DIRECT SCALING OF HPLC SEPARATIONS TO UPLC® TECHNOLOGY FOR THE ANALYSIS OF POLAR COMPOUNDS

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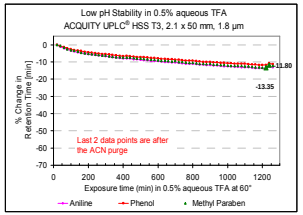
INTRODUCTION

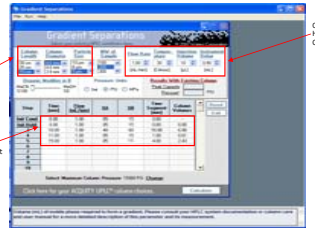
Performing fast and efficient separations of polar compounds without compromising resolution is challenging. Waters ACQUITY UPLC® HSS T3 columns successfully meet this challenge because they are designed to retain and separate polar compounds in a UPLC environment. Just like Atlantis® T3 HPLC columns, HSS (high strength silica) T3 UPLC columns use a trifunctional C_{18} alkyl phase bonded at a ligand density that enhances polar compound retention. As a result, these columns are capable of operating at 100% aqueous conditions with minimal dewetting.

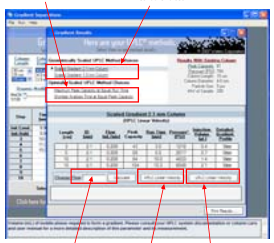
Since ACQUITY UPLC® HSS T3 columns benefit from the same lifetime, peak shape, and stability seen with Atlantis® T3 columns, methods are easily transferred between HPLC and UPLC technology. Possessing the ability to separate polar compounds faster with ACQUITY UPLC® HSS T3 columns allows for more efficient method development and chromatographic condition screening.

LOW pH STABILITY



ACQUITY UPLC® CALCULATOR

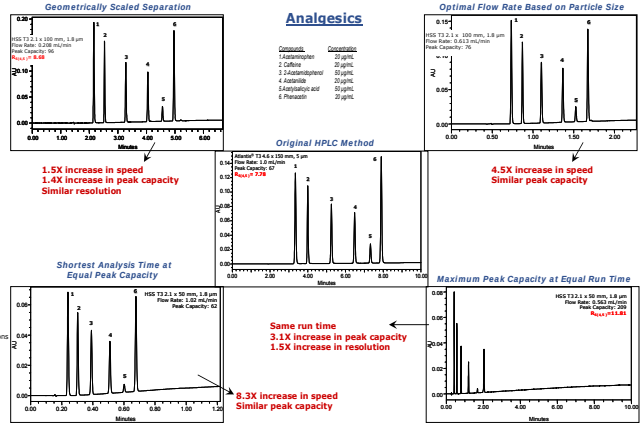




APPLICATIONS

Analgesics

Compounds	Concentration
1.Acetaminophen	20 µg/mL
2. Caffeine	20 µg/mL
3.4-Acetamidophenol	50 µg/mL
4. Acetanilide	20 µg/mL
5.Acetylsalicylic acid	50 µg/mL
6. Phenacetin	20 µg/mL



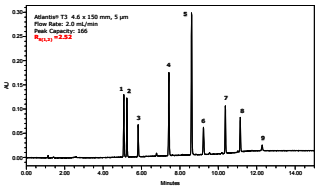
Comparison of ACQUITY UPLC® calculator options. Mobile phase A was 0.1% HCOOH in H_2O. Mobile phase B was 0.1% HCOOH in ACN. The gradient (HPLC) started with 15% B, then increased to 60% B in 10 minutes. Flow rate was 1.0 mL/min. UV @ 260 nm. HPLC column was Atlantis® T3 4.6 x 150 mm, 5 µm. The mixture was prepared in water. The injection volume was 10 µL.

The gradient (UPLC Linear Velocity) started with 15% B, then increased to 60% B in 1.13 minutes. Flow rate was 0.613 mL/min. UV @ 260 nm. 30 °C. The UPLC columns were ACQUITY UPLC® HSS T3 2.1 x 50 mm, 1.8 µm OR 2.1 x 100 mm, 1.8 µm. The injection volume was 0.7 µL (50 mm) or 1.4 µL (100 mm).

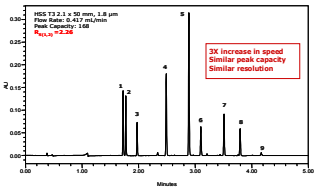
Polar and Non-polar Compounds

Compounds	Concentration
1. Acetanilide	20 µg/mL
2. Triamcinolone	30 µg/mL
3. Hydrocortisone	15 µg/mL
4. 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b] pyridine-3-carbonitrile	15 µg/mL
5. 3-Aminofluoranthene	35 µg/mL
6. 6α-Methyl-17α-hydroxyprogesterone	25 µg/mL
7. 2-Bromofluorene	15 µg/mL
8. Perylene	20 µg/mL
9. Naphtho[2,3-a]pyrene	20 µg/mL

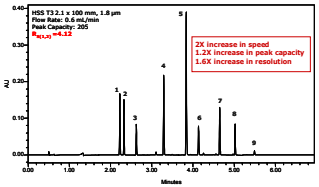
Original HPLC Method



Geometrically Scaled Gradient (50 mm length HSS T3 column)



Manually Input Flow Rate (100 mm length HSS T3 column)



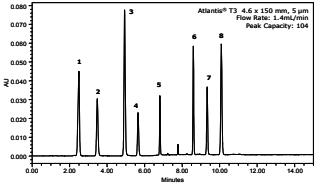
Mobile phase A was 0.1% HCOOH in H_2O. Mobile phase B was 0.1% HCOOH in ACN. The gradient (HPLC) had an initial hold at 0% B for 0.5 minutes, then increased to 100% B at 10 minutes and hold 100% B for 5 minutes. Flow rate was 2.0 mL/min. UV @ 254 nm. The HPLC column was Atlantis® T3 4.6 x 150 mm, 5µm. The injection volume was 10 µL.

The gradient (HPLC Linear Velocity) had an initial hold at 0% B for 0.33 minutes, then increased to 100% B at 6.67 minutes and hold 100% B until 10.0 minutes Flow rate was 0.417 mL/min. UV @ 254 nm. The UPLC columns were ACQUITY UPLC® HSS T3 2.1 x 100 mm or 2.1 x 50 mm, 1.8 µm. The mixture was prepared in 0.1% HCOOH in water/0.1% HCOOH in ACN (50/50). The injection volume was 0.7 µL (50 mm) or 1.4 µL (100 mm).

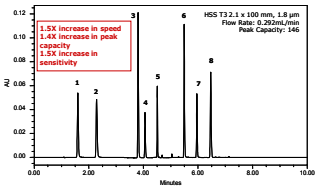
Water Soluble Vitamins

Compounds	Concentration
1. L-ascorbic acid	25 µg/mL
2. Nicotinic acid	10 µg/mL
3. Thiamine	25 µg/mL
4. Pyridoxal	50 µg/mL
5. Pyridoxine	50 µg/mL
6. Folic acid	45 µg/mL
7. Caffeine	10 µg/mL
8. Riboflavin	10 µg/mL

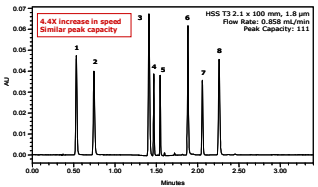
Original HPLC Method



Geometrically Scaled Gradient (100 mm length HSS T3 column)



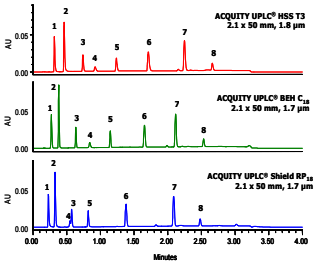
Optimal Flow Rate Based on Particle Size (UPLC Linear Velocity)



Mobile phase A was 0.1% TFA in H_2O. Mobile phase B was ACN. The gradient (HPLC) started with 0% B, then increased to 3% B in 4 minutes, to 15% B at 6 minutes and 20% B at 15 minutes. Flow rate was 1.4 mL/min. UV @ 260 nm. The HPLC column was Atlantis® T3 4.6 x 150 mm, 5 µm. The mixture was prepared in water. The injection volume was 10 µL.

The gradient (UPLC Linear Velocity) started with 0% B, then increased to 3% B in 0.9 minutes, to 15% B at 1.36 minutes and 20% B at 3.40 minutes. Flow rate was 0.858 mL/min. UV @ 260 nm. The UPLC column was ACQUITY UPLC® HSS T3 2.1 x 100 mm, 1.8 µm. The injection volume was 1.4 µL.

COMPARISON OF UPLC CHEMISTRIES FOR CARDIAC DRUG COMPOUND RETENTION



Mobile phase A was 10 mM ammonium formate in H_2O, pH 3.0. Mobile phase B was methanol. The gradient started at 15% B and increased to 75% B in 2.5 minutes, then held at 75% B for 0.5 minutes. Flow rate was 0.8 mL/min. UV @ 270 nm. 30 °C. Injection volume was 1.0 µL. Peaks: 1 = Procainamide, 2= Procaine, 3= Pindolol, 4= Lidocaine, 5= Disopyramide, 6= Propranolol, 7= Nifedipine, 8= Nimodipine. The mixture was prepared in MeOH/10 mM ammonium formate in water, pH 3.0 (15/85)

CONCLUSIONS

- ACQUITY UPLC® HSS T3 columns exhibit superior stability at low pH conditions.
- HPLC methods developed on Atlantis T3 columns were successfully scaled to ACQUITY UPLC® HSS T3 columns.
- The ACQUITY UPLC® calculator gives several options for method transfer from HPLC to UPLC, depending on the goal of the final separation.
- For methods transferred to ACQUITY UPLC® HSS T3 columns from Atlantis® T3, greater peak capacity was achieved in less time (3-8X reduction in run time).
- Sensitivity increases of up to 50% were observed when HPLC methods were transferred to UPLC.
- ACQUITY UPLC® HSS T3 columns are suitable for analysis of extremely polar compounds in a high pressure regime.

50 YEAR LEGACY

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