

Unique C18 Packings for the Retention and Separation of Highly Polar Compounds

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Summary

The selectivity of Grace® VisionHT™ column phases is discussed in relation to that of more traditional C18 phases. The unique surface structure and bonding technology has assured that the phase has kept pace with more recently developed materials to retain its position as a key alternative to classic C18 phases in the market today.

Introduction

Although RP HPLC has been a routine analytical technique since the early 1970s, developments in packing material technology are still advancing. Material development, column hardware technology, instrument design and market needs have collectively contributed to significant advancements in HPLC performance.

The first generation type A silica materials were very useful and their shortcomings with bases, for instance, were overcome by the addition of mobile phase additives to suppress acidic silanol activity. This led to the development of base deactivated type A silicas and then inert silicas, often made from organic sols. These were characterized by a low level of acidic silanols and a very low level of trace metals. The metal content of type A silicas was a characteristic of the particular brand and often indicated the production method and raw materials. Thus, material could have for example, 150ppm calcium or <10, 2000ppm sodium or <20, several hundred ppm iron or aluminium or <10 depending on the brand. Aluminium added stability at high mobile phase pH but gave very poor chromatography with chelating compounds. Bonding technology was generally geared to producing phases with a high coverage and a high degree of end-capping to give a minimal level of free, accessible silanols. Over the last 10 years, polar pharmaceutical compounds have come to the forefront and can pose severe problems for classic C18 materials best suited to the separation of non- to moderately polar compounds. Shorter chain RP phases can be used but very short chain materials (<C4) suffer stability problems at low and high pH (<pH2 and >pH8). Polar embedded phases (such as carbamate and amide) and AQ phases have been introduced which give improved polar retention and are stable in 100% aqueous mobile phases. Hybrid phases and bidentate phases have also been introduced for general RP applications.

Grace takes a novel approach. Base-deactivated phases and high coverage inert materials typically give good chromatography with most analytes, including bases, but also give similar selectivity. The new Grace® VisionHT™ column phases, C18 Classic and C18 Polar versions, give markedly different selectivity with polar analytes and therefore can give orthogonal selectivity to a standard high coverage, end-capped C18 or C8. In addition, because of the pure nature of the surface and the uniform covering of inert vicinal silanols, peak shape with chelators and bases is generally very good (although highly polar bases can produce some degree of tailing).

Results and Discussion

The C18 Polar benefits of Grace® VisionHT™ columns have been discussed previously^{1,2}. By examining data on the chromatography of substituted benzene derivatives, the C18 Polar effect of various substituents can be determined relative to methyl benzene (toluene), e.g., chlorobenzene would have 33% greater retention than expected, phenol 85% and benzylamine 837% when compared to toluene on a high coverage phase bonded on the same base silica. The Grace® VisionHT™ Classic column phase has equivalent values of 7%, 42% and 154%.

An example of the improvement in resolution possible from the C18 Polar effect is seen in Figure A¹, a pharmaceutical test mix run at pH 7.

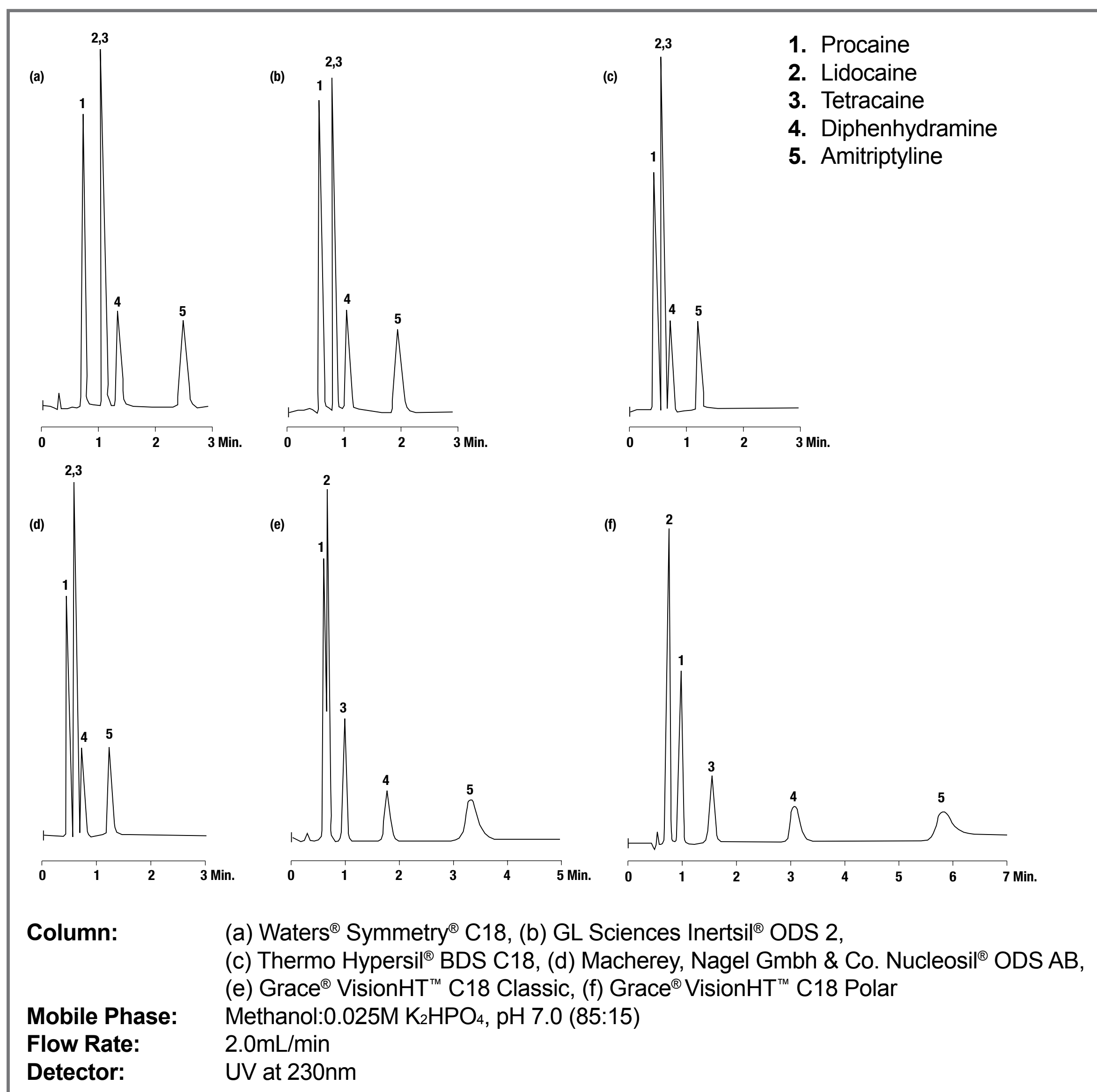


Figure A

The Grace® VisionHT™ C18 Classic column chromatogram shows a marked increase in retention for the bases compared to the more conventional C18 columns. Selectivity is starting to change. The Grace® VisionHT™ C18 C18 Polar column chromatogram shows a further increase in retention and change in selectivity. The difficult pair of lidocaine and tetracaine are now fully resolved away from the solvent front and with good peak shape. Note the movement of procaine and tetracaine. Also note that it is not necessary to fully end-cap a phase to obtain good peak shape. In fact, with an inert, fully hydrated silica, non-end capped phases often give better peak shape due to absence of lone silanols and the presence of hydrogen bonded vicinal silanol groups.

A further example differentiating the Grace® VisionHT™ C18 Classic column separation from the Grace® VisionHT™ C18 Polar column separation, which is due solely to the C18 Polar effect, is shown in Figure B¹. Here the base silicas and bonding technology are identical with just a bonding methodology difference producing the polar effect. The pindolol in particular shows marked increase in retention due to the interaction between the very basic isopropylamine, the arylamine and the aliphatic hydroxyl groups with the surface silanols.

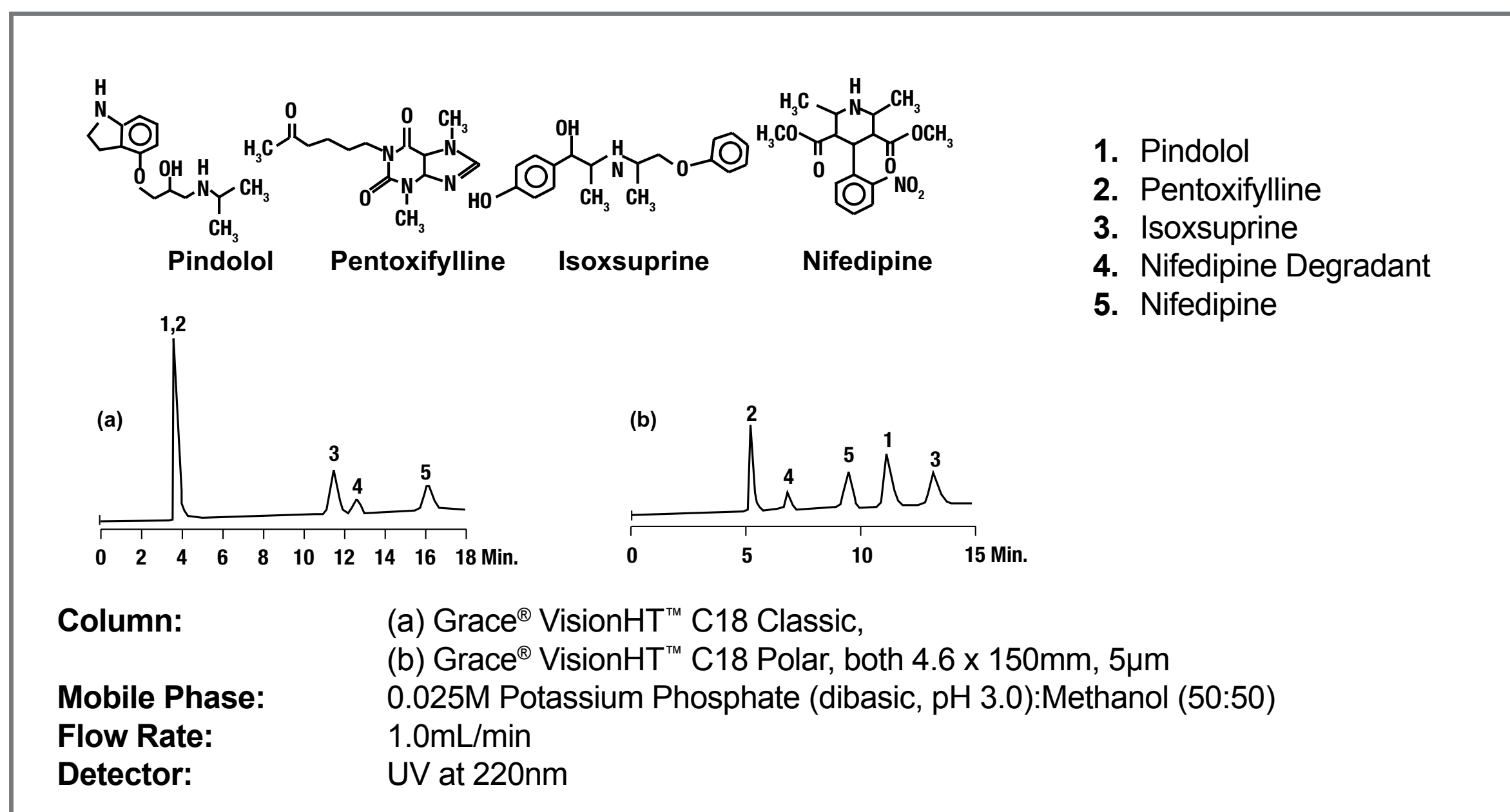


Figure B

Some of the Grace® VisionHT™ column phases are characterized by their low surface coverage. The Grace® VisionHT™ C18 column phase has a carbon level of 6% from a 210m²/gm silica and the C18 Polar a carbon level of 5%. This compares approximately to 10% for a fully bonded and end-capped phase with a similar surface area. A typical type B silica with a surface area of 300m²/gm would have a carbon level of 17%. This reduced hydrophobicity leads to a reduced reversed-phase mechanism retention and shorter elution times for a given mobile phase for non-polar compounds. In the case of moderately polar compounds, the reduced non-polar retention but increased polar retention on the residual silanols could balance each other out, but for polar molecules the lower non-polar retention would be more than compensated for by the enhanced polar retention. This is illustrated in Figure C² of carboxylic acids of varying polarity. The Grace® VisionHT™ C18 Polar phase gives good peak shapes and baseline resolution, the latter having a slightly shorter retention time of less than 5 minutes. The fully bonded phase of similar surface area gives good shaped peaks and baseline resolution but has a run time of 13 minutes and the assay may benefit from the use of gradient elution. Thus, the Grace® VisionHT™ C18 Classic and C18 Polar columns give much reduced run times which will increase throughput.

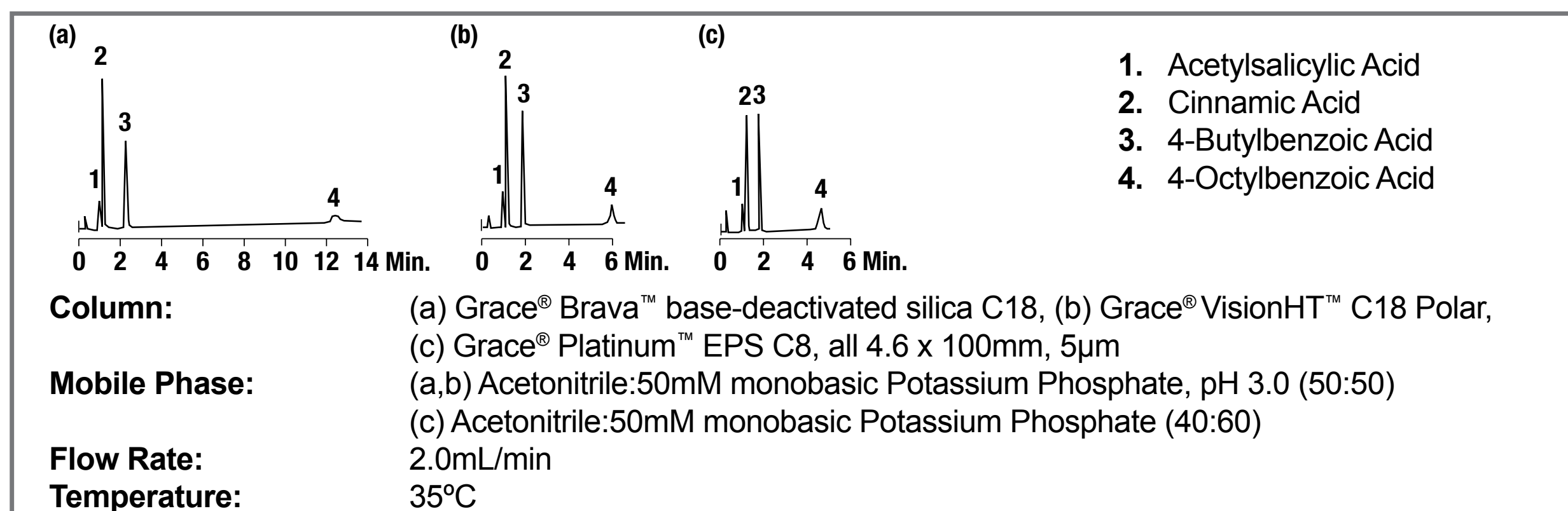


Figure C

In Figure D², separation of anticonvulsants, the classic C18 Grace® VisionHT™ column phase gives a better chromatogram by controlling the movement of the lamotrigine (2) and carbamazepine (4) peaks. In the latter case, resolution is maintained but peak order is changed. In the former the peak is not fully resolved from phenobarbitone (3). Dropping the level of organic modifier to pull peaks (2) and (3) apart is possible but would lead to a longer overall run time for the separation.

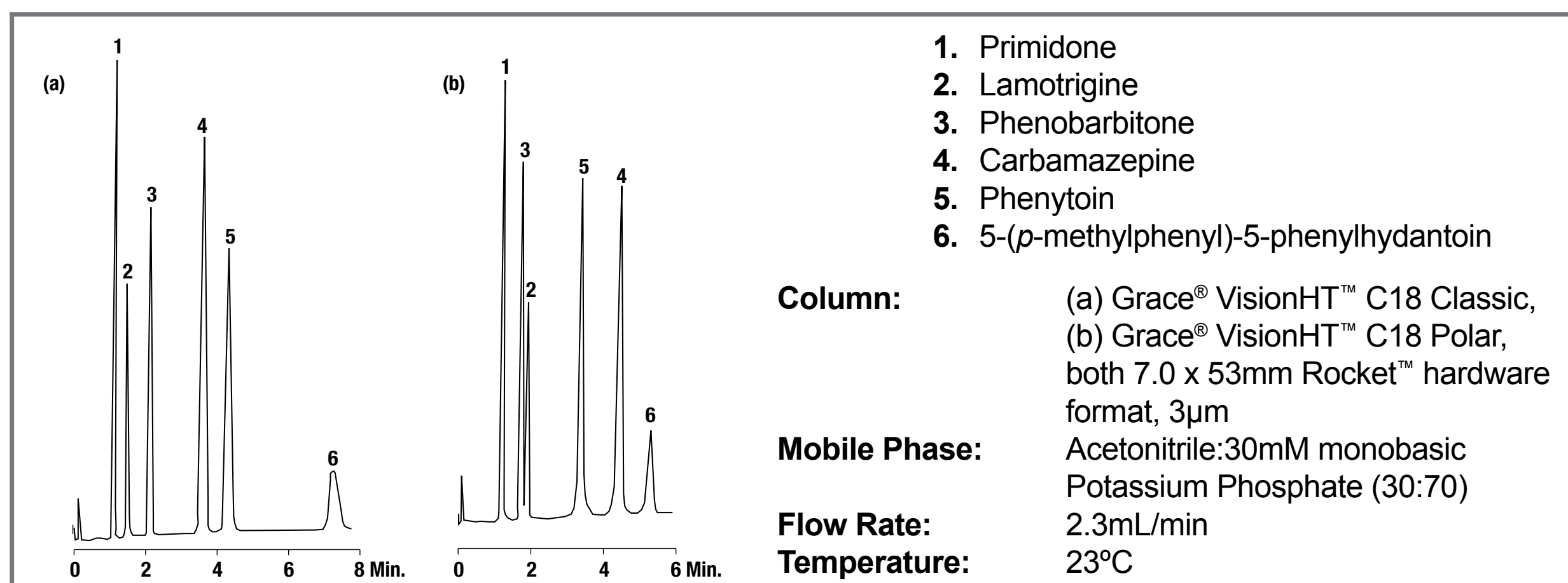


Figure D

The Grace® VisionHT™ column phases thus rely on 2 main separation mechanisms generated by the bonded alkyl groups and the residual silanols. An increase in the coverage of alkyl groups would lead to a proportionate reduction in the level of silanols. This could lead to reproducibility issues. However, due the bonding technology employed, the phases are very reproducible and RSDs for production are in the region of 2 – 3.4%. Ion exchange effects can be reduced by the use of buffers of the correct pH and molarity. Both volatile and non-volatile buffers in the range 1 to 10 may be used at typical levels of 25 – 50mmol.

Reproducibility is shown graphically in Figure E, with reproducibility for acids, bases and neutrals.

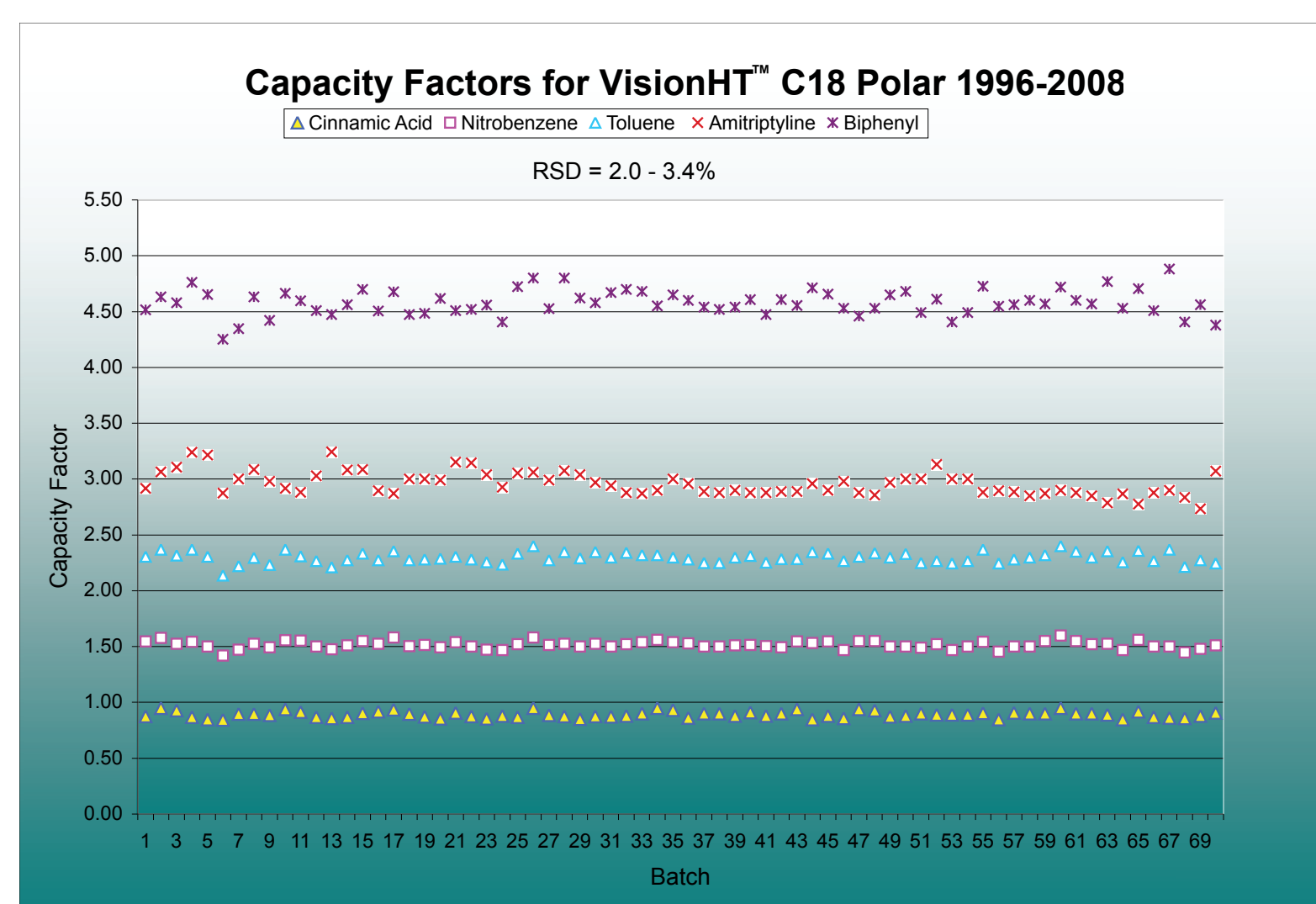


Figure E

Because of the reproducibility of the bonding, development of Grace® VisionHT™ C18 Polar column phases with surface coverages of 0.25, 0.5, 1.0 (C18 Polar), 1.5 and 2.0 $\mu\text{mole}/\text{m}^2$ were prepared and compared. Figure F shows the retention factors for a series of analytes for the 5 phases and Figure G the respective chromatograms. It can be seen that the non-polar molecules, biphenyl and toluene show the expected linear relationship based on carbon level while the more polar compounds show a plateau at higher coverage. This leads to pronounced changes in the polar/non-polar selectivity between polars and non-polar compounds. Note the relative movement of amitriptyline.

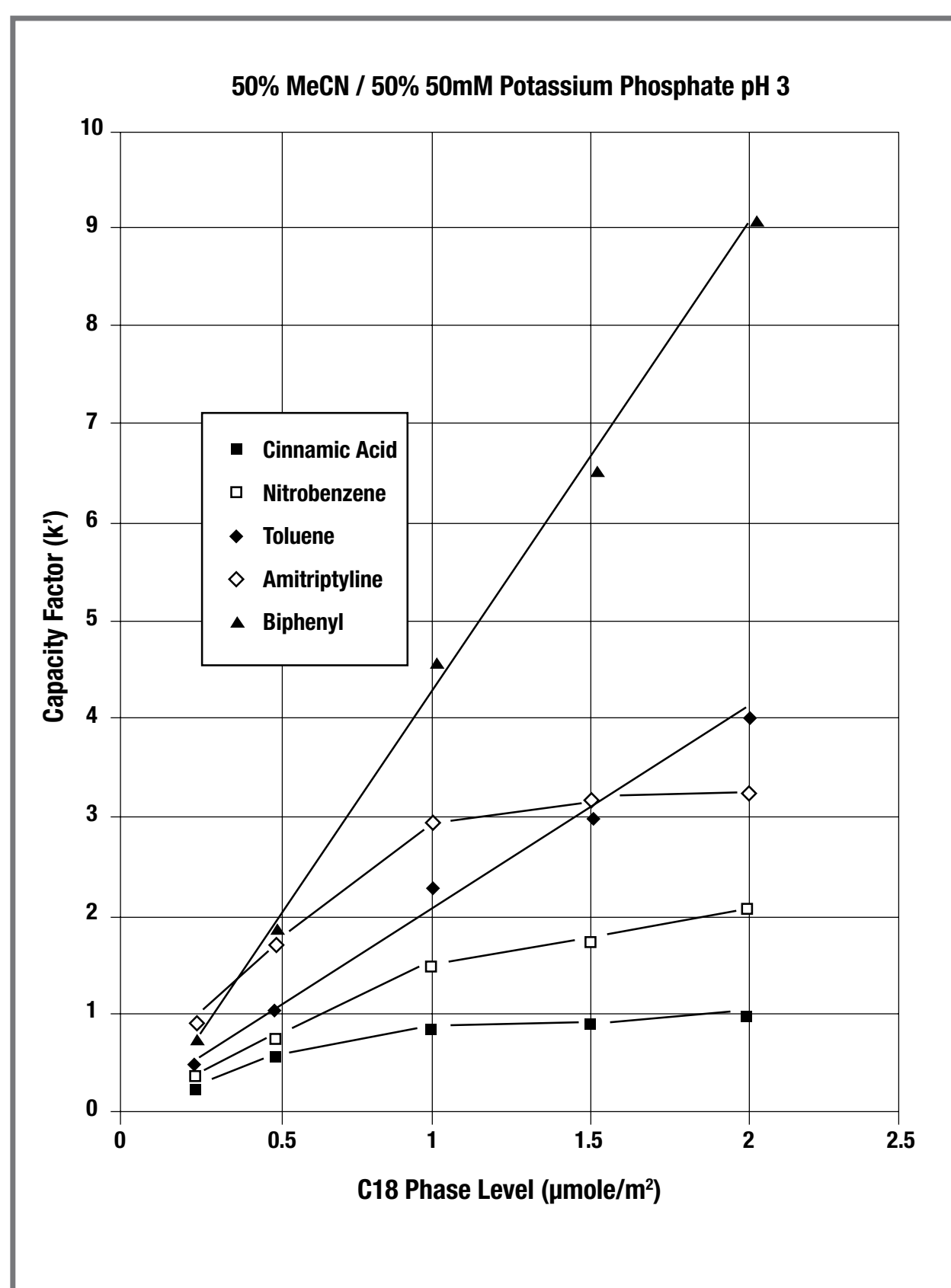


Figure F

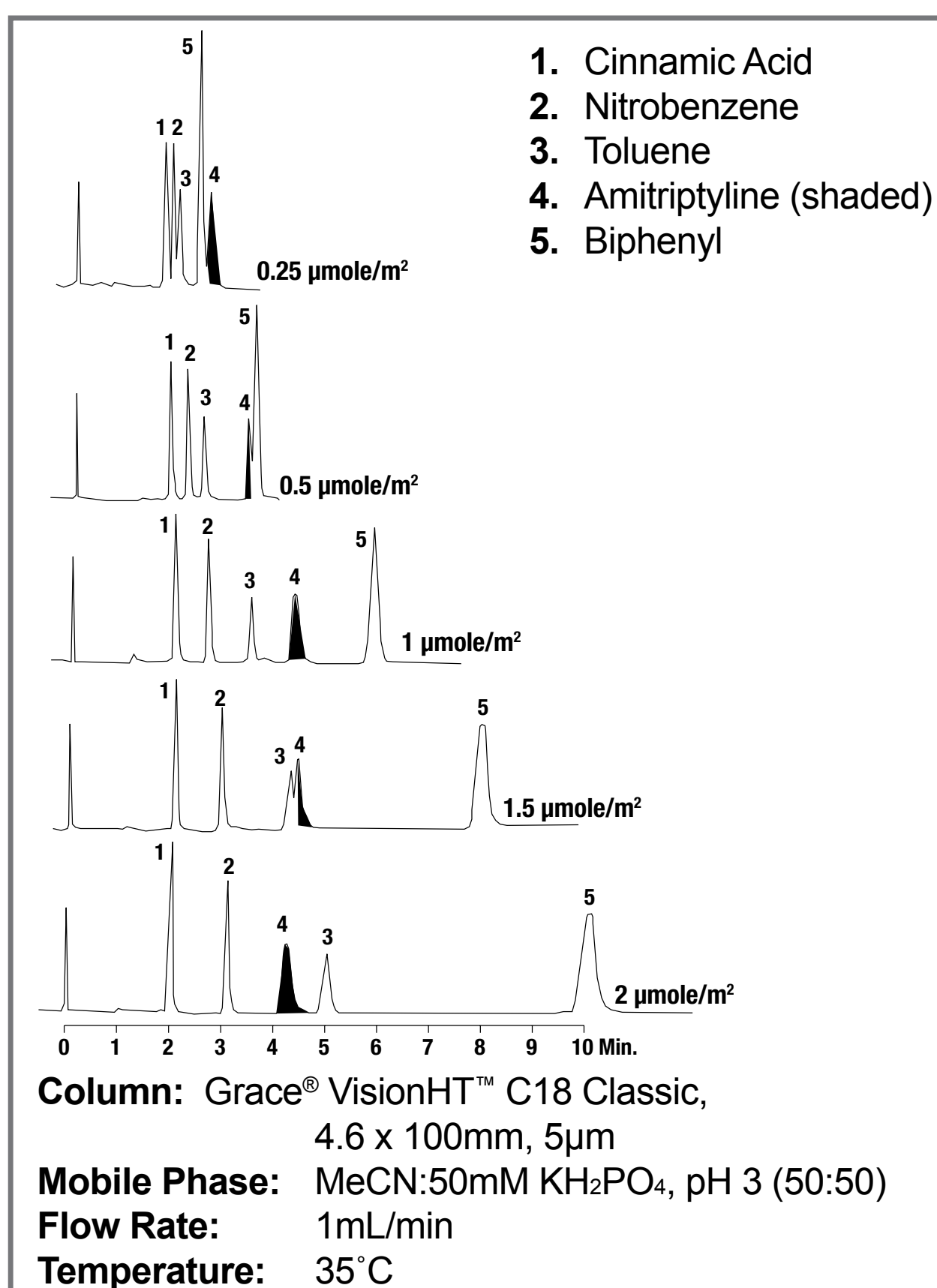


Figure G

The use of 2 different controlled retention mechanisms of course is in addition to the normal LC variables of mobile phase strength and pH. Analyte ionization is an important LC parameter.

Figure H shows the variation in elution order with pH for a series of bases on a Grace® VisionHT™ C18 Polar column. We can observe that the order changes with pH. Peak shape at pH 3 and 10 is better than at pH 7 under the elution conditions shown due to the pKa effect indicating the advantage of choosing elution pH conditions carefully.

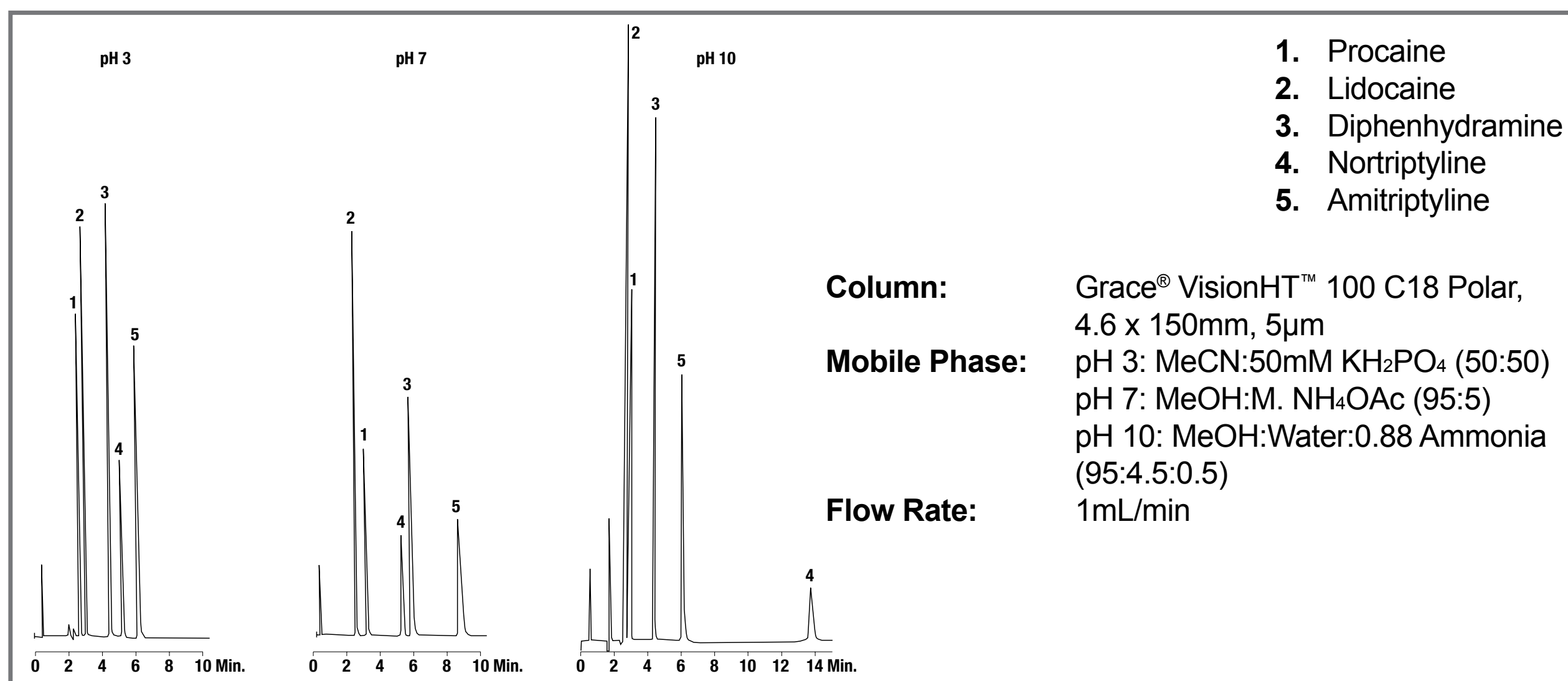


Figure H

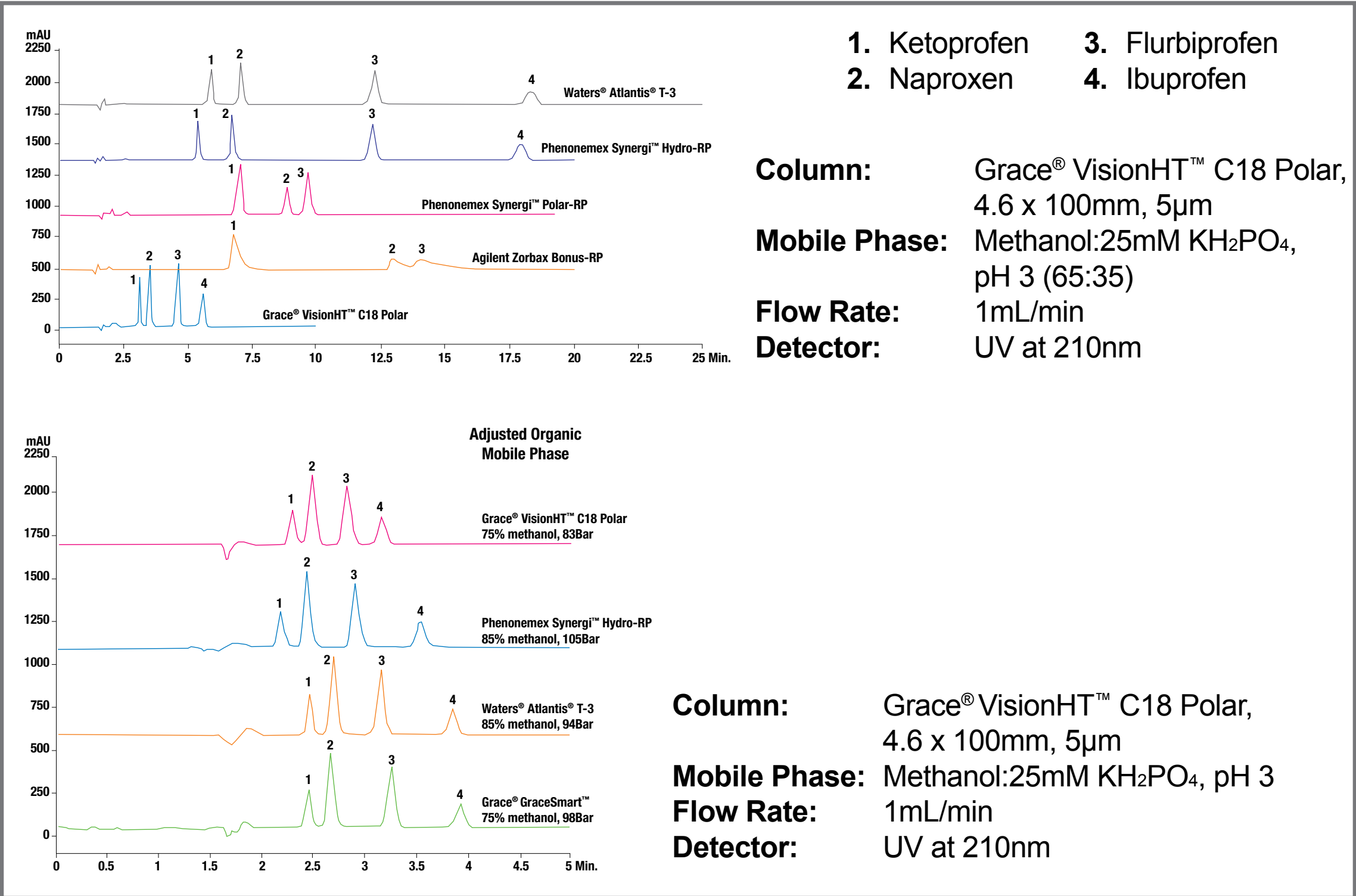
Recently, several new phases have been introduced from major manufacturers targeting the polar molecule field. Four samples will be considered, anti-inflammatories (Figure I), Ketoprofen degradants (Figure J), antibiotics (Figure K) and some similar synthetic peptides³ (Figure L). The Grace® VisionHT™ C18 Polar column is compared with a polar end-capped column, for extreme retention of non-polar and extremely polar alkyl compounds, an ether linked phenyl with polar end-capping, a sterically protected and highly endcapped polar embedded (amide) phase and 2 new fully bonded and endcapped C18 columns.

The ketoprofen degradant example (Figure J) shows the increased retention of the polar degradants and the lower retention for the less polar degradants, reducing run time while not compromising resolution.

In the example of the antibiotics (Figure K), the majority of the phases will not fully resolve cephalixin and cepharadine but cephalothin has a long retention. Reducing the organic level to slow the chromatogram down should help with the GraceSmart™ column but the others would benefit from the use of gradient elution. Re-equilibration time would of course increase the overall run cycle time in all these gradient cases.

The peptide comparison (Figure L) shows that the peptides have a much wider range of elution on the Grace® VisionHT™ C18 column compared to competitor columns. This allows much more scope for resolution of degradant material.

Anti-inflammatories



Because of the mixed mode separation mechanism with the Grace® VisionHT™ C18 Polar column phase, it can show both reversed-phase and normal-phase activity with certain polar molecules. This is shown in Figure M with uracil. At very low and zero acetonitrile levels we see increased retention due to a reversed-phase mechanism, retention reaches a minimum with increasing acetonitrile, then starts to increase at very high percentages of the organic solvent using a normal-phase mechanism. The low coverage allows utilization of this property in that the materials are stable in 100% aqueous mobile phases, the C18 chains not being liable to phase dewetting.

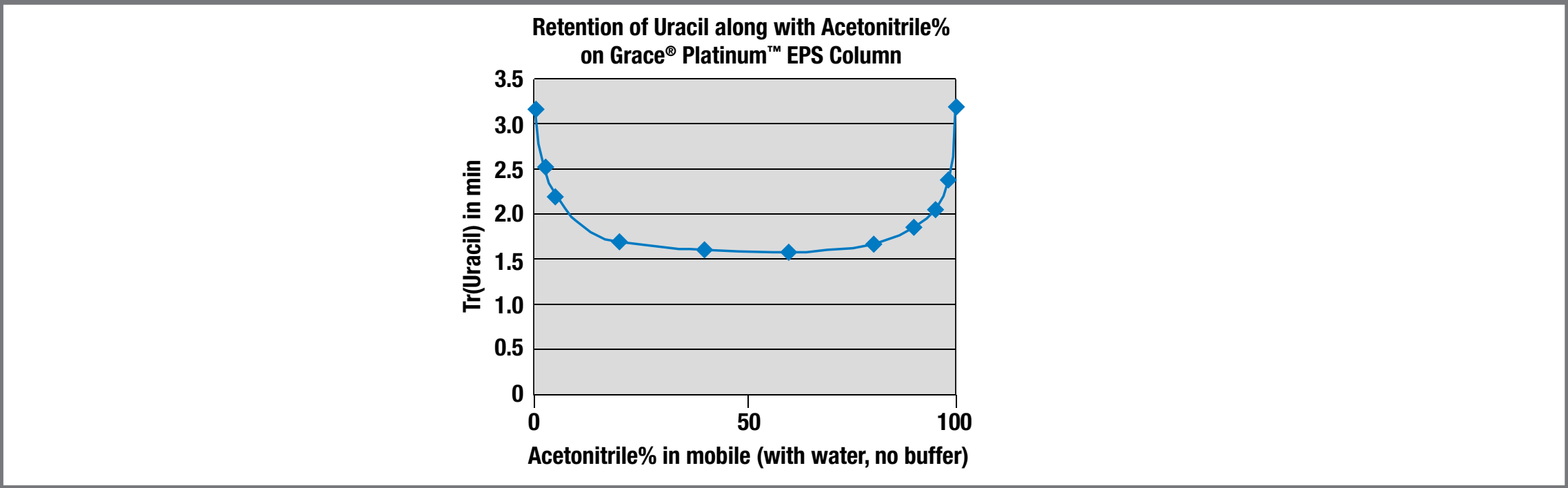


Figure M

From the work of Snyder et al^{4,5} on column comparisons, the separation mechanism contributions of hydrophobicity, steric effects, silanol effects (acidic and non-acidic) and ion-exchange effects can be determined. From a plot of hydrophobic interaction (H term) vs base interaction (C term at pH 7), a positioning of phases relative to each other can be determined and compared.

This is shown in Figure N for a selection of popular phases. It can be seen that Grace® VisionHT™ C18 Classic and Grace® VisionHT™ C18 Polar column phases are positioned differently and well away from standard fully bonded materials and many new materials designed for polar analyte chromatography. The Grace® VisionHT™ C18 Polar column phase has a high base interaction but a relatively low hydrophobic interaction effect. Compare the location of the embedded phase Grace® Alltima™ HP C18 amide, Waters® Xterra™ C18 RP, Grace® VisionHT™ C18 Classic, Phenomenex Luna® C18, Waters® Atlantis® T3, Grace® Alltima™ HP C18 HL column phases etc. The further away the columns are from one another the greater the selectivity difference.

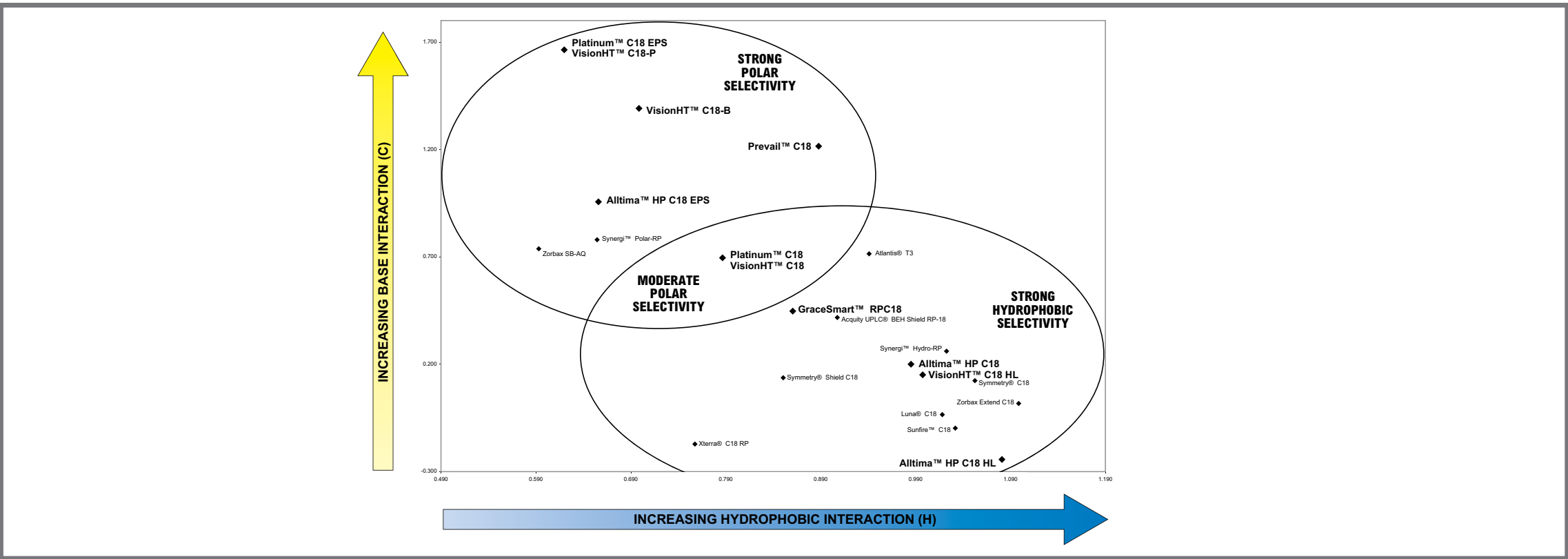


Figure N

To increase the application range, Grace® VisionHT™ column phases are available in particle sizes in 1.5µm, 3µm, 5µm, and 10µm.

Conclusions

It can be seen that the Grace® VisionHT™ column range of chromatography media, in particular the C18 Polar phases, are excellent choices for columns orthogonal in selectivity to classic C18, high coverage C18 columns. Their very reproducible mixed mode separation mechanism, coupled with traditional solvent and pH selectivity variables, give a product ideally suited to pharmaceutical development.

References

1. LC-GC Intl, July 1998, I Chappell, S.C. McGee and R.J. Weigand
2. LC-GC NA, Jan 2002, I Chappell
3. Grace Poster 2008, Fast Separations of Peptides Using Small and Large Pore Sub-Two Micron Columns, Reno Nguyen, Scott Anderson, Mark Jacyno, Wendy Luo, Ian Chappell, Linda Xu, and Joyce Wang
4. "The "Hydrophobic-subtraction" Model of Reversed-phase Column Selectivity", L.R. Snyder, J.W. Dolan and P.W. Carr, J. Chromatogr. A, 1060 (2004) 77 -116 .
5. "A New Look at the Selectivity of Reversed-phase HPLC Columns", L.R. Snyder, J.W. Dolan and P.W. Carr, Anal. Chem., 79 (2007) 325 -3262 .

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