 GHz, extended dynamic range, *m/z* 1,700
Polarity	Positive
Gas Temperature	210 °C
Drying Gas (Nitrogen)	13 L/min
Nebulizer Gas	45 psi
Sheath Gas	250 °C, 12 L/min
Capillary Voltage	3,500 V (+), 3,000 V (-)
Nozzle Voltage	500 V
Fragmentor	160 V
Oct 1 Rf Vpp	750 V
Acquisition Conditions	Dynamic MRM
Agilent 1290 Infinity II LC	
Analytical Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 x 100 mm, 1.9 µm, narrow bore LC column (part number 695675-902)
Column Temperature	60 °C
Injection Volume	1 µL
Autosampler Temperature	5 °C
Needle Wash	15 sec in wash port, MeOH: IPA (1:1, v/v)
Mobile Phase	A) water: MeOH (9:1, v/v) with 10 mM ammonium acetate and 10 µM medronic acid B) ACN: MeOH: IPA (3:2:5, v/v/v) with 10 mM ammonium acetate
Flow Rate	0.4 mL/min
Gradient Program	**Time (min) % B** 0 62 2.0 84 8.0 86 8.1 93 10.0 100 12.9 100 13.0 62
Stop Time	20 minutes
Post Time	0 minutes

For rapid evaluation and comparison of each parameter's impact on lipid extraction efficiency, the total phospholipid profile was monitored using precursor ion scan mode. The scan was set to the *m/z* 184.1 fragment (protonated phosphocholine) with scan range *m/z* 100 to 1,300 by LC/TQ detection.

Sample preparation

Human plasma was prepared based on a protein precipitation followed with Bond Elut Lipid Extraction SPE for lipid extraction, isolation, and purification. The general workflow on the 96-well plate is similar to that on the 1 mL cartridge, but with different specific operations for batch processing on the 96-well plate.

A) Stable labeled internal standard spiking

For the stable labeled internal standards recovery study, plasma samples were prespiked with UltimateSPLASH One internal standard. Prespiked plasma samples were prepared at 2.5 to 15 µg/mL, depending on the lipid. 100 µL of UltimateSPLASH One internal standard was dried down and reconstituted in 1 mL of SRM 1950 plasma. This mixture was vortexed and sonicated for 10 minutes on ice and stored at −80 °C until use. To prepare the postspike samples, 100 µL of UltimateSPLASH One internal standard was dried down and then reconstituted in 1 mL of n-BuOH: MeOH (1:1 v/v). This solution was vortexed and ultrasonicated for 10 minutes and was used to reconstitute dried matrix blank plasma samples.

For the lipid profiling study, all human plasma samples were extracted following the Bond Elut Lipid Extraction plate protocol and then reconstituted in a solution containing EquiSPLASH internal standard in n-BuOH: MeOH (1:1 v/v). To prepare the reconstituted solution, 100 µL EquiSPLASH internal standard was dried down and then reconstituted in 1 mL of n-BuOH: MeOH (1:1 v/v). This mixture was vortexed and ultrasonicated for 10 minutes on ice.

B) Bond Elut Lipid Extraction plate protocol

Figure 1 shows the SPE procedure step by step for human plasma preparation for lipidomics study. Some precautions are highlighted as below.

- Offline protein precipitation (PPT) should be used by conducing the PPT extraction in a separate collection plate, instead of in Bond Elut Lipid Extraction plate directly. This is for ease for following treatment to improve lipids extraction efficiency.
- The entire sample homogenate after PPT extraction should be transferred to Bond Elut Lipid Extraction plate. This is to prevent the potential lipids loss bonded by protein precipitates.
- Sonication on ice was used to improve the lipids extraction efficiency and also to prevent labile lipid molecules degradation caused by heat generated during sonication.
- A liquid handler was used for sample mixing and transferring steps on 96-well plate. If similar equipment is not available in lab, a multi-channel pipette is necessary for the options on 96-well plate.
- Washing solution was added to the sample collection to rinse the wells. The additional rinsing ensures that no precipitates stick to the well wall, preventing sample loss due to an incomplete transfer.
- To prevent plastic contamination, the 2 mL glass coated collection plate or plate with 2 mL glass insert should be used for sample collection.
- A mid-drying step may be necessary in the middle of sample elution. It is to prevent sample cross contamination resulted by large volume eluent overflow or splashing.
- Heat sealing plate mat is highly recommended to prevent sample contamination.

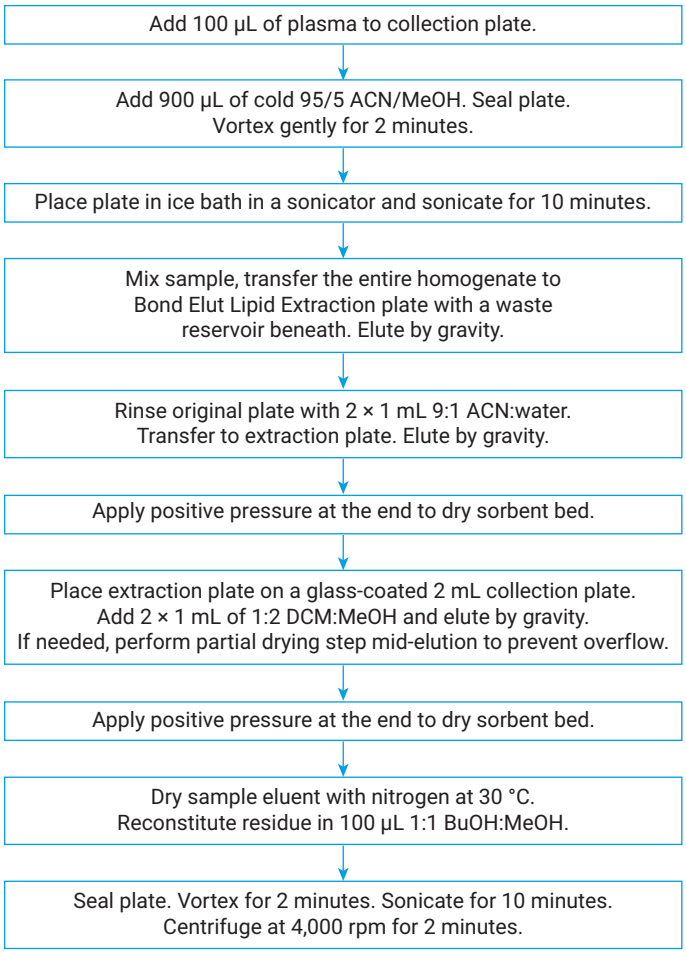

Figure 1. Human plasma preparation procedure for lipidomics study using Bond Elut Lipid Extraction plate.

Results and discussion

SPE method background cleanliness using Bond Elut Lipid Extraction

For untargeted lipidomics studies that use aggressive solvents like DCM, contamination introduced during sample preparation is a concern. Therefore, a plastic container should be avoided for sample preparation. For the Bond Elut Lipid Extraction 96-well plate SPE method, it is unavoidable that the sample will encounter plastic surfaces. However, the Bond Elut Lipid Extraction format and sorbent packing allow a smooth and fast elution. Therefore, the contact time between the elution solvent and plastic surface on plate is limited. The use of a glass-coated collection plate or plate with glass inserts prevents the eluent from interacting with the plastic surface on the collection plate. The fast drying after elution also limited the contact time. A reagent blank is run through the entire procedure to verify cleanliness, and the results are shown in Figure 2.

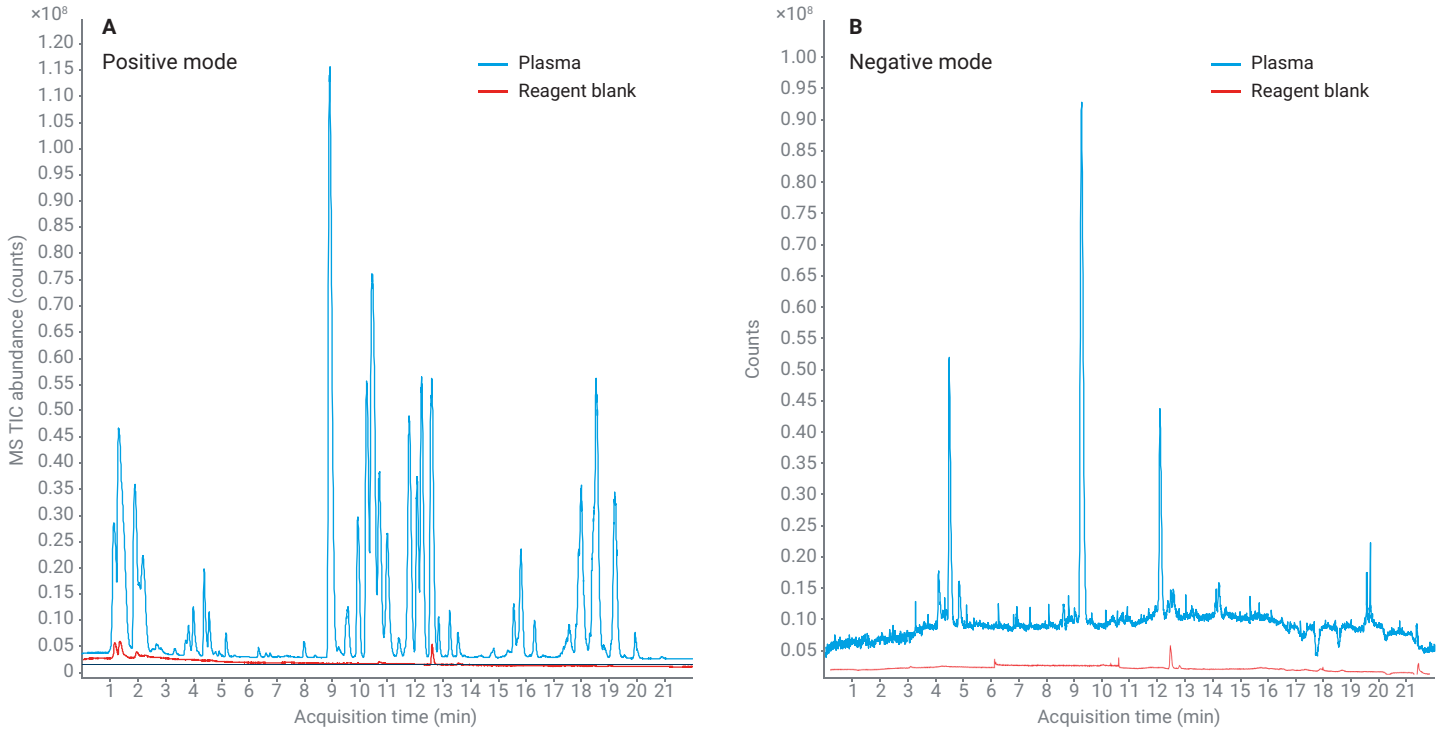

Figure 2. MS TIC chromatograms of SPE plasma extract (blue) versus reagent blank (red) on LC/Q-TOF positive mode (A) and negative mode (B).

Performance comparison between Bond Elut Lipid Extraction using 96-well plates versus 1 mL cartridges

Previous work demonstrated method performance for human plasma lipidomics studies using a Bond Elut Lipid Extraction SPE method on a 1 mL cartridge,[6] and also a thorough comparison between the SPE method and common LLE methods.[5] This study focuses on the performance comparison between the Bond Elut Lipid Extraction method on a 96-well plate and on a 1 mL cartridge. The SPE method comparison includes three aspects: the total ion chromatogram (TIC) comparison for plasma extract using both positive mode and negative, the stable labeled lipid internal standards recoveries, and the lipid profiling results.

Figure 3 shows the TICs for human plasma extracted by the Bond Elut Lipid Extraction method on a 1 mL cartridge and on a 96-well plate. It is clear from the TIC comparison that the general pattern of the lipids extracted by each method is similar, with some noticeable differences for lipid abundances. For example, the triacylglycerols (TG) profiles within retention time (RT) window 17 to 20 minutes appear to have a similar pattern for both methods, but with a higher abundance of sample by the 96-well plate SPE method. Similarly, the TIC chromatograms in the RT window 8 to 14 minutes, where primarily phosphatidylcholines (PC) and sphingomyelins (SM) elute, again show a similar pattern with higher abundances for the 96-well plate sample. For early eluting polar lipids in the RT window 1 to 6 minutes, a similar pattern is once more observed with variations for the abundances for samples prepared by the two SPE methods.

Generally, a higher abundance of lipids was achieved by the 96-well plate SPE method. Contributions to the variations include:

- Improved flow, controlled by gravity on the Bond Elut Lipid Extraction 96-well plate, allows for more efficient lipid retention and elution than on the 1 mL cartridge.
- Small sorbent bed mass differences cause slight variations for lipid retention and elution. A 40 mg amount of EMR—Lipid sorbent is packed for the 1 mL cartridge, while 60 mg of sorbent is packed for the 96-well plate to accommodate the square-well format.

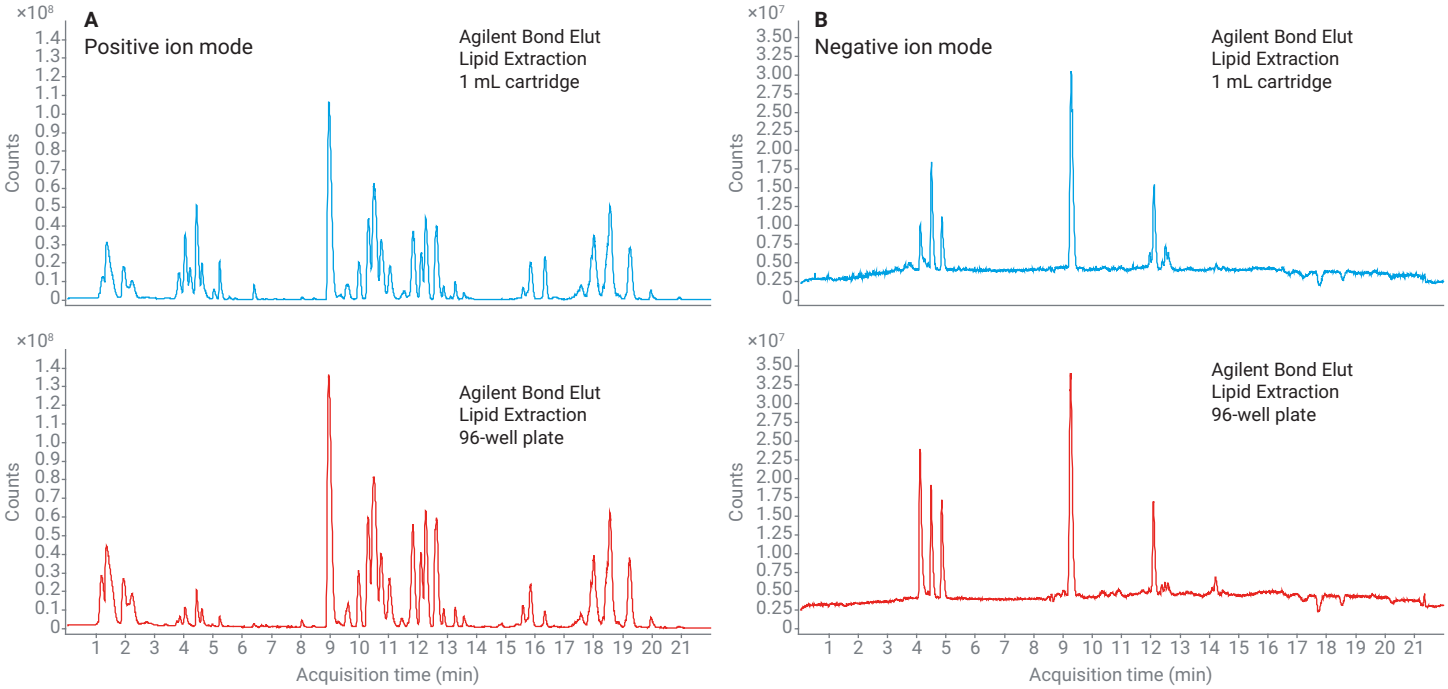

Figure 3. MS TIC chromatograms of plasma extracted by Agilent Bond Elut Lipid Extraction method on a 1 mL cartridge (top) versus a 96-well plate (bottom). Chromatograms were collected under positive mode (A) and negative mode (B) by LC/Q-TOF.

The recovery of 63 deuterium-labeled lipids, representing lipids from 15 classes, was assessed using an LC/TQ quantitative method (Figure 4). The results demonstrate that the SPE extraction using both cartridges and plates yields greater than 70% recovery for all lipid classes. The average recoveries show good consistency for both SPE methods. Less than a 5% difference was demonstrated between the two SPE methods for all lipid classes, except for lysophosphoserine (LPS) and PC, which were 5 to 10% difference. These differences are acceptable for analytical method deviation.

A comparison of the different identified lipid classes distribution is shown in Figure 5, using the Bond Elut Lipid Extraction 96-well plate SPE method versus the Bond Elut Lipid Extraction 1 mL cartridge SPE method. The results show high similarity based on the proportions and concentrations of the identified lipid compound classes.

The lipid profiling study also shows comparable results for samples from the Bond Elut Lipid Extraction SPE method using 96-well plates and the SPE method using 1 mL cartridges. Table 3 lists the number of identified individual lipid compounds and the summed concentrations within each class identified in positive and negative mode. The total number of individual lipids in each class is less than the maximum number of peaks detected in the accurate retention time and mass database due to the stringency filter applied to the analysis.

Simplified workflow and matrix cleanup

Compared to traditional LLE methods used for lipidomics sample preparation, the Bond Elut Lipid Extraction method significantly simplifies the entire workflow with time and labor savings. For LLE methods, repeat extractions are used to improve the lipid extraction efficiency and to remove unwanted sample matrix co-extractives such as salts.

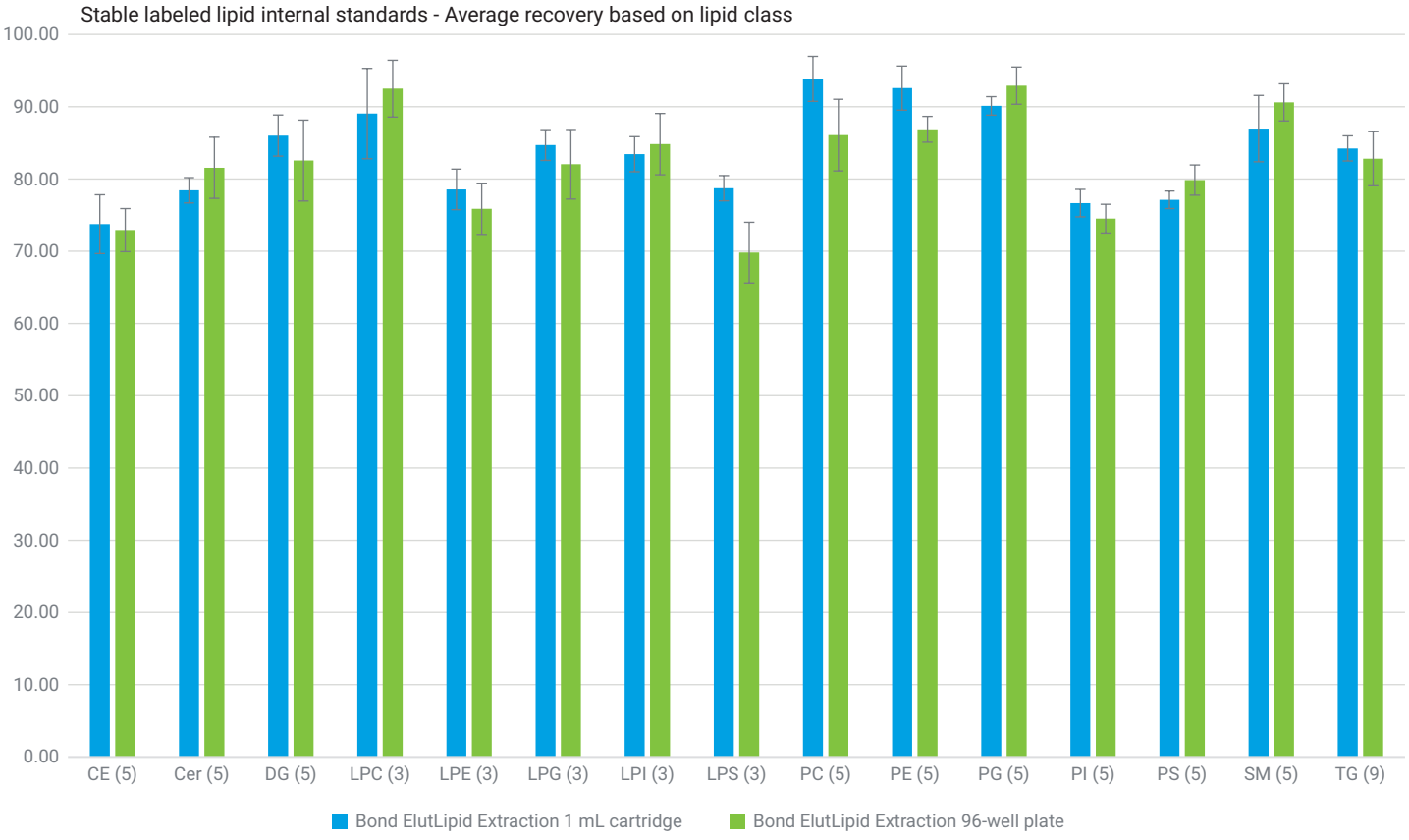

Figure 4. Average recovery of deuterium-labeled lipid internal standards by lipid class based on a targeted LC/TQ workflow. Each bar represents the average of (n) lipids from the lipid class. Lipid classes include: cholesterol esters (CE), ceramides (Cer), diacylglycerides (DG), lysophosphocholine (LPC), lysophosphoethanolamine (LPE), lysophosphoglycerol (LPG), lysophosphoinosotol (LPI), lysophosphoserine (LPS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylgycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM), and triacylglycerol (TG). The number of individual lipids in each class is shown parenthetically.

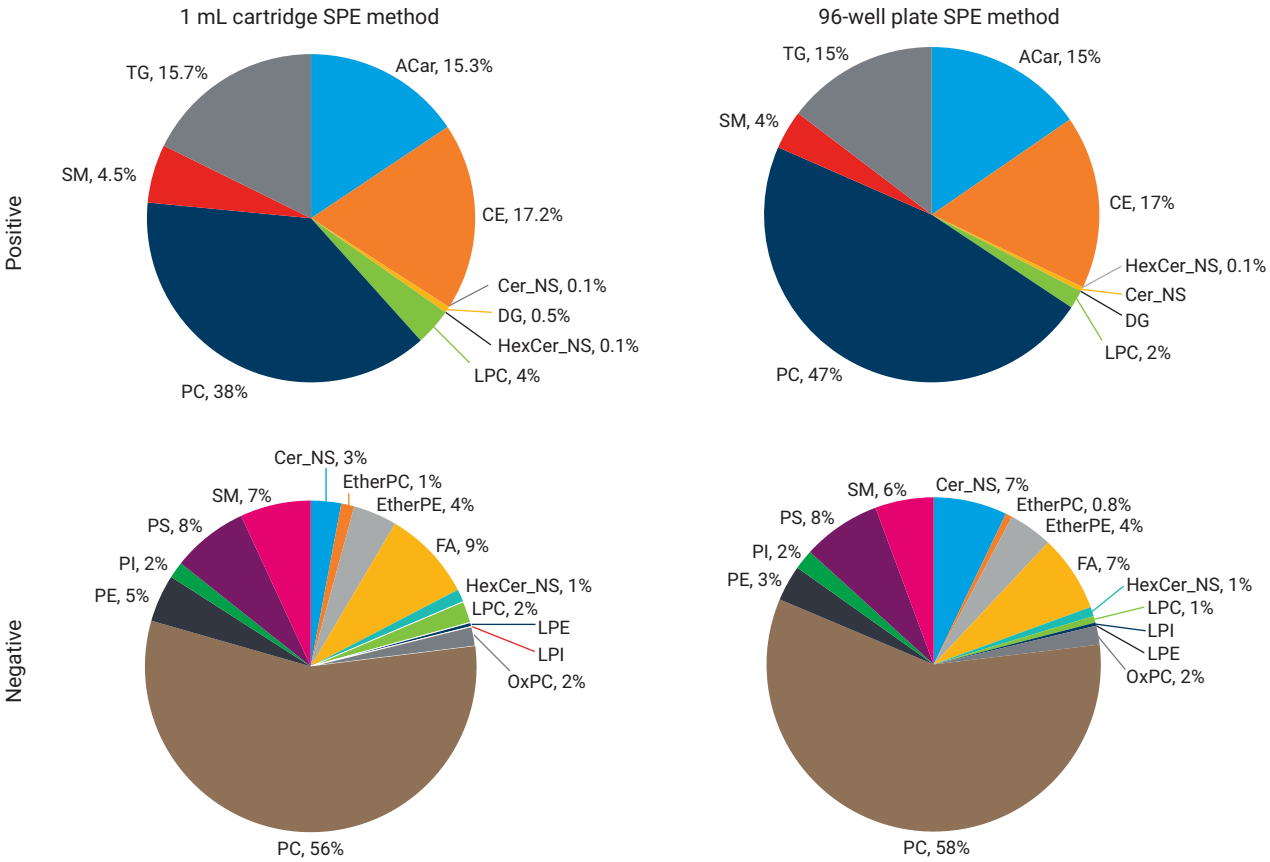

Figure 5. Average lipid class distribution based on calibrated molar concentration (nmol/L) from the LC/Q-TOF profiling study. Profiling results were the average of five preparation replicates with five MS replicates for each preparation sample.

This procedure can be time consuming and labor intensive as it involves multiple steps of phase separation, organic phase transfer, and sample drying. For the Bond Elut Lipid Extraction method, the sorbent provides selective interaction with lipid compounds, which allows for relatively harsh washing with 9:1 ACN:water. With the loading and washing steps, sample matrix can be removed efficiently and conveniently, especially unwanted salts and other interferences without long aliphatic chains.

The SPE procedures are simply additions of solvents to cartridges or wells of plates, which do not require high skill or specialized technique. Therefore, the methods can be performed by any operator and yield similar results. The batch processing on the 96-well plate further improves ease-of-use for the workflow, and thus increases sample preparation productivity significantly. The 96-well plate SPE procedure is also well suited for automation. Further work is underway to optimize procedures for automated SPE lipid extraction.

Conclusion

A simple, rugged, and reliable SPE method using Agilent Bond Elut Lipid Extraction 96-well plates was verified for human plasma sample preparation for lipidomics analysis. The SPE method using a 96-well plate was demonstrated to provide phenomenal lipid identification for untargeted analysis in human plasma for both the number of identified lipids and peak intensity. The evaluation for stable labeled internal lipid standards demonstrated excellent recovery for all major lipid classes. Compared to the SPE method using 1 mL cartridges, the 96-well plate SPE method provides further time and effort savings, and thus increases sample preparation productivity. The improved product format and sorbent packing in the plate wells ensure easy flow and elution by gravity, which makes the 96-well plate easily compatible with automation platforms.

Table 3. Number of identified lipids and corresponding summed concentration.

MS Mode	Lipid Class	Bond Elut Lipid Extraction SPE 1 mL Cartridge		Bond Elut Lipid Extraction SPE 96-Well Plate	
		Summed Concentration (nmol/mL)	Number of Identified Lipids	Summed Concentration (nmol/mL)	Number of Identified Lipids
Positive	ACar	1.12	9	1.61	8
	CE	1.32	9	1.75	9
	Cer_NS	3.34×10^{-5}	5	1.50×10^{-4}	7
	DG	4.35×10^{-2}	8	4.54×10^{-2}	8
	HexCer_NS	5.05×10^{-6}	2	1.86×10^{-5}	2
	LPC	0.26	61	0.19	57
	PC	2.73	75	4.93	80
	SM	0.41	38	0.39	40
	TG	1.27	31	1.53	31
Negative	Cer_NS	2.20×10^{-2}	22	7.56×10^{-2}	25
	EtherPC	9.43×10^{-3}	13	6.76×10^{-3}	10
	EtherPE	3.17×10^{-2}	4	4.51×10^{-2}	3
	FA	6.57×10^{-2}	3	7.88×10^{-2}	4
	HexCer_NS	9.27×10^{-3}	2	9.58×10^{-3}	2
	LPC	1.57×10^{-2}	13	6.75×10^{-3}	11
	LPE	9.27×10^{-3}	2	3.34×10^{-3}	4
	LPI	9.32×10^{-5}	2	2.66×10^{-5}	1
	OxPC	1.41×10^{-2}	3	1.94×10^{-2}	3
	PC	0.42	33	0.62	34
	PE	3.44×10^{-2}	8	3.68×10^{-2}	9
	PI	1.19×10^{-2}	11	2.04×10^{-2}	10
	PS	5.51×10^{-2}	6	8.07×10^{-2}	6
	SM	5.12×10^{-2}	17	6.01×10^{-2}	17
Total		–	377	–	381

References

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5. Apffel A.; Zhao L.; Sartain M. J. A Novel Solid Phase Extraction Sample Preparation Method for Lipidomic Analysis of Human Plasma Using Liquid Chromatography/Mass Spectrometry. *Metabolites* **2021**, *11*, 294–313.
6. Apffel A.; Zhao L. Lipidomic Analysis of Human Plasma Using Bond Elut Lipid Extraction with the Agilent 6545 LC/Q-TOF. *Agilent Technologies application note*, publication number 5994-1783EN, **2020**.

Appendix

Table A1. Stable labeled lipid internal standard (UltimateSPLASH One) dMRM parameters using LC/TQ detection in positive mode.

Compound Name	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (V)	Retention Time (min)
CE 14-1 d7	619.6	376.4	10	11.274
CE 16-1 d7	647.7	376.4	10	11.628
CE 18-1 d7	675.7	376.4	5	12.066
CE 20-3 d7	699.7	376.4	10	11.722
CE 22-4 d7	725.7	376.4	10	11.778
Cer d18-1_d7_16-1	543.6	525.5	8	5.062
	543.6	271.2	20	
Cer d18-1_d7_18-1	571.6	553.5	8	6.261
	571.6	271.2	28	
Cer d18-1_d7_20-1	599.6	581.5	8	7.755
	599.6	271.2	24	
Cer d18-1_d7_22-1	627.6	609.5	8	9.168
	627.6	271.2	32	
Cer d18-1_d7_24-1	655.7	637.6	12	9.684
	655.7	271.3	28	
DG 17-0_14-1 d5	575.5	540.5	20	6.897
	575.5	332.3	20	
DG 17-0_16-1 d5	603.6	332.2	20	8.61
	603.6	316.2	20	
DG 17-0_18-1 d5	631.6	596.5	20	9.436
	631.6	332.3	20	
DG 17-0_20-3 d5	655.6	620.4	20	9.209
	655.6	332.4	20	
DG 17-0_22-4 d5	664.6	394.2	20	9.327
	664.6	332.3	20	
LPC 15-0 d5	487.4	184	30	1.696
	487.4	104	24	
LPC 17-0 d5	515.4	184	30	2.191
	515.4	104	24	
LPC 19-0 d5	543.4	184	28	2.648
	543.4	104	28	
LPE 15-0 d5	445.3	304.3	12	1.718
	445.3	184	30	
LPE 17-0 d5	473.3	332.3	20	2.215
	473.3	184	30	
LPE 19-0 d5	501.4	483.4	8	2.661
	501.4	360.3	20	
LPG 15-0 d5	476.3	458.2	0	1.757
	476.3	304.4	8	
LPG 17-0 d5	504.3	468.1	8	2.252
	504.3	332	20	
LPG 19-0 d5	532.4	496.3	8	2.692
	532.4	360.3	16	

Compound Name	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (V)	Retention Time (min)
LPI 15-0 d5	564.3	546.3	5	1.484
	564.3	528.3	10	
LPI 17-0 d5	592.4	574.3	5	1.844
	592.4	556.3	10	
LPI 19-0 d5	620.4	602.4	5	2.287
	620.4	584.4	10	
LPS 15-0 d5	489.3	384.3	6	1.399
	489.3	304.3	16	
LPS 17-0 d5	517.3	412.3	16	1.856
	517.3	332.3	16	
LPS 19-0 d5	545.4	440.4	16	2.31
	545.4	360.4	20	
PC 17-0_14-1 d5	723.6	184	28	4.595
	723.6	125.1	50	
PC 17-0_16-1 d5	751.6	184	28	5.529
	751.6	125.1	50	
PC 17-0_18-1 d5	779.6	184	28	6.8
	779.6	125	48	
PC 17-0_20-3 d5	803.6	184	32	6.216
	803.6	124.9	50	
PC 17-0_22-4 d5	829.7	184	28	6.709
	829.7	124.8	50	
PE 17-0_14-1 d5	681.5	540.5	20	4.718
	681.5	184	20	
PE 17-0_16-1 d5	709.6	568.6	20	5.714
	709.6	184	20	
PE 17-0_18-1 d5	737.6	596.5	20	7.074
	737.6	184	20	
PE 17-0_20-3 d5	761.6	620.6	16	6.45
	761.6	184	20	
PE 17-0_22-4 d5	787.6	646.6	20	6.937
	787.6	184	20	
PG 17-0_14-1 d5	729.5	540.5	10	3.774
	712.5	540.5	10	
PG 17-0_16-1 d5	757.6	568.5	10	4.369
	740.5	568.5	10	
PG 17-0_18-1 d5	785.6	596.6	20	5.213
	768.6	596.6	10	
PG 17-0_20-3 d5	809.6	620.6	10	4.831
	792.6	620.6	10	
PG 17-0_22-4 d5	835.6	646.6	20	5.134
	818.6	646.6	20	
PI 17-0_14-1 d5	817.6	540.5	25	3.636
	800.5	540.5	10	
PI 17-0_16-1 d5	845.6	568.5	20	4.206
	828.6	568.5	15	
PI 17-0_18-1 d5	873.6	596.6	10	4.996
	856.6	596.5	10	

Compound Name	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (V)	Retention Time (min)
PI 17-0_20-3 d5	897.6	620.6	20	4.657
	880.6	620.6	15	
PI 17-0_22-4 d5	923.6	646.6	15	4.927
	906.6	646.6	15	
PS 17-0_14-1 d5	725.5	540.5	20	3.69
	725.5	296.3	32	
PS 17-0_16-1 d5	753.5	568.5	20	4.291
	753.5	324	32	
PS 17-0_18-1 d5	781.6	596.5	24	5.1
	781.6	352.3	36	
PS 17-0_20-3 d5	805.6	620.4	20	4.776
	805.6	332.4	32	
PS 17-0_22-4 d5	831.6	646.5	16	5.307
	831.6	332.4	40	
SM d18-1_16-1_d7	710.6	193.2	28	4.107
	710.6	125	50	
SM d18-1_18-1_d7	738.7	193.2	28	4.931
	738.7	125	50	
SM d18-1_20-1_d7	766.7	193.2	24	5.92
	766.7	125	50	
SM d18-1_22-1_d7	794.7	193.2	24	7.363
	794.7	125	50	
SM d18-1_24-1_d7	822.7	193.2	28	9.018
	822.7	125	50	
TG 14-0_14-0_13-0 d5	731.7	500.5	15	10.71
	731.7	486.5	19	
TG 14-0_14-0_15-1 d5	757.7	512.5	23	10.727
	757.7	500.4	20	
TG 14-0_14-0_17-1 d5	785.7	540.5	15	10.973
	785.7	500.5	15	
TG 16-0_16-0_15-1 d5	813.8	556.5	23	11.257
	813.8	540.5	27	
TG 16-0_16-0_17-1 d5	841.8	568.5	23	11.542
	841.8	556.5	19	
TG 16-0_16-0_19-2 d5	867.8	594.6	19	11.56
	867.8	556.5	23	
TG 18-1_18-1_17-1 d5	893.8	608.6	23	11.56
	893.8	594.6	19	
TG 18-1_18-1_19-2 d5	919.8	620.6	19	11.597
	919.8	608.6	35	
TG 18-1_18-1_21-2 d5	947.9	648.6	35	11.883
	947.9	608.6	31	

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