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

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INTRODUCTION

The comprehensive lipidomic analysis of various biological tissues is a challenging task due to the extreme complexity of individual lipid classes varying in their structure, attached functional groups, polarity, dissociation and ionization behavior.

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 Premier System with 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).

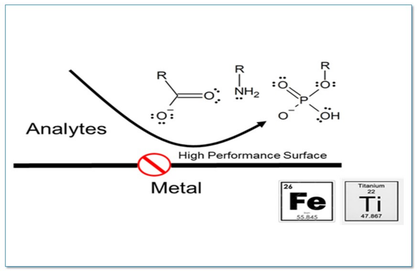

Figure 1. *Hybrid sutface technology (HST) or Premier Solution employs an inert chemical surface treatment to mitigate metal-analyte interactions(1).*

METHODS

Sample Preparation

- Samples were prepared in one vial and divided to two vials for each conventional and ACQUITY Premier system
- Same mobile phase throughout the analysis
- All experiments performed within 2-3 days
- Serial dilution with three replicates

LC/MS Conditions

LC System:	ACQUITY UPLC I-Class and **ACQUITY Premier System**
Detection:	Synapt-XS
Column(s):	ACQUITY UPLC CSH C18 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 for 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 for 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., *Using Hybrid Organic-Inorganic Surface Technology to Mitigate Analyte Interactions with Metal Surfaces in UHPLC.* Anal Chem 2021, 93(14), 5773-5781.

[2] Isaac G. and Plumb R., *ACQUITY Premier LC Technology significantly improves sensitivity, peak shape and recovery for phosphorylated and carboxylate lipids.* Waters Application Note 720007092, Jan 2021.

RESULTS and DISCUSSION

Chemical Structure of Investigated Lipid Classes

Figure 2. *The chemical structure of analysed lipid standards (A) Glycerophosphates including 16:0/0:0 lysophosphatidic acid (LPA (16:0/0:0)) and 16:0/18:1 phosphatidic acid (PA(16:0/18:1)). (B) Glycerophosphoserines including 18:1/0:0 lysophosphatidylserine (LPS(18:1/0:0)) and 16:0/18:1 Phosphatidylserine (PS(16:0/18:1)).*

Lysophosphatidic Acid, LPA(16:0/0:00)

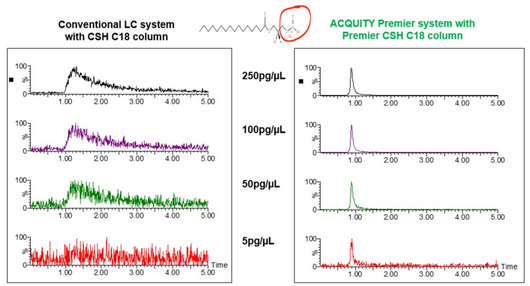

Figure 3. *Negative mode base peak extracted ion chromatogram at a concentration range of 5-250 pg/µL for LPA(16:0/0:0) m/z 409.2355 using conventional system/CSH C18 column and AQUITY Premier System/CSH C18 column. The increase in sensitivity can be clearly seen for LPA (16:0/0:0) at a lower concentration level (especially at 50 and 5 ng/mL) as evidenced by the noticeably improved peak intensity and shape for the Premier solution compared to the conventional system.*

Phosphatidylserine, PS(16:0/18:1)

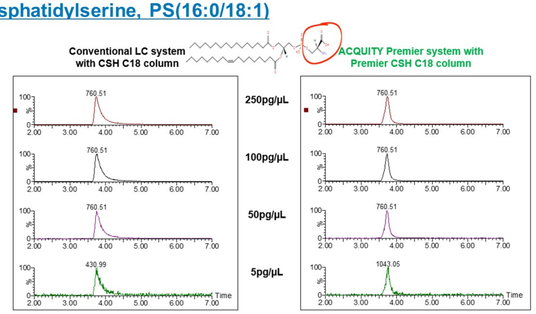

Figure 4. *Negative mode base peak extracted ion chromatogram at a concentration range of 5-250 pg/µL for PS(16:0/18:1) m/z 760.5129 using conventional system/CSH C18 and AQUITY Premier System/CSH C18 column.*

Avanti Polar Lipids Egg Chicken PA Extract

The developed method was applied for the analysis of Avanti Polar Lipids egg chicken phosphatidic acid extract.

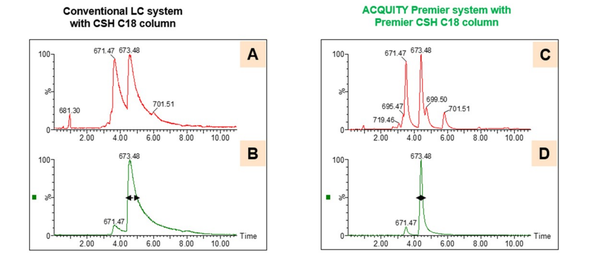

Figure 5. *Avanti polar lipids egg chicken PA extract (10 ng/µL) measured using (A) conventional system/CSH C18 column (B) Premier System/CSH C18 column. Corresponding extracted ion chromatogram of PA(16:0_18:1) at m/z 673.481 using (C) conventional system/CSH C18 column with average peak FWHM 34.0 sec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

Analyte loss to metal surfaces is problematic for compounds containing electron rich moieties such as phosphate and carboxylate groups and is especially troublesome for compounds present at low concentrations. The Premier Solution or Hybrid sutface technology (HST) improves peak area recovery of these compounds.

12-Fold Increase in Peak Intensity and Improved Peak Shape for LPA(16:0/0:0) With ACQUITY Premier System and CSH C18 Column

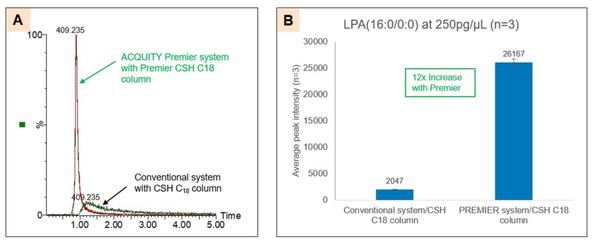

Figure 6. *The peak intensity obtained for LPA(16:0/0:0) at a concentration of 250pg/µL using conventional system and ACQUITY Premier System*
(A) Overlayed extracted ion chromatogram obtained using the two configurations. (B) Bar graph showing a 12-fold increase in signal intensity for LPA(16:0/0:0) 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.

30-Fold Increase in Peak Intensity and Improved Peak Shape for LPA(16:0/0:0) With ACQUITY Premier System and BEH Amide Column

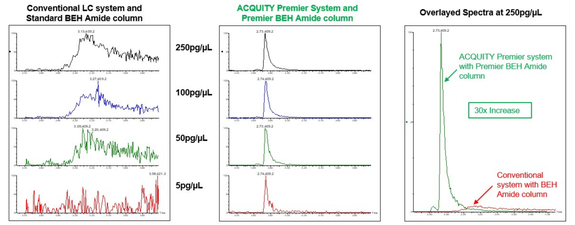

Figure 7. *A side-by-side comparison of negative mode base peak extracted ion chromatogram using conventional system/BEH Amide column (on the left) and ACQUITY Premier system/BEH Amide column (in the middle) at different concentration levels and an overlayed chromatogram at 250ng/mL. The ACQUITY Premier System & column provided 30 times increase in peak intensity compared to the conventional system and column.*

25-Fold Increase in Peak Intensity and Improved Peak Shape for Cer-1-Phosphate (d18:1/16:0) with ACQUITY Premier System and BEH Amide Column

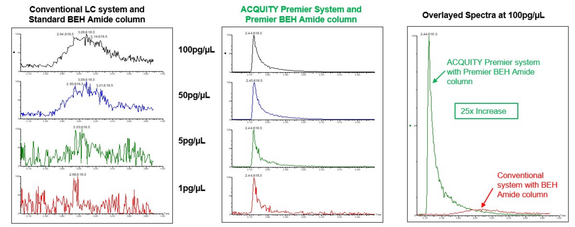

Figure 8. *A side-by-side comparison of negative mode base peak extracted ion chromatogram for 18:1/16:0 Ceramide phosphate using conventional system/BEH Amide column (on the left) and ACQUITY Premier system/BEH Amide column (in the middle) at different concentration levels and an overlayed chromatogram at 250ng/mL. The ACQUITY Premier System & column provided 25 times increase in peak intensity compared to the conventional system and column.*

CONCLUSION

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

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