Retention and Separation of Highly Polar Compounds on a Novel HPLC Phase

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Summary

The selectivity of Grace® Platinum™ 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 standard 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.

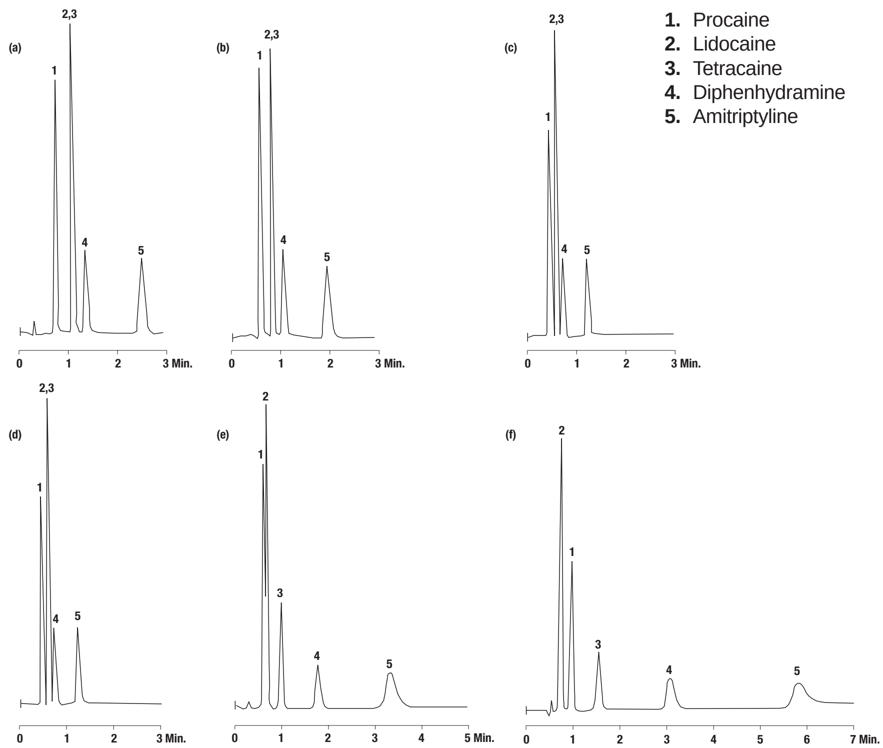
The first generation type A silica materials were very useful and their shortcomings with bases, for instance, were overcome by the addition of mobile phase additives to suppress acidic silanol activity. This led to the development of base deactivated type A silicas and then inert silicas, often made 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 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 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.

In the late 1990s, a novel approach was taken by Alltech, now a part of Grace, with the introduction of their Platinum™ column product line[1]. 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® Platinum™ column phases, standard and EPS versions, give markedly different selectivity with polar analytes and therefore can give orthoganol 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). Development work on the Grace® Platinum™ column range has continued and it is still a leading orthoganol phase compared to a standard high coverage material.

Results and Discussion

The EPS (Extended Polar Selectivity) benefits of Grace® Platinum™ columns have been discussed previously[1,2] By examining data on the chromatography of substituted benzene derivatives, the EPS 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 standard Grace® Platinum™ column phase has equivalent values of 7%, 42% and 154%.

An example of the improvement in resolution possible from the EPS effect is seen in Figure A[1], a pharmaceutical test mix run at pH 7.



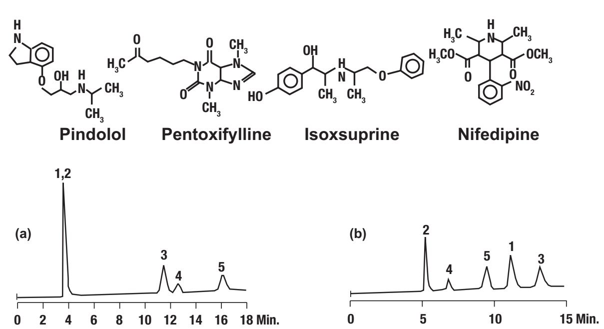
1. Procaine
2. Lidocaine
3. Tetracaine
4. Diphenhydramine
5. Amitriptyline

Column:	(a) Waters® Symmetry® C18, (b) GL Sciences Inertsil® ODS 2, (c) Thermo Hypersil® BDS C18, (d) Macherey, Nagel Gmbh & Co. Nucleosil® ODS AB, (e) Standard Grace® Platinum™ C18, (f) Grace® Platinum™ EPS C18
Mobile Phase:	Methanol:0.025M K_2HPO_4, pH 7.0 (85:15)
Flow Rate:	2.0mL/min
Detector:	UV at 230nm

Figure A

The Grace® Platinum™ C18 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® Platinum™ C18 EPS 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® Platinum™ C18 column separation from the Grace® Platinum™ C18 EPS column separation, which is due solely to the EPS effect, is shown in Figure B[1]. Here the base silicas and bonding technology are identical with just a bonding methodology difference producing the EPS 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.

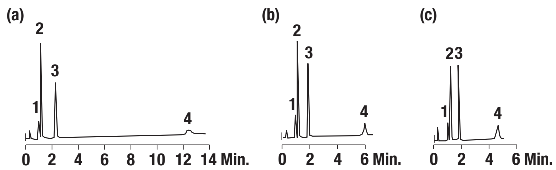


1. Pindolol
2. Pentoxifylline
3. Isoxsuprine
4. Nifedipine Degradant
5. Nifedipine

Column:	(a) Standard Grace® Platinum™ C18, (b) Grace® Platinum™ EPS C18, both 4.6 x 150mm, 5µm
Mobile Phase:	0.025M Potassium Phosphate (dibasic, pH 3.0):Methanol (50:50)
Flow Rate:	1.0mL/min
Detector:	UV at 220nm

Figure B

Grace® Platinum™ column phases are characterized by their low surface coverage. The Grace® Platinum™ C18 column phase has a carbon level of 6% from a 210m²/gm silica and the C18 EPS 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 enhaced polar retention. This is illustrated in Figure C[2] of carboxylic acids of varying polarity. Both the Grace® Platinum™ EPS C18 and C8 column phases give 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® Platinum™ columns give much reduced run times which will increase throughput.

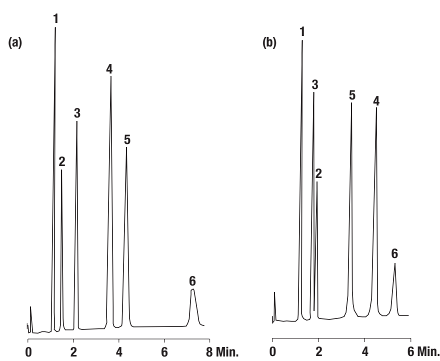


1. Acetylsalicylic Acid
2. Cinnamic Acid
3. 4-Butylbenzoic Acid
4. 4-Octylbenzoic Acid

Column: (a) Grace® Brava™ base-deactivated silica C18, (b) Grace® Platinum™ EPS C18, (c) Grace® Platinum™ EPS C8, all 4.6 x 100mm, 5µm
Mobile Phase: (a,b) Acetonitrile:50mM monobasic Potassium Phosphate, pH 3.0 (50:50)
(c) Acetonitrile:50mM monobasic Potassium Phosphate (40:60)
Flow Rate: 2.0mL/min
Temperature: 35ºC

Figure C

Grace® Platinum™ column phases are available with 2 different degrees of coverage so that the optimum EPS effect can be utilized. In Figure D[2], separation of anticonvulsants, the standard Grace® Platinum™ 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.



1. Primidone
2. Lamotrigine
3. Phenobarbitone
4. Carbamazepine
5. Phenytoin
6. 5-(*p*-methylphenyl)-5-phenylhydantoin

Column: (a) Grace® Platinum™ C18, (b) Grace® Platinum™ EPS C18, both 7.0 x 53mm Rocket™ hardware format, 3µm
Mobile Phase: Acetonitrile:30mM monobasic Potassium Phosphate (30:70)
Flow Rate: 2.3mL/min
Temperature: 23ºC

Figure D

The Grace® Platinum™ 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 for batches to 2008.

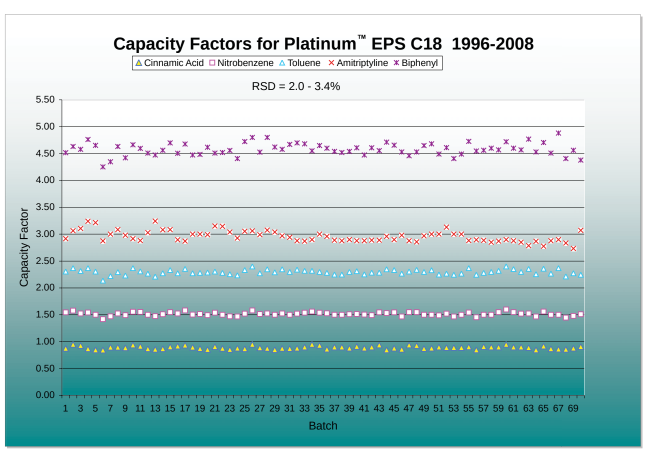


Figure E

Because of the reproducibility of the bonding, development of Grace® Platinum™ EPS column phases with surface coverages of 0.25, 0.5, 1.0 (standard EPS), 1.5 and 2.0 µmoles/m² 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.

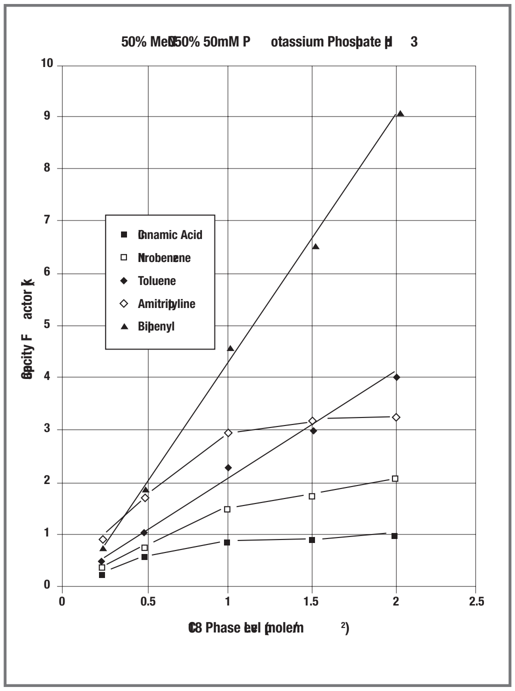
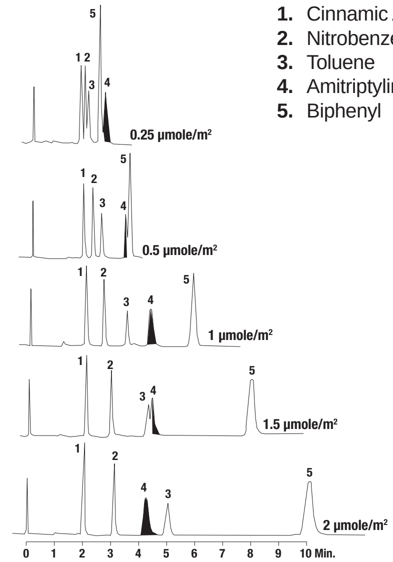


Figure F



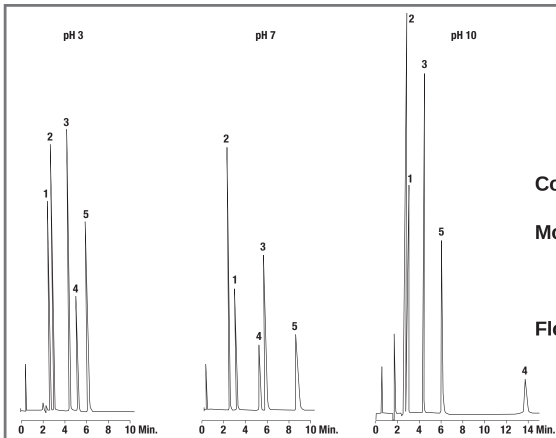
1. Cinnamic Acid
2. Nitrobenzene
3. Toluene
4. Amitriptyline (shaded)
5. Biphenyl

Column: Grace® Platinum™ C18, 4.6 x 100mm, 5µm
Mobile Phase: MeCN:50mM KH_2PO_4, pH 3 (50:50)
Flow Rate: 1mL/min
Temperature: 35˚C

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® Platinum™ EPS C18 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.



1. Procaine
2. Lidocaine
3. Diphenhydramine
4. Nortriptyline
5. Amitriptyline

Column: Grace® Platinum™ 100 EPS C18, 4.6 x 150mm, 5µm
Mobile Phase: pH 3: MeCN:50mM KH_2PO_4 (50:50)
pH 7: MeOH:M. NH_4OAc (95:5)
pH 10: MeOH:Water:0.88 Ammonia (95:4.5:0.5)
Flow Rate: 1mL/min

Figure H

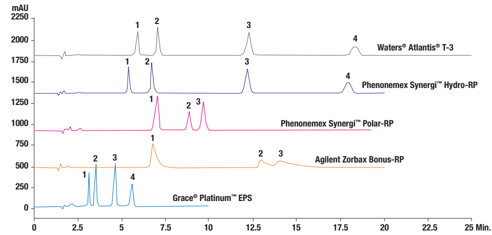
Recently, several new phases have been introduced from major manufacturers targeting the polar molecule field. Four samples will be considered, anti-inflammatories (Figure I), Ketoprofen degradants (Figure J), antibiotics (Figure K) and some similar synthetic peptides[3] (Figure L). The Grace® Platinum™ EPS C18 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 cephalexin 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.

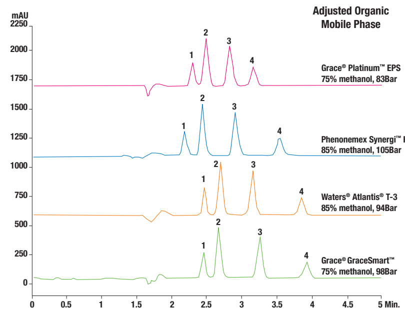
The peptide comparison (Figure L) shows that the peptides have a much wider range of elution on the Grace® Platinum™ C18 (Grace® VisionHT™ C18-P) column compared to competitor columns. This allows much more scope for resolution of degradant material.

Anti-inflammatories



1. Ketoprofen
2. Naproxen
3. Flurbiprofen
4. Ibuprofen

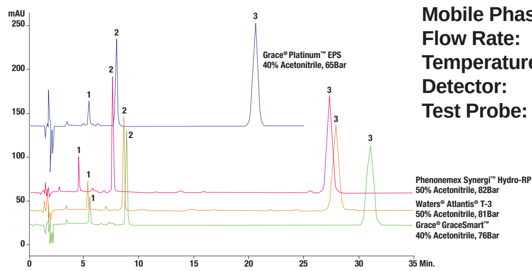
Column: C18, 4.6 x 100mm, 5µm
Mobile Phase: Methanol:25mM KH_2PO_4, pH 3 (65:35)
Flow Rate: 1mL/min
Detector: UV at 210nm



Column: C18, 4.6 x 100mm, 5µm
Mobile Phase: Methanol:25mM KH_2PO_4, pH 3
Flow Rate: 1mL/min
Detector: UV at 210nm

Figure I

Ketoprofen Degradants



Mobile Phase: Acetonitrile:0.2% Formic Acid in Water
Flow Rate: 1mL/min
Temperature: 30ºC
Detector: UV at 230nm
Test Probe: Ketoprofen degraded

Figure J

Antibiotics

1. Cephalexin
2. Cepharadine
3. Cephalothin

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

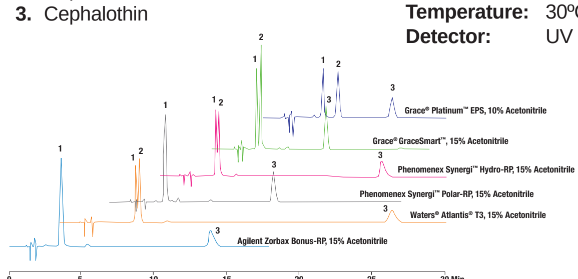
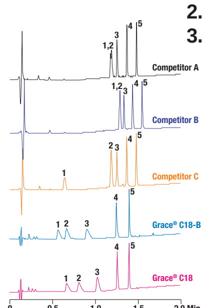


Figure K

Similar Synthetic Peptides



1. Ac-RGGGGLGLGK-amide (911 Da)
2. RGAGGLGLGK-amide (883 Da)
3. Ac-RGAGGLGLGK-amide (926 Da)
4. Ac-RGVGGLGLGK-amide (954 Da)
5. Ac-RGVVGLGLGK-amide (996 Da)

Column: C18, Small Pore, Sub2µm, 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, 15), (3.0, 80)
Flow Rate: 0.8mL/min
Detector: UV at 215nm
Column Temperature: 45°C
Column Backpressure: 6000 to 7500 psi

Figure L

Because of the mixed mode separation mechanism with the Grace® Platinum™ EPS 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.

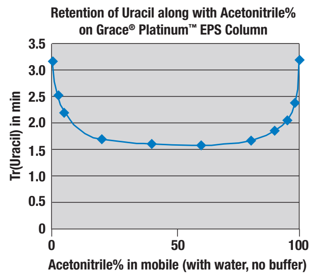


Figure M

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® Platinum™ and Grace® Platinum™ EPS column phases are positioned differently and well away from standard fully bonded materials and many new materials designed for polar analyte chromatography. The Grace® Platinum™ EPS 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® Platinum™ C18, 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.

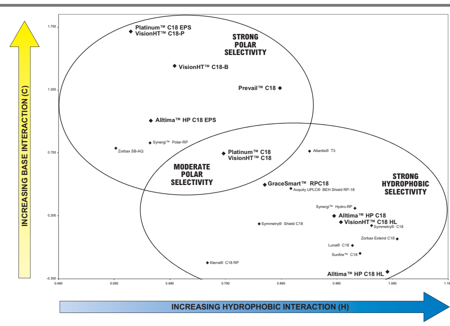


Figure N

To increase the application range, Grace® Platinum™ column phases are available in particle sizes in 1.5µm, 3µm, 5µm, and 10µm. The 1.5µm phases are branded under the VisionHT™ name (VisionHT™ C18 is Platinum™ C18 and VisionHT™ C18-P is Platinum™ EPS C18). The 3, 5, and 10µm particles are available in columns from micro-bore through analytical to preparative.

Conclusions

It can be seen that the Grace® Platinum™ column range of chromatography media, in particular the EPS phases, are excellent choices for columns orthogonal in selectivity to standard, 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

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