

New Reversed-Phase Materials for the Separation of Polar Acidic Compounds

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Bonnie Alden, Melvin Blaze, Cheryl Boissel, Darryl W. Brousmiche, Jessica Field, Kenneth Glose, Nicole Lawrence, Donna Osterman, Thomas H. Walter
Waters Corporation, Consumables R&D, Milford MA USA

INTRODUCTION

Reversed-phase (RP) liquid chromatography is widely used for the analysis of both neutral and ionizable analytes. However, the separation of polar acidic compounds still poses a significant challenge for the currently available RP columns, with many analytes of interest being poorly retained. Solutions can be found, but they may not be ideal for many users. Reversed-phase methods using ion-pairing reagents may require long equilibrations or additional steps for sample derivatization may be needed. However, many existing approaches are not compatible with mass spectrometry (MS) detection.

Charged Surface Hybrid (CSH™) columns have been shown to retain and separate polar acidic analytes, such as those found in the tricarboxylic acid (TCA) cycle using MS-compatible mobile phases.[1] The CSH packing materials are based on 130 Å bridged-ethyl hybrid (BEH) particles and incorporate a controlled low level of positively charged surface groups, which impart different selectivity for ionized analytes compared to conventional reversed-phase columns.

To explore further increasing the retention of polar acidic analytes, bonded phases were prepared using BEH particles with pore diameters ranging from ca 60 to 140 Å. The pore diameter was varied to study its impact on analyte retention and compatibility with 100% aqueous mobile phases, which can be a challenge for small pore size C₁₈ bonded phases.[2] The particles were bonded either with C₁₈ groups (RP Controls) or with C₁₈ groups and a new positive charge modifier (RP/AX). We will show the performance of these materials for the retention and separation of polar acids using MS-compatible mobile phases.

References

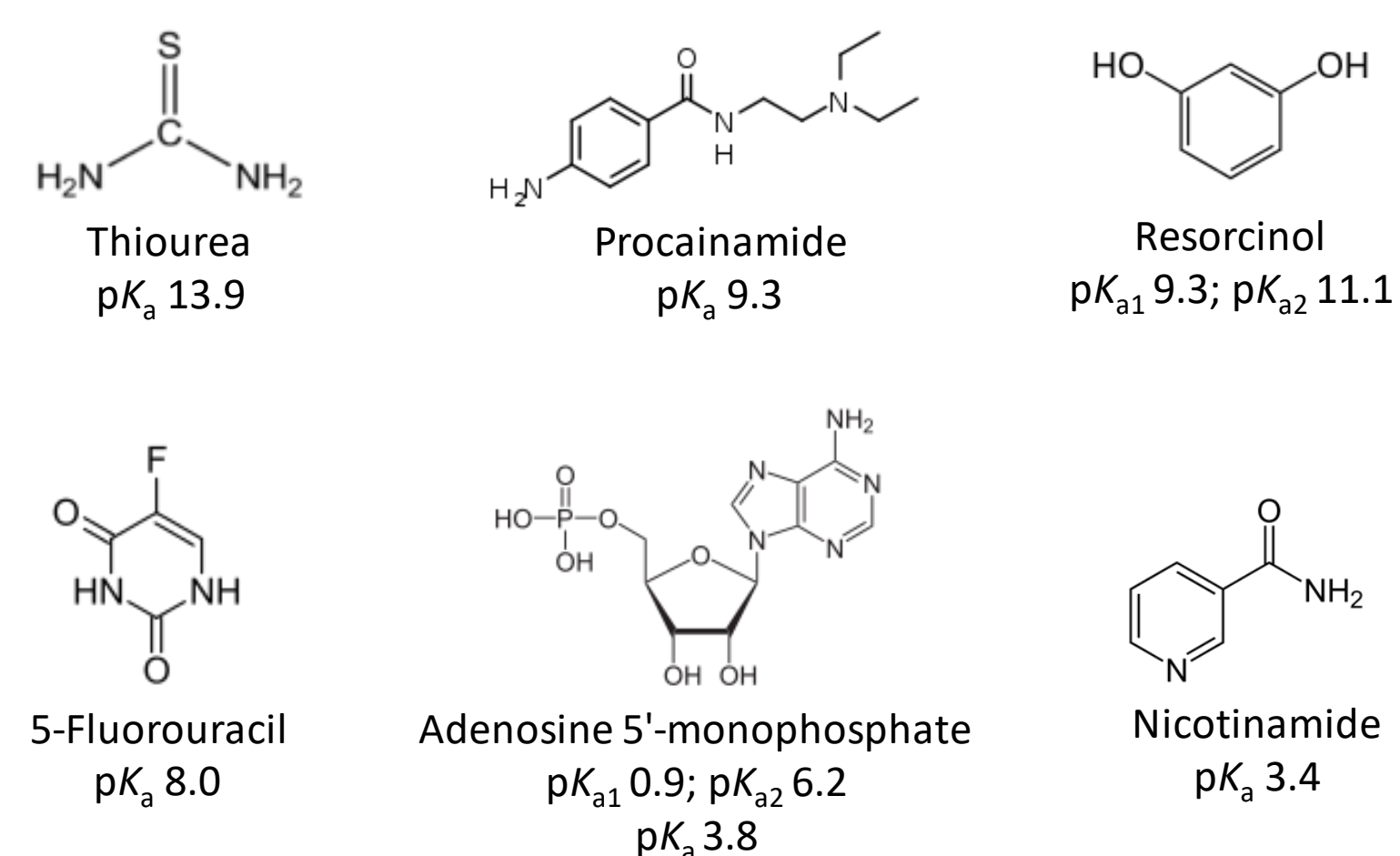
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METHODS

Retention: An ACQUITY UPLC I-Class system with an ACQUITY Photodiode Array Detector was used with column configuration of 2.1 x 50 mm. Detection was by UV @ 230 nm or 254 nm. The isocratic separations were carried out using a mobile phase of 10 mM ammonium formate (aq) at pH 3. The flow rate was 200 µL/minute and the column temperature was controlled at 30°C. The void marker was thiourea, eluted using 100% acetonitrile.

Highly Aqueous Mobile Phase Compatibility: Using the above UPLC system and chromatographic conditions, four separations of the sample were carried out. Next the flow was stopped for 10 minutes allowing the back-pressure on the column outlet to decrease. The flow was then restarted. The % Dewet is calculated using the retention time before the zero-flow segment and the retention time for the first injection after the pump flow was restarted.

Buffer Concentration and pH: An ACQUITY H-Class PLUS system with an ACQUITY Photodiode Array Detector was used with column configuration of 2.1 x 50 mm. Detection was by UV @ 230 nm or 254 nm. The flow rate was 200 µL/minute and the column temperature was controlled at 30°C. The void marker was thiourea, eluted using 100% acetonitrile. A set of six model analytes was used to evaluate the effect of buffer concentration and buffer pH on retention of the analytes



RESULTS

Pore diameters from 66 to 138 Å were evaluated to determine retention for a neutral polar analyte, thymine, and compatibility in highly aqueous mobile phases. The retention factor for thymine on a 138 Å control batch was 4.1. Prototype RP batches of 95 Å, 79 Å, and 66 Å achieved higher retention factors; 6.7, 7.6, and 9.2, respectively. While the smaller pore diameter BEH RP materials gave the greatest retention, their compatibility with 100% aqueous mobile phases decreased (Fig. 1). The pore diameter of 95 Å gave increased retention vs. 130 Å RP materials and with the addition of the new charge modifier, improved the compatibility with 100% aqueous mobile phases.

A BEH 95 Å particle (BEH95) with the RP/AX bonding was evaluated for retention characteristics using a set of polar analytes. Two studies were run; one using different concentrations (Fig. 2a and 2b) of ammonium formate. The second studied a pH range of 2.5 to 8.5 (Fig. 3a and 3b). The use of the BEH particle extended the operating range vs. silica based bonded-phases.[3]

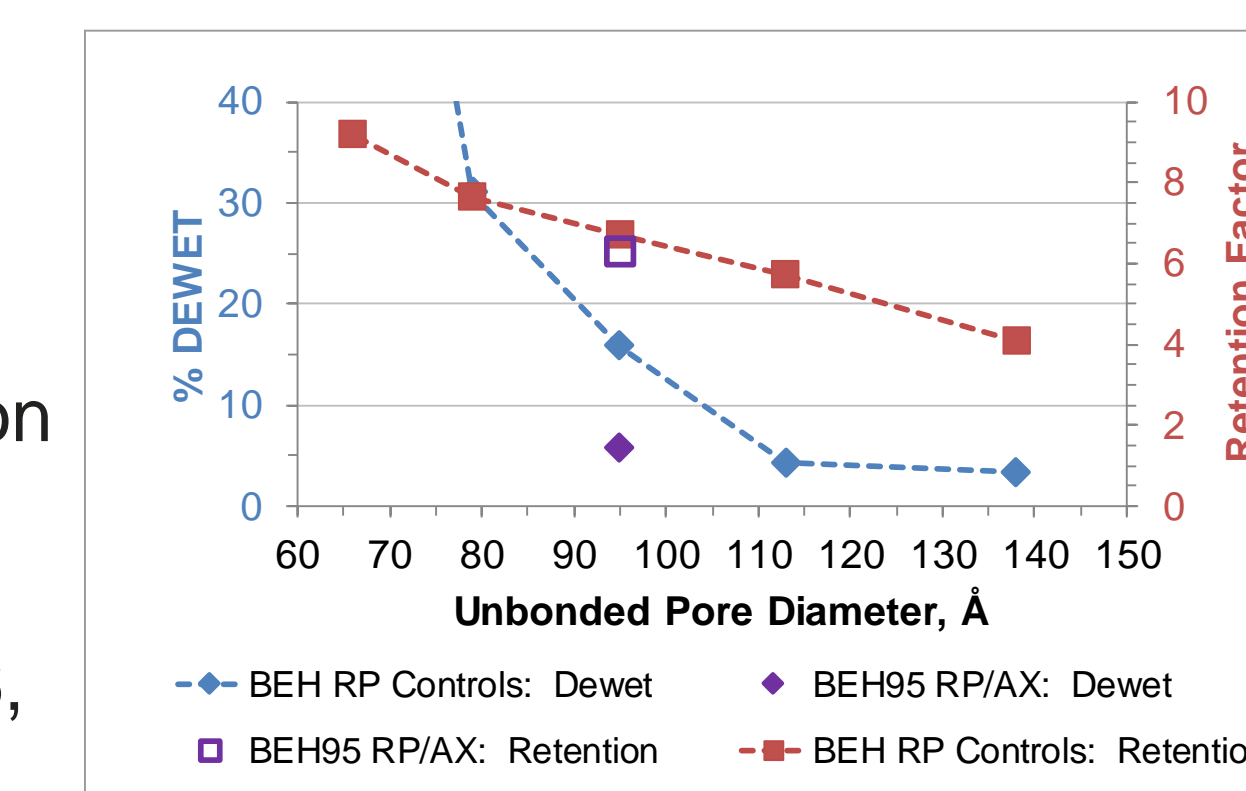


Figure 1. Comparison of Retention and % Dewet for Thymine for Different Pore Diameter BEH particles

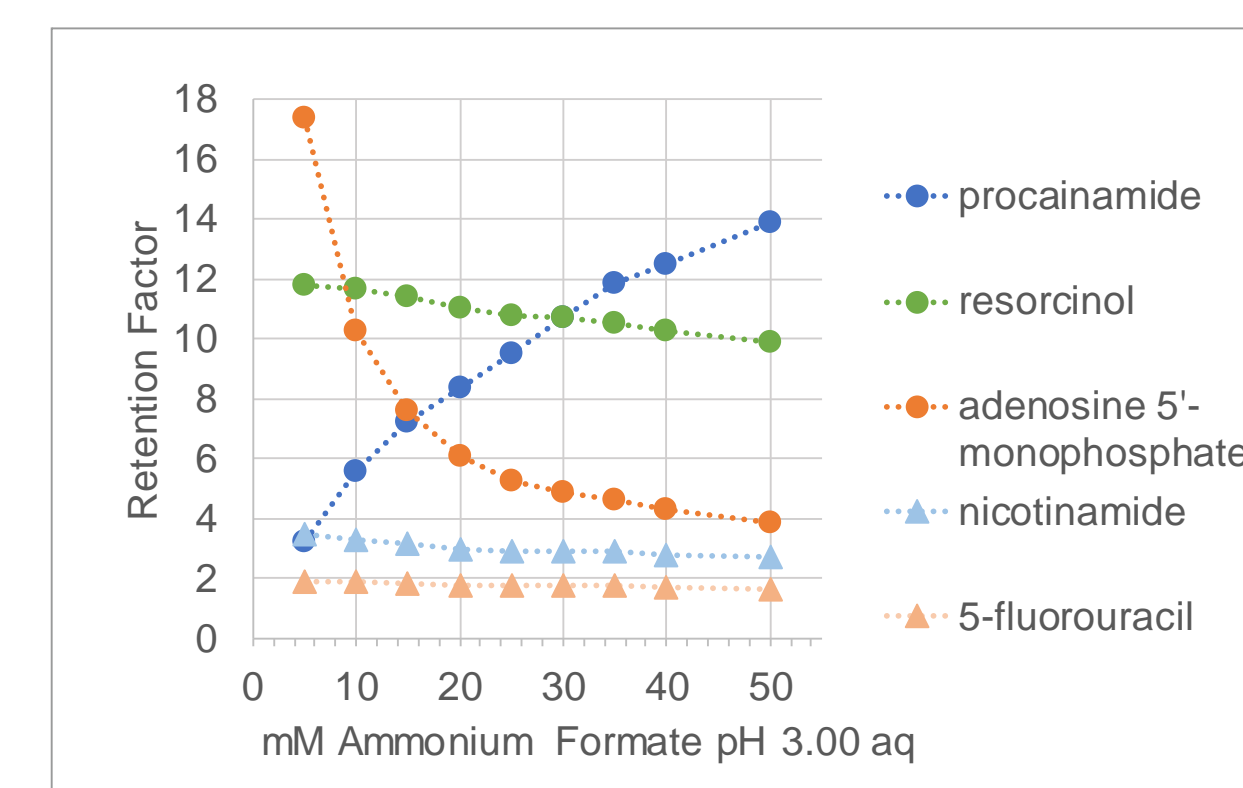


Figure 2a. Retention Factor vs. Buffer Concentration at pH 3.00

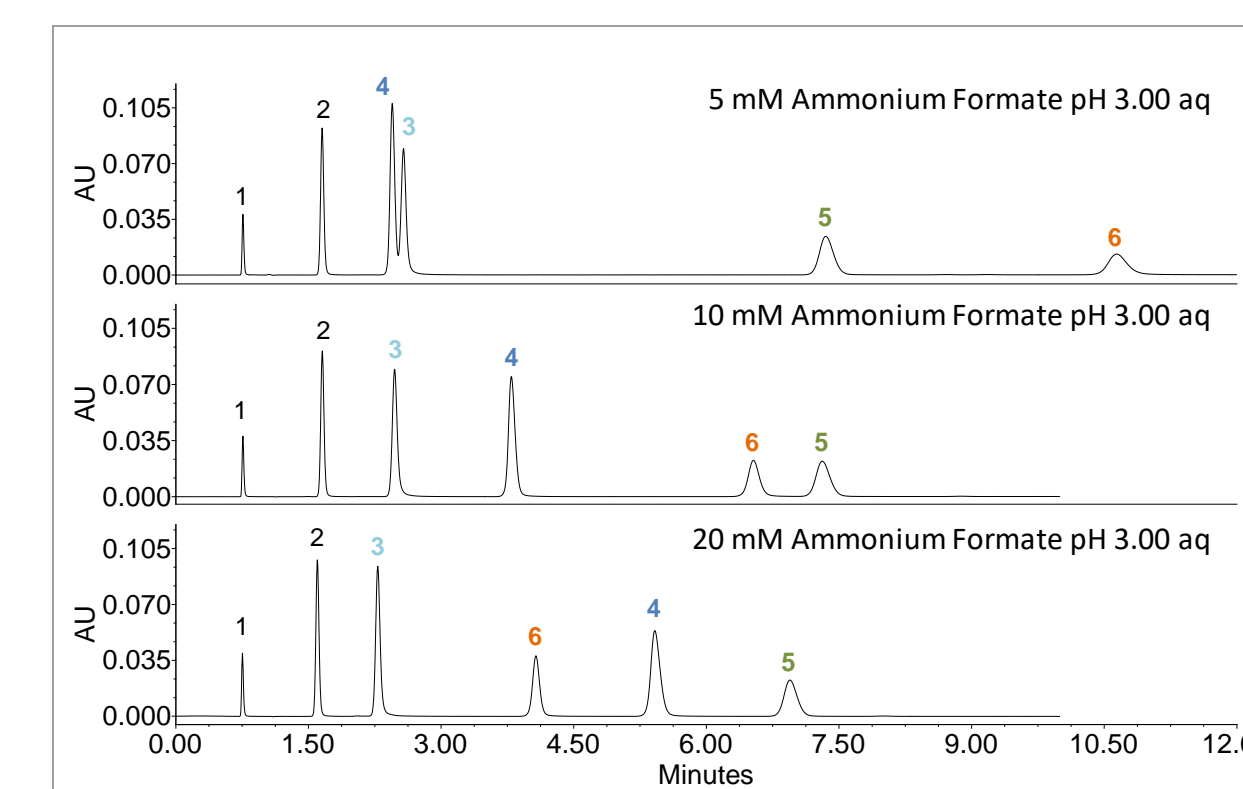


Figure 2b. Retention Factor vs. Buffer Concentration at pH 3.00
Peak ID-1) thiourea, 2) 5-fluorouracil, 3) nicotinamide, 4) procainamide, 5) resorcinol, 6) adenosine 5'-monophosphate

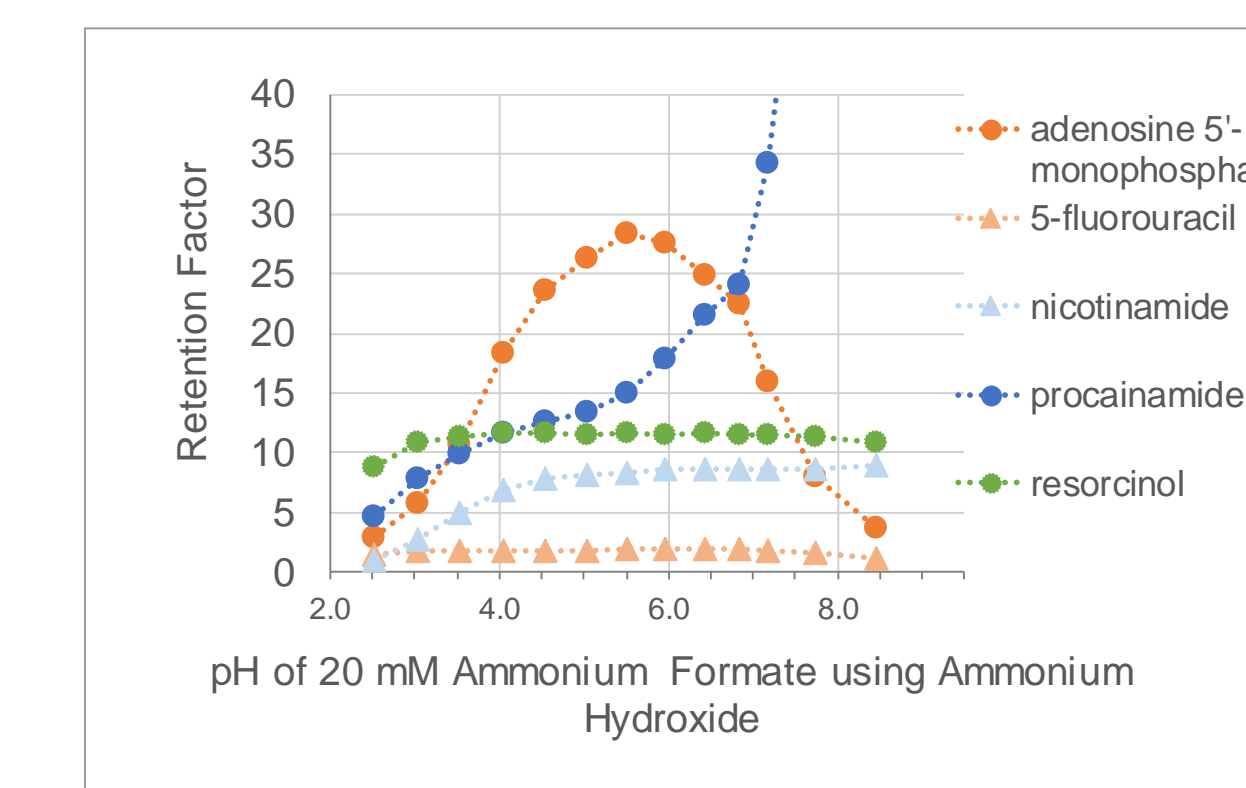


Figure 3a. Retention Factor vs. Buffer pH

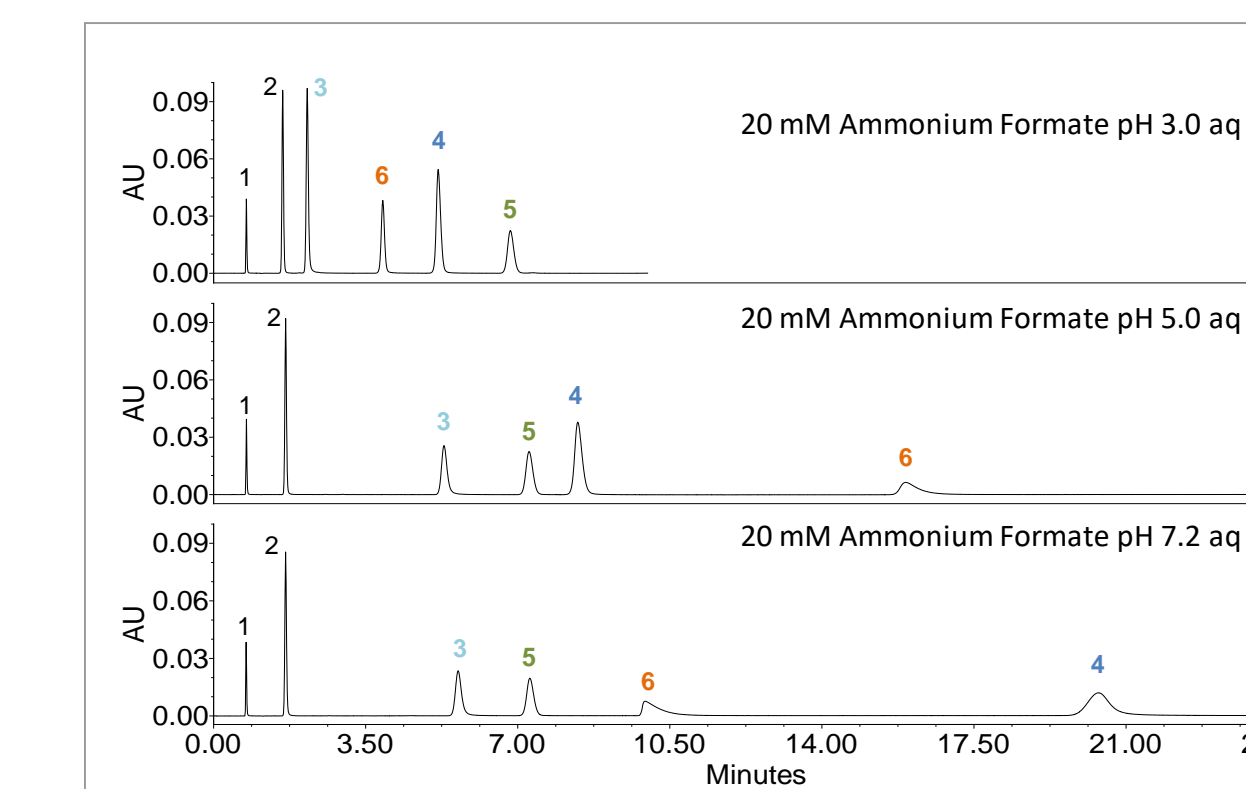


Figure 3b. Retention Factor vs. Buffer pH
Peak ID-1) thiourea, 2) 5-fluorouracil, 3) nicotinamide, 4) procainamide, 5) resorcinol, 6) adenosine 5'-monophosphate

DISCUSSION

Using a mobile phase of 10 mM ammonium formate (aq) at pH 3 and isocratic separation conditions, the retention for the model analytes was compared on the BEH95 RP/AX prototype, CSH C₁₈, and BEH C₁₈ columns. The BEH95 RP/AX prototype has a charge modifier that is protonated at pH < 8, and the charge modifier on CSH C₁₈ materials is protonated at pH < 5. At pH 3, the surface modifiers are positively charged, increasing the retention of negatively charged analytes, like adenosine 5'-monophosphate (AMP). The BEH95 RP/AX prototype had the highest retention vs. a traditional RP material, such as BEH C₁₈, which had the least retention of AMP. The opposite effect is observed for the basic analyte, procainamide. (Fig. 4). For this analyte, the positively charged surface repels the protonated base, resulting in decreased retention time. The BEH95 RP/AX prototype had the least retention and the BEH C₁₈ column gave the greatest retention. Selectivity and retention for the ionizable analytes can be optimized by adjusting pH and buffer concentration.

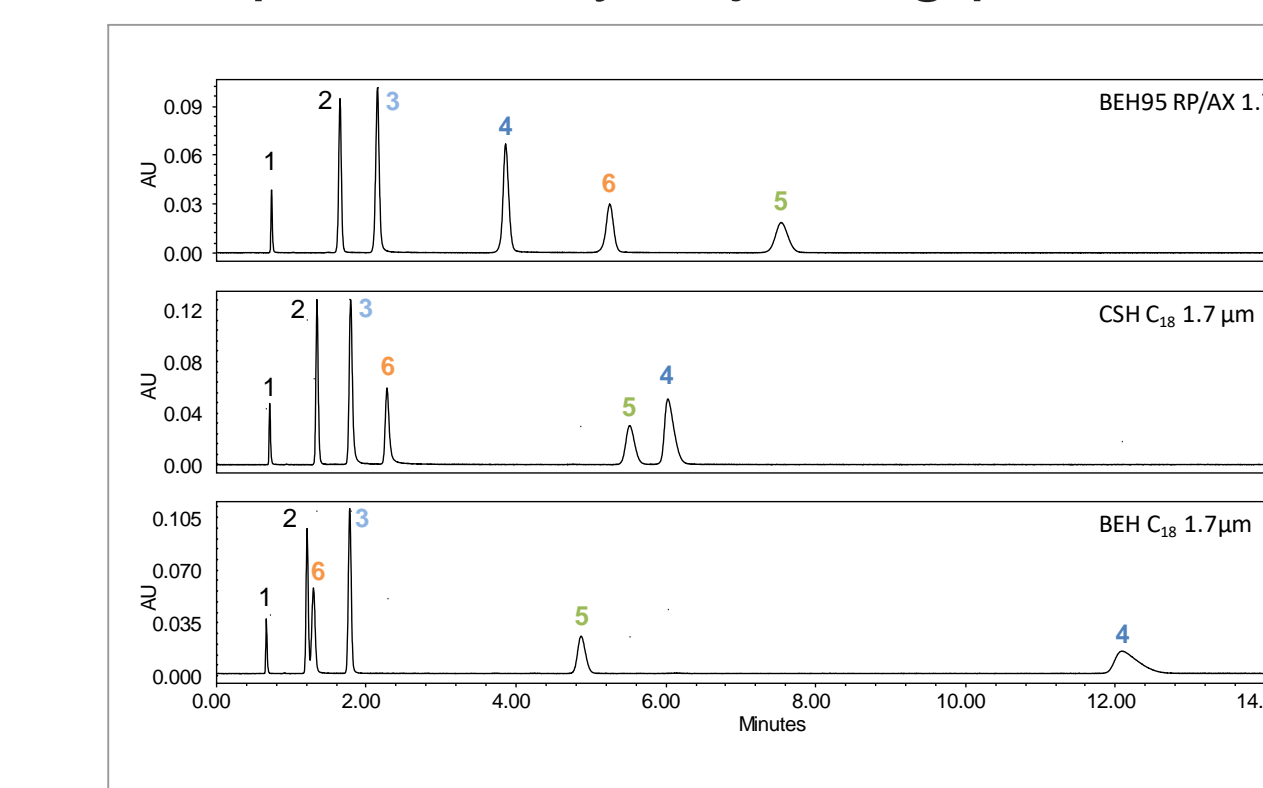


Figure 4. Retention Comparison
Peak ID-1) thiourea, 2) 5-fluorouracil, 3) nicotinamide, 4) procainamide, 5) resorcinol, 6) adenosine 5'-monophosphate

CONCLUSIONS

- The BEH 95 Å particle and a RP/AX bonding chemistry achieves high retention for acidic and neutral analytes without sacrificing compatibility in highly aqueous mobile phases.
- Acidic analyte retention increases from: BEH C₁₈ < CSH C₁₈ < BEH95 RP/AX.
- Basic analyte retention decreases from: BEH C₁₈ > CSH C₁₈ > BEH95 RP/AX.