A Comparison of Phospholipid Removal Methods & Plates

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Introduction

In an effort to help further streamline method development for mass spectroscopy based testing at ARUP Laboratories, we have evaluated the efficiency of seven different commercially available phospholipid removal plates. Our study compared the performance of these plates to each other, to liquid-liquid extraction using non-miscible organic solvents and protein precipitation extractions. Our goal was to rank the performance of these phospholipid removal methods in terms of reduction of lipids, and the ease of use on automated liquid handlers that are currently in use in our labs. Ultimately, the choice of extraction technique will depend heavily on the chemical properties of the analyte(s) in the test, therefore this ranking should serve only as a starting point for our future method development endeavors.

Method

Extraction

The following phospholipid removal sample plates were tested :

Phenomenex Phree™ Phospholipid Removal Plate, 8E-S133-TGB, 96-well plate sample volume 25-400 µL

Phenomenex Novum™ SLE, 8E-E138-FGA, MINI 96-well plate 300 µL volume (sample + diluent 1:1)

Phenomenex Strata DE, 8-S325-FGB, 96-well plate 200 µL volume (sample + diluent 1:1)

Biotage ISOLUTE® PLD+ Protein and Phospholipid Removal Plate, 918-0050-P01, 96-well plate 500 µL volume (sample + diluent)

Biotage ISOLUTE® SLE+ Supported Liquid Extraction, 820-0400-T, 400 µL tube volume (sample + diluent 1:1)

Waters Oasis® PRiME HLB µElution Plate, 186008052, 96-well plate sample volume 10-375 µL

Agilent Captiva EMR-Lipid Plate, 5190-1000, 96-well plate sample volume 20-200 µL

We also tested the following extraction techniques:

Protein Precipitation

Liquid-Liquid Extraction.

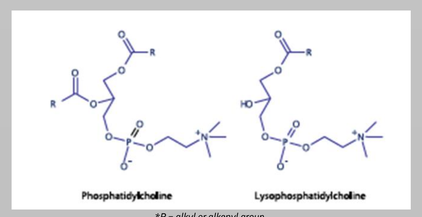
Extractions were performed per the manufacturer's instructions that came with each product using the same lot of blank plasma.

100 µL of Blank Plasma, pooled from ~ 20 individuals, obtained from ARUP Reagent Lab, P-31000.

The samples were dried down at 40 ºC and reconstituted with 1 mL of mobile phase A and B mixed in a ratio to match the starting conditions of the analytical gradient.

The samples were analyzed in triplicate over two days.

To check for carryover a solution blank was injected after each sample.



*R = alkyl or alkenyl group

Method

Disposables

Column: Phenomenex Kinetex 2.6 µm C18 100 Å, 50X2.1 mm, OOB-4723-AN.

Mobile Phase A: 5mM Ammonium Formate, 0.05% Formic Acid in Lab water

Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Analysis

The following instrumentation and conditions were used for each experiment:

Agilent 1260 pump, 1260 column heater, and 1260 degasser stack.

A PAL auto-sampler was used to inject the samples.

5 µL injection volume was used.

LC Gradient

Min	Flow (µL/Min)	A%	B%
0.0	600	95	5
0.5	600	95	5
3.0	600	2	98
4.0	600	2	98
4.1	600	95	5
5.5	600	95	5

MS Parameters

Electro Spray Ionization in the Positive Mode

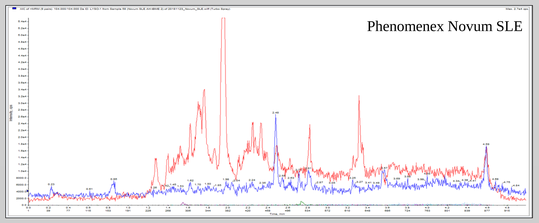
Temperature	550
Curtain Gas™	30
Collision Gas	9
Ion Source Gas 1	50
Ion Source Gas 2	50
IonSpray™ Voltage	5500

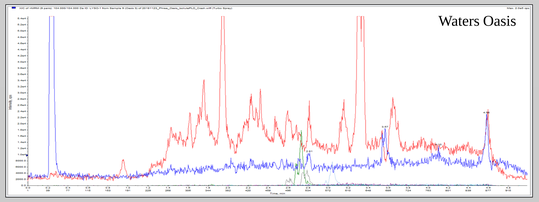
The following nine transitions were monitored for phospholipids and lysophospholipids.

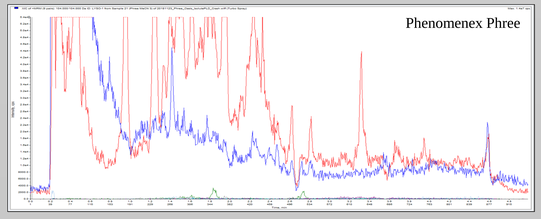
Q1	Q2	DP	CE	EP	CXP
104.0	104.0	165	7	9	12
184.0	184.0	165	7	9	12
496.0	184.0	80	43	9	12
522.0	184.0	80	43	9	12
524.0	184.0	80	43	9	12
704.0	184.0	80	43	9	12
758.0	184.0	80	43	9	12
786.0	184.0	80	43	9	12
806.0	184.0	80	43	9	12

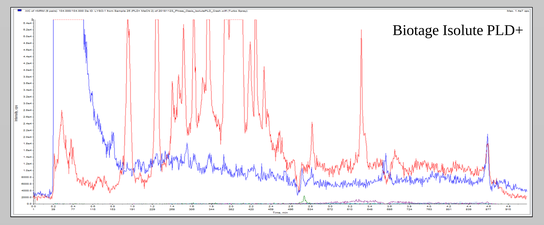
Results

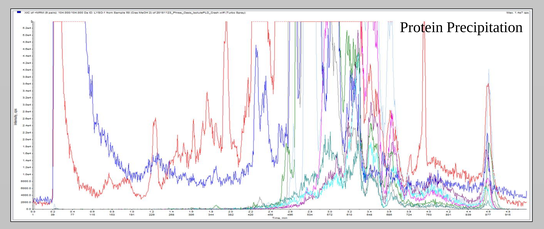
Representative chromatograms for each plate type: All nine transitions from the previous table are included in the chromatograms below. All chromatograms are normalized to 5.5e4 intensity and a 5 minute run time.

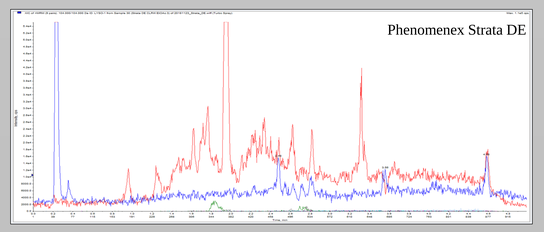




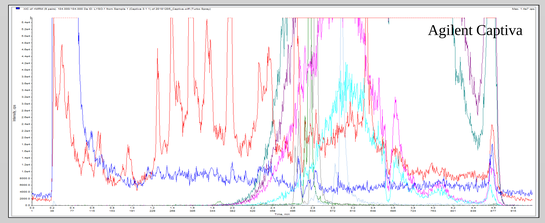


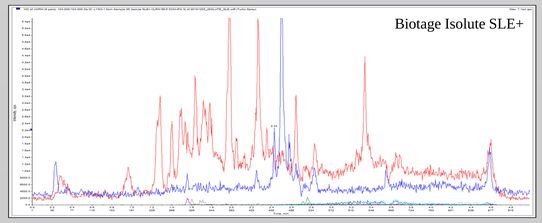


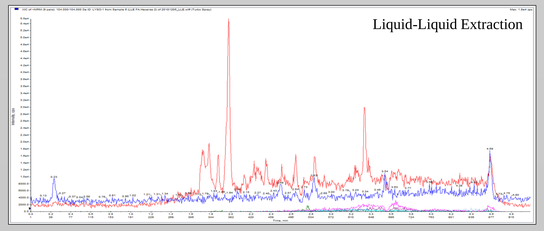




Results Cont.







Evaluation Criteria

Phospholipid removal efficiency

The most important goal for our laboratory was the removal of phospholipids. We evaluated this based on normalizing the chromatograms to the same scale and visually reviewing the peak areas for the different phospholipid transitions.

Ease of use

Length of time per extraction, ability to automate on a liquid handler, and amounts of laboratory supplies used for each preparation.

Ease of Automation

It is important to us to embrace technologies that can be easily scaled up and automated.

Conclusion

Below is a table that shows our final ranking based on the criteria listed above:

Phospholipid Removal Efficiency	Ease of Use	Ease of Automation (96-Well Plate on a Liquid Handler)
ISOLUTE® PLD+	Captiva	Protein Precip
Phree™	Phree™	ISOLUTE® PLD+
Novum™ SLE	ISOLUTE® PLD+	Captiva
Strata DE	Protein Precip	Phree™
LLE	Strata DE	Novum™ SLE
ISOLUTE® SLE+	Oasis®	Strata DE
Oasis®	Novum™ SLE	Oasis®
Captiva	ISOLUTE® SLE+	ISOLUTE® SLE+
Protein Precip	LLE	LLE