Application of Hybrid Surface Technology for Improving Sensitivity and Peak Shape of Phosphorylated and Carboxylate Lipids

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Introduction

- Phosphorylated and carboxylate lipid species are metal sensitive and can readily absorb to stainless steel surfaces within the flow path of LC systems. This process can lead to poor peak shape, low recovery, and reduction in sensitivity.
- Here we present the ACQUITY™ Premier System with ACQUITY Premier CSH™ C18 Column also called hybrid surface technology (HST) that can significantly improve sensitivity, peak shape and recovery of phosphorylated and carboxylate lipids compared to standard stainless-steel surface ACQUITY UPLC™ I-Class and CSH C18 Column. The Premier solution mitigates analyte interactions with metal surfaces (Figure 1).

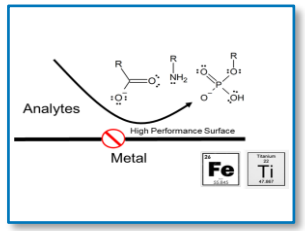

Figure 1.Hybrid sutface technology (HST) or Premier Solution employs an inert chemical surface treatment to mitigate metal-analyte interactions (1).

METHODS

LC/MS Conditions

LC System:	ACQUITY UPLC I-Class and **ACQUITY Premier**
Detection:	Synapt-XS™
Column(s):	ACQUITY UPLC CSH C_{18} and **ACQUITY Premier CSH C18** (2.1x100 mm, 1.7µm) for RP ACQUITY UPLC BEH Amide and **ACQUITY Premier BEH™ Amide** (2.1x100 mm, 1.7µm) for HILIC
Column Temp.:	55 °C (CSH C18 RP) and 55 °C (BEH Amide)
Flow Rate:	400 µL/min (CSH C18 RP) and 600 µL/min (BEH Amide)
Mobile Phase A and B (CSH C18 RP):	600/390/10 (ACN/Water/1M aqueous ammonium formate) in 0.1% FA 900/90/10 (IPA/ACN/1M aqueous ammonium formate) in 0.1% FA
Mobile Phase A and B (BEH Amide):	95/5 (ACN/Water) in 10mM Ammonium Acetate 50/50 (ACN/Waters) in in 10mM Ammonium Acetate

References
[1] DeLano M et al., J., Anal Chem 2021, 93(14), 5773-5781.
[2] Isaac G. and Plumb R., Waters Application Note 720007092, Jan 2021.

Results

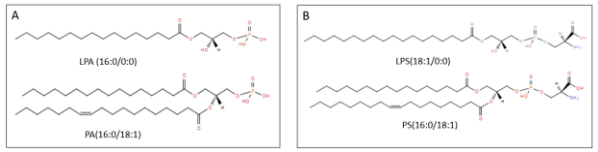

Figure 2. Chemical structure of analyzed phosphorylated and carboxylate lipids.

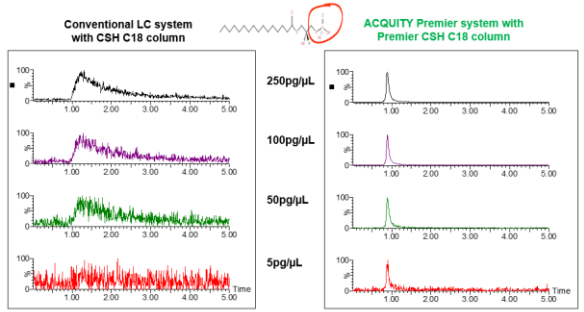

Figure 3. Negative mode base peak extracted ion chromatogram at a concentration range of 5-250 pg/µL for LPA(16:0/0:0) m/z 409.2355 using conventional system/CSH C18 column and ACQUITY Premier System/CSH C18 Column.

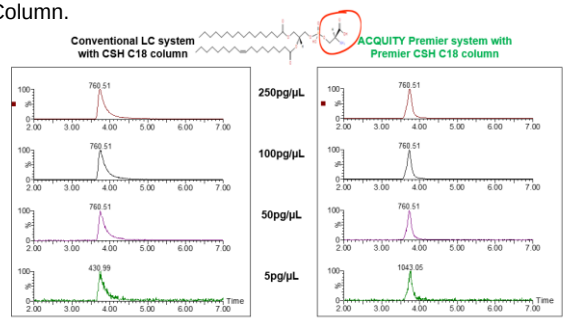

Figure 4. Negative mode base peak extracted ion chromatogram at a concentration range of 5-250 pg/µL for PS(16:0/18:1) m/z 760.5129 using conventional system/CSH C18 and ACQUITY Premier System/CSH C18 Column.

Results

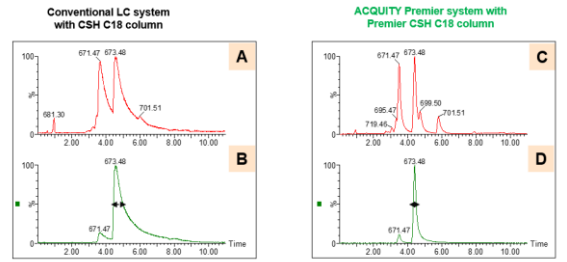

Figure 5. Avanti polar lipids egg chicken PA extract (10 ng/µL) measured using (A) conventional system/CSH C18 column (B) ACQUITY Premier System/CSH C18 column. Corresponding extracted ion chromatogram of PA(16:0_18:1) at m/z 673.481 using (C) conventional system/CSH C18 column with average peak FWHM 34.0 sec (n=3) and (D) ACQUITY Premier System/CSH C18 Column with average peak FWHM 10.8 sec (n=3).

Abundance Plots and Improved Peak Shape of Selected Phosphorylated and Carboxylate Lipid Classes

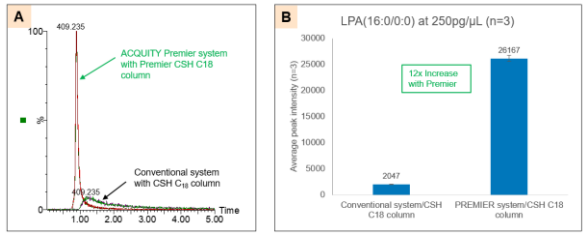

Figure 6. The peak intensity obtained for LPA(16:0/0:0) at a concentration of 250pg/µL using conventional system and ACQUITY Premier System
(A) Overlayed extracted ion chromatogram obtained using the two configurations. (B) Bar graph showing a 12-fold increase in signal intensity for LPA(16:0/0:0) with the ACQUITY Premier System compared to conventional system. A significant increase in signal intensities are observed with the ACQUITY Premier Solution for all investigated phosphorylated and carboxylate lipids.

Results

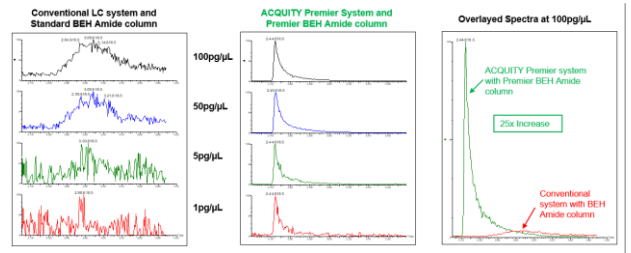

Figure 7. A side-by-side comparison of negative mode base peak extracted ion chromatogram for 18:1/16:0 ceramide phosphate using conventional system/BEH Amide column (on the left) and ACQUITY Premier System/BEH Amide column (in the middle) at different concentration levels and an overlayed chromatogram at 250ng/mL. The ACQUITY Premier System & Column provided 25 times increase in peak intensity compared to the conventional system and column.

Conclusions

- Routine analysis of phosphate and carboxylate containing challenging lipids without the need for additives, modifiers, or dedicated methods.
- Increased sensitivity, recovery, and reproducibility. AQUITY Premier System and Column increased signal intensity by 25-30 times.
- Improved peak shape and reduced tailing by minimizing analyte-surface interaction. AQUITY Premier System and Column reduced peak tailing by 65-80%.
- Increased lipidomics coverage by simultaneous analysis of phosphorylated and carboxylate lipids in addition to other lipid classes.