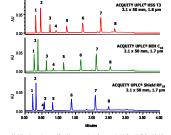
## THE SCIENCE OF WHAT'S POSSIBLE. Jane Xu, Kenneth J. Fountain, Pamela C. Iraneta, Diane M. Diehl Waters Corporation, Milford, MA 01757, USA Geometrically Scaled UPLC Optimally Scaled Method Choice INTRODUCTION Polar and Non-polar Compounds Water Soluble Vitamins Performing fast and efficient separations of polar Concentration 25 µg/mi Compounds Concentratio L-ascorbic acid 25 upini compounds without compromising resolution is Nicotinic acid 10 µg/ml challenging, Waters ACOUITY UPLC® HSS T3 columns Thiamine 25 µg/ml Column volum Pyridoxal Pyridoxine 50 µg/mL 50 µg/mL successfully meet this challenge because they are 15 jąini. 30 joini designed to retain and separate polar compounds in a 6. 6o-Methyl-17o-hydroxy one 25 µg/mL Folic sold 45 µpInL 15 µgint. 20 µgint. Caffeine 10 µa/mL UPLC environment. Just like Atlantis® T3 HPLC 8. Perylete Riboflevin 10 up ml columns, HSS (high strength silica) T3 UPLC columns 9 Abritho/7 Lainean 20 united Original HPLC Method use a trifunctional C18 alkyl phase bonded at a ligand -Original HPLC Method density that enhances polar compound retention. As Geometrically scaled Atlantis® T3 4.6 x 150 mm, 5 µm Flow Rate: 1.4mL/mi 0.05 gradient (HPLC Linear a result, these columns are capable of operating at Atlantis® T3 4.6 x 150 mm, 5 µm Flow Rate: 2.0 mL/min Peak Capacity: 166 0.07 Peak Capacity: 3 100% aqueous conditions with minimal dewetting. Optimal flow rate Since ACOUITY UPLC® HSS T3 columns benefit from based on particle size 0.050 (UPLC Linear the same lifetime, peak shape, and stability seen with 200 Atlantis® T3 columns, methods are easily transferred 0.0 0.030 hetween HPLC and UPLC technology. Possessing the ability to separate polar compounds faster with manually input scaling based on particle flow rate size 0.00 ACQUITY UPLC® HSS T3 columns allows for more 2.0 2.50 efficient method development and chromatographic APPLICATIONS condition screening. 4 00 8.00 10.00 12.00 200 6.01 .... Geometrically Scaled Separation Optimal Flow Rate Based on Particle Size Analgesics Geometrically Scaled Gradient (100 mm length HSS T3 column) LOW pH STABILITY Geometrically Scaled Gradient (50 mm length HSS T3 column Concentratio. 20 µg/mL 20 µg/mL 50 µg/mL 50 µg/mL 20 µg/mL 20 µg/mL HSS T3 2 1 x 100 mm 1 8 um Low pH Stability in 0.5% aqueous TFA HSS T3 2.1 x 50 mm, 1.8 µm Flow Rate: 0.417 mL/min Peak Capacity: 168 Flow Rate: 0.292mL/min Peak Capacity: 14 1.Acetamicphen 2. Cafeire 3. 2.Acetamidpheno ACQUITY UPLC® HSS T3, 2.1 x 50 mm, 1.8 µm 1.5X increase in speed 0.10 J 1.4X increase in peak 4 Acetaniide 5Acetvisalcvic acid SX incre 3X increase in speed 0.09 CONCLUSIONS nsitivity nilar resolution Time in ₹ 0.0 13.35 0.50 1.50 200 360 4.00 100 Original HPLC Method 0.04 40 % 1 5Y increase in sneed 4 5¥ increase in cneed 1.4X increase in peak capacity Similar peak capacity Last 2 data points are after UPLC® HSS T3 columns. Similar resolution the ACN purple 8.00 200 400 600 800 1000 1200 Exposure time (min) in 0.5% aqueous TEA at 60' Manually Input Flow Rate (100 mm length HSS T3 column) Optimal Flow Rate Based on Particle Size (UPLC Linear Velocity) - Dhenol Methyl Dorohan Aniline Shortest Analysis Time at HSS T3 2.1 x 100 mm, 1.8 µm Flow Rate: 0.6 mL/min HSS T3 2.1 x 100 mm, 1.8 µm Flow Rate: 0.858 mL/min Peak Capacity: 111 **ACOUITY UPLC® CALCULATOR** Faual Peak Canacity 2.00 400 Maximum Peak Capacity at Equal Run Time ak Canarity: 205 ISS T3 2.1 x 50 mm, 1.8 µm Flow Rate: 1.02 mL/mi increase in speed reduction in run time). 2X increase in neak canacity Same run time 0.04 ₹ 0.20-4 3.1X increase in peak capacity to UPLC. 1.5X increase in resolution 0.03 ACOUITY UPLC<sup>®</sup> HSS T3 columns are suitable 0.02

aradient (UPLC Linear Velocity) started with 0% B, then increased to 3% B in 0.9 minute

to 15% B at 1.36 minutes and 20% B at 3.40 minutes. How rate was 0.858 mil/minutes 260 nm. The UPLC column was ACOUTY UPLC® HSS 132.1 x 100 mm. 1.8 um. The injection volume was 1.4 µL

## COMPARISON OF UPLC CHEMISTRIES FOR CARDIAC DRUG COMPOUND RETENTION



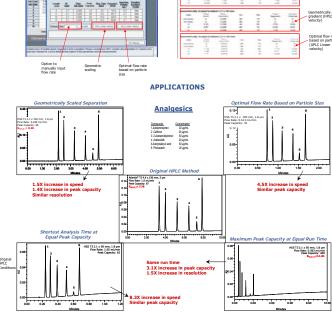
Mobile phase A was 10 mM ammonium formate in H<sub>2</sub>O, pH 3.0. Mobile phase B was methanol. The gradient started at 15% B and increased to 75% B in 2.5 minutes, then held at 75% B for 0.5 minutes. Flow rate was 0.8 mL/min. UV @ 270 nm. 30 °C. Injection volume was 1.0 µL. Peaks: 1 = Procainamide, 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)

- ACOUITY UPLC<sup>®</sup> HSS T3 columns exhibit superior stability at low pH conditions.
- HPLC methods developed on Atlantis T3 columns were successfully scaled to ACQUITY
- The ACQUITY UPLC<sup>®</sup> 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<sup>®</sup> HSS T3 columns from Atlantis® T3, greater peak capacity was achieved in less time (3-8X
- · Sensitivity increases of up to 50% were observed when HPLC methods were transferred
- for analysis of extremely polar compounds in a high pressure regime.



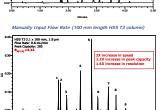
## DIRECT SCALING OF HPLC SEPARATIONS TO UPLC® TECHNOLOGY FOR THE ANALYSIS OF POLAR COMPOUNDS





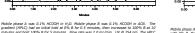
Comparison of ACQUITY UPLC® calculator options. Mobile phase A was 0.1% HCOOH in H2O. Mobile phase B was 0.1% HCOOH in ACN. The gradient (HPLC) started with 15% B, then increased to 60% B in 10 minutes. Flow rate was 1.0 mL/min. UV @ 260 nm. HPLC column was Atlantis® T3 4.6 x 150 mm, 5 µm. The mixture was prepared in water. The injection volume was 10 ul

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).



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<sup>®</sup> T3 4.6 x 150 mm, Sµm. The Injection volume was 10 µL.

The gradient (HPLC Linear Velocity) had an initial hold at 0% B for 0.33 minutes, the Ine gradient (HHL Linear Velocity) nad an initial nold at 0% 8 for 0.33 minutes, men increased to 100% 8 at 6.67 minutes and hold 100% 8 until 10.0 minutes. Flow rate was 0.417 mL/min. UV @ 254 nm. The UPLC columns were ACQUITY UPLC% HSS T32.1 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).



Mobile phase A was 0.1% TFA in H<sub>2</sub>O. Mobile phase B was ACN. The gradient (HPLC) started recome plasse w was 0.2% if w in rBQC recome plasse is was w.o.t. the glassies (PrEC) states 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 mi/min. UV @ 260 m. The HPLC column was Atlantis<sup>®</sup> 13 4.6 x 150 mm, 5 m. The mixture was prepared in water. The injection volume was 10 µ.

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