CONSIDERATIONS WHEN DESIGNING A BONDED PHASE FOR POLAR ANALYTES

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ISC 2016 31st International Symposium on Chromatography
August 28th—September 1, 2016 University College Cork, Ireland
Poster P01-001-101 *Contact: Bonnie_A_Alden@Waters.com*

INTRODUCTION

Due to its reproducibility and broad applicability, reversed-phase chromatography is a widely used method of analysis for a variety of compounds. However, one difficult issue still exists, the low retention of polar compounds. In many cases, to achieve sufficient retention, highly aqueous (>90% water) mobile phases are required. Traditional ca. 100 Å reversed-phase materials with high surface concentrations of hydrophobic (C_{18}) groups do not give reproducible results under these conditions due to variability in the penetration of the highly aqueous mobile phase into the pores of the stationary phase (1). We have specifically designed a bonded phase on solid-core silica particles for the retention of polar analytes and compatibility with highly aqueous mobile phases (CORTECS T3).

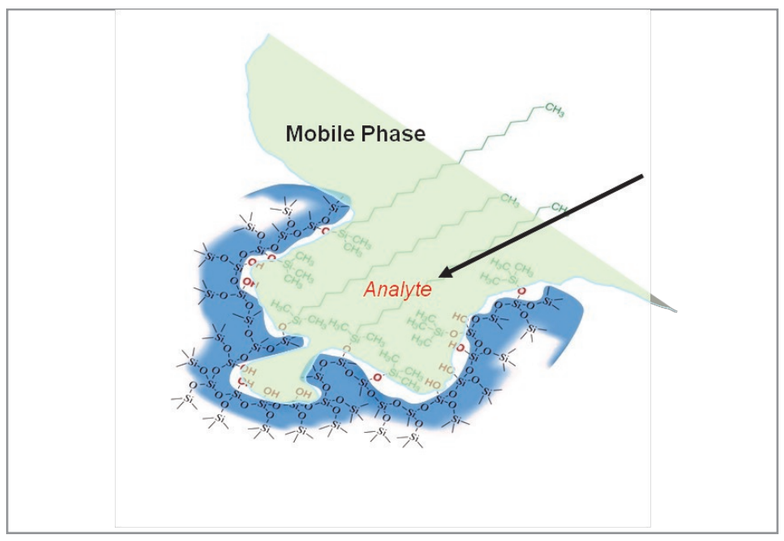

Figure 1. Proper wetting of bonded chromatographic surface

METHODS

Analysis Conditions

Waters Corporation (Milford, MA, USA) Empower 3® Chromatography Data Software was used for instrument control, data acquisition, and processing. The chromatographic system was an ACQUITY UPLC H-Class which consisted of a Sample Manager with Flow Through Needle, Quaternary Solvent Manager, ACQUITY PDA, a CM-A Column Manager with active preheating. Column temperatures were set to 30° C. In all cases, the flow rate was 200 µL/minute. The test sample used to assess the initial retention and subsequent % loss of retention was a 3 µL injection of thiourea (10 µg/mL) and thymine (12 µg/mL). Retention times were an average of five injections. Thiourea (10 µg/mL) prepared in 100 % acetonitrile (ACN) was used as the void volume (V_o) marker. The mobile phase used for the determination of retention loss was 10 mM ammonium formate, pH 3, prepared in 100 % HPLC grade water. The column configuration used was 2.1 x 50 mm.

Protocol

Wet Stationary Phase
Acetonitrile (40 min)

Determine Void Volume Time

Equilibrate
Methanol/H_2O (50:50) (30 min)

Equilibrate
10 mM Ammonium Formate, pH 3 in 100 % H_2O

Determine Thymine Retention

Stop Flow for 10 Minutes

Restart Flow
10 mM Ammonium Formate, pH 3 in 100 % H_2O

Determine Thymine Retention

RESULTS

ACQUITY UPLC HSS C_{18} 1.8 µm and ACQUITY UPLC HSS T3 1.8 µm columns were chosen as fully porous benchmarks and to demonstrate the effect of surface coverage. ACQUITY UPLC HSS C_{18} is a high coverage bonded-phase with a C_{18} surface concentration of 3.2 µmol/m^2. Using the previously described protocol, a retention loss of 100 % was calculated for thymine. Reducing the C_{18} surface concentration, as in ACQUITY UPLC HSS T3, to 1.6 µmol/m^2, the % loss in thymine retention is only 7 % (n=5).

To investigate this phenomenon on solid-core materials, the CORTECS UPLC 1.6 µm product family and several prototype solid-core materials were evaluated and % loss of retention and the initial retention factor for thymine were calculated for each material listed in Table 1.

Table 1. Properties of Bonded Phases

	PD, Å	Charge Modifier	C18 Concentration	% tR Loss	k Thymine
ACQUITY UPLC HSS C18	100	NA	3.2	101	3.73
ACQUITY UPLC HSS T3	100	NA	1.6	7	4.89
SC-C18-E	160	NA	1.7	4	2.22
SC-C18-F	160	NA	2.1	6	2.26
SC-C18-G	160	NA	2.5	7	2.21
SC-C18-B	120	LOW	2.3	5	2.67
SC-C18-C	120	MEDIUM	2.3	4	2.72
SC-C18-D	120	HIGH	2.0	3	2.63
SC-C18-A	90	MEDIUM	2.3	12	2.52
CORTECS UPLC C18	90	NA	2.7	88	2.69
CORTECS UPLC C18+	90	LOW	2.3	23	2.92
CORTECS UPLC C8	90	NA	3.4	98	1.75
CORTECS UPLC Phenyl	90	NA	3.2	1	1.96
CORTECS UPLC T3	120	NA	1.6	5	2.78
CORTECS UPLC Shield RP18	90	NA	3.2	3	1.44

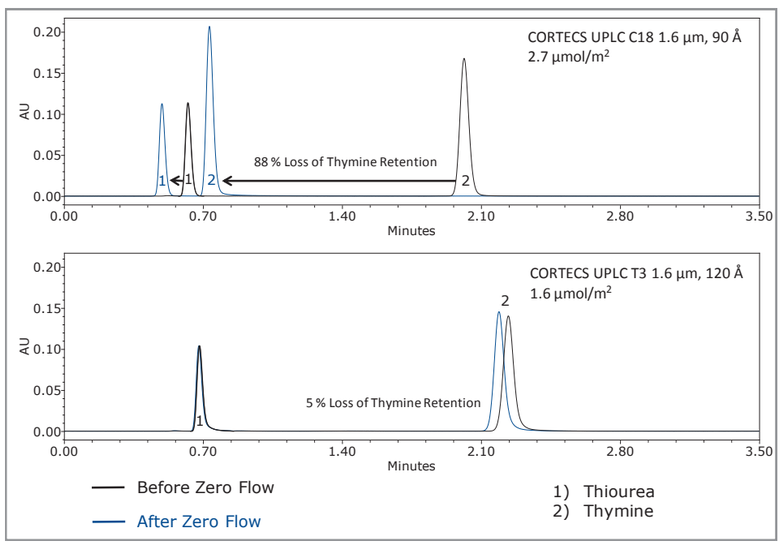

Figure 2. Effect of 10 minutes of Zero Flow using 100 % aqueous mobile phase on two bonded phases differing in pore size and C_{18} surface concentration

One series of materials was prepared on 160 Å 2.7 µm solid-core silica to investigate the effect of varying the C_{18} surface concentration and a second series was prepared on 120 Å 2.7 µm solid-core (SC) particles to investigate the level of charge modifier. Both resulted in lower % losses in t_R for thymine than the control (SC-C18-A), which used 90 Å 2.7 µm solid-core particles with a 2.3 µmol/m^2 C_{18} surface concentration and a medium level of charge modifier.

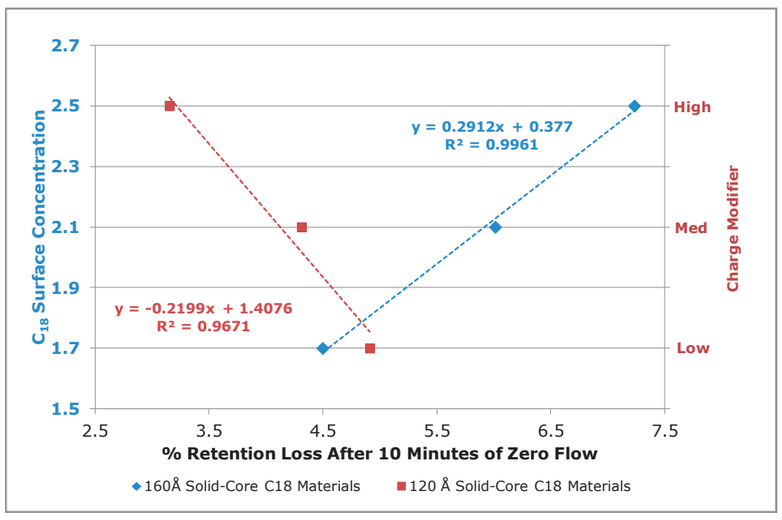

Figure 3. Effect of C_{18} surface concentration on 160 Å 2.7 µm solid-core bonded phases

References

1. T. H. Walter, P. Iraneta and M. Capparella *J. Chrom. A* **1075**, 2005, 177.

DISCUSSION

Using highly aqueous mobile phases can lead to poor reproducibility due to mobile phase extrusion from the pores.[1] From these data, there are at least four factors influencing the amount of retention loss observed. The pore diameter, ligand concentration, ligand type and surface charge each play a role in the wetting of reversed-phase materials.

CORTECS UPLC C_{18} and CORTECS UPLC C_8 columns were evaluated. The % losses in retention for thymine were 88 % and 98 %, respectively. These were expected results based on the small pore diameter of the base particle and high surface concentrations of the bonded phases. A 2.7 µm solid-core silica particle was prepared with 160 Å pores, and three prototype C_{18} materials (SC-C18-E, SC-C18-F, SC-C18-G) were prepared using 1.7 µmol/m^2, 2.1 µmol/m^2, and 2.5 µmol/m^2 of C_{18}. The % losses in thymine retention were 7 % or lower, comparable to the performance of ACQUITY UPLC HSS T3 and shows the C_{18} surface concentration impact on % loss of retention (R^2 0.996) (Figure 3). A CORTECS UPLC C_{18}+ column exhibited a 23 % loss in retention compared to 88 % on CORTECS UPLC C_{18}. The improvement in wetting is explained by a lower C_{18} surface concentration, 2.3 µmol/m^2, and the presence of a positive charge modifier. The % loss in is halved to 12 % when the charge is increased (SC-C18-A). Since both pore enlargement and surface charge enhance compatibility with 100 % aqueous mobile phases, a series of three prototype C_{18} materials (SC-C18-B, SC-C18-C, SC-C18-D) were prepared using 120 Å solid-core silica particles and a varied amount of the charge modifier. The % losses in retention were 5% or lower, with an R^2 of 0.9671 for the amount of modifier versus the % retention loss.

One surprising result was obtained for a full coverage material prepared on 90 Å solid-core silica particles: the CORTECS UPLC Phenyl phase lost only 1 % of its retention for thymine. Also, as previously demonstrated (1), the embedded polar group bonded phase in CORTECS UPLC Shield RP_{18} showed only 3 % loss of retention. However, it is important to note while these materials are compatible with 100% aqueous mobile phases, CORTECS T3 exhibits approximately 30% more retention for polar analytes. In the end, a stationary phase with a controlled surface concentration of C_{18}, no surface charge modifier and a pore diameter of 120 Å achieved the best balance of retention for polar analytes and minimal dewetting.

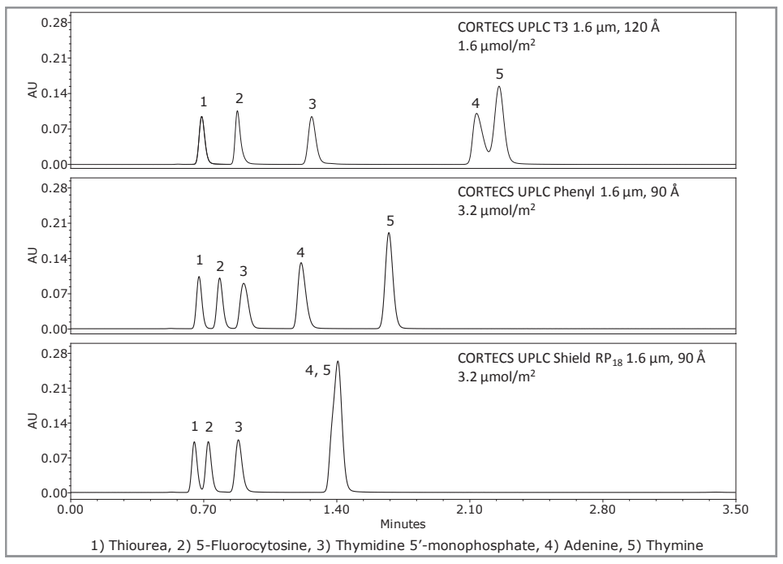

Figure 4. Comparison of CORTECS bonded phases for polar analyte retention using 100 % aqueous mobile phase

CONCLUSION

- **The design of CORTECS UPLC T3 considered the retention for polar analytes with compatibility in 100 % aqueous mobile phases, balancing these attributes to achieve the optimum performance.**
- **Choice of ligand type, ligand concentration, surface charge and particle pore diameter all significantly influence wettability of RPLC stationary phases.**
- **Increasing pore diameter to 120 Å or higher significantly reduces the impact of C_{18} surface concentration on the % retention loss.**
- **A charge modifier reduces the retention losses observed for moderate C_{18} surface concentration materials, even when 90 Å solid - core materials particles are used.**

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