

# Factors Affecting the Compatibility of Reversed-Phase Chromatographic Materials with Highly Aqueous Mobile Phases

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## INTRODUCTION

Reversed-phase (RP) liquid chromatography is a widely used method of analysis in a variety of application fields primarily due to its reproducibility and broad applicability. However, the use of RP liquid chromatography for separating highly polar analytes remains very challenging. To obtain adequate retention for such analytes, very weak mobile phases (containing mostly water) must be used. Unless used carefully, traditional reversed-phase materials based on < 100 Å pore diameter particles with high surface concentrations of hydrophobic (C<sub>8</sub> or C<sub>18</sub>) groups may not give reproducible results under these conditions due to the lack of penetration of the highly aqueous mobile phase into the pores of the stationary phase (1, 2).

Our goal for this research was to evaluate the characteristics required to promote retention of polar analytes, when using highly aqueous mobile phases (>90% water) on a RP column. We have investigated the effects of the pore diameter, bonded phase surface concentration, and the addition of a positively charged surface modifier on polar analyte retention and dewetting.

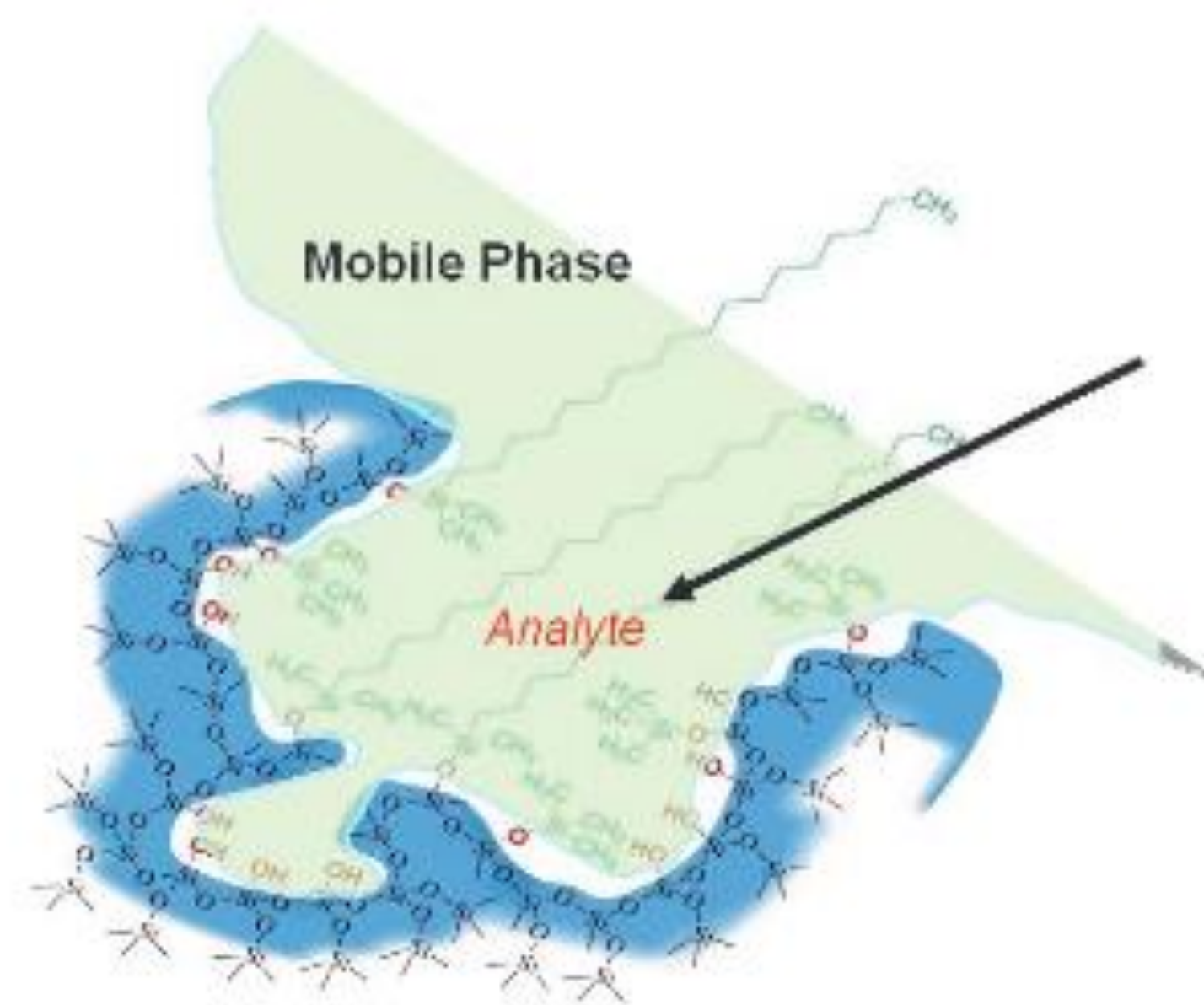
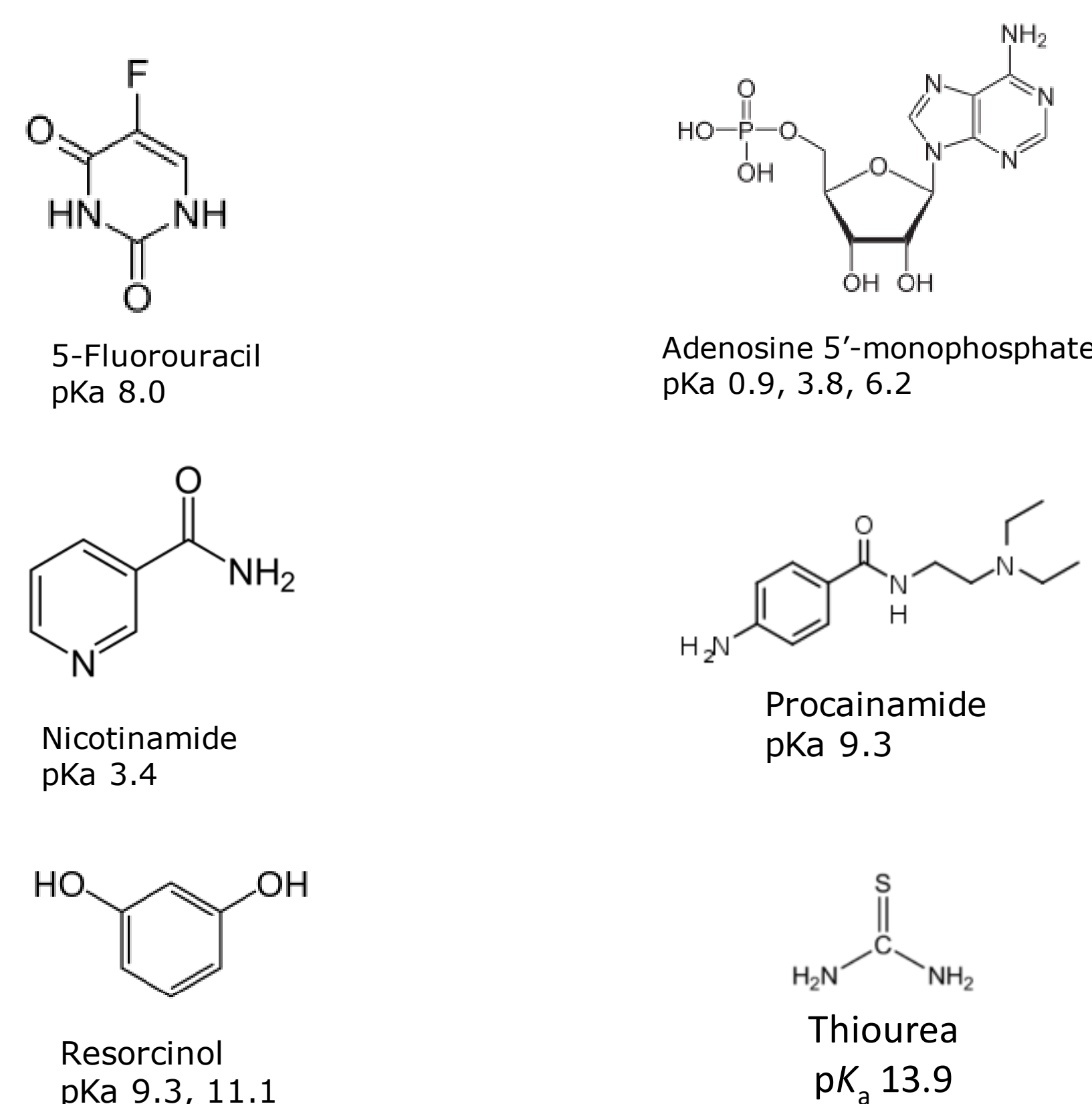


Figure 1. Proper wetting of bonded chromatographic surface

## METHODS

**Retention:** An ACQUITY UPLC® I-Class system with an ACQUITY Photodiode Array Detector was used with column configurations of 2.1 x 50 mm. Detection was by UV @ 254 nm. The isocratic separations were carried out in a 100% aqueous 10mM Ammonium Formate, pH 3 mobile phase with a flow rate of 0.2 mL/min and column temperature controlled at 30°C. The sample contained 5.0 µg/mL Thiourea, 12.5 µg/mL 5-Fluorouracil, 25.0 µg/mL Nicotinamide, 37.5 µg/mL Procainamide, 12.5 µg/mL Adenosine 5'-monophosphate, and 125.0 µg/mL Resorcinol. Four consecutive separations of the polar mixture were carried out and retention times of the last three were used to calculate retention factors. The void marker was thiourea, eluted using 100% acetonitrile.

**Aqueous Mobile Phase Compatibility:** Using the above system and conditions, the flow is stopped for 10 minutes allowing the backpressure on the column outlet to decrease. The flow is then restarted. The % tR Loss is calculated using the retention time before the zero-flow segment and the retention time for the first injection after restart.



## RESULTS

A new charged surface modifier was added to increase the retention of anionic polar analytes and for the ability to operate in 100 % aqueous mobile phases. Evaluations were conducted on Bridged Ethyl Hybrid (BEH) material with pore diameters of 66-138 Å.

	Properties of bonded phases			Results	
	PD, Å	Charge Modifier	C <sub>18</sub> Concentration µmol/m <sup>2</sup>	% tR Loss	k Thymine
RP-Control-A	138	N/A	1.6	3.3	4.1
RP-Control-B	113	N/A	1.7	4.3	5.7
RP-Control-C	95	N/A	1.5	4.7	6.5
RP-Control-D	79	N/A	1.5	30.9	7.6
RP-Control-E	66	N/A	1.6	99.6	9.2

In addition to pore diameter, C<sub>18</sub> surface concentrations from 1.4 - 1.8 µmol/m<sup>2</sup> and different charge modifier surface concentrations were also investigated.

	Properties of bonded phases			Results	
	PD, Å	Charge Modifier	C <sub>18</sub> Concentration µmol/m <sup>2</sup>	% tR Loss	k Thymine
RP/AX-B	95	Low	1.4	2.8	5.6
RP/AX-C	95	Low	1.8	6.5	5.8
RP/AX-A	95	Medium	1.6	3.7	5.6
RP/AX-D	95	High	1.4	1.9	5.4
RP/AX-E	95	High	1.8	4.3	5.6

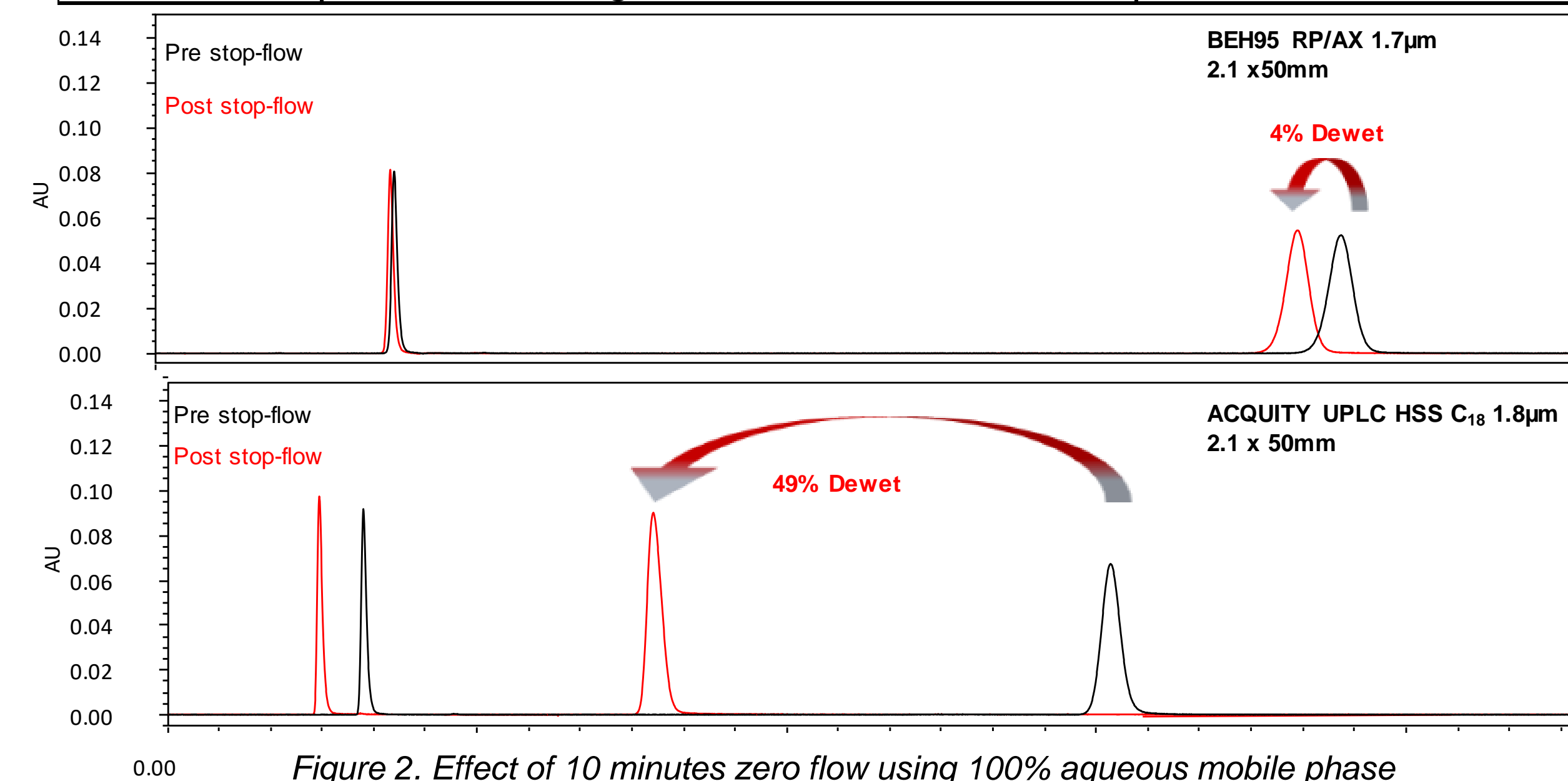


Figure 2. Effect of 10 minutes zero flow using 100% aqueous mobile phase

## DISCUSSION

To obtain increased retention of highly polar analytes, highly aqueous mobile phases are often needed. Not all reversed-phase columns are compatible and a loss of retention will occur when the flow is stopped and restarted. The pore diameter, C<sub>18</sub> bonding concentration, and surface charge each play a role in the wetting of reversed-phase materials. The use of an intermediate C<sub>18</sub> surface concentration coupled with the addition of a positive charge modifier makes the BEH95 RP/AX prototype compatible with highly aqueous mobile phases. An experiment was conducted in which the flow of a 100% aqueous mobile phase was stopped and restarted after 10 minutes on a 2.1 x 50 mm, 1.7 µm column. The decrease in retention for Thymine was only 4%. This was 12x lower than the 49% retention loss observed for ACQUITY UPLC HSS C<sub>18</sub> of equivalent particle and column size. (Figure 2.)

## CONCLUSIONS

- C<sub>18</sub> bonded phases on BEH particles with pore sizes < 100Å show significant retention losses after stopping and restarting the flow of 100% aqueous mobile phases.
- Adding a charge modifier reduces the retention losses allowing a 95Å BEH particle bonded with 1.6 µmol/m<sup>2</sup> C<sub>18</sub> groups to be compatible with 100% aqueous mobile phases.

## References

1. T. H. Walter, P. Iraneta and M. Capparella *J. Chrom. A* **1075**, 2005, 177.
2. F. Gritti, D. Brousmiche, M. Gilar, T. H. Walter and K. Wyndham *J. Chrom. A* **1596**, 2019, 41.