ADVANCES IN STATIONARY PHASE CHEMISTRY FOR LC METHODS DEVELOPMENT

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INTRODUCTION

Method developers employ several tools to achieve optimum separations for their compounds of interest, including different columns, mobile phases, and software packages. Ideally, the optimum method is obtained using the minimum number of screening runs possible. The most common methods development approach is to screen multiple columns and mobile phases to cover as much of the selectivity space as possible prior to optimization, which can be performed manually or with software.

In this work, we introduce new column chemistries built on Charged Surface Hybrid (CSH™) technology for routine UPLC and HPLC separations. The practical advantages to these columns include superior peak shape and loading of basic compounds in low ionic strength mobile phases, rapid re-equilibration after exposure to different mobile phase pH, and increased selectivity. In addition, these CSH columns are incorporated into a novel methods development strategy that employs new high pressure LC instrumentation and integrated optimization software. Finally, methods developed on these columns can be transferred between UPLC, HPLC, and preparative platforms while still maintaining separation selectivity.

METHODS

All separations were performed on either an ACQUITY UPLC® or ACQUITY UPLC H-Class system equipped with an ACQUITY PDA detector. Where indicated, an ACQUITY SQD was utilized to obtain mass spectrometric data. The aqueous mobile phases were either 0.1% formic acid (pH ~ 2.7) or 0.1% ammonium hydroxide (pH ~ 10.2). The organic mobile phases were acetonitrile and methanol. The column dimensions, gradient conditions, column temperature, and injection parameters are specified in the figure captions.

CSH™ TECHNOLOGY

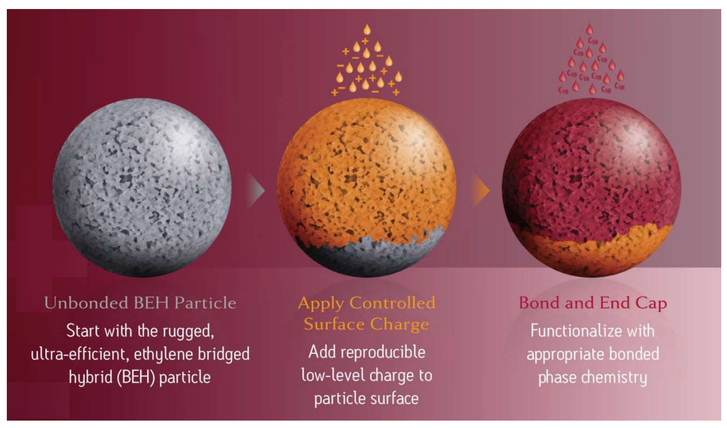


Figure 1. Bonding process for Charged Surface Hybrids (CSH Technology). The presence of a reproducible, low level charge on the particle surface alleviates problems encountered in acidic, low ionic strength mobile phases (i.e., overloading, retention time drifting), while maintaining predominantly reversed-phase behavior.

SELECTIVITY

The selectivity factor (S)[1] measures the selectivity difference between column chemistries under a given set of conditions. The value is determined by measuring and plotting the retention factors (k_g in a gradient) of analytes run on two columns under the same chromatographic conditions. Higher selectivity values indicate a higher degree of orthogonality.

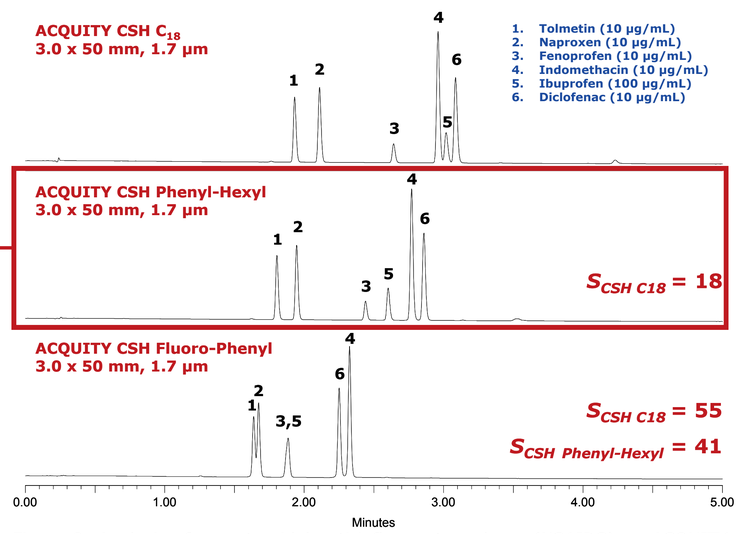


Figure 2. Analysis of non-steroidal anti-inflammatory drugs (NSAIDS) on ACQUITY CSH columns. Mobile phase A is water, mobile phase B is acetonitrile, and mobile phase C is 2% HCOOH in water. Gradient is from 25 to 80% B in 5 min with a constant 5% C. ACQUITY UPLC H-Class system. UV detection at 270 nm. 10 µL injection, 30 °C. Flow rate is 1.2 mL/min.

TRANSFERABILITY ACROSS PARTICLE SIZES

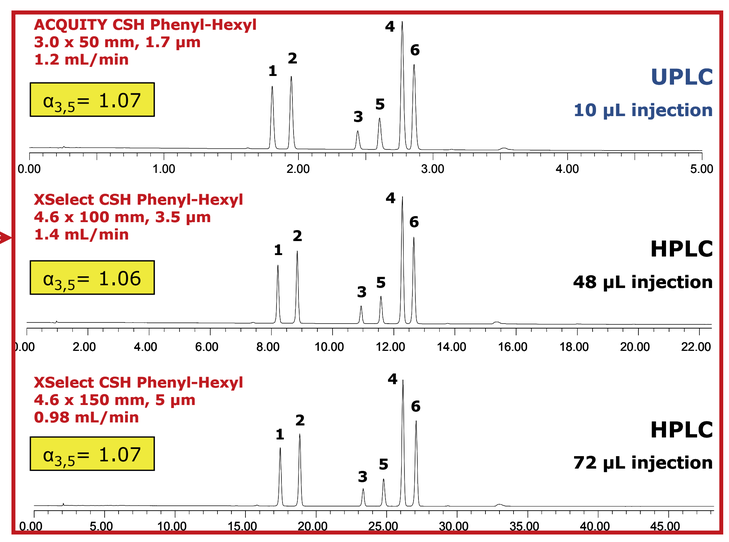


Figure 3. Analysis of non-steroidal anti-inflammatory drugs (NSAIDS) by UPLC and HPLC. Mobile phase A is water, mobile phase B is acetonitrile, and mobile phase C is 2% HCOOH in water. For UPLC, the gradient is from 25 to 80% B in 5 min with a constant 5% C. The gradient times for HPLC were scaled proportionally to maintain the same number of column volumes throughout the gradient ACQUITY UPLC H-Class system. UV detection at 270 nm. Column temperature is 30 °C.

PEAK SHAPE IN FORMIC ACID

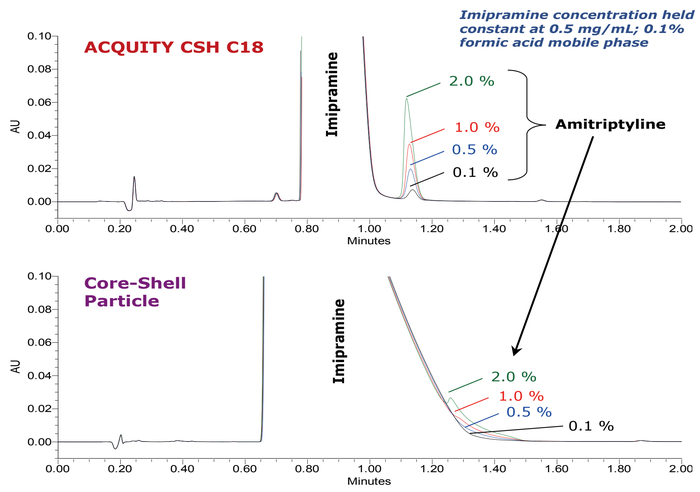


Figure 4. Trace-level analysis of amitriptyline on ACQUITY CSH and Core-Shell particles. Imipramine concentration held constant at 0.5 mg/mL. Mobile phase A is water, mobile phase B is acetonitrile, and mobile phase C is 2% HCOOH in water. Gradient is from 25 to 35% B in 2 min with a constant 2.5% C. ACQUITY UPLC H-Class system. UV detection at 254 nm. 5 µL injection, 40 °C. Flow rate is 0.6 mL/min. Column dimensions were 2.1 x 50 mm, 1.7 µm for both chromatograms.

RETENTION TIME DRIFT

Conventional high purity reversed-phase columns have low surface charges at low pH. Therefore, very small changes in surface charge may cause a large change in retention for ionized analytes. This effect is worsened by the use of low ionic strength mobile phases. The result is a change in the retention of ionized analytes due to exposure to mobile phases of different pH.[2-4]

The ability to maintain consistent selectivity and peak shape after exposure to different mobile phases is particularly important during method development. ACQUITY CSH and XSelect™ columns are resistant to changes in selectivity for ionized analytes after exposure to low ionic strength mobile phases with drastically different pH.

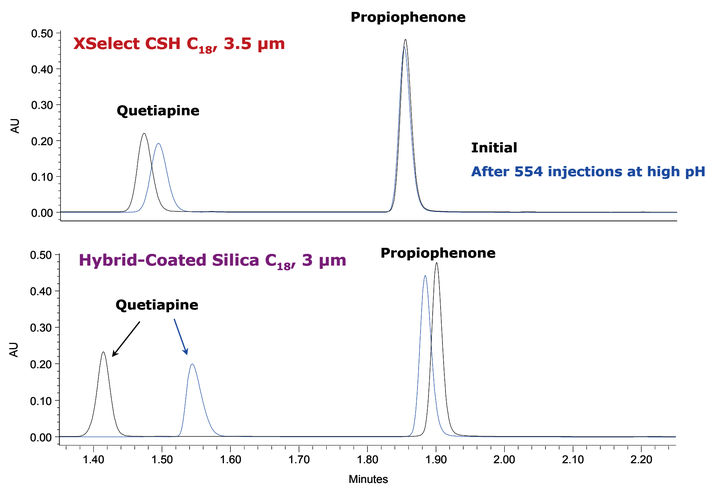


Figure 5. Comparison of XSelect CSH C_{18} and a hybrid-coated silica C_{18} particle in a formic acid mobile phase before and after exposure to high pH mobile phases. Low pH mobile phase 0.1% HCOOH. High pH mobile phase is 0.1% NH_4OH. Gradient is from 5 to 95% methanol in 2.5 min. ACQUITY UPLC system. UV detection at 254 nm. 5 µL injection, 30 °C. Flow rate is 0.6 mL/min. Column dimensions were 2.1 x 50 mm for both chromatograms.

QUALITY BY DESIGN (QbD) IN METHODS DEVELOPMENT

UPLC® Technology

- **Sub-2 µm columns combined with low dispersion instrumentation give higher resolution separations, faster**

ACQUITY CSH™ Columns

- **Wide range of selectivity**
- **Resistance to retention time drift in pH switching applications**
- **More robust separations in low ionic strength mobile phases (e.g., formic acid)**

Fusion AE™ Software

- **Applies DOE approach to method development using simple templates**
- **Facilitates data interpretation**
- **Automates sample and method set creation in Empower**

Case Study: Mirtazapine

The following case study uses the US Pharmacopeia resolution mixture for mirtazapine, which contains the main active ingredient and 7 related impurities at trace levels in the sample. The goal is to develop a robust method that adequately separates all trace impurities so that they can be accurately quantified. The method development process is automated using UPLC and Fusion AE software so that the optimum conditions are achieved without extensive experimentation. The ACQUITY UPLC HSS C_{18} SB column is added as the fourth column in the method development screening protocol due to its ability to retain extremely polar bases, as well as provide alternate selectivity to the three CSH columns.

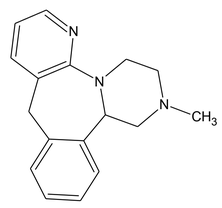
Screening Conditions

System: ACQUITY UPLC with column manager and PDA
(Empower™ 2; Fusion AE™)

Columns (2.1 x 50 mm)
1. ACQUITY CSH C_{18}, 1.7 µm
2. ACQUITY CSH Fluoro-Phenyl, 1.7 µm
3. ACQUITY CSH Phenyl-Hexyl, 1.7 µm
4. ACQUITY UPLC HSS C_{18} SB, 1.8 µm

Aqueous mobile phases
—A1: 0.1% HCOOH in water
—A2: 0.1% NH_4OH in water
Organic Solvents
—B1: Acetonitrile
—B2: Methanol

Flow rate: 0.5 mL/min
Column oven temperature: 30°C
Gradient: 5 – 95%B
UV detection @ 290 nm
Injection volume: 2 µL on 20 µL loop (PLNO mode)



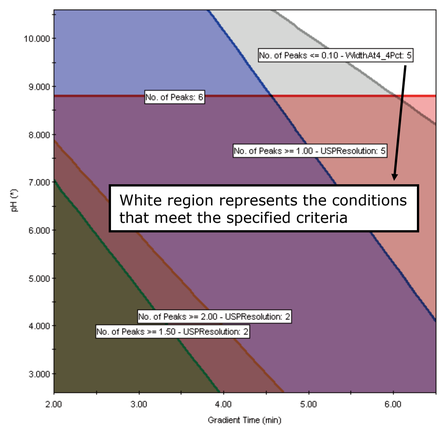
Mirtazapine (1 mg/mL)

$C_{17}H_{19}N_3$

Formula weight = 265.4

pKa = 7.1

Screening Results



Study Variable Data

Study Variable Name	Optimizer Answer Level Setting
Gradient Time	5.13
Organic Solvent Type	Acetonitrile
pH	10.600
Column Type	CSH HL C18

Column, pH, and elution solvent determined for optimization (see below)

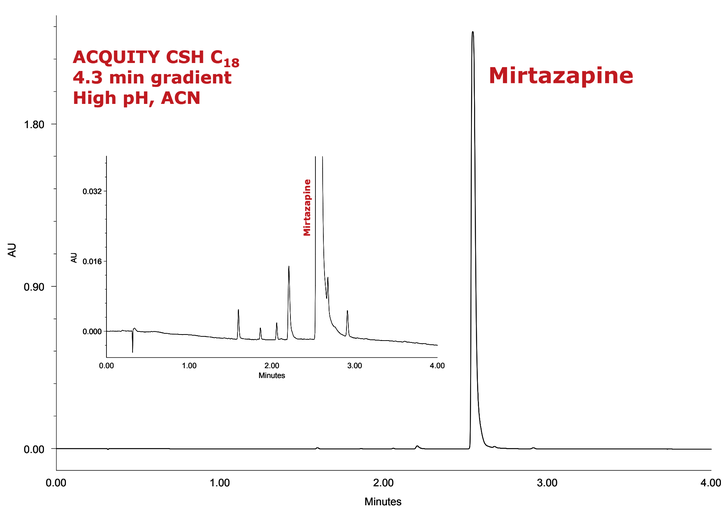


Figure 6. Analysis of mirtazapine resolution mixture (USP) using similar conditions to the optimized conditions predicted by Fusion AE software based on initial screening. The inset of the figure shows a zoomed in view of the trace-level impurities related to mirtazapine.

Method Optimization

Optimization Parameters

Flow rate (set window 0.2 –0.7 mL/min)
Gradient end point (set window 60 –95% acetonitrile)
Gradient time (set window 2 -6.5 minutes)
Column temperature (set window: 30 –45 °C)

Optimizer Answer #4: 5 of 35

Study Variable Data

Study Variable Name	Optimizer Answer Level Setting
Pump Flow Rate	0.700
Gradient Time	6.50
Final % Organic	77.98
Oven Temperature	45.0

Optimized Method

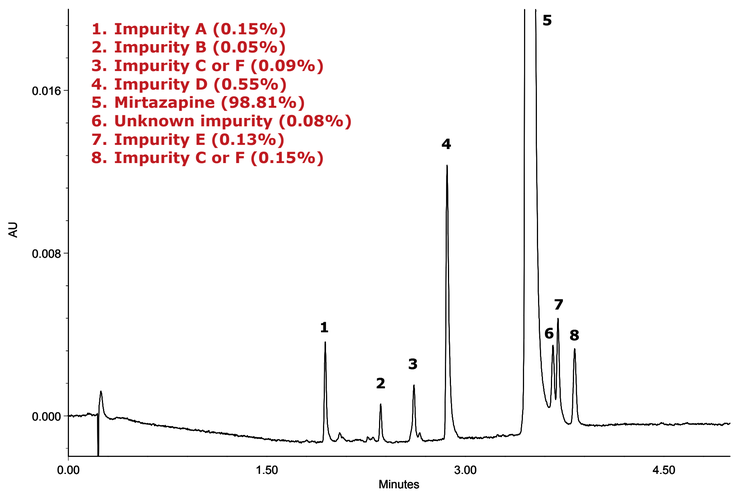


Figure 7. Optimized separation of mirtazapine resolution mixture (USP) based on the conditions predicted by Fusion AE software. Peak area percents for each component are listed in the figure. Impurities C and F have the same molecular formula, and thus cannot be differentiated without pure standards. These are not readily available through the USP.

CONCLUSIONS

- **ACQUITY CSH and XSelect columns provide a new generation of stationary phases for routine UPLC and HPLC separations.**
- **The selectivity differences between the three CSH column chemistries are large enough to cover a wide range of the separation space, even with low ionic strength mobile phases.**
- **XSelect and CSH columns give the same selectivity for a given separation independent of the particle size.**
- **The fixed positive charge on the CSH particle surface allows higher loading and better peak shape in formic acid, allowing better separation and quantitation of trace components.**
- **The resistance to pH switching drift makes ACQUITY CSH and XSelect columns ideal for routine method development.**
- **The combination of CSH columns, UPLC Technology, and Fusion AE method development/optimization software can be used to develop robust methods with minimal user intervention.**

References

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2. Marchand, D. H.; Williams, L. A.; Dolan, J. W.; Snyder, L. R. *J. Chromatogr. A* **2003**, *1015*, 53.
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