Phree™

Phospholipid Removal Plates

Remove Phospholipids

Remove Protein

No Method Development

phenomenex®
. . . breaking with tradition℠

One Quick Method, Three Big Advantages

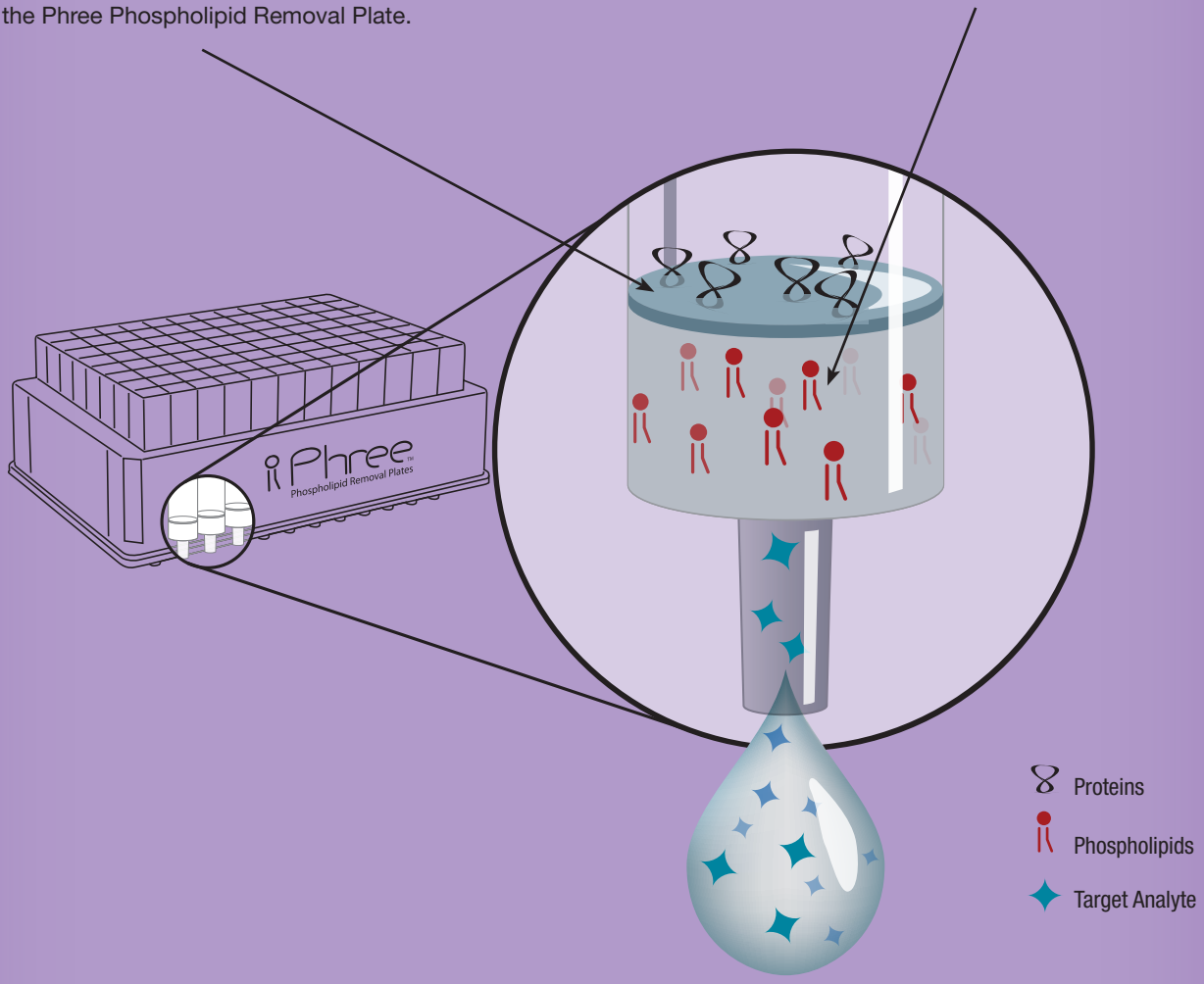
1. **Remove Proteins** from 96 samples at once
2. **Eliminate Phospholipids** that cause ion suppression and increase your column lifetime!
3. **No Method Development;** one method for acids, bases, and neutrals

Remove Proteins

Solvent Shielding Technology™ prevents dripping of organic solvent, allowing for protein precipitation within the wells of the Phree Phospholipid Removal Plate.

Eliminate Phospholipids

The Phree sorbent selectively removes phospholipids from precipitated plasma samples.



Be Free of Phospholipids and Proteins

Phree™ Phospholipid Removal Plates

"We have demonstrated that Phree Phospholipid Removal Plates resulted in >98% decrease in the peak area of the six phospholipids we monitored in rat plasma compared to traditional protein crash. We highly recommend the use of these plates for high throughput LC-MS/MS bioanalytical sample preparation."

Nina Khoshaba
WIL Research

3 Steps Above Traditional Protein Precipitation

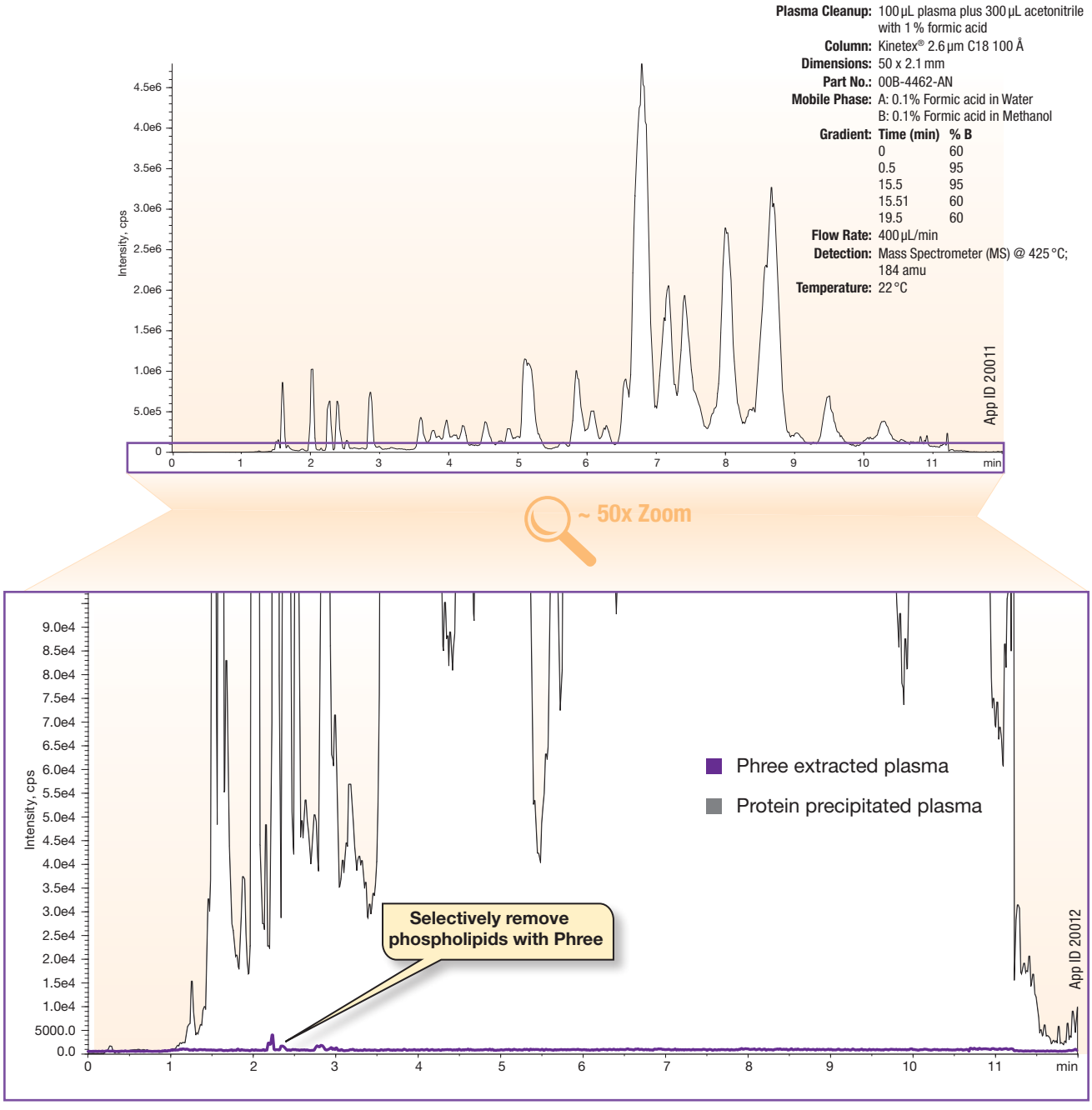
1. Protein Precipitation Does Not Remove Phospholipids

Endogenous phospholipids are a primary source of ion suppression and resulting matrix effects in bioanalytical LC/MS work. Ion suppression caused by the presence of phospholipids can result in:

- irreproducible results
- quantitation issues
- loss in method sensitivity
- matrix to matrix bias

Total Phospholipid Profile

Protein Precipitation vs. Phree™ Phospholipid Removal Plates

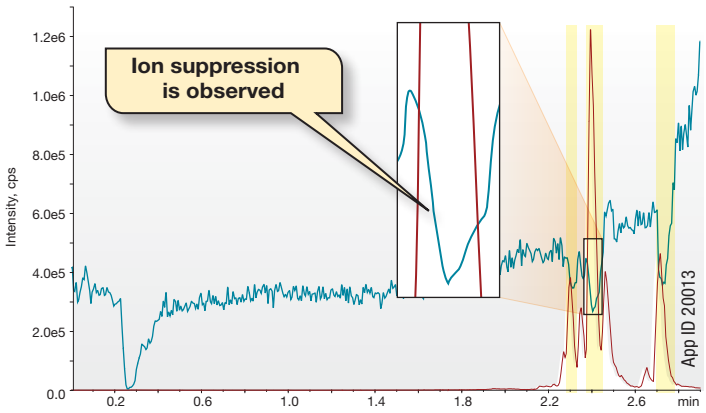


Phospholipid profile monitored using *m/z 184-184*

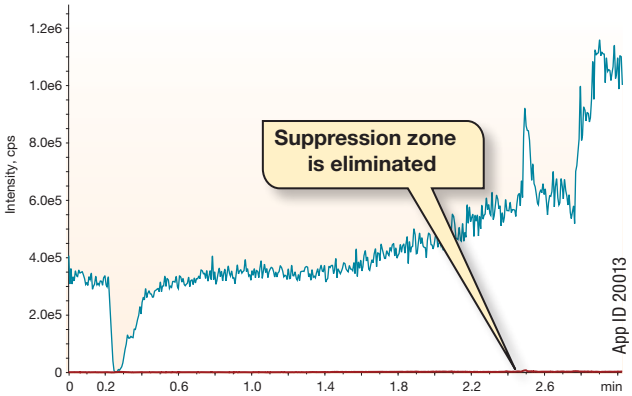
2. Reduce Ion Suppression

The presence of phospholipids in plasma samples produces zones of ion suppression that correlate exactly with the phospholipid elution profile when analyzed via mass spectrometer (MS).

Protein Precipitated Plasma



Phree Extracted Plasma



Suppression Zone

Phospholipids *m/z 184-184*

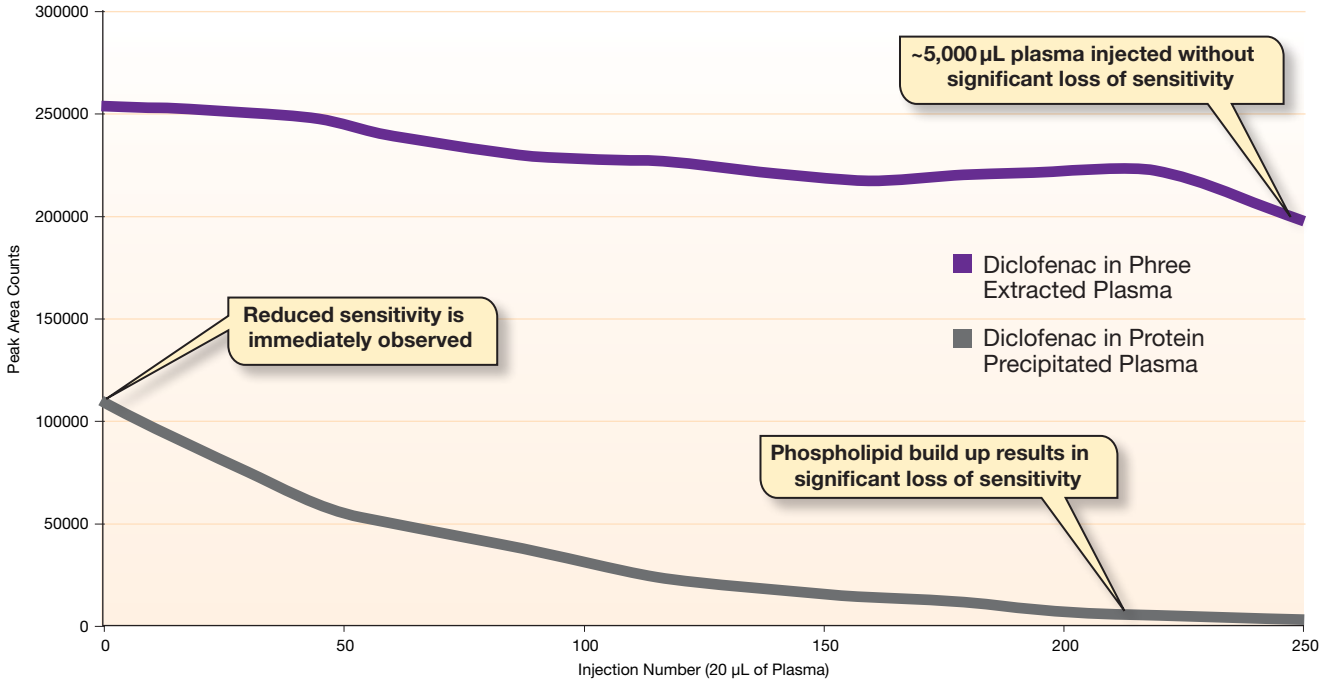
Amoxapine *m/z 314-271*

Amoxapine was infused post-column to establish an ion suppression/enhancement profile with both protein precipitated plasma (left) and Phree extracted plasma (right), showing that Phree can successfully reduce ion suppression.

3. Maximize Sensitivity and Column Lifetime

Phospholipids reduce the sensitivity of the MS signal and shorten column lifetime when they build up over time.

Column Sensitivity after 250 Injections

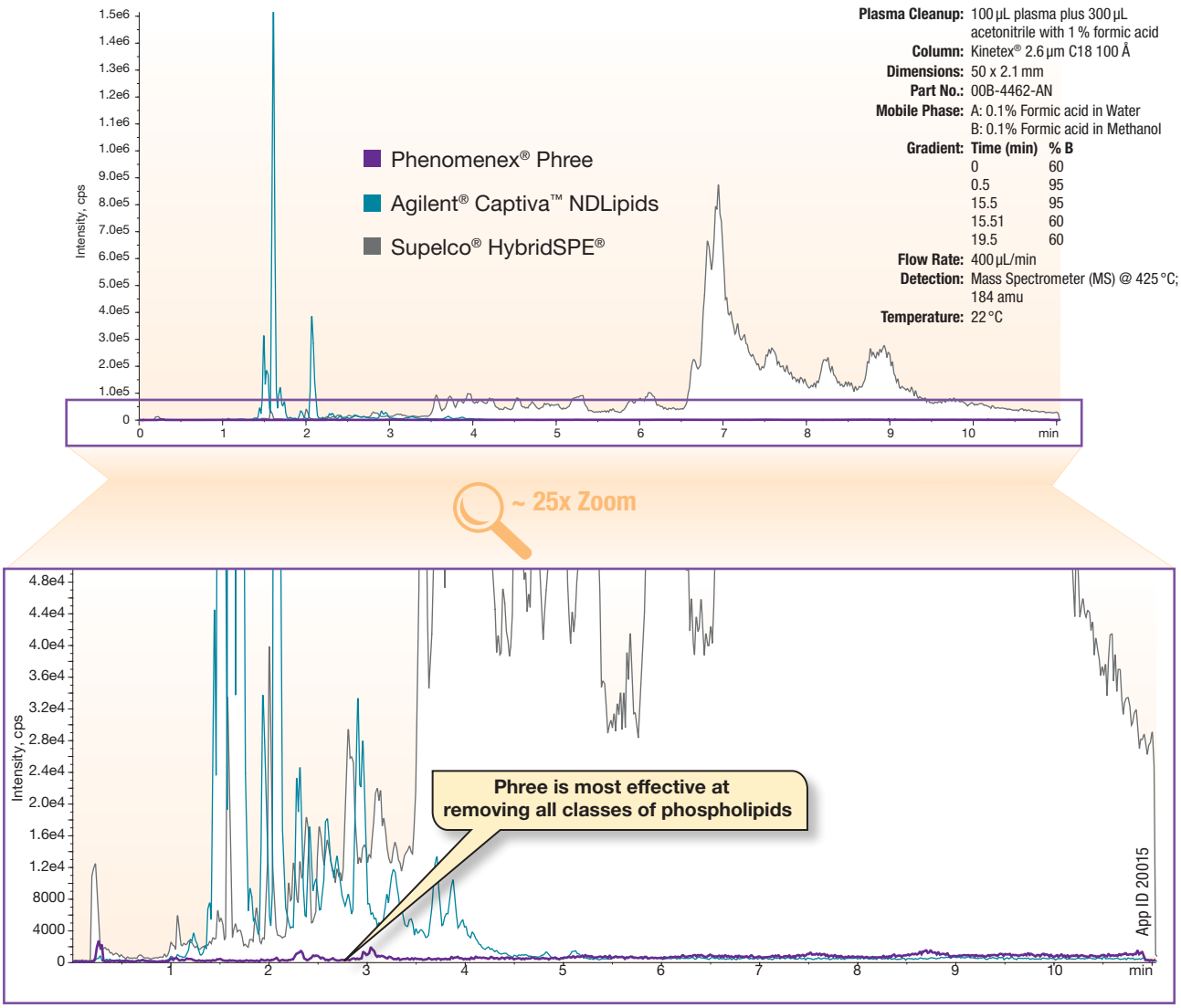


To assess the effect of phospholipid build up, repetitive 20 µL injections of diclofenac in protein precipitated plasma versus diclofenac in Phree extracted plasma were made.

An Improved Phospholipid Removal Solution

Remove All Classes of Phospholipids

Lysophosphatidylcholines and phosphatidylcholines both contribute to matrix effects. Remove all classes of phospholipids using Phree™ Phospholipid Removal Plates.



Phospholipid profile monitored using *m/z 184-184*

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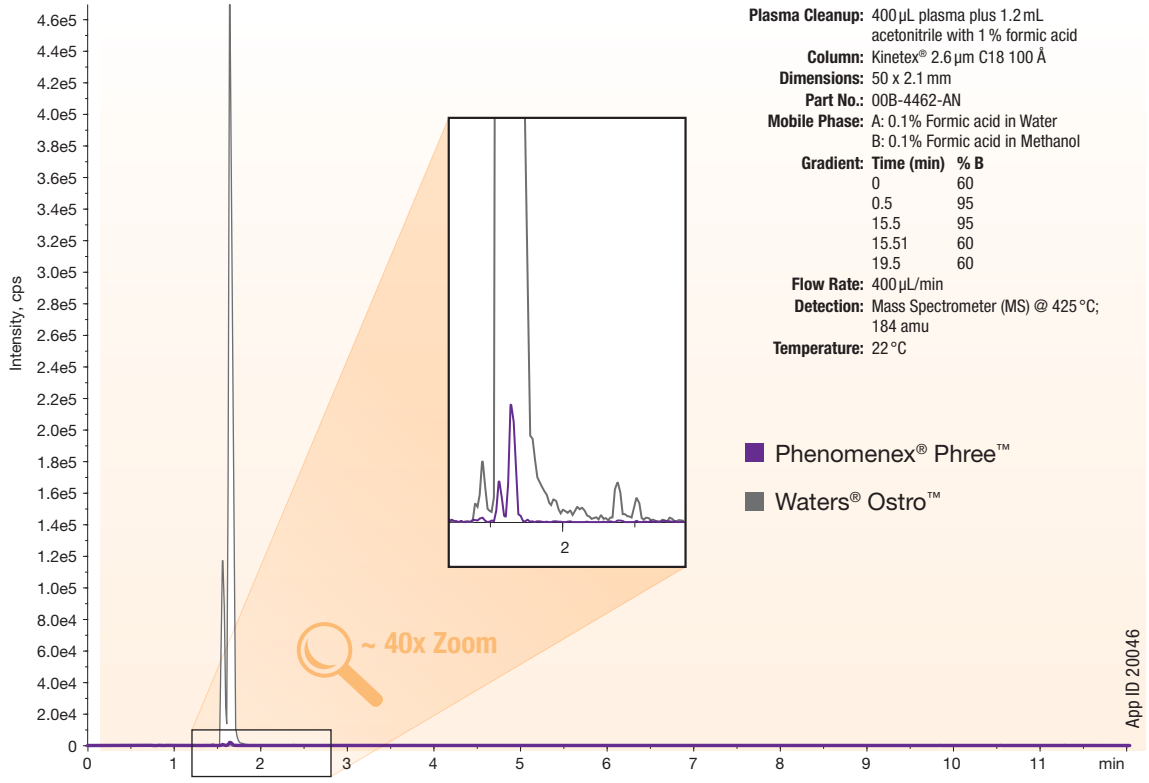
Supelco and HybridSPE are registered trademarks of Sigma-Aldrich Co.
Comparative separations may not be representative of all applications.

Extended Phospholipid Capacity

The Phree sorbent has an extended capacity for phospholipids, allowing you to load up to 400 µL of plasma without significant breakthrough of phospholipids.

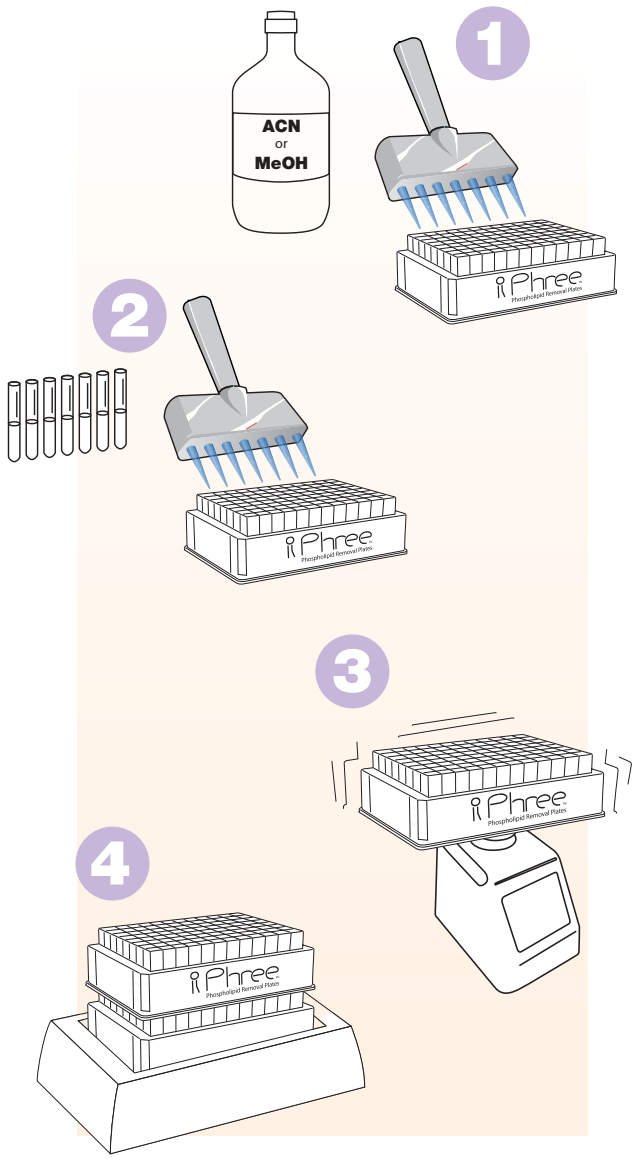


Phospholipid profile monitored using *m/z 184-184*

Waters Corporation states that the true maximum recommended plasma volume that can be processed using the Ostro plate is 350 µL due to well volume limitations. Ostro is a trademark of Waters Corporation. Comparative separations may not be representative of all applications.

Skip the Method Development

One Method for Acids, Bases, and Neutrals



1. Dispense

Organic solvent into the wells of the Phree™ Phospholipid Removal Plate in a volume of 3-4x the volume of the intended plasma sample. Maximum volume of organic solvent should not exceed 1.2 mL. Recommended organic solvents are listed below.

2. Add

25 µL – 400 µL plasma directly into the organic solvent in each well of the Phree Phospholipid Removal Plate. Maintain a final ratio of 3:1 to 4:1 organic solvent:plasma.

3. Vortex*

2 minutes at maximum possible speed, taking care not to allow cross contamination. Sample can stand for up to 25 minutes.

4. Filter

Centrifuge: Place the Phree Phospholipid Removal Plate on top of a collection plate and centrifuge at 500 g for 5 minutes or until filtrate is collected.

Vacuum: Place the Phree Phospholipid Removal Plate onto a suitable 96-well sample manifold or robot. Ensure that a 96-well collection plate is positioned inside the manifold or under the Phree Phospholipid Removal Plate. Vacuum at 2-7 inch Hg for up to 5 minutes or until filtrate is collected.

Positive Pressure: Place the Phree Phospholipid Removal Plate on top of a collection plate and apply 2-5 psi using a positive pressure manifold.

* When used with a liquid-handling instrument or automation, aspirate/dispense cycles may be used to promote in-tip mixing and precipitation. This will ensure complete precipitation and filtration. Vortexing is not necessary when in-tip mixing is performed.

Recommended Organic Solvents

Solvent	Solvent Volume	Plasma Volume
Acetonitrile with 1 % Formic acid	300 µL	100 µL
Methanol with 1 % Formic acid	400 µL	100 µL

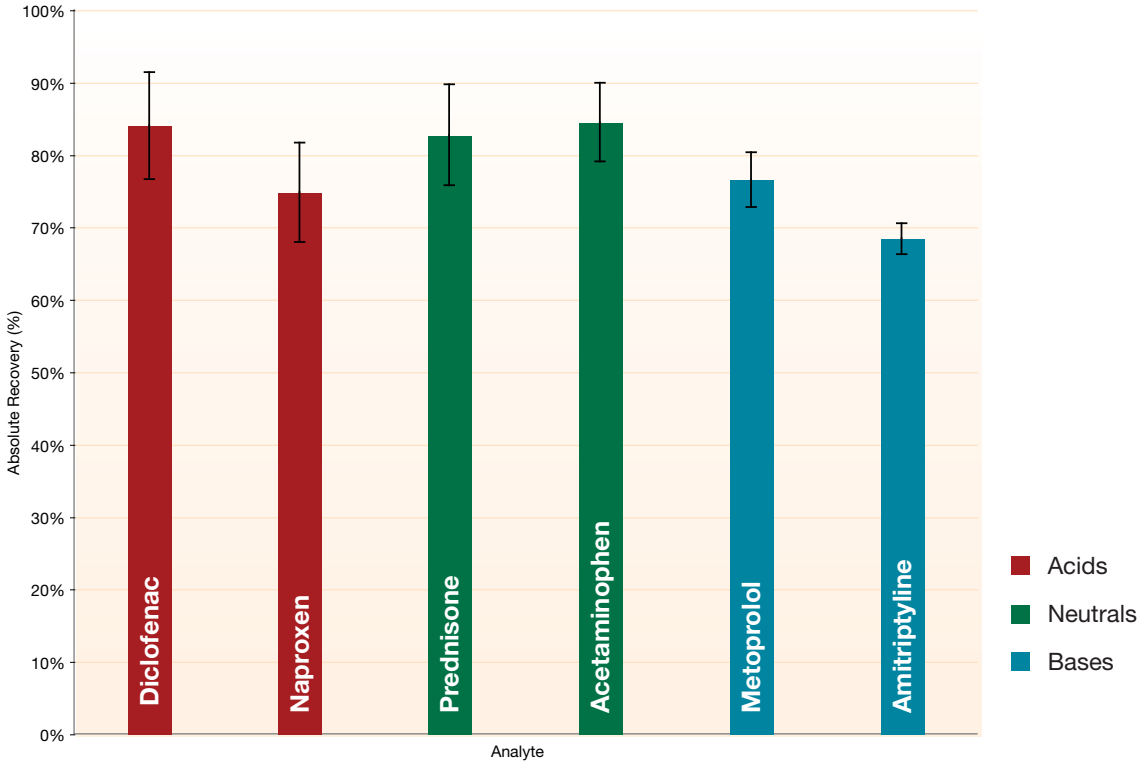
Tip

Phenomenex collection plates and sealing mats pair perfectly with Phree Phospholipied Removal Plates. Go to page 11 for a complete list of collection plates and sealing mats.

High Recoveries of Target Analytes

The Phree general protocol has been developed to produce high recoveries of acids, bases, and neutrals while simultaneously removing all classes of phospholipids.

Absolute Recoveries of Acids, Bases, and Neutrals



Recovery data was obtained by calculating the average absolute recoveries of analytes extracted from 3 Phree plates.

Sample Preparation Specialists are Ready to Assist You

Two Levels of Method Development Support

Level 1

Contact one of our dedicated sample preparation specialists for immediate method development assistance.

Level 2

Send your sample to our analytical services group for custom method development. Visit **www.phenomenex.com/PhenoLogix** for more information.

Get Started

Speak with your Sample Preparation Specialist:

By phone: **310-212-0555** or your local Phenomenex representative

By email: **Support@phenomenex.com**

> "Phenomenex's prompt support is very important for us to achieve our work. I thank them for their persistent support and innovative products."
>
> Allena Ji
> Wyeth

Order Phree Now!

Part No.	Description	Unit	Price
8E-S133-TGB	Phree Phospholipid Removal 96-Well Plates	2/box	
Collection Plates (deep well, polypropylene)			
AH0-7192	Strata® 96-Well Collection Plate 350 µL/well	50/pk	
AH0-7193	Strata 96-Well Collection Plate 1 mL/well	50/pk	
AH0-7194	Strata 96-Well Collection Plate 2 mL/well	50/pk	
AH0-8635	Strata 96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk	
AH0-8636	Strata 96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk	
AH0-7279	Strata 96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk	
Sealing Mats			
AH0-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk	
AH0-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk	
AH0-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk	
AH0-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk	
AH0-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk	
AH0-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk	
AH0-7362	Sealing Tape Pad	10/pk	
Vacuum Manifold			
AH0-8950	Strata 96-Well Plate Manifold, Universal with Vacuum Gauge	ea	

guarantee

If Phree Phospholipid Removal Plates do not perform as well or better than your current phospholipid removal plate, return the product with your comparative data within 45 days for a FULL REFUND.

Tip

For more information about Phenomenex sample preparation products, visit **www.phenomenex.com/sampleprepinfo**

iPhree™

Phospholipid Removal Plates

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium
t: +31 (0)30-2418700
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: (800) 543-3681
f: (310) 328-7768
info@phenomenex.com

Denmark
t: 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: 09 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

India
t: 040-3012 2400
f: 040-3012 2411
indiainfo@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

Norway
t: 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: (800) 541-HPLC
f: (310) 328-7768
info@phenomenex.com

Sweden
t: 08 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

All other countries:
Corporate Office USA
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

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