Waters™

응용 자료

Improved Bioanalytical Performance Using a Simplified 2-Step Oasis™ PRiME HLB Protocol with an Ultra-Short UPLC Column for UPLC-MS/MS

Nikunj Tanna, Chelsea Plummer, Tom Walter

Waters Corporation, United States

Published on September 12, 2025

Abstract

In bioanalytical workflows, sample preparation is a critical step that can significantly impact data quality and system robustness. This study demonstrates how a fast, 2-step Oasis PRiME HLB solid-phase extraction (SPE) protocol offers multiple benefits over protein precipitation (PPT) in plasma for key analytical performance metrics. Using a new ultra-short UPLC™ column format (2.1 x 10 mm) to maximize speed and throughput, the phospholipid removal efficiency, impact on system pressure, sensitivity, area counts, and column lifetime are evaluated. The results show that this combination of a simple 2-step SPE method and ultra-short UPLC columns can enable fast, clean, and more reliable bioanalytical data acquisition while significantly improving speed and throughput for sample analysis.

Benefits

- >90% reduction in residual phospholipid content compared to PPT using Oasis PRiME HLB sample cleanup

- Fast, one-minute method enabled by the use of an ultra-short column (2.1 x 10 mm)
- Minimal impact on observed signal, MS sensitivity, and system pressure maintained at up to 5000 injections assisted by cleaner sample prep with Oasis PRiME HLB
- Fast and simple sample prep workflow with an automation-friendly 2-step SPE protocol with 2 fewer steps and 35% faster than PPT

Introduction

Bioanalytical laboratories are under increasing pressure to deliver high-throughput, high-quality results in both discovery and development lab environments without compromising robustness and reproducibility. During method development, scientists must balance cleanliness, speed, and cost when selecting a sample preparation strategy. Although protein precipitation is cost-effective and widely used, it often fails to sufficiently remove matrix components, such as phospholipids, which can compromise assay performance, column longevity, and instrument uptime.

In contrast, Oasis PRiME HLB is a novel reversed-phase SPE sorbent designed to simplify method development while maintaining high analyte recovery and cleanliness by removing >95% of phospholipids. Oasis PRiME HLB's 2-step "load and elute" protocol reduces sample preparation time by up to 35% compared to PPT, which requires vortexing, centrifugation, and supernatant transfer.

In this study, a 2-step Oasis PRiME HLB protocol described previously[1] was used for plasma samples examining a panel of 14 analytes with varied physico-chemical properties. The performance was benchmarked against PPT to assess the impact on analytical metrics critical to LC-MS bioanalysis.

Additionally, the sample preparation protocol was paired with a new ultra-short UPLC column to further increase throughput. The combination of rapid cleanup and fast chromatographic separation can improve laboratory efficiency, without sacrificing performance.

Experimental

All analytes used in the panel were procured from Fisher Scientific (Hampton, NH). All solvents, acids and bases used were purchased from Millipore Sigma (St. Louis, MO). Oasis PRiME HLB µElution plates, ACQUITY™ Premier Columns, ACQUITY Premier UPLC System and Xevo™ TQ Absolute Mass Spectrometer were all obtained from Waters™ Corporation.

Table 1. Analyte Panel used in Study

LC Conditions

LC system:	ACQUITY Premier UPLC I-Class System
Vials:	96-well Sample Collection Plate, 700 µL Round well
Column:	Prototype 2.5 µm HSS T3 2.1 x 10 mm Column
Column temperature:	55 °C
Sample temperature:	10 °C
Injection volume:	5 µL
Flow rate:	500 µL/min
Mobile phase A:	0.1% Formic acid in 100% Water
Mobile phase B:	0.1% Formic acid in 100% Acetonitrile
Gradient:	5–95% B over 40 seconds. Total run time 1 minute

MS Conditions

MS system:	Xevo TQ Absolute Mass Spectrometer

Ionization mode:	ESI positive
Acquisition mode:	MRM
Capillary voltage(kV):	2
Desolvation gas flow (L/Hr):	1000
Desolvation temperature (°C):	600
Cone gas flow (L/Hr):	150
Collision gas flow (L/Hr):	0.2
Nebulizer (Bar):	7

Data Management

Instrument control software:	MassLynx™ (v4.2)
Quantification software:	TargetLynx™ (v4.2)

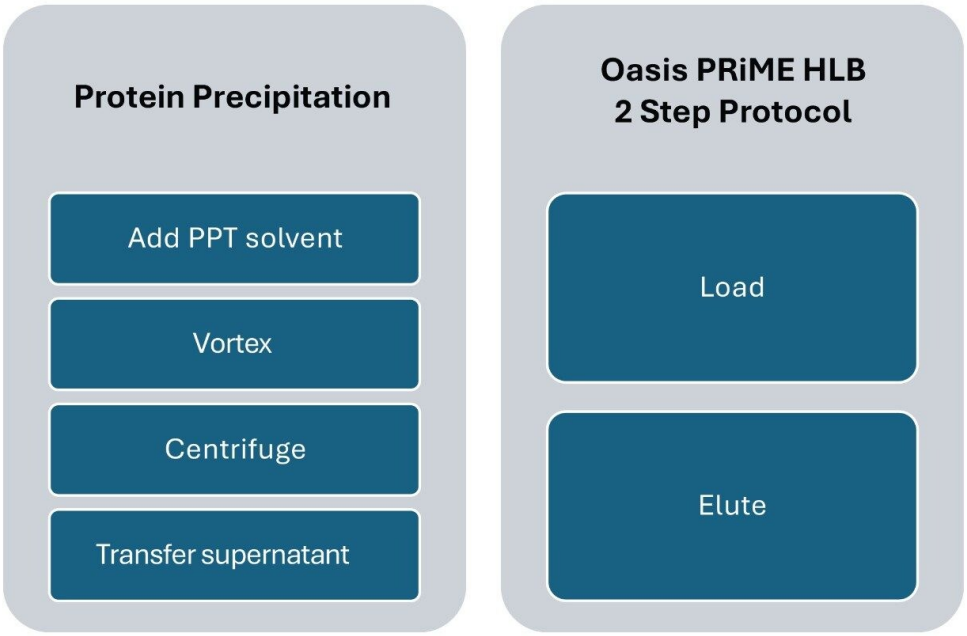

Figure 1. Sample preparation workflows.

Results and Discussion

Comparative data are presented for the following key performance parameters:

- Phospholipid levels
- Impact on system pressure
- Impact on sensitivity
- Relative area count trends
- Total injections on column

The 2-step Oasis PRiME HLB protocol demonstrated significantly improved phospholipid cleanup compared to protein precipitation. Phospholipids are a major contributor to matrix effects in LC-MS/MS assays, which cause ion suppression, decreased sensitivity, can impact robustness, and even clog columns leading to decreased

lifetimes. As shown in Figure 2, the total ion chromatograms (TICs) for precursors of *m/z* 184 fragments (representing total phospholipid content) have much lower signal intensity, approximately 4x (<90%), in PRiME HLB-processed samples, indicating more effective matrix removal.

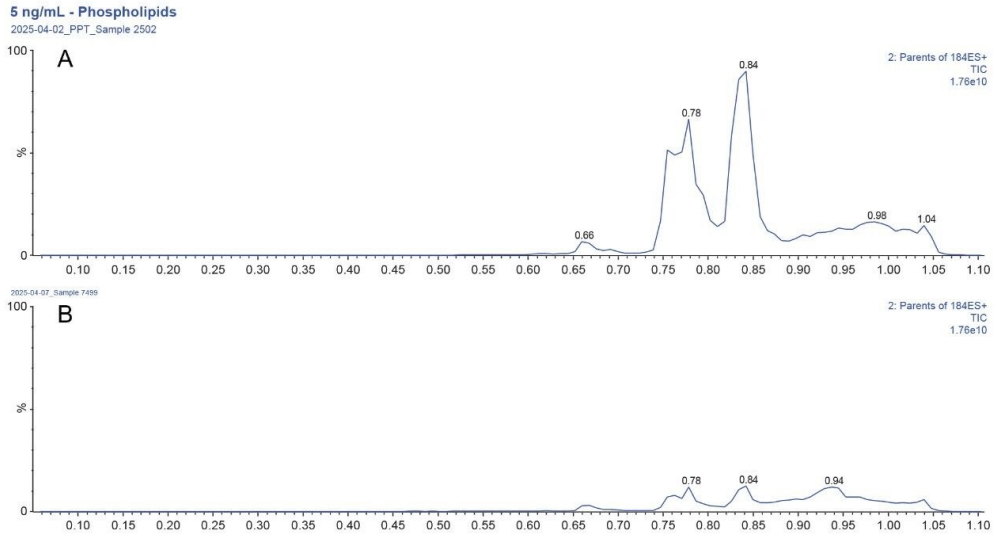

Figure 2. Total ion chromatogram (TIC) of the residual phospholipid background with A) PPT sample prep and B) Oasis PRiME HLB 2-step sample prep.

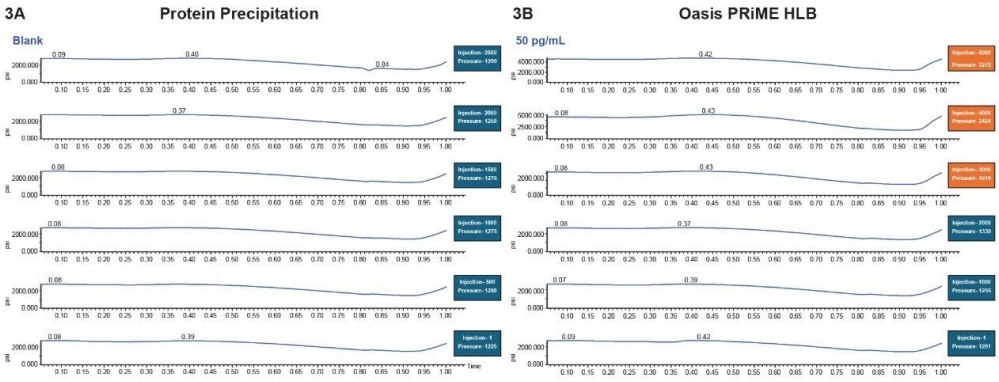

Figure 3. System pressure trace comparison when using PPT vs Oasis PRiME HLB sample preparation.

System pressure is another critical parameter in high-throughput LC-MS/MS workflows. Gradual increases in pressure over time are caused by matrix buildup on the column and can signal the need for maintenance or even column replacement. These disruptions not only cost time and money, but also introduce risk in regulated studies. The prototype ultra-short column showed good performance for over 2000 injections even with relatively dirty PPT samples. However, with sample extracted using Oasis PRiME, the total number of injections on the columns were >5000, thereby increasing the column lifetime by >2.5x. Figure 3 shows a pressure trace comparison across 1000s of injections for both PPT and SPE. For both techniques, through 2500 injections, the impact to system pressure is minimal. For samples extracted using PPT, the loss of analyte signal after 1000 injections was observed, and by 2000 injections, the area counts were reduced by 70%. In contrast, for samples prepared using SPE, the system pressure does increase, but it was possible to maintain consistent sensitivity and area counts over 5000 injections, as displayed in Figures 4 and 5.

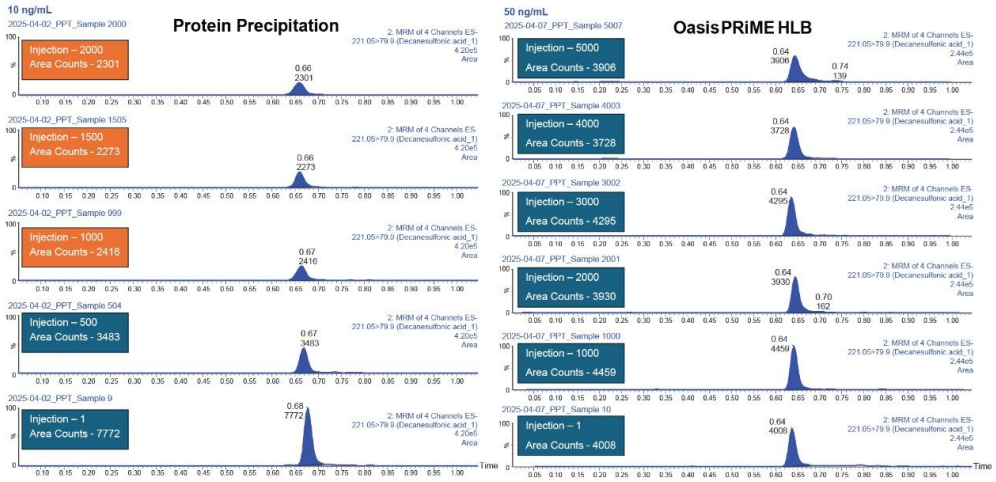

Figure 4. Area counts for a representative compound, 1-decansulfonic acid, for up to 2000 injections for PPT and 5000 injections for Oasis PRiME HLB prepped samples.

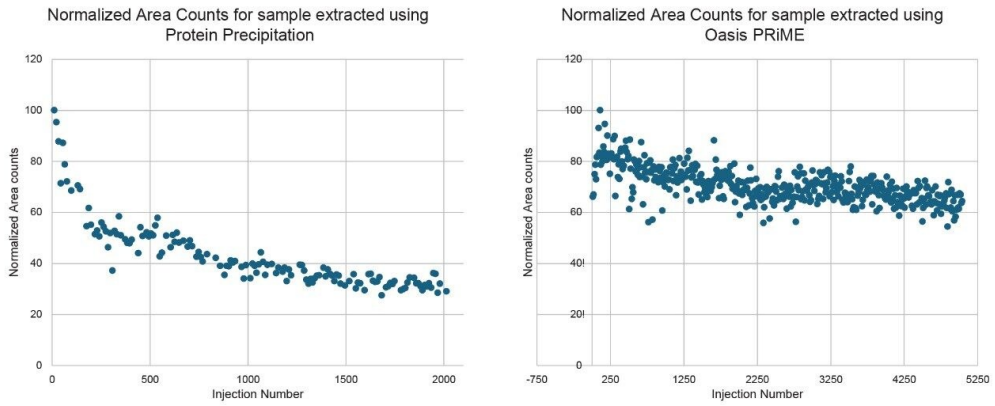

Figure 5. Normalized area counts for 1-decanesulfonic acid prepared by PPT vs 5000 injections for samples prepared using the Oasis PRiME HLB 2-step protocol.

Figure 4 demonstrates the impact that sample cleanliness has on signal consistency in area counts across multiple injections. In Figure 4, a representative analyte from the panel is compared across 1-2000 injections for PPT and 1-5000 injections for Oasis PRiME HLB SPE. For SPE, even after 5000 injections, the variability is low (< 7%), while PPT shows a 45% decrease in signal intensity after 500 injections. Figure 5 trends the area counts data injection by injection where this can be quickly interpreted to show a fast drop off in signal for PPT with a relatively consistent sensitivity indicated by the area counts when SPE is the sample preparation method. This improved performance enables greater confidence in results without the need for frequent recalibration or troubleshooting, which is important in both discovery and regulated environments.

The use of a new ultra-short UPLC column in this study also contributed to maintaining fast cycle times without sacrificing data quality. Shorter columns with optimized particle technology enable one-minute methods while still delivering sharp peaks and high sensitivity, as demonstrated in Figure 4. Despite the high injection counts, the column maintained consistent performance, suggesting that even in high-throughput workflows, rapid separations can be achieved reliably when paired with cleaner extracts from SPE.

Conclusion

This study highlights the advantages of using a simplified 2-step Oasis PRiME HLB SPE protocol over traditional protein precipitation for plasma sample preparation in bioanalysis. Clear improvements in phospholipid removal, system pressure stability, MS sensitivity, and reproducibility of area counts, without the need for complex or time-consuming sample prep protocols were observed. When combined with ultra-short (2.1 x 10 mm) UPLC columns, this workflow enables fast, clean and reliable data acquisition, making it ideally suited for high-throughput bioanalytical applications. The use of a quick, fast, and clean sample prep with Oasis PRiME HLB allows for more injections on column in high-throughput workflows (>1 minute UPLC methods), perfect for labs seeking to increase efficiency without compromising analytical quality.

References

1. Tanna N., Plummer, C. Oasis PRiME HLB – The Fastest Way to Clean Samples, Waters Application Note, 720008684 <https://www.waters.com/nextgen/global/library/application-notes/2025/oasis-prime-hlb-the-fastest-way-to-clean-samples.html> , February 2025.

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/acquity-uplc-i-class-plus-system.html>

Xevo TQ Absolute XR Triple Quadrupole Mass Spectrometer <https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-systems/xevo-tq-absolute.html>

MassLynx IVD MS Software <https://www.waters.com/nextgen/global/products/in-vitro-diagnostics-solutions/clinical-diagnostics-software/masslynx-ivd-ms-software.html>

720009018, September 2025