A REVIEW OF WATERS HYBRID PARTICLE TECHNOLOGY.

Part 3. Charged Surface Hybrid (CSH) Technology and Its Use in Liquid Chromatography

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Waters

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CURRENT STATE OF REVERSED-PHASE SEPARATIONS

Since its early beginnings, the science of chromatographic separations has seen a steady progression of technological advances.[1] Improvements in both chromatographic instruments and stationary phases have led to leaps in separation performance. One of the most significant recent advances in stationary phases was the introduction of hybrid organic/inorganic particles.[2-4] Hybrid-based packing materials offer an extended usable pH range compared to that of silica-based packing materials, as well as excellent peak shape and high efficiency. In addition to advances in stationary phase chemistry, the availability of columns containing sub-two micron particles has allowed for dramatic improvements in the speed, sensitivity and resolution of LC separations.[5] However, despite these advances, new challenges continue to emerge, particularly as the result of the increasing use of liquid chromatography/mass spectrometric (LC/MS) methods.

Unlike the mobile phases commonly used with optical detectors (e.g., phosphate buffers), a fundamental requirement for LC/MS is the use of volatile mobile phases. The preference today is to use additives such as formic acid, acetic acid, or ammonia instead of buffers like ammonium formate, ammonium acetate, or ammonium bicarbonate. Solutions of these additives have lower buffering capacity and lower ionic strength than traditional buffers. The desire for low-ionic-strength mobile phases is driven by the need for the higher MS sensitivity that is gained by the lack of charge competition for analytes of interest. Additives are also preferred in purification applications because they are easier to remove from the isolated product fraction.

However, careful examination of chromatographic data using these low-ionic-strength mobile phases has revealed unexpected behavior for charged analytes on high-purity packing materials. These include:

- Unexpectedly high tailing factors for analytical mass loads of basic analytes in low pH mobile phases due to mass overload[6]
- Slow equilibration at low pH[7a,b]
- Retention time shifts in low pH mobile phases after exposure to high pH (e.g., > pH 7) mobile phases[7b]

The development of charged surface hybrid (CSH™) materials was aimed at improving performance with acidic, low ionic strength mobile phases, while increasing the selectivity options available to method development scientists. The research behind this development has led to an in-depth understanding of the limitations of currently available reversed-phase columns. In designing chromatographic materials specifically for methods using MS-compatible mobile phases, the challenge is not only to maintain—but to improve upon—the attributes of reproducibility, maximized efficiency, increased selectivity, and extended pH stability. We have achieved these goals with CSH particle technology.

INTRODUCING CSH TECHNOLOGY

The foundation of CSH Technology is our patented BEH Technology™ (ethylene-bridged-hybrid) particle.[3] BEH particles have been prepared in a cGMP, ISO:9001 certified high-volume manufacturing facility since 2004. Using these optimized particles has allowed us to focus our attention on new surface-modification approaches. Recognizing that surface charge has a major impact on the behavior of ionized analytes,[8] we developed a new surface-modification process that allows the introduction of a reproducible, low-level positive surface charge in acidic mobile phases (see Figure 1). In basic mobile phases, ionization of silanol groups creates a negative surface charge. The optimization of the surface charge was a key consideration in the development of CSH Technology. The goal was to alleviate the above-mentioned problems encountered in acidic, low-ionic-strength mobile phases, while maintaining predominantly reversed-phase behavior.

The bonded ligands for the ACQUITY UPLC® CSH and XSelect™ column families were carefully chosen to produce excellent peak shape, high efficiency, complementary selectivities, and chemical stability. CSH C_{18}, Phenyl-Hexyl and Fluoro-Phenyl columns incorporate trifunctional bonding chemistries. CSH C_{18} and Phenyl-Hexyl columns are end capped using a proprietary process that ensures excellent peak shapes and chemical stability.

The CSH Fluoro-Phenyl chemistry is not end capped in order to maximize its unique selectivity. CSH columns are available in several different particle sizes, enabling seamless scalability. ACQUITY UPLC CSH columns (1.7 µm particle size) are optimized for ultra-performance liquid chromatography (UPLC®) separations. XSelect high performance liquid chromatography (HPLC) columns (3.5 and 5 µm particle sizes) are designed to offer outstanding performance for analytical and preparative separations.

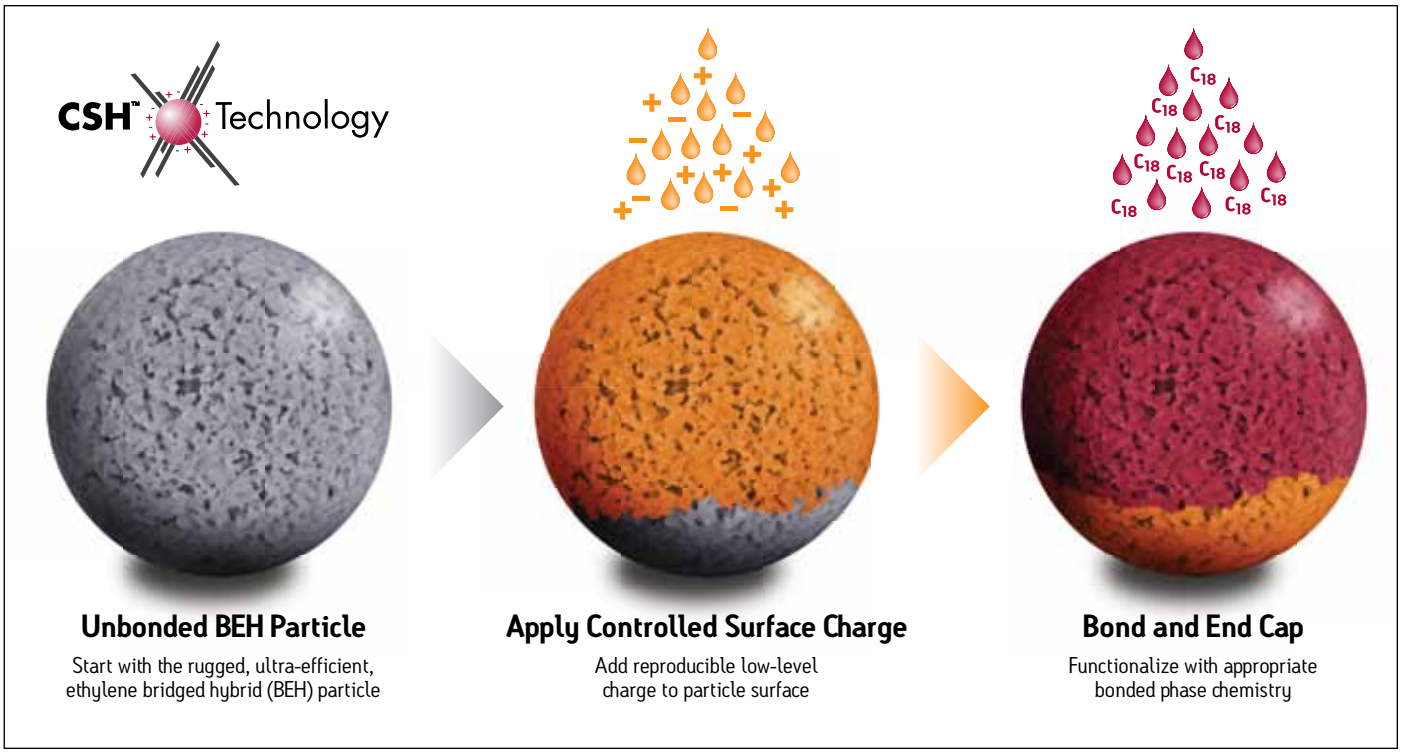

Figure 1: Schematic depiction of the CSH Technology process. Starting with an unbonded BEH particle (left), a small controlled charge is applied to the BEH particle surface (middle). The CSH particle is then bonded and sometimes end capped (right). The bonded material is then chromatographically tested (see Figure 3). CSH Technology is incorporated into the ACQUITY UPLC CSH and XSelect column families.

OPTIMIZING PERFORMANCE IN ACIDIC, LOW-IONIC-STRENGTH MOBILE PHASES

Tailing Factors and Loading Capacity for Bases

Poor peak shape and low loading capacity for basic analytes at low pH is an issue for many commercially available reversed-phase columns. While the cause of this problem is not completely understood, the conclusion of several studies is that variations in the surface charge of the chromatographic materials lead to the observed differences in chromatographic performance. Extensive knowledge of the overloading behavior for neutral analytes exists in the literature.[9] The mass loading capacity for charged analytes has long been recognized as being orders of magnitude lower than for neutral analytes. Loss of over 50% of the column efficiency is seen even at very low sample amounts. These extremely low loading capacities can differ by over an order of magnitude depending on the ionic strength of the mobile phase,[10] the use of ion-pairing reagents such as TFA,[11] the specific surface area of the packing,[12] or the particular column brand.[13] The impact of mass overload on peak shape under analytical load condition on XSelect CSH™ C_{18}, AMT HALO™ C_{18} and Gemini®-NX C_{18} columns is shown in Figure 2.

As seen in Figure 2, the HALO C_{18} and Gemini-NX C_{18} columns give poor peak shapes for the bases metoprolol (peak 1) and amitriptyline (peak 3). The base papaverine was included in the mix because it has a very high molar absorptivity; this allows its use at a lower concentration. Because of the lower sample concentration for papaverine, it does not appear to suffer from overload. The symmetrical peak shape observed for papaverine on all three columns confirms that the poor peak shape for the other bases is due to mass overloading. Such overloading limits the accuracy and sensitivity of MS analyses of basic compounds. The XSelect CSH C_{18} column (Figure 2 top) was designed to not suffer from this limitation. The peak capacities (P_c) are shown in Figure 2 to permit a quantitative comparison. Of these three stationary phases, only XSelect CSH C_{18} can take advantage of the efficiency gains from the use of < 5 µm packings when using formic acid mobile phases for basic analytes. The overloaded peak shapes for metoprolol and amitriptyline seen in Figure 2 would obscure any benefit from an increase in column efficiency arising from the use of smaller particles.

The effect of surface charge was studied on many prototype materials during the development of CSH Technology. Shown in Figure 3 are the results of experiments in which samples from the same batch

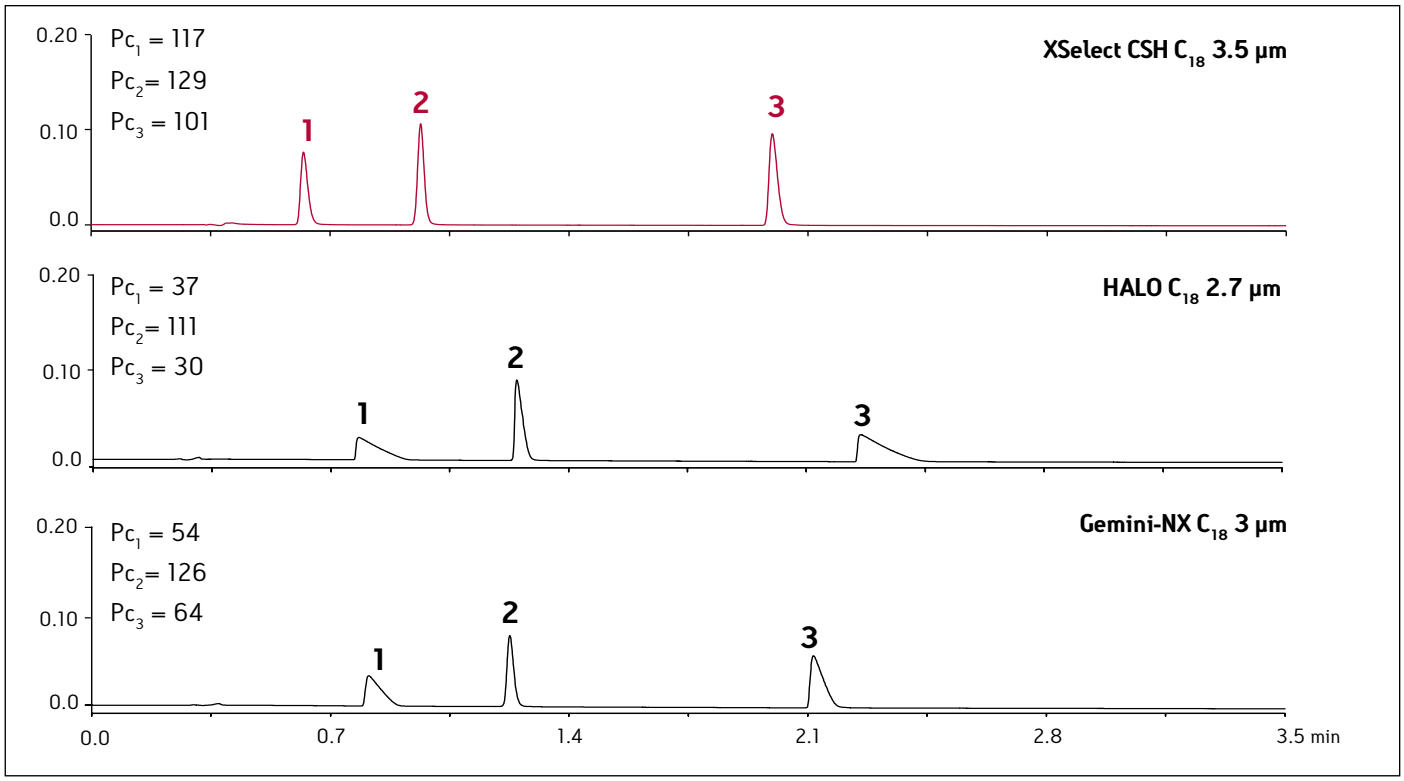

Figure 2: Comparison of peak shape and peak capacity (P_c) for bases on three 2.1 x 50 mm C_{18} columns. Gradient: A: 0.1% formic acid in water; B: acetonitrile; 15 – 65% B linear in 4.6 minutes. Temperature: 30 °C. Flow rate: 0.4 mL/min. Sample: 2 µL injection. Detection: 260 nm. Analytes: (1) metoprolol tartrate (200 ng/µL); (2) papaverine (10 ng/µL); (3) amitriptyline (50 ng/µL). System: ACQUITY UPLC.

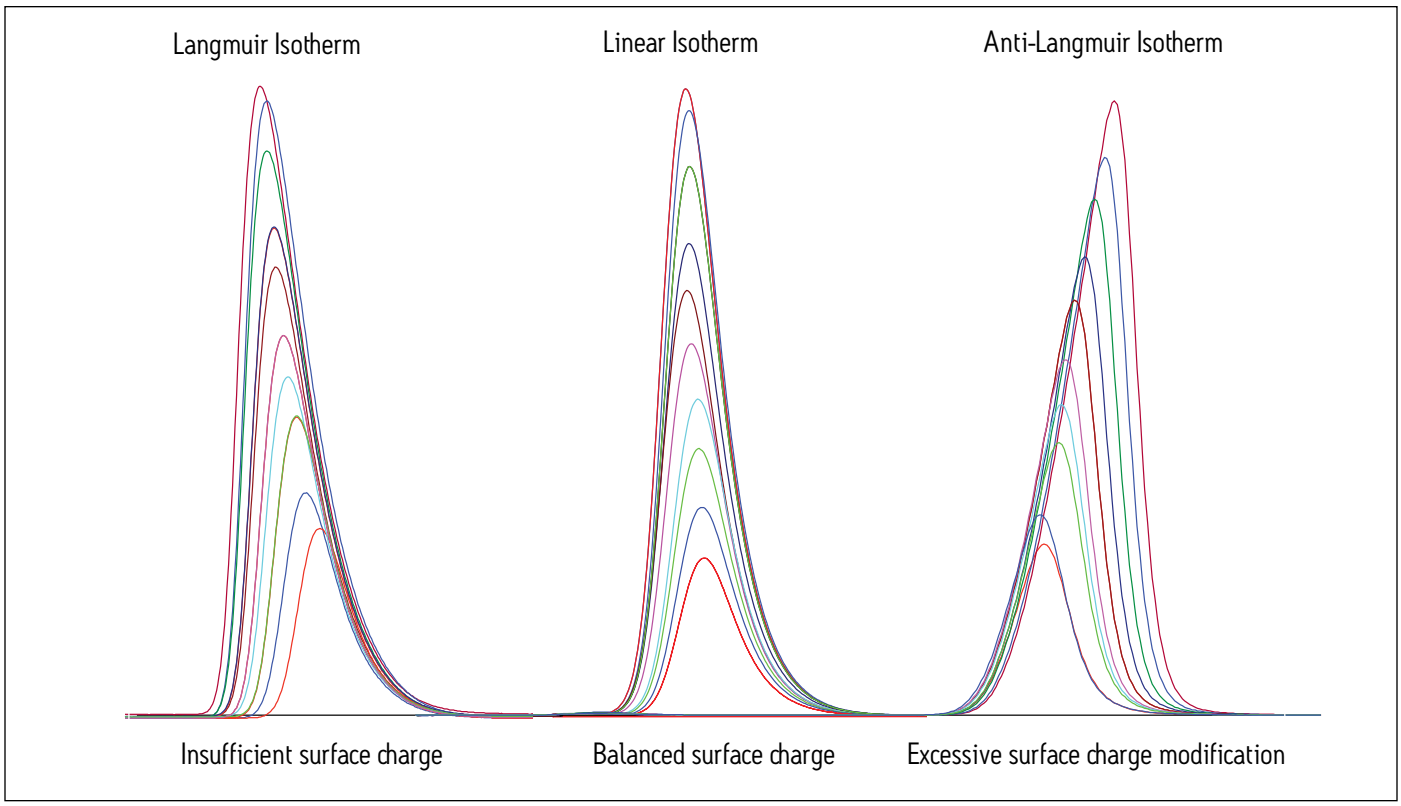

Figure 3: Comparison of isocratic loading behavior for amitriptyline on 4.6 x 150 mm columns containing three different CSH Technology research materials. Amitriptyline on-column load range: 0.3 – 1.2 µg. Injection volume: 20 µL. Mobile phase: 0.05% TFA in 40% acetonitrile. Flow rate: 1.0 mL/min. Temperature: 30 °C. Detection: 230 nm. System: Alliance® 2695.

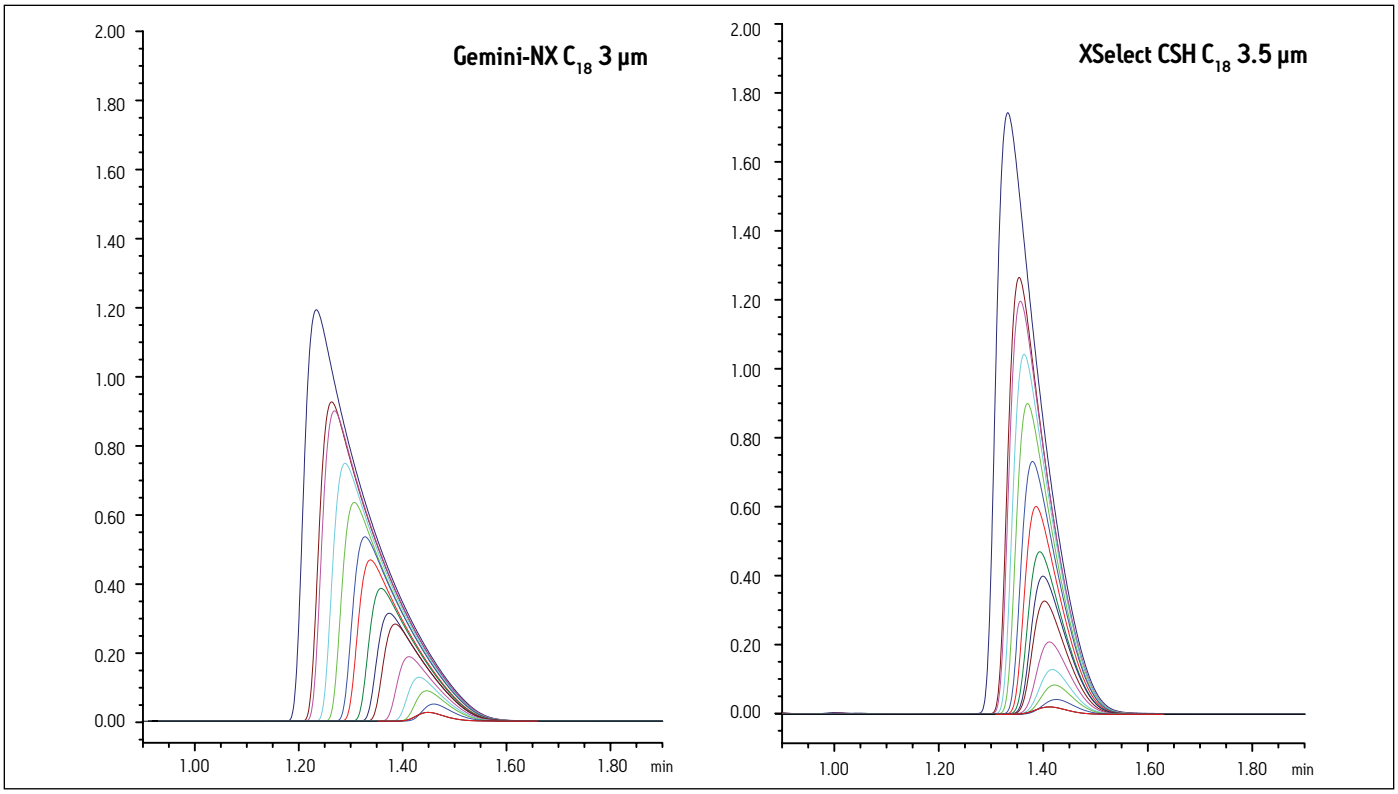

Figure 4: Comparison of isocratic loading behavior for amitriptyline on Gemini-NX C_{18} and XSelect CSH C_{18} columns (both 2.1 x 50 mm). Amitriptyline on-column load range: 0.05–6 µg. Injection volume: 1.5 µL. Mobile phase: 0.05% TFA in 39% (Gemini-NX C_{18}) or 37% (XSelect CSH C_{18}) acetonitrile. Flow rate: 0.2 mL/min. Temperature: 30 °C. Detection: 260 nm. System: ACQUITY UPLC.

of BEH particles had their surface modified with a positive charge at two progressively higher levels. The three materials were then bonded (C_{18}) and end capped. The non-charge modified material (shown at left) serves as a control. Figure 3 shows that the overloaded peak profiles at increasing sample concentrations for the three packings exhibit tailing/Bi-Langmuirian peak shape (with no charge modification), nearly symmetrical Gaussian/linear peak shape (with a small, controlled amount of charge), and fronting/Anti-Langmuirian peak shape (with a much larger amount of charge); these suggest convex, linear, and concave Langmuir isotherms, respectively.[9a] CSH Technology uses an optimized surface charge to give high efficiencies for loads that far exceed those attainable on ordinary reversed-phase columns.

The result of this optimization is shown in Figure 4. The retention factors for amitriptyline were matched on both 2.1 x 50 mm columns. The on-column loads ranged from 0.05 to 6 µg and were delivered in 1.5 µL injections using the mobile phase as the sample diluent. It is clear that the XSelect CSH C_{18} column maintains nearly linear-isotherm behavior for amitriptyline at mass loads that approach those used in purification applications.

Retention Time Changes after High pH Exposure

A second issue encountered with MS-compatible mobile phases involves changes in the retention of ionized analytes due to exposure to mobile phases of different pH.[7b,14] Although the exact mechanism that produces these changes is not known, it has been proposed that slow surface equilibration is to blame. Because conventional high-purity reversed-phase columns have much reduced surface charge at low pH, very small changes in surface charge may cause a large change in retention for ionized analytes. This effect is exacerbated by the use of low-ionic-strength mobile phases. The change in selectivity is not due to loss of bonded phase because the change is reversible, and no loss of retention is observed for neutral analytes. Storage and/or equilibration of columns in the low-pH mobile phase (allowing time for diffusion) will eventually return them to their original selectivity. This slow equilibration does not occur at elevated pH because of the relatively high concentration of deprotonated silanols.

The ability to maintain consistent selectivity and peak shape after exposure to different mobile phases is particularly important during method development. One approach to method development is to select a column and to acquire chromatograms using different organic solvents and different pH values to find the optimum separation

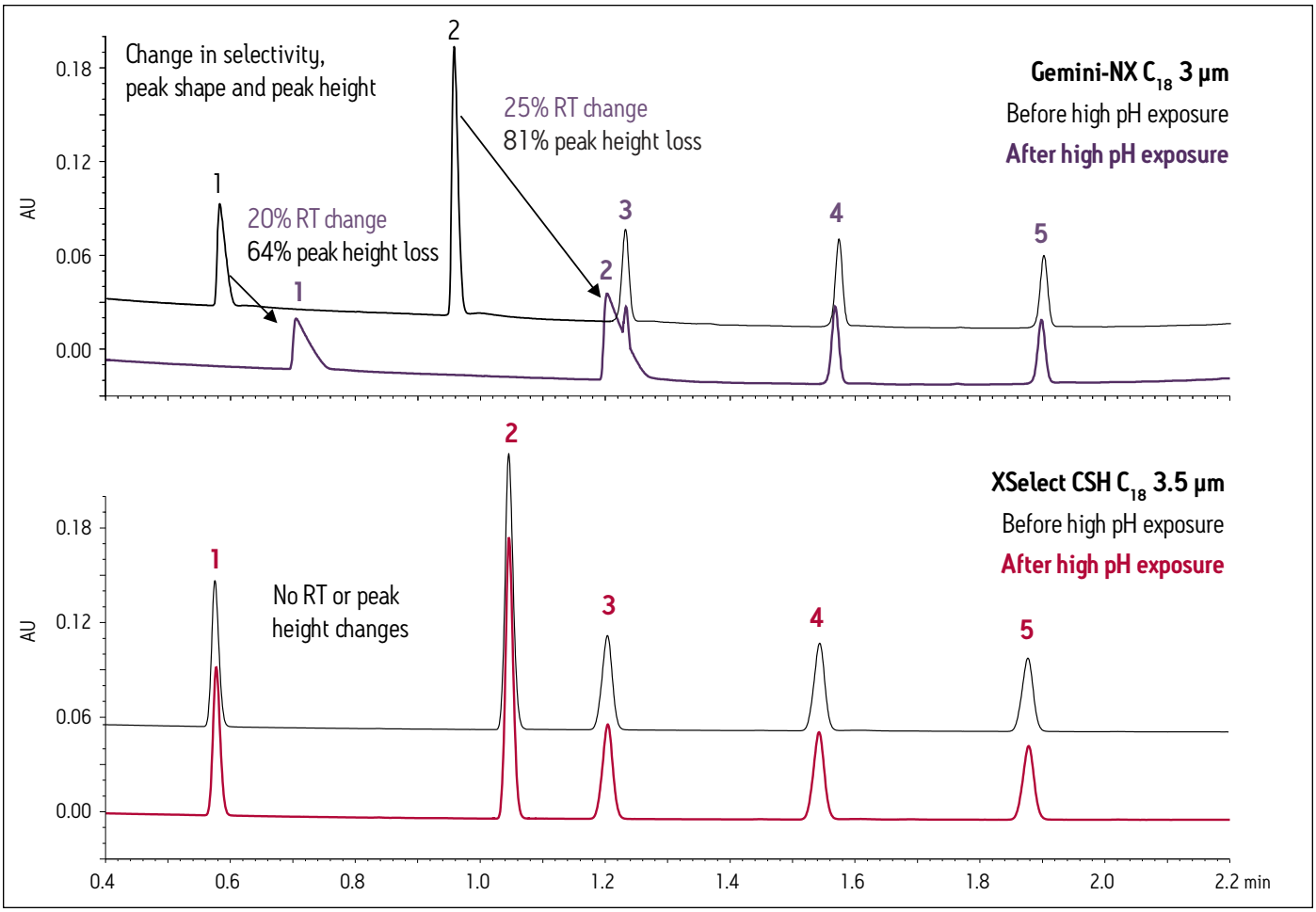

Figure 5: Separations on Gemini-NX C_{18} (top) and XSelect CSH C_{18} (bottom) columns (both 2.1 x 50 mm) before and after exposure to a pH 10 mobile phase. Gradient: A: 0.1% formic acid in water; B: acetonitrile; 5 to 95% B linear in 2.5 minutes. Temperature: 30 °C. Injection volume: 2 µL. Detection: 260 nm. Flow rate: 0.8 mL/min. Analytes: (1) metoprolol; (2) amitriptyline; (3) dimethylphthalate; (4) diethylphthalate; (5) dipropylphthalate. System: ACQUITY UPLC.

conditions. The exposure to multiple mobile phases changes the selectivity of the separation on many reversed-phase columns, resulting in a method that cannot be reproduced later on new, previously unused columns.

This inconsistent selectivity can also affect separations performed on open access systems utilizing both high and low pH separation methods. Substantial changes in retention for ionized analytes may result in confusion and/or the transfer of inaccurate information.

An illustration of the change in selectivity and peak shape that occurs in formic-acid gradients is shown in Figure 5 for a Gemini-NX C_{18} column in contrast to the result for an XSelect CSH C_{18} column. The two columns were tested using the same protocol. The chromatograms in Figure 5 were obtained before and after 7 cycles; each cycle included alternately 7 injections in a 0.1% formic acid/acetonitrile gradient followed by 17 injections in a 10 mM ammonium bicarbonate (pH 10)/acetonitrile gradient. Both acidic and pH 10 gradients ran from 5 to 95% acetonitrile in 2.5 minutes.

In Figure 5 the Gemini-NX C_{18} column shows a 20% change in retention and 64% loss in peak height for metoprolol and a 25% change in retention and 81% loss in peak height for amitriptyline after being exposed to pH 10 ammonium bicarbonate gradients. In addition, the peak shape for the bases has significantly degraded on the Gemini-NX C_{18} column. Loss of stationary phase or column efficiency is not the problem as confirmed by the relatively unchanged peak shape and retention for the three phthalates. On the XSelect CSH C_{18} column there were no significant changes in retention or peak shape for any of the five analytes. These data indicate that, unlike the Gemini-NX C_{18} column, the same XSelect CSH C_{18} column can be used in method development screens of high and low pH gradient conditions with the assurance that the method will provide the same chromatography as on an unused column.

REPRODUCIBILITY

The reproducibility of commercially available reversed-phase columns has been the subject of investigation by several research groups and column manufacturers. In comparisons of the reproducibility of neutral/base relative retentions using buffers at pH 2.7 and 7, high-purity reversed-phase columns showed greater variability in the low pH mobile phase.[15] The reproducibility for ionizable analytes is substantially worse when using low-ionic-strength mobile phases, such as those containing additives (e.g., formic acid) rather than buffers.

By controlling the surface charge under low pH conditions, CSH Technology provides more reproducible batch-to-batch selectivity. During QC testing of ACQUITY UPLC CSH and XSelect stationary phases, selectivity is monitored using both isocratic and gradient separations of a mixture of analytes using a pH 3 mobile phase. Shown in Figure 6 is an overlay of a gradient separation on nine different batches of ACQUITY UPLC CSH and XSelect CSH C_{18} packing materials. In this overlay, different particle sizes were also selected to demonstrate not only batch-to-batch reproducibility but also the scalability of the manufacturing process from 1.7 to 5 µm particle sizes.

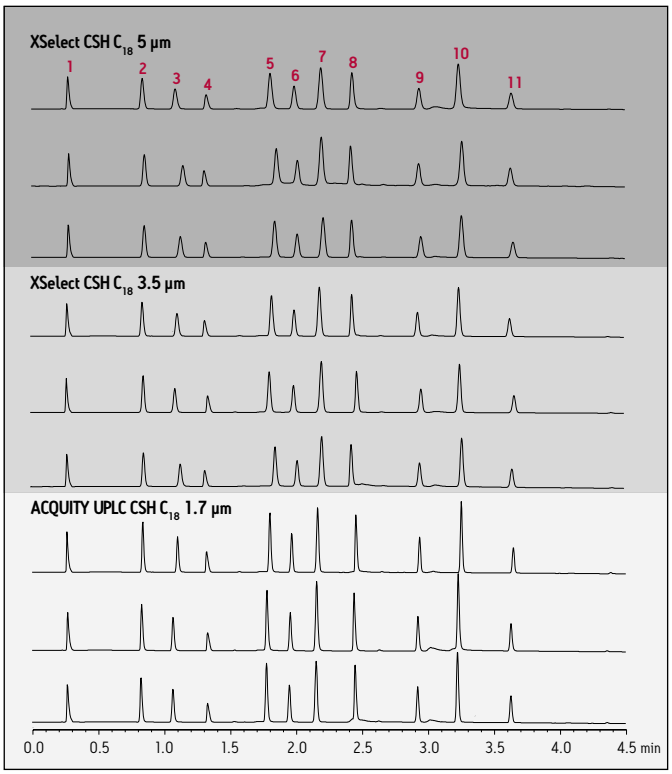

Figure 6: ACQUITY UPLC CSH and XSelect CSH C_{18} reproducibility and scalability. Gradient separations on 2.1 x 50 mm columns, containing nine different batches of CSH C_{18} material across three (1.7, 3.5, and 5 µm) particle sizes. Gradient: A: 15.4 mM ammonium formate, pH 3; B: acetonitrile; 5 to 90% B linear in 5 minutes. Temperature: 30 °C. Injection volume: 5 µL. Detection: 254 nm. Flow rate: 0.5 mL/min. Analytes: (1) thiourea; (2) resorcinol; (3) 2-nitrobenzoic acid; (4) metoprolol; (5) 2-chlorobenzoic acid; (6) 3-nitrophenol; (7) 2-nitrophenol; (8) amitriptyline; (9) diethylphthalate; (10) fenoprofen; (11) dipropylphthalate. System: ACQUITY UPLC.

ACID STABILITY

With their intended use in mind, the ACQUITY UPLC CSH and XSelect bonded-phase chemistries were carefully designed to ensure excellent acid stability. To demonstrate their stability, the three XSelect chemistries and three other recently introduced C_{18} columns were challenged using an accelerated 0.5% TFA stability test.[16] The loss of retention was monitored using methyl paraben. The challenge and test mobile phase, 0.5% TFA in water, was used at a flow rate of 1.4 mL/min on 2.1 x 50 mm columns. The columns were maintained at 60 °C throughout the testing. Methyl paraben was injected every 20 minutes for a total of 61 injections. After the 61st injection, all columns were checked for retention loss due to dewetting by purging them in 100% acetonitrile and re-equilibrating them in aqueous 0.5% TFA to confirm the retention for the 61st injection. There was no evidence of retention loss due to dewetting. As shown in Figure 7, the results indicate that the XSelect columns have much better stability than the other three C_{18} columns under these acidic test conditions. This is important since the ACQUITY UPLC CSH and XSelect family of columns is designed to provide superior performance in acidic mobile phases.

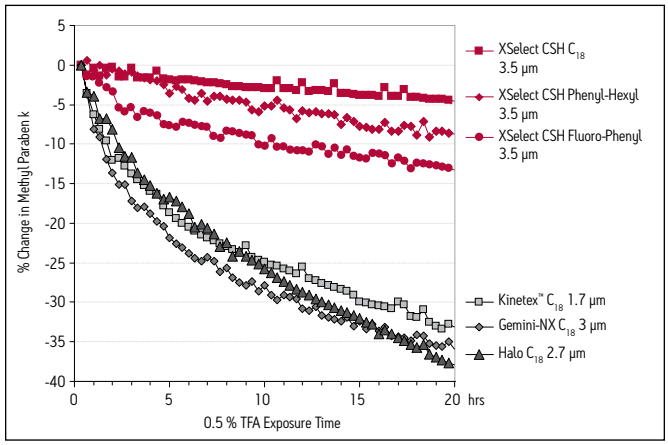

Figure 7: Results of accelerated acid stability testing for six columns, showing the percent change in the retention factor (k) of methyl paraben versus the time the columns were exposed to 0.5% TFA (pH ~ 1.3) at 60 °C.

BASE STABILITY

The recommended upper pH limit for most bonded-silica-based materials is pH 8. The generally accepted failure mechanism for bonded-silica-based materials tested up to pH 10 is base-catalyzed dissolution of the underlying silica particle.[17] The failure mode experienced by chromatographers under these conditions is an abrupt and catastrophic loss of column efficiency due to bed collapse. The association between the sudden loss of column efficiency and particle dissolution is supported by the discovery of a 1–10 mm-deep void upon visual inspection of the column inlet after testing.

Although efficiency loss is an appropriate measure of stability for silica-based packing materials, testing at pH > 10 and elevated temperature is required to evaluate the stability of hybrid packing materials. At pH > 10 on bonded hybrid packing materials, particle dissolution is no longer the most significant mode of failure—bonded-phase loss becomes the determining factor for stability.

An accelerated pH 12.3 test at 50 °C was used to optimize high-pH stability during the development of BEH Technology. The test involves a 1.8 hour thermal equilibration period for the column in the test mobile phase, acquisition of initial retention and efficiency data using the test mobile phase at 0.43 mL/min, purging the column in the challenge mobile phase (0.02 N NaOH in water) at 0.85 mL/min, washing for 10 minutes to remove the sodium hydroxide, then equilibration and testing in the test mobile phase. All columns were held at a constant 50 °C during the entire test protocol. The flow rates given above are for 3.0 mm i.d. columns and were scaled appropriately for other dimensions. The sequence of test-challenge-wash is repeated until the column fails or reaches 80 hours. Failure in this test is deemed as either > 50% loss of efficiency or > 50% loss of retention or both. Because the loss of efficiency is not governed solely by the kinetics of particle dissolution but also by the original mechanical stability of the column, it is not as reproducible as retention loss. The loss of bonded phase and retention portends loss of efficiency due to accelerated particle dissolution from increased surface exposure.

The homogeneous organosilane hybrid particles of the ACQUITY UPLC BEH and XBridge™ column families are the same particles used as a support for the ACQUITY UPLC CSH and XSelect column families, respectively. As shown in Figure 8, the XBridge packings exhibit the best high-pH stability of the tested columns. This is not surprising since the bonding strategies used for XBridge/ACQUITY UPLC BEH products were optimized for high-pH stability. However, these strategies limit selectivity differences available at low pH. The bonding strategies used for the ACQUITY UPLC CSH and XSelect column families were optimized for selectivity differences at low pH. This slightly reduces their high-pH stability relative to that of XBridge packings. Nevertheless, their high-pH stability greatly exceeds that of XTerra® MS C_{18} and is better than that of Gemini-NX C_{18}. The recommended upper pH limit for the CSH C_{18} and Phenyl-Hexyl columns is 11.

The columns in Figure 8 were on the system for different lengths of time, depending on their failure rate. Once removed from the system at the end of testing, the columns were opened, and the void depth at the inlet was measured. The voids in the XBridge and XSelect columns were less than 2 mm; the corresponding void depth for the Gemini-NX C_{18} column was 22 mm. The substantial difference between the size of the void in the Gemini-NX column and that of an XSelect or XBridge column is most likely due to the difference between a hybrid-coated silica particle (Gemini-NX) and a homogeneously polymerized hybrid particle (XSelect and XBridge). Once the coating of the hybrid-coated silica particle is penetrated, the underlying silica is rapidly dissolved.

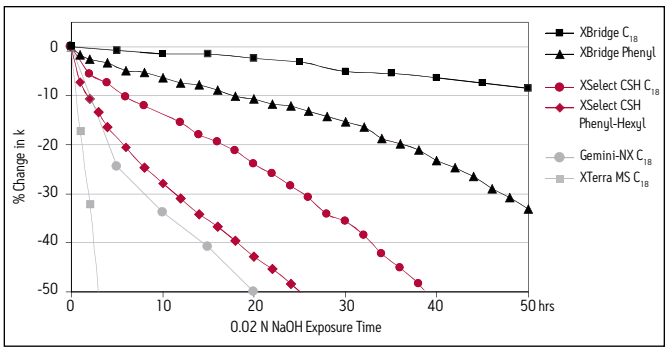

Figure 8: Results of accelerated base stability testing for six columns, showing the percent change in retention factor (k) versus the time the columns were exposed to 0.02 N NaOH (pH 12.3) at 50 °C. Test analyte and mobile phase: butylparaben in methanol: 0.1% formic acid in water 30:70 (v/v); or decanophenone in acetonitrile:water 50:50 (v/v). Challenge mobile phase: 0.02 N NaOH in water. Gemini-NX C_{18} and XBridge C_{18} tested in 4.6 x 50 mm column format, XTerra MS C_{18} tested in 3.0 x 50 mm column format, all other columns were tested in a 3.0 x 30 mm column format.

The ACQUITY UPLC CSH and XSelect CSH Fluoro-Phenyl chemistry was not included in the columns tested at pH 12.3. This is because the CSH Technology Fluoro-Phenyl-bonded hybrid was intentionally not end capped in order to preserve its unique selectivity in low-pH mobile phases. The benefits of end capping for high pH stability have been reported in the literature[18] and confirmed in our research laboratory. The recommended upper pH limit of the CSH Fluoro-Phenyl chemistry is 8.

SELECTIVITY

As sample complexity increases, chromatographic separations require higher resolution. In spite of improvements in resolution through the use of sub-2-micron packing materials, sometimes analytes are still not adequately resolved from each other or from other matrix components. The current separation selectivity space covered by reversed-phase columns leaves a substantial amount of room for the development of novel packing materials. Offering a family of chemistries that provide substantial differences in selectivity was one of the design considerations for CSH columns.

Thirty years ago, Horváth et al. studied selectivity differences through the use of kappa-kappa plots. These described the energetics of retention between column pairs as homoenergetic or heteroenergetic.[19] More recently, tests based on linear solvation-energy relationships, such as the Snyder-Dolan (S-D) hydrophobic subtraction approach,[20] have been used to select similar columns or those with very different selectivity. A simple way to quantify selectivity differences, very similar to Horvath's kappa-kappa plots, is to plot the retention factors for analytes on column pairs. A linear regression gives a correlation coefficient that is close to 1 for columns that exhibit essentially the same selectivity and close to 0 for columns that exhibit orthogonal selectivity. Shown in Figure 9 are retention data plotted for the columns that make up the XBridge family of reversed-phase packings (bottom) and the new XSelect family of columns (top) against the selectivity offered by XBridge C_{18}. The more scatter around the regression line, the larger the selectivity differences. A measure of the selectivity differences that has been previously described[21] uses the square of the correlation coefficient (R^2) to calculate the selectivity distance, S, between column pairs.

$$\textit{Selectivity (S)} = 100 \times \sqrt{1-R^2}$$

Under these mobile phase conditions, the S-value provided by the XSelect family versus XBridge C_{18} is 49, which is almost two times greater than that obtained for the XBridge family (S = 16).

In the example shown in Figure 9, a single linear regression was performed using the retention data for all reversed-phase column chemistries in each family. A more detailed approach is to calculate S-values for the individual column pairs to generate individual selectivity distances between each pair. These distances can then be joined together to create multidimensional forms that provide a visual representation of the selectivity space covered by various groupings or families of columns. Examples are shown in Figure 10 for the XBridge and XSelect HPLC column families. It is clear through comparison that the space covered by the XSelect family is substantially greater than that covered by the XBridge family.

One of the challenges in designing stationary phases with large selectivity differences is maintaining acceptable batch-to-batch reproducibility. Many commercially available columns with unique selectivities suffer from significant variability. Avoiding this problem was a key consideration in the design of the ACQUITY UPLC CSH and XSelect phases. Shown in Figure 11 is an overlay of gradient separations on columns containing nine different batches of XSelect CSH Fluoro-Phenyl materials. As with the results shown earlier for the ACQUITY UPLC CSH and XSelect CSH C_{18} chemistry, these batches include three different particle sizes: 1.7, 3.5, and 5 µm. The overlay demonstrates that, even across different particle sizes, the same selectivity is maintained for the phase that shows the most unique selectivity (compared to corresponding ACQUITY UPLC CSH C_{18} and XSelect CSH C_{18}, respectively).

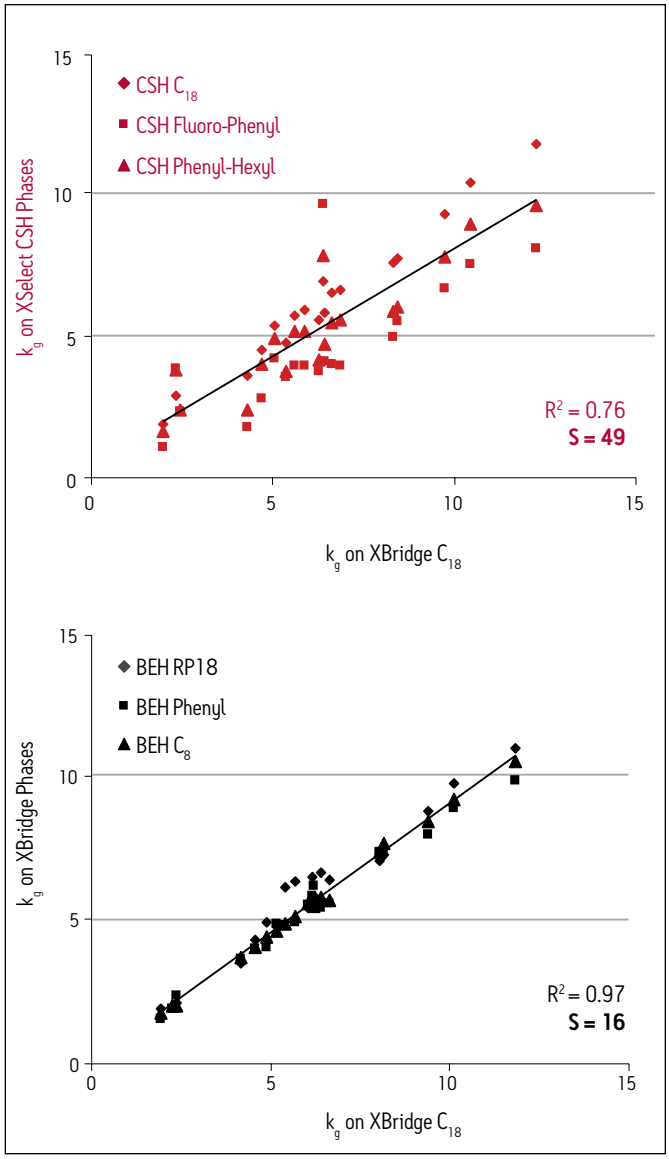

Figure 9: Plots of gradient retention factors (k_g) on XSelect (top) or XBridge (bottom) columns versus gradient retention factors on an XBridge C_{18} column. Conditions as in Figure 6, except using the following analytes: 2-nitrobenzoic acid; 2-chlorobenzoic acid; pyrenesulfonic acid; fenoprofen; metoprolol; papaverine; propranolol; amitriptyline; berberine; resorcinol; 2-nitrobenzyl alcohol; 2-chlorophenol; fluoxetine; caffeine; diethylphthalate; dipropylphthalate; 2-nitrophenol; 3-nitrophenol; 4-nitrophenol; and thiourea as void marker. The more scatter around the regression line, the larger the selectivity differences.

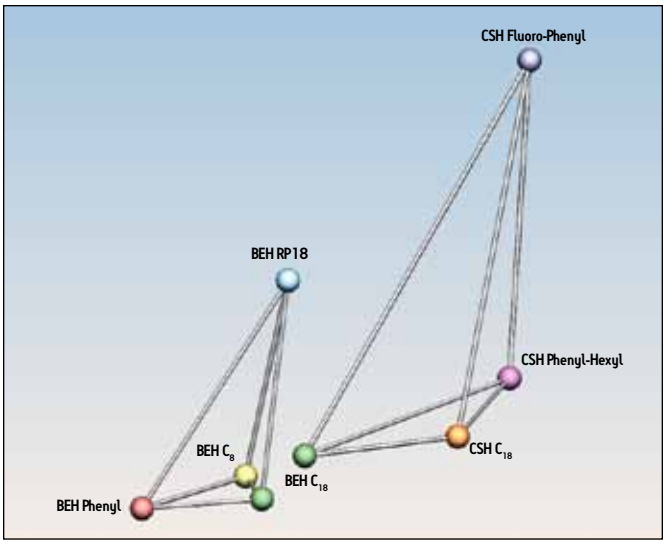

Figure 10: Representations of the selectivity differences between chemistries in the XBridge and XSelect HPLC column families. The analytes and mobile phase are the same as given in Figure 9 except that berberine and pyrenesulfonic acid were excluded from the calculations. The distances between vertices represent the S values between each pair of chemistries. Greater distances between the points indicate larger selectivity differences.

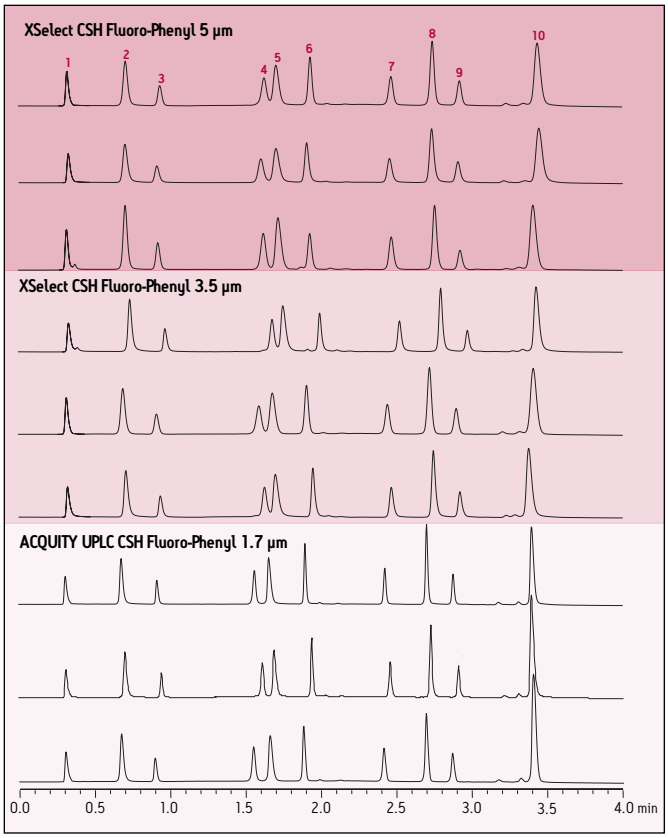

Figure 11: ACQUITY UPLC CSH Fluoro-Phenyl (1.7 µm) and XSelect CSH Fluoro-Phenyl (3.5 and 5 µm) reproducibility and scalability. Gradient separations on 2.1 x 50 mm columns containing nine different batches of CSH Technology Fluoro-Phenyl representing three (1.7, 3.5, and 5 µm) paricle sizes. Gradient: A: 15.4 mM ammonium formate, pH 3; B: acetonitrile; 5 to 90% B linear in 5 minutes. Temperature: 30 °C. Injection volume: 5 µL. Detection: 254 nm. Flow rate: 0.5 mL/min. Analytes: (1) thiourea; (2) resorcinol; (3) metoprolol; (4) 3-nitrophenol; (5) 2-chlorobenzoic acid; (6) amitriptyline; (7) diethylphthalate; (8) fenoprofen; (9) dipropylphthalate; (10) pyrenesulfonic acid. System: ACQUITY UPLC.

CONCLUSIONS

CSH Technology addresses the key issues encountered when reversed-phase columns are used with acidic, low-ionic-strength (MS-compatible) mobile phases. Problems with low efficiency and tailing peaks for basic analytes are greatly reduced. In addition, slow equilibration effects observed when the mobile-phase pH is changed are alleviated. ACQUITY UPLC CSH and XSelect columns, incorporating CSH Technology, offer a new level of performance for acidic MS-compatible mobile phases. With outstanding batch-to-batch reproducibility, extended pH stability, and a wide range of selectivities, these columns offer a robust platform for chromatographic method development. With particle sizes ranging from 1.7 to 5 µm, separations using these new columns may be seamlessly scaled between UPLC and analytical and/or preparative HPLC separations.

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