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 metal oxide surfaces and 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® Class and CSH C18 column. The Premier solution mitigates analyte interactions with metal surfaces (Figure 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.
- Setaal dilution with three replicates.

LC/MS Conditions
- LC System: Acquity UPLC® Class and ACQUITY Premier System
- Detector: Quattro Micro Xevo MS
- Columns: ACQUITY UPLC® BEH Amide and ACQUITY Premier BEH Amide 2.6 μm, 150 × 2.1 mm, 100 A
- Flow rates: 0.25 mL/min (ACQUITY UPLC®) and 0.35 mL/min (ACQUITY Premier System)
- Mobile Phase A: 0.1% formic acid in water
- Mobile Phase B: 0.1% formic acid in acetonitrile
- Injection volume: 1 μL
- Cone voltage: 30 V (Acquity UPLC®) and 50 V (ACQUITY Premier System)
- APCI source temperature: 450°C
- Electron impact (E.I.) mass range: 50–2000 m/z
- Instrument solvent flow rate: 600 mL/min
- LC-MS operating conditions: 25°C

RESULTS and DISCUSSION

Chemical Structure of Investigated Lipid Classes

Avanti Polar Lipids Egg Chicken PA Extract

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

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 surface 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

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

25-Fold Increase in Peak Intensity and Improved Peak Shape for Cer1-Phosphatidylcholine (c18:1/16:0) with ACQUITY Premier System and BEH Amide 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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