

IMPROVED PERFORMANCE OF MODERN MS-COMPATIBLE REVERSED-PHASE/ANION-EXCHANGE MIXED-MODE HPLC COLUMNS

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INTRODUCTION

Challenges in polar acids separations

- Poor retention on traditional reversed-phase (RP) columns
- Poor mass spectrometry (MS) compatibility of ion-pairing reagents
- The 'column bleed' problem with existing mixed-mode columns

Introducing Atlantis™ PREMIER BEH C₁₈ AX Column¹

- pH-stable bridged-ethyl hybrid (BEH) base particle and bonded phase (pH 2 ~ 10)
- High surface area (270 m²/g) for improved overall retention
- Minimal dewetting when used with highly aqueous mobile phases
- Anion-exchange groups (pK_a ~ 7.5) in a controlled low surface concentration for improved retention of ionized acids
- Novel column hardware for improved recovery of metal-sensitive analytes

METHODS

Polar Mixture Separations (Figures 1)

Instrument: ACQUITY UPLC I-Class System — PDA Detector, Empower 3 CDS
Column size: 2.1 × 50 mm Column temp.: 30 °C
Injection volume: 1.5 µL
Separation condition: 10 mM ammonium formate pH 3.00 (aq) at 0.2 mL/min
UV detection: 254 nm

% Matrix Effects (Figure 2)

Instrument: ACQUITY UPLC H-Class System — Xevo TQD-MS, MassLynx 4.2
LC-ESI-MS method Conditions:
Column size: 2.1 × 150 mm Column temp.: 50 °C
MP for positive ESI-MS: Acetonitrile and 0.1 % (v/v) formic acid in water (pH: 2.7)
MP for negative ESI-MS: Acetonitrile and 10 mM ammonium formate in water (pH: 6.4)
Mobile phase flow rate: 0.2 mL/min, combined with MS infusion of test analytes at 20 µL/min

$$\text{Matrix Factor (MF)} = \frac{\text{Peak area of Analyte from post-Column infusions}}{\text{Peak area of Analyte from post-Union infusions}}$$

$$\% \text{ Matrix Effect (ME)}: \begin{cases} (MF - 1) \times 100, & MF > 1 \\ (1 - MF) \times 100, & MF < 1 \end{cases}$$

RESULTS

Improved retention for acids

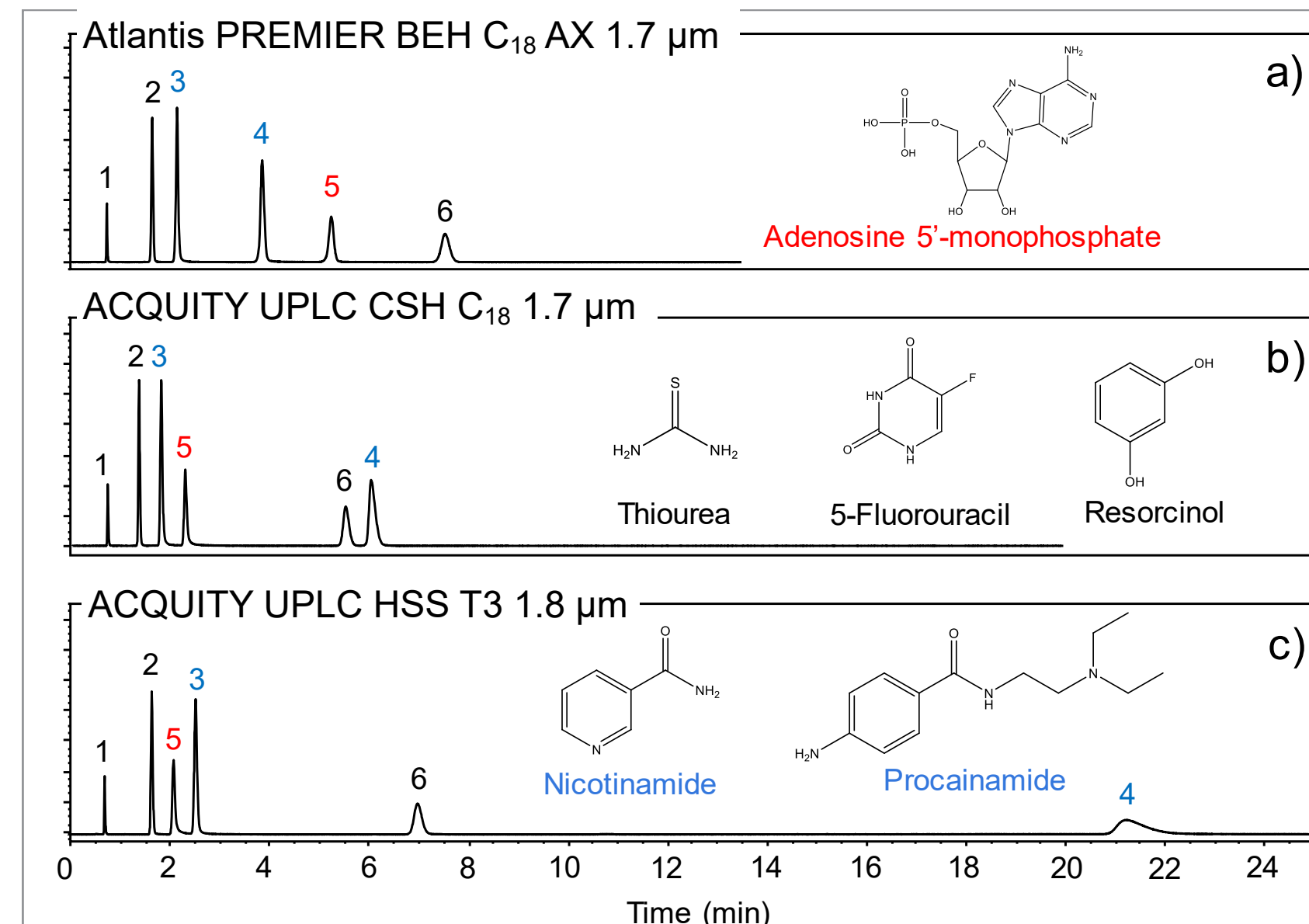


Figure 1. Isocratic separations of polar analytes at pH 3.00.

Charge status
Positively charged
Negatively charged
Neutral

- 1) Thiourea, 2) 5-Fluorocytosine, 3) Nicotinamide, 4) Procainamide, 5) Adenosine 5'-monophosphate, 6) Resorcinol

Improved recovery for metal-sensitive analytes²

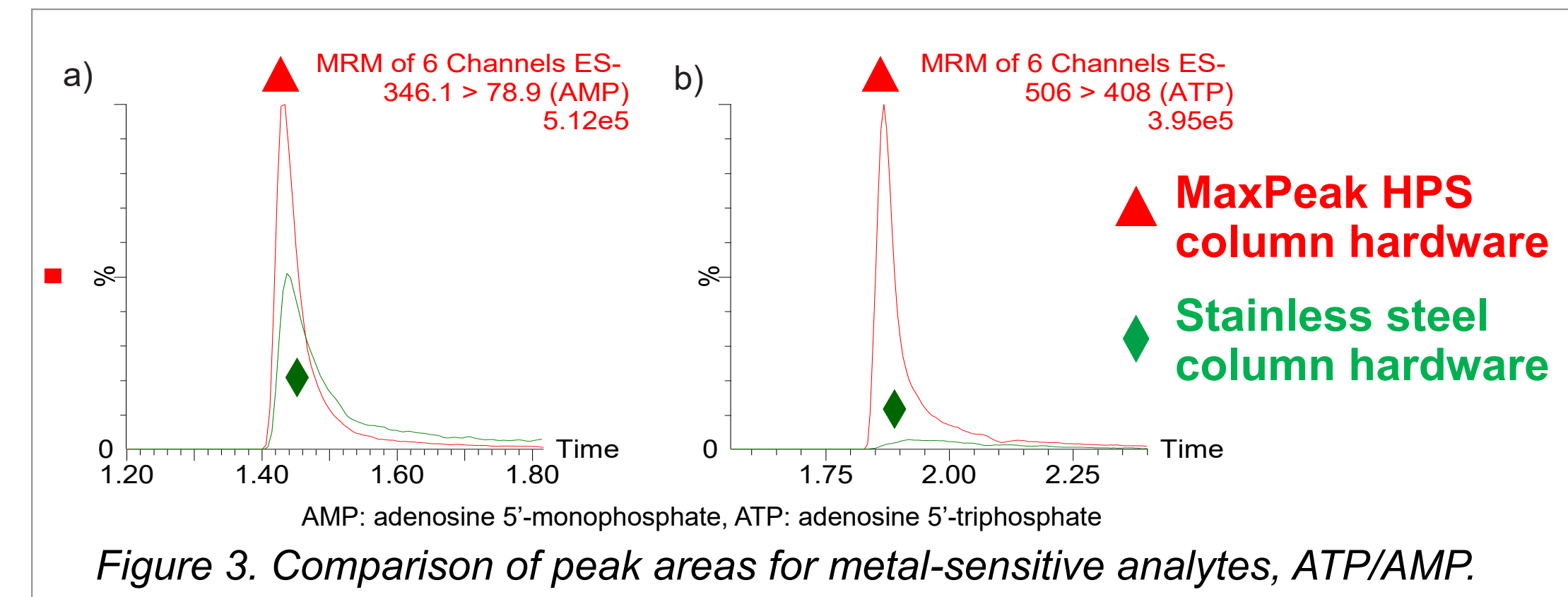


Figure 3. Comparison of peak areas for metal-sensitive analytes, ATP/AMP.

CONCLUSIONS

- Compared to conventional RP stationary phases, when used with mobile phase pH values < 8, Atlantis BEH C₁₈ AX shows increased retention of anions, such as ionized acids, and decreased retention of cations, such as protonated bases.
- MS signal suppression or enhancement caused by bonded-phase hydrolysis products observed for analytes in positive ESI and negative ESI modes were significantly lower than two leading mixed-mode RP/AX columns.
- MaxPeak HPS hardware provides improved-recovery, better peak shape and increased signal intensity and analysis reproducibility for metal-sensitive analytes.

References

1. Walter, T. H. *et al*, A New Mixed-Mode Reversed-Phase/Anion-Exchange Stationary Phase Based on Hybrid Particles, *Waters Application Note*, 720006742EN, 2020
2. Smith, K. M. and Rainville, P., Utilization of MaxPeak High Performance Surfaces and the Atlantis PREMIER BEH C18 AX Column to Increase Sensitivity of LC-MS Analysis, *Waters Tech Brief* 720006745EN, 2020

Low matrix effect due to bonded phase hydrolysis products

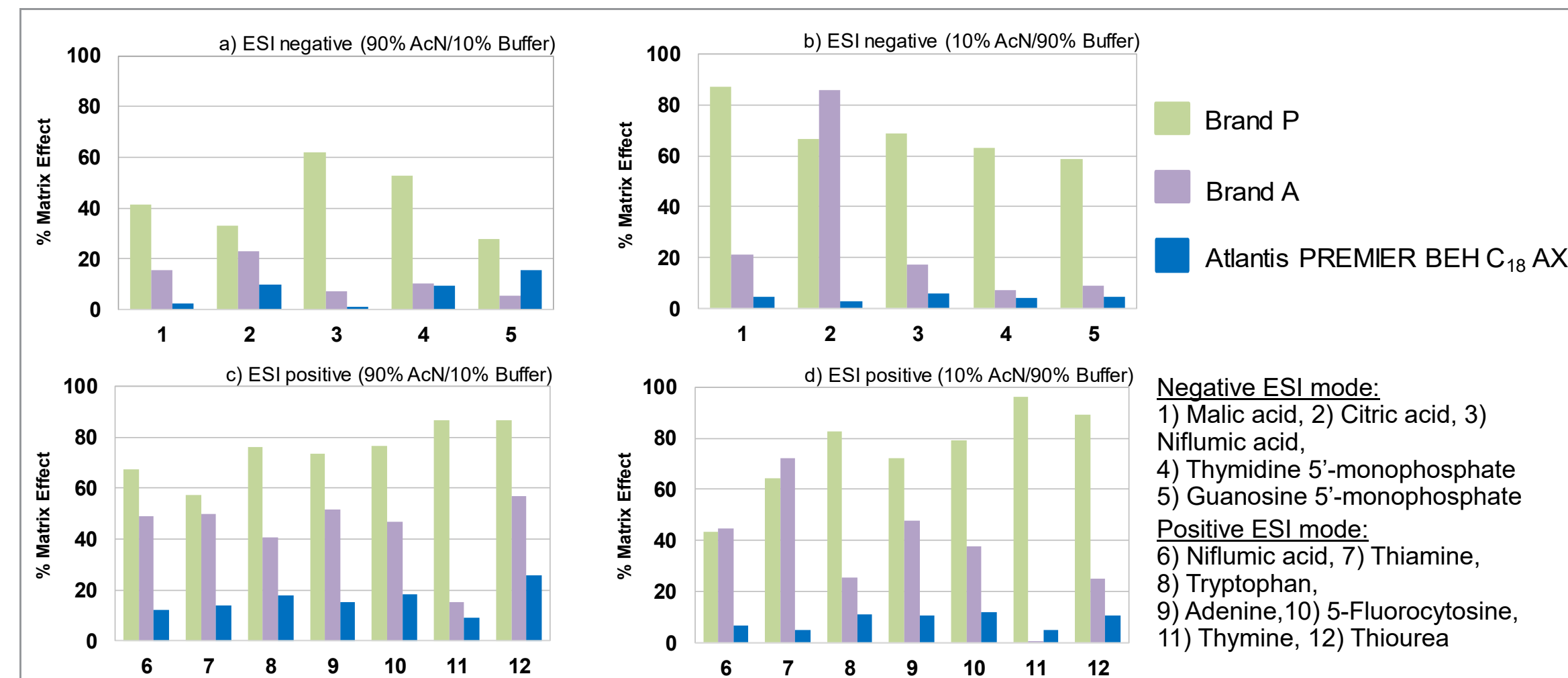


Figure 2. % Matrix Effect in negative and positive ESI modes.