Phospholipid and Protein Removal in One Simple SPE Process Prevents Matrix-Based Signal Suppression

- Resprep PLR SPE removes both proteins and phospholipids in one easy, high-efficiency procedure.
- Avoid signal suppression by removing interfering phospholipids from the sample matrix.
- No method development—straightforward and effective sample preparation for acids, bases, and neutral compounds.

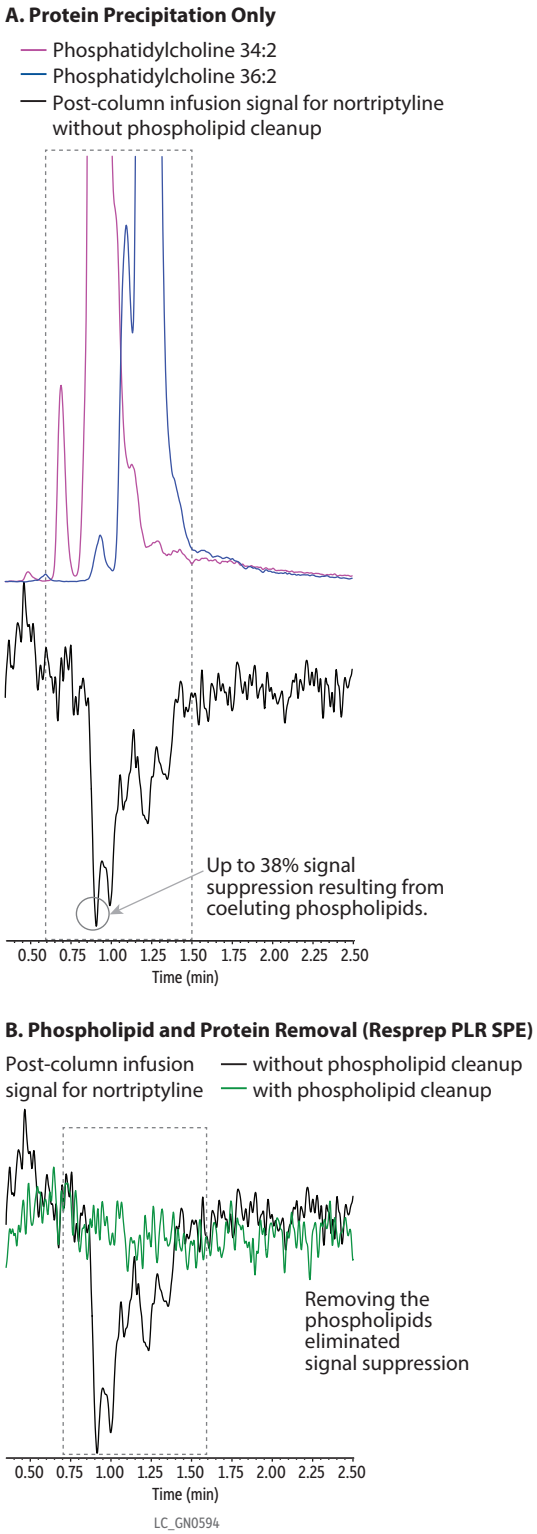
Whether you are analyzing whole blood, plasma, or serum, sample matrix components can interfere with analysis, decreasing both accuracy and sensitivity. Removing proteins through precipitation and filtration is a very common strategy for preventing problems, but with this procedure the sample still contains a high concentration of phospholipids. While LC-MS/MS analytical methods will not detect phospholipids if the ions they produce are not monitored, their presence can still affect the analytical results and the overall cleanliness of the instrument.

How to Avoid Phospholipid Signal Suppression

If phospholipids in the sample coelute with analytes of interest, they can affect target analyte ionization, which can cause suppression and lower overall analytical sensitivity. To illustrate signal suppression, we established a post-column infusion of nortriptyline and monitored the steady signal that the constant flow of analyte created. Then, we injected a sample of protein-precipitated plasma onto the column and monitored the presence of phospholipids as well as their effect on the nortriptyline signal. Figure 1A shows how certain phospholipids significantly reduced the nortriptyline response (by 38% in this case). In situations where a target analyte coelutes with phospholipids, use of internal standards can help account for phospholipid signal suppression and further method development can often eliminate the coelution. However, when phospholipid and protein removal can be done concurrently in the same process normally used to remove proteins alone, the question becomes "why try to design a method around something I can easily remove from my sample?"

Resprep PLR SPE products allow you to do just that: one simple SPE workflow removes proteins through precipitation and filtration, while phospholipids are selectively retained in an innovative sintered composite material. Figure 1B illustrates the effectiveness of removing phospholipids along with proteins using a Resprep PLR 96-well plate (cartridges are also available). Comparing again to a continuous post-column infusion of nortriptyline, we can see that the signal is suppressed with protein precipitation alone, but when the sample phospholipids are removed along with the proteins, the signal is no longer compromised. Higher accuracy and sensitivity can be obtained by avoiding phospholipid signal suppression.

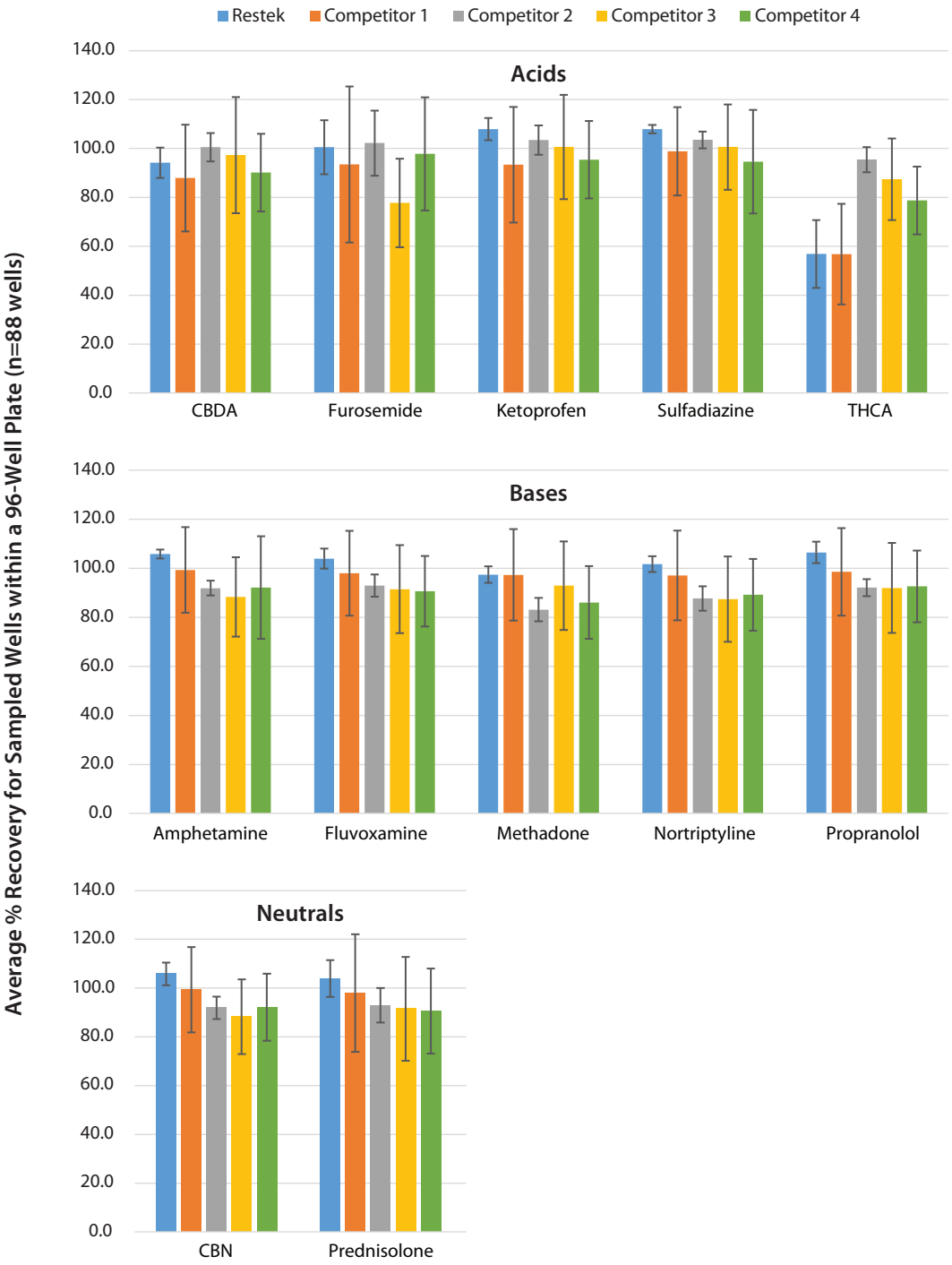
Figure 1: Protein precipitation alone does not prevent phospholipid signal suppression (A), but simultaneous phospholipid and protein removal with Resprep PLR SPE eliminates this problem (B).



Excellent Recoveries for Wide Range of Compounds with No Method Development

Simultaneous phospholipid and protein removal using Resprep PLR SPE cartridges or 96-well plates can improve sensitivity by reducing phospholipid signal suppression, but its broad applicability offers an additional benefit: less time spent on method development. Simple to use and effective for a wide variety of compound chemistries, Resprep PLR SPE provides reliable cleanup without the need for compound-specific method development. As shown in the competitor comparison in Figure 2, good recoveries were obtained in plasma with a high degree of analytical precision for acidic, basic, and neutral compounds. Tight manufacturing controls ensure consistently strong performance, as demonstrated by the accurate and precise recovery results obtained in plasma for all three target compound categories across three independent lots (Table I).

Figure 2: Evaluation of Analyte Recovery for a Single 96-Well Plate by Manufacturer*



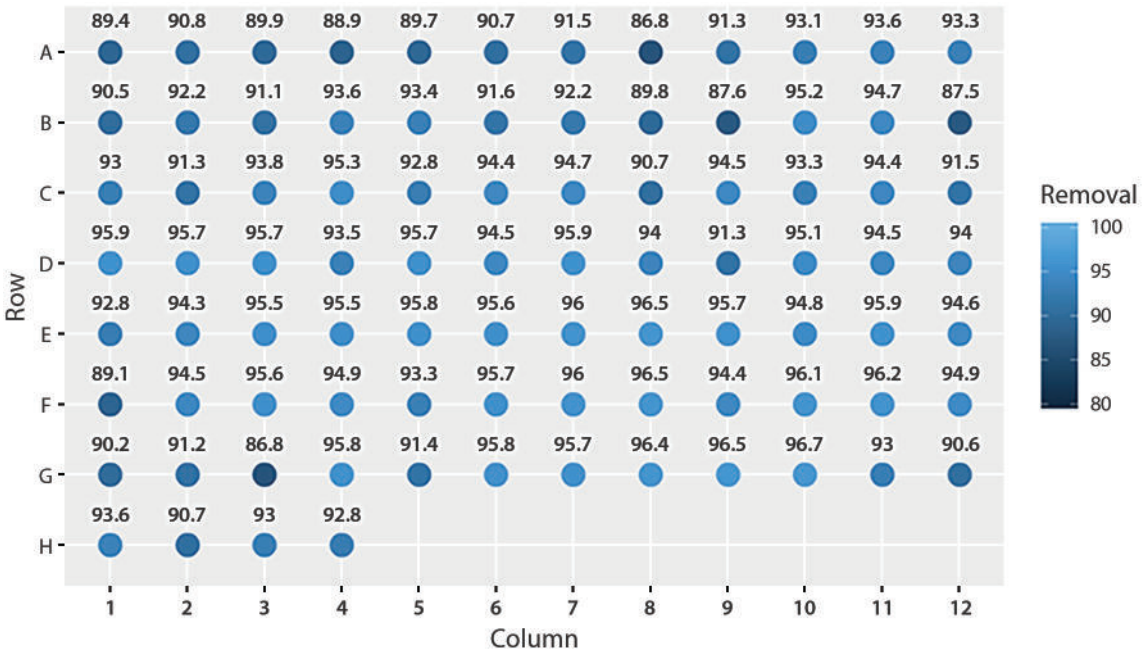
*Fortification levels given in Table I. Error bars represent ±%RSD.

Table I: Grand average percent recoveries for acids, bases, and neutral compounds across three different lots of Resprep PLR 96-well plates (3 plates per lot).

Compound Class	Compound	Fortification Level (ng/mL)	Grand Average of % Recoveries (n = 787 wells)	Standard Deviation	%RSD
Acids	CBDA	150	94.1	9.3	9.9
	Furosemide	250	105.3	17.2	16.4
	Ketoprofen	125	105.4	7.3	6.9
	Sulfadiazine	25	108.2	5.7	5.3
	THCA	100	68.9	9.6	14.0
Bases	Amphetamine	15	106.8	5.6	5.3
	Fluvoxamine	50	104.3	6.4	6.1
	Methadone	5	99.4	6.0	6.1
	Nortriptyline	15	101.2	6.0	6.0
	Propranolol	25	105.0	6.2	5.9
Neutrals	CBN	100	87.7	9.1	10.4
	Prednisolone	125	105.0	10.6	10.0

The reliable performance demonstrated here is due the robust design of Resprep PLR SPE products. A top frit is used to capture proteins, while the sorbent bed below is a specially designed porous polymer matrix that is co-sintered to silica particles that have bound ligands that retain phospholipids. The end result is a solid matrix for phospholipid and protein removal that—unlike loose sorbent beds—is not susceptible to channeling. Figure 3 shows the resulting performance: high levels of phospholipids are removed consistently across all tested wells in a representative 96-well plate. This means your target analytes can be effectively recovered and analytical accuracy will not be affected by phospholipid signal suppression. In addition, your instrument and overall productivity will benefit from not injecting high concentrations of phospholipids that will eventually reduce LC column lifetime and increase MS downtime as the ion source becomes contaminated.

Figure 3: Representative phospholipid removal performance for a typical Resprep PLR 96-well plate.



In conclusion, switching from protein precipitation alone to simultaneous phospholipid and protein removal with Resprep PLR SPE is an easy way to obtain more accurate, reliable results for clinically relevant substances in whole blood, plasma, or serum. The simple sample preparation process is effective for a wide range of analytes, so excellent results can be obtained for acids, bases, and neutral compounds without time and resources being spent on additional method development. In addition to removing interferences that could affect your data, Resprep PLR SPE ensures that less contamination is injected into your column and mass spectrometer, which results in less downtime for maintenance.

Resprep PLR SPE Products

- Remove both proteins and phospholipids from biological samples in one easy, high-efficiency procedure.
- Avoid signal suppression by removing interfering phospholipids from the sample matrix.
- No method development—straightforward and effective sample preparation for acids, bases, and neutral compounds.
- Offered in 96-well plate format for high-throughput or automated workflows and in cartridge format for lower throughput applications.
- 3-way versatility for filtration—compatible with all common devices:
  - Vacuum manifolds
  - Positive pressure manifolds
  - Centrifugation

Simultaneously remove phospholipids and proteins in a single, simple procedure with Resprep PLR (phospholipid removal) SPE products. Whole blood, serum, and plasma all contain proteins and phospholipids that can interfere with target analytes and hasten the need for instrument maintenance. It's important to remove them from samples prior to analysis, and Resprep PLR SPE cartridges or 96-well plates make this an easy task by combining protein precipitation and phospholipid removal in one sample preparation process. No analyte-specific method development is required because the same procedure can be used for samples containing acids, bases, or neutral compounds. From preparation to analysis, Restek is proud to offer workflow solutions that help provide accurate, reliable results.

Description	qty.	cat.#
Resprep PLR SPE Cartridge, 25 mg/1 mL cartridge	100-pk.	28300
Resprep PLR SPE 96-Well Plate, 25 mg/2 mL each well	ea.	28301

Well Plates

- Polypropylene plates with round-bottom wells reduce liquid retention; conical bottom provides optimal recovery of reagents.
- Nunc shared wall technology allows increased well volume for optimum storage capacity and improved mixing.
- Round well shape is ideal for applications that require vortexing.
- Ideal for sample collection, storage, sampling, and combinatorial chemistry and library applications.
- Fits most autosampler compartments.
- All microplates manufactured by Nunc meet the recommendation of American National Standards Institute (ANSI) (ANSI/SBS 1-2004).

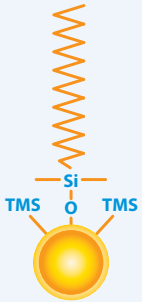
Description	Well Shape	Well Bottom	qty.	cat.#
0.45 mL 96-Well Plates	round	conical	20-pk.	26497
0.45 mL 96-Well Plates	round	conical	case of 120	26496
1.3 mL 96-Well Plates	round	round	5-pk.	26495
1.3 mL 96-Well Plates	round	round	case of 50	26494
2.0 mL 96-Well Plates	round	round	5-pk.	26493
2.0 mL 96-Well Plates	round	round	case of 60	26492

Resprep VM-96 Vacuum Manifold for 96-Well Plates

- Heavy-duty, stainless steel and aluminum body stays in place and does not slide like lighter models.
- Viewing window allows easy observation of plate height and drip rate.
- Durable O-ring and gaskets resist solvent damage and provide leak-free seals time after time.
- Precision-manufactured parts assemble quickly and easily, with perfect alignment of well plate and collection plate.
- Customize plate height to your exact requirements; precision height adaptor and five shims in a range of thicknesses allow easy, accurate configuration.
- Works with any manufacturer's well plates and collection plates for solid phase extraction (SPE), supported liquid extraction (SLE), protein precipitation (PPT), and filtration.

Description	qty.	cat.#
Resprep VM-96 vacuum manifold	ea.	25858

Column Characteristics:



Stationary Phase Category: C18, octadecylsilane (L1)
Ligand Type: End-capped C18
Particle: 1.8 µm, 2.7 µm, or 5 µm superficially porous silica (SPP or "core-shell")
Pore Size: 90 Å
Carbon Load: 9% (1.8 µm), 7% (2.7 µm), 5% (5 µm)
End-Cap: yes
Surface Area: 125 m^2/g (1.8 µm), 130 m^2/g (2.7 µm), or 100 m^2/g (5 µm)
Recommended Usage:
pH Range: 2.0–8.0
Maximum Temperature: 80 °C
Maximum Pressure: 1,034 bar/15,000 psi* (1.8 µm), 600 bar/8,700 psi (2.7 µm); 400 bar/5,800 psi (5 µm)

* For maximum lifetime, recommended maximum pressure for 1.8 µm particles is 830 bar/12,000 psi.

Properties:
- Compatible with moderately acidic to neutral mobile phases (pH 2–8).
- Excellent data quality in food, environmental, bioanalytical, and other applications.

Switch to a C18 when:
- You need a general-purpose column for reversed-phase chromatography.
- You need to increase retention of hydrophobic compounds.

Raptor C18 LC Columns (USP L1)

- A traditional end-capped C18 ideal for general-purpose use in reversed-phase chromatography.
- Wide pH range (2–8) provides excellent data quality for many applications, matrices, and compounds.
- Offers the highest hydrophobic retention of any Raptor phase.
- Part of Restek's Raptor LC column line featuring 1.8, 2.7, and 5 µm SPP core-shell silica.

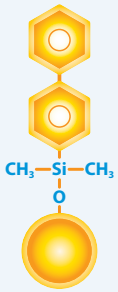
Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9304232	—	—
50 mm	9304252	930425E	—
100 mm	9304212	930421E	—
150 mm	9304262	—	—
2.7 µm Columns			
30 mm	9304A32	9304A3E	9304A35
50 mm	9304A52	9304A5E	9304A55
100 mm	9304A12	9304A1E	9304A15
150 mm	9304A62	9304A6E	9304A65
5 µm Columns			
30 mm	—	930453E	—
50 mm	9304552	930455E	9304555
100 mm	9304512	930451E	9304515
150 mm	9304562	930456E	9304565
250 mm	—	—	9304575

Raptor Biphenyl LC Columns (USP L11)

- Ideal for bioanalytical testing applications like drug and metabolite analyses.
- Heightened selectivity and retention for compounds that are hard to resolve or elute early on C18 and other phenyl chemistries.
- Limits ionization suppression and allows simple, MS-friendly mobile phases.
- Part of Restek's Raptor LC column line featuring 1.8, 2.7, and 5 µm SPP core-shell silica.

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9309232	—	—
50 mm	9309252	930925E	—
100 mm	9309212	930921E	—
150 mm	9309262	—	—
2.7 µm Columns			
30 mm	9309A32	9309A3E	9309A35
50 mm	9309A52	9309A5E	9309A55
100 mm	9309A12	9309A1E	9309A15
150 mm	9309A62	9309A6E	9309A65
5 µm Columns			
30 mm	—	930953E	—
50 mm	9309552	930955E	9309555
100 mm	9309512	930951E	9309515
150 mm	9309562	930956E	9309565
250 mm	—	—	9309575

Column Characteristics:



- Stationary Phase Category: Phenyl (L11)
- Ligand Type: Biphenyl
- Particle: 1.8 µm, 2.7 µm, or 5 µm superficially porous silica (SPP or "core-shell")
- Pore Size: 90 Å
- Carbon Load: 7% (1.8 µm), 7% (2.7 µm), 5% (5 µm)
- End-Cap: yes
- Surface Area: 125 m^2/g (1.8 µm), 130 m^2/g (2.7 µm), or 100 m^2/g (5 µm)
- Recommended Usage:
  - pH Range: 2.0 to 8.0
  - Maximum Temperature: 80 °C
  - Maximum Pressure: 1,034 bar/15,000 psi* (1.8 µm), 600 bar/8,700 psi (2.7 µm); 400 bar/5,800 psi (5 µm)

* For maximum lifetime, recommended maximum pressure for 1.8 µm particles is 830 bar/12,000 psi.

Properties:
- Increased retention for dipolar, unsaturated, or conjugated solutes.
- Enhanced selectivity when used with methanolic mobile phase.
- Ideal for increasing sensitivity and selectivity in LC-MS analyses.

Switch to a Biphenyl when:
- Limited selectivity is observed on a C18.
- You need to increase retention of hydrophilic aromatics.

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Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

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