Unique Selectivity Improves Separation of Polar Compounds by HPLC and UHPLC

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Abstract

Here we discuss a group of C18 phases with complementary selectivity that is ideal for separating everything from small polar molecules to larger proteins/ peptides. These phases, combined with unique hardware demonstrate the separation benefits of the universal VisionHT™ column platform.

Introduction

Over the last 10 years, polar pharmaceutical compounds have come to the forefront and can pose severe problems for standard 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 that give improved polar retention and are stable in 100% aqueous mobile phases. Base-deactivated phases and high coverage inert materials typically give good chromatography with most analytes, including bases, but also give similar selectivity.

Grace® Vision HT™ column phases give markedly different selectivity with polar analytes and therefore can give orthoganol selectivity to a standard highly 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 very good. For maximum versatility, the Grace® Vision HT™ column phases are available in several different particle sizes: 1.5µm, 3µm, 5µm, and 10µm.

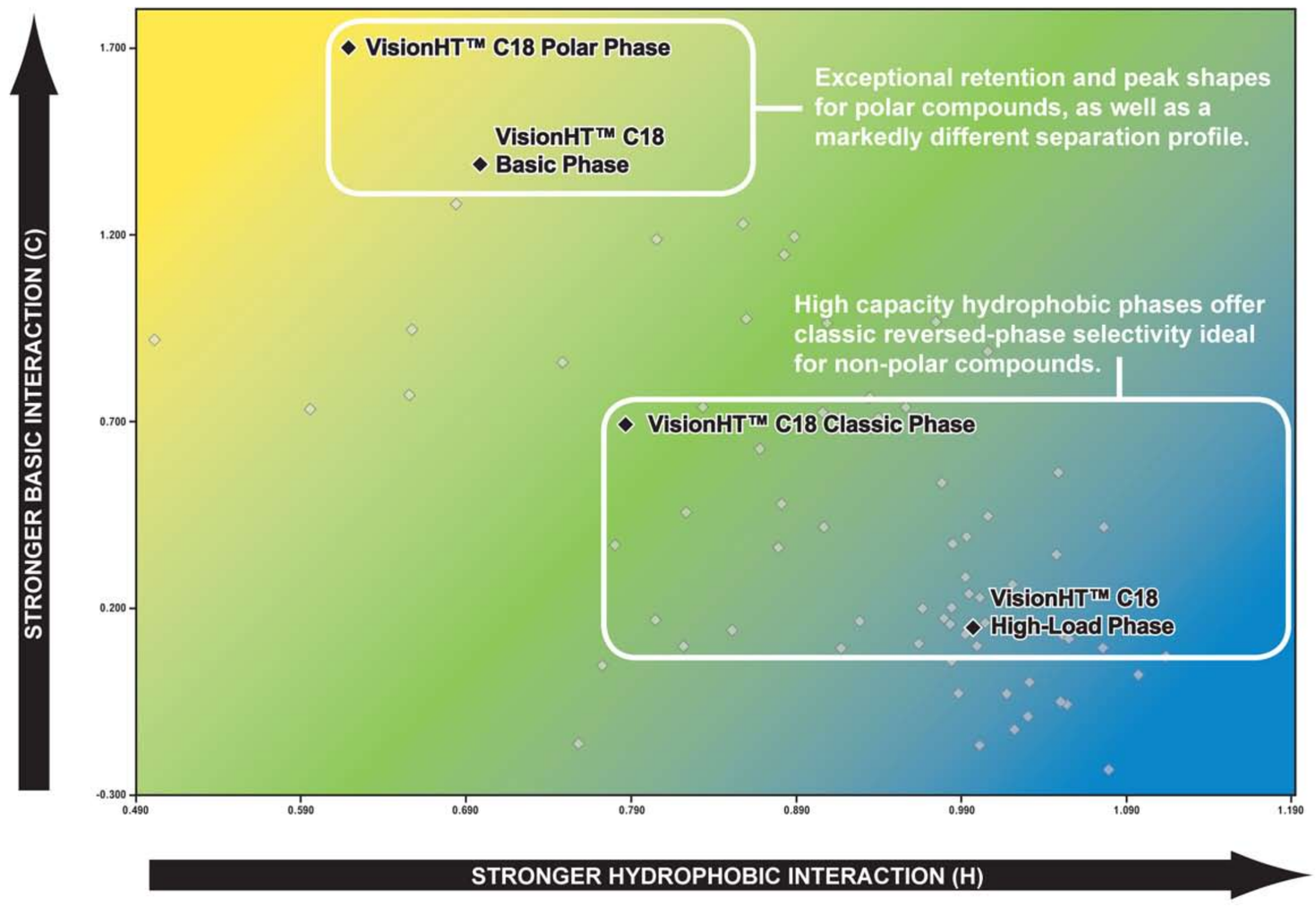
Results and Discussion

While most manufacturers thoroughly cover the silica surface of their reversed phase columns to minimize silanol interactions, Grace takes a unique approach by controlling the silica exposure to dramatically change the selectivity compared to traditional highly endcapped C18 columns.

From the work of Snyder, et al[1,2], on column selectivity 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 for a selection of popular phases.

It can be seen that Grace® VisionHT™ C18 Classic, C18 Basic, and C18 Polar column phases are positioned differently and well away from standard fully bonded/end-capped 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, allowing it to retain polar compounds much better than more hydrophobic phases.

Grace® Vision HT™ Reversed-Phase Chemistries Span the Full Polarity Spectrum



Orthogonal phases found at opposite ends of the polarity spectrum produce a maximum change in selectivity.

Figure 1

The Vision HT™ C18 Phases Offer Unique Selectivity Compared to Traditional Highly Endcapped C18 Phases

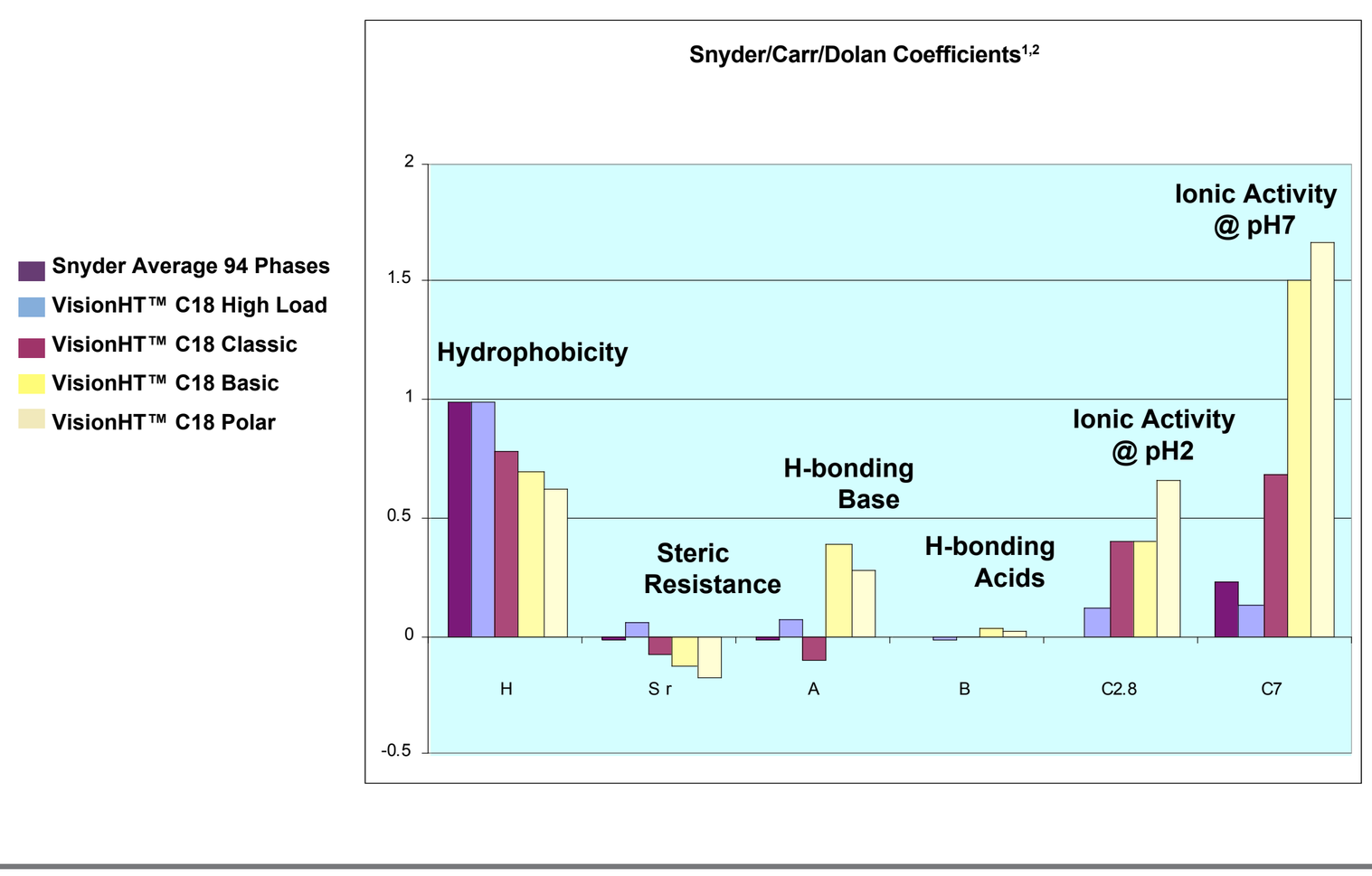
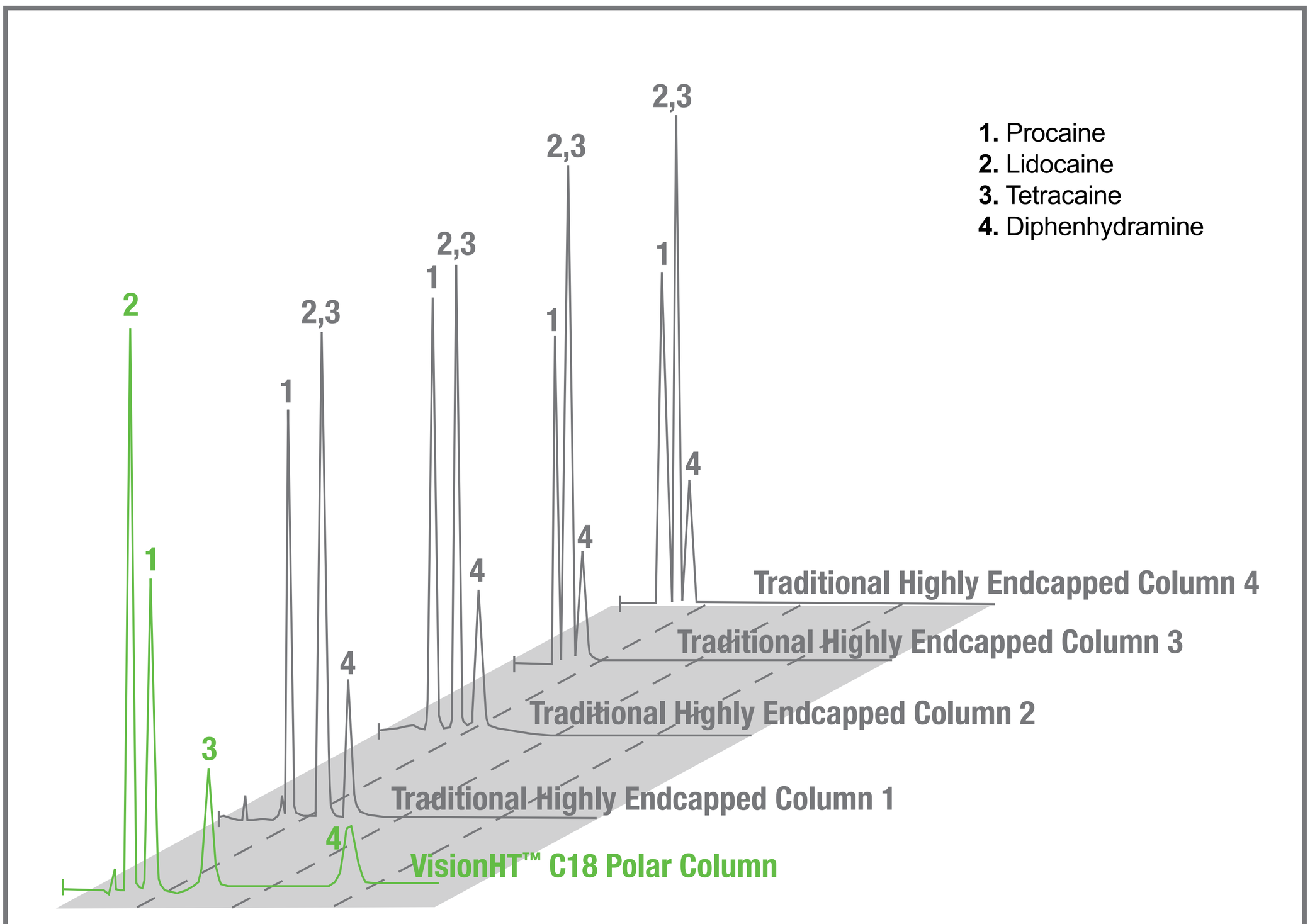


Figure 2

The Grace® VisionHT™ C18 Polar Column Separates Highly Polar Pharmaceuticals Better than Traditional Highly Endcapped C18 Phases

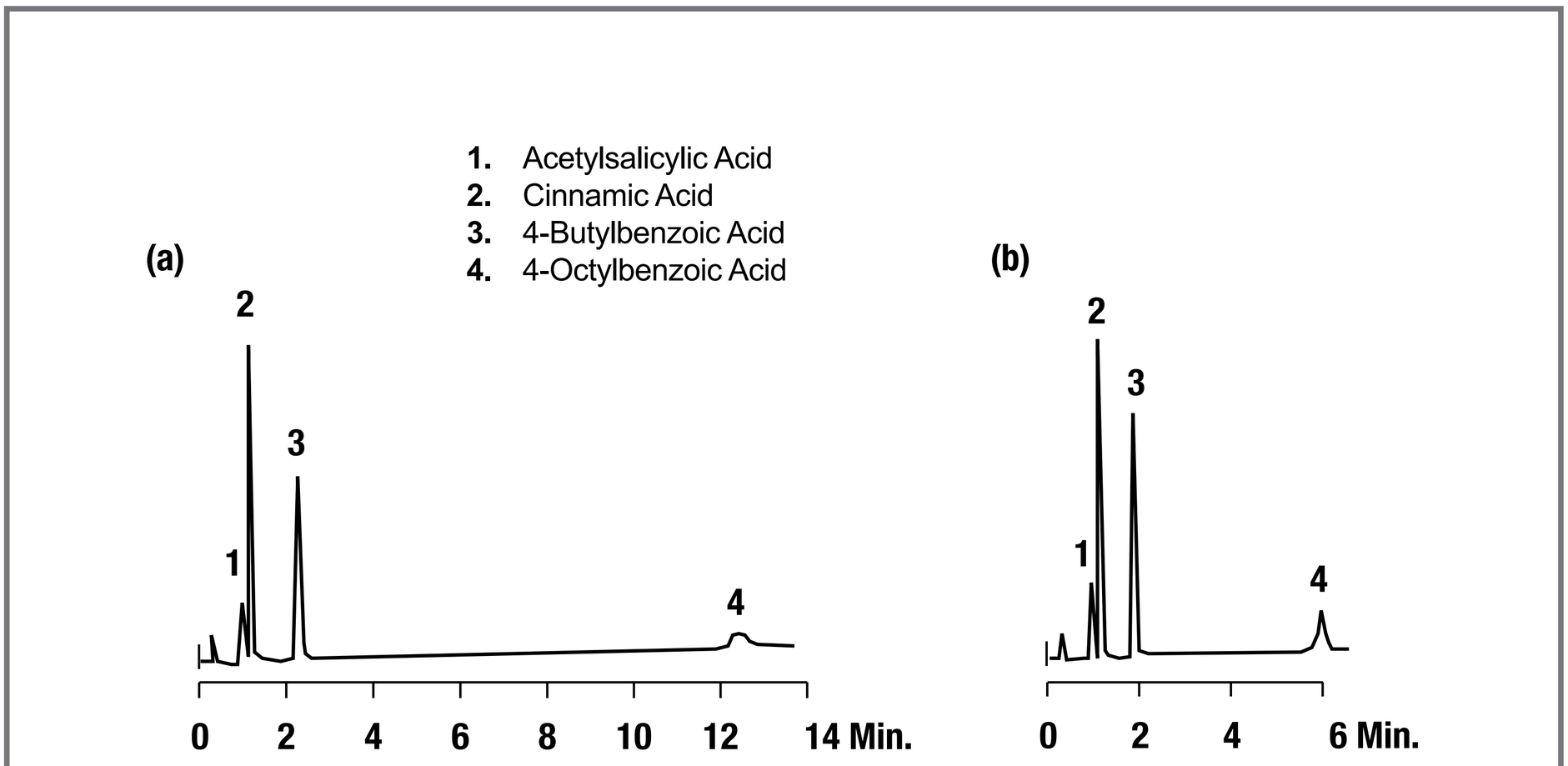


Mobile Phase: Methanol:0.025M K_2HPO_4, pH 7.0 (85:15)
Flow Rate: 2.0mL/min
Detector: UV at 230nm

Figure 3

In **Figure 3**, the Grace® VisionHT™ C18 Polar column shows an increase in retention and change in selectivity from the 4 traditional columns. The difficult pair of lidocaine and tetracaine is fully resolved away from the solvent front and with good peak shape. Note the movement of procaine and tetracaine. It is not necessary to fully end-cap a phase to obtain good peak shape. In fact, with an inert, fully hydrated silica, non endcapped phases often give better peak shape due to absence of lone silanols and the presence of hydrogen bonded vicinal silanol groups.

The Grace® VisionHT™ C18 Polar Column Can Reduce Run Times While Maintaining Good Resolution and Peak Shapes



Column: (a) Traditional highly endcapped C18, (b) Grace® VisionHT™ C18 Polar
Mobile Phase: Acetonitrile:50mM monobasic Potassium Phosphate, pH 3.0 (50:50)
Flow Rate: 2.0mL/min
Temperature: 35ºC

Figure 4

The Grace® VisionHT™ C18 Polar column phase has a carbon level of 5% from a 210m^2/gm silica. This compares approximately to 10% for a fully bonded and endcapped phase with a similar surface area. A typical type B silica with a surface area of 300m^2/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 nonpolar 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 the separation of carboxylic acids of varying polarities in **Figure 4**. The Grace® VisionHT™ C18 Polar phase gives good peak shapes and baseline resolution, the last peak having a retention time of 6 minutes. The fully bonded phase of similar surface area gives good peak shapes 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 Polar columns give reduced run times, increasing throughput.

Capacity Factors for Grace® VisionHT™ C18 Polar Columns Over 70 Batches Show Excellent Reproducibility

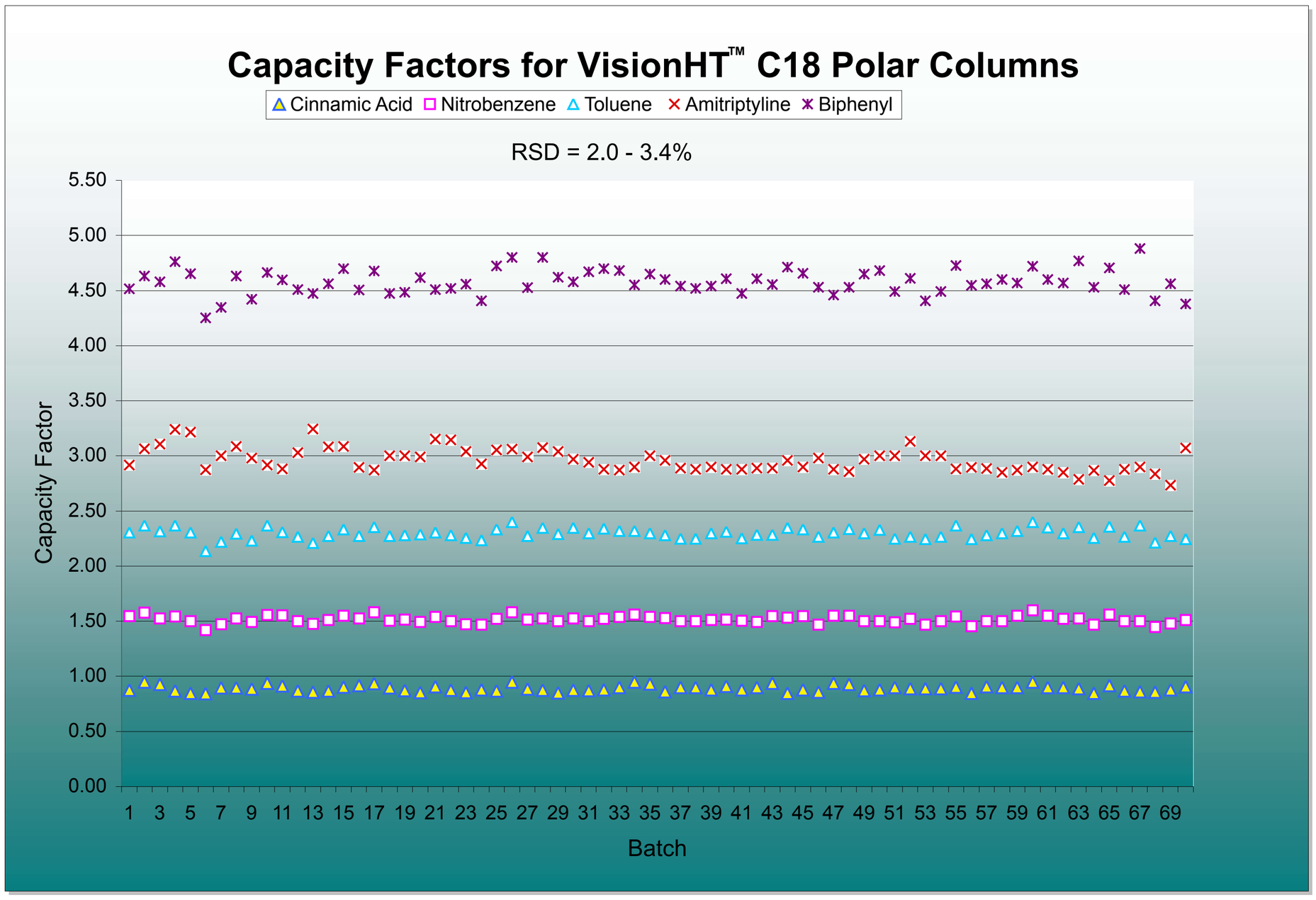
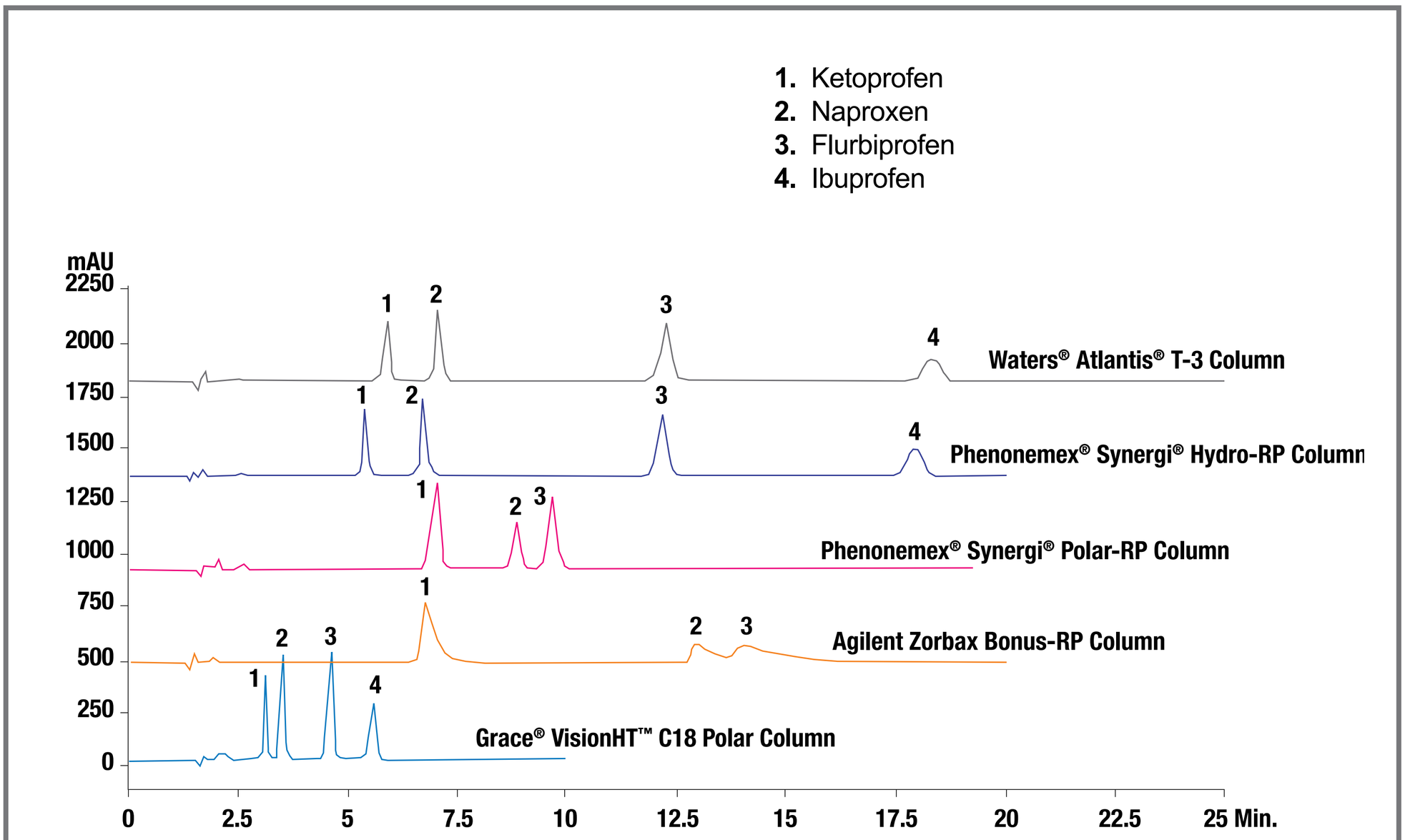


Figure 5

Reproducibility of these phases is excellent with RSD values for acids, bases and neutrals being between 2.0 and 3.4% over many batches. This fine control over the polar / non-polar selectivity is important and complements the normal LC variables of mobile phase strength and pH. Reproducibility over 70 batches is shown graphically in **Figure 5**.

The VisionHT™ C18 Polar Column Separates Anti-inflamatories Faster than Other Polar Phases

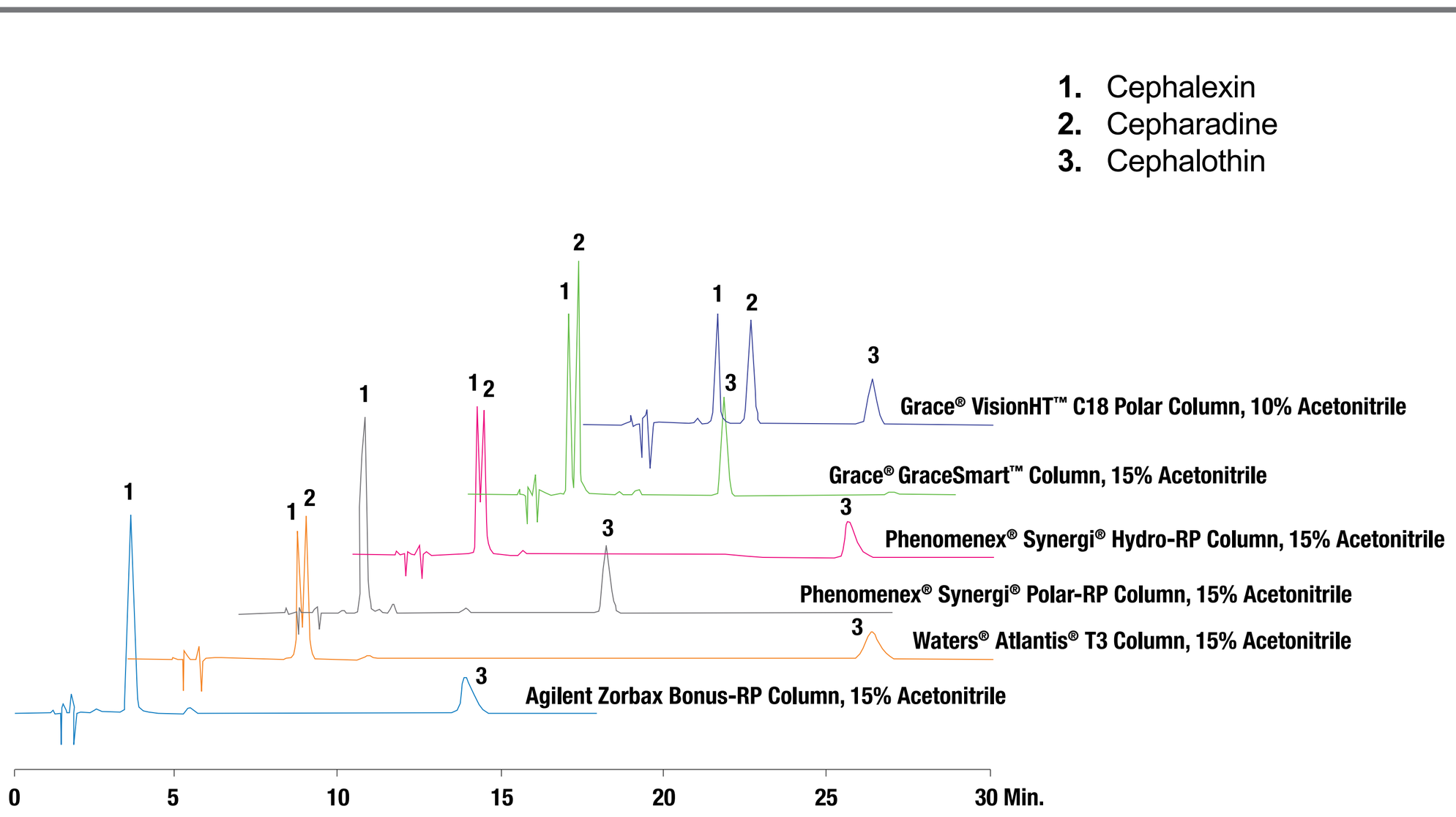


Column: Grace® VisionHT™ C18 Polar, 4.6 x 100mm, 5µm
Mobile Phase: Methanol:25mM KH_2PO_4, pH 3 (65:35)
Flow Rate: 1mL/min
Detector: UV at 210nm

Figure 6

Figure 6 shows a faster separation of anti-inflammatory drugs on the Vision HT™ C18 Polar column compared to other manufacturers' polar phases.

The VisionHT™ C18 Polar Column Separates Antibiotics Faster and With Greater Resolution than Other Polar Phases



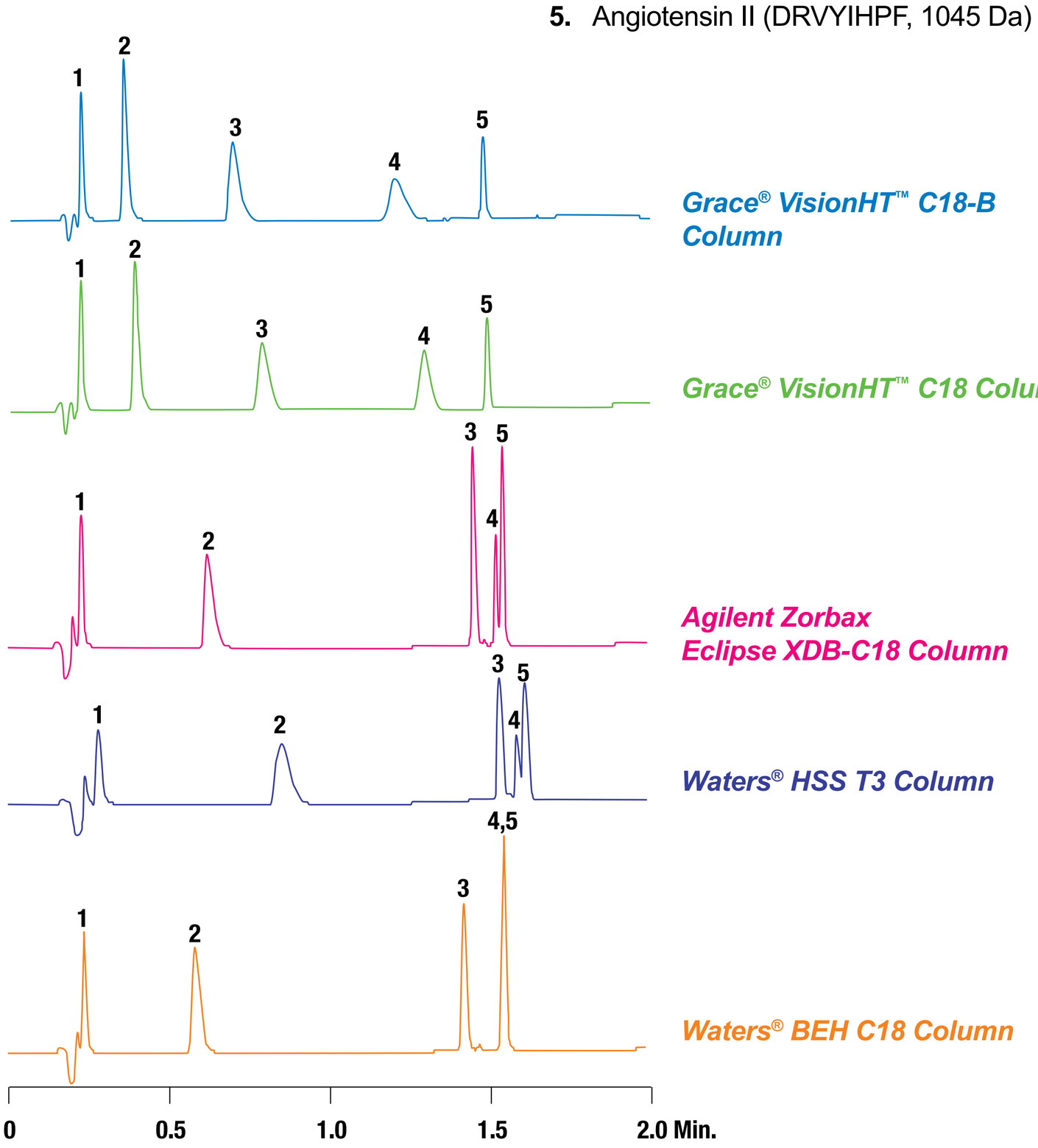
Mobile Phase: Acetonitrile:25mM KH_2PO_4, pH 7 in Water
Flow Rate: 1mL/min
Temperature: 30ºC
Detector: UV at 254nm

Figure 7

In **Figure 7**, the majority of the phases will not fully resolve cephalexin and cepharadine and cephalothin has a long retention. The Vision HT™ C18 Polar column resolves the three compounds and exhibits good peak shapes.

VisionHT™ C18 Columns are Available in 1.5µm Packings to Increase Speed While Maintaining Resolution for Complex Samples

1. GY (238 Da)
2. VYV (379 Da)
3. Met Enkephalin (YGGFM, 573 Da)
4. Leu Enkephalin (YGGFL, 555 Da)
5. Angiotensin II (DRVYIHPF, 1045 Da)



Column Dimension:	1.5µm, 2.1 x 50mm
HPLC System:	Agilent 1200 RRLC
Mobile Phase A:	0.1% TFA in Water
Mobile Phase B:	0.085% TFA in 80:20 Acetonitrile:Water
Gradient (min,%B):	(0.0, 5), (1.0,100), (2,100)
Flow Rate:	0.8mL/min.
Detector:	UV at 215nm
Injection Volume:	10µL
Column Temperature:	45°C
Column Backpressure:	508bar

Figure 8

In **Figure 8**, the Vision HT™ C18 and C18-B columns show better resolution for the five peptides compared to the traditional highly endcapped columns, indicating a potential for increased resolution with complex samples.

Conclusion

We have shown that the Grace® VisionHT™ C18 column phases offer unique selectivity covering the full polarity spectrum and are excellent alternatives to traditional highly endcapped phases for separating polar compounds. These phases, combined with unique hardware demonstrate the separation benefits of this column platform.

References

1. “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 .
2. “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 .