Reliable Reproducibility and More Selectivity from Superficially Porous Particles

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Superficially porous particle technology is no longer new. There are many more options available now, and new phases are often released. These columns are being adopted by more chromatographers every day as a way to save time and increase lab throughput. Nonetheless, many chromatographers are still using conventional 3.5µm and 5µm columns when they could be seeing significant time savings and improved resolution from superficially porous technology. In this article Agilent Poroshell 120 columns were used to illustrate some of the benefits that superficially porous particles provide.

A brief history of superficially porous particles

Superficially porous particles (SPPs) are different from traditional fully porous particles in that they have a solid core surrounded by a thin porous shell. There are four types of porous particles used in analytical scale LC columns.

- Sub-3 µm superficially porous particles - the most popular modern SPPs that typically operate at up to 600 bar. They are available in various column id such as 4.6, 3.0 and 2.1mm.
- Sub-2 µm superficially porous particles - smaller core-shell particles that can be operated at ultrahigh pressure (e.g. 1000 bar). They are only available in 2.1mm id columns.
- Sub-2 µm fully porous particles - the smallest fully porous particles, developed for ultrahigh pressure separations and mainstream for UHPLC. They are typically available in narrow-bore columns such as 2.1 or 3.0mm id.
- Traditional fully porous particles - the most popular fully porous particles for HPLC applications, with particle sizes from 2.5 to 5 µm. They can be operated at up to 600 bar and are available in various column ids such as 4.6, 3.0 and 2.1mm.

The original intention of designing superficially porous particles was to reduce the analyte diffusion length inside the particle, while not reducing column backpressure. The development of SPPs began four decades ago when Horvath and Lipsky [1] made very large pellicular particles for ion-exchange separations. Since then, such particles have gone through several cycles of development. The major technology breakthrough occurred in 2006 when Kirkland and co-workers commercialised modern sub 3 µm SPPs. The success of these columns revitalised research interest. Currently, multiple manufacturers offer columns packed with sub 3 µm SPPs: 2.7 µm Halo from Advanced Material Technologies, 1.7 µm and 2.6 µm Kinetex from Phenomenex, and 2.7 µm Poroshell 120 from Agilent Technologies. However, the list of commercially available superficially porous particles is likely to grow.

Due to their exceptional chromatographic efficiency, superficially porous particles quickly gained popularity in analytical labs [2]. It was soon appreciated that the particles could also be used on traditional HPLC if the instrument was carefully optimised, another factor that helped their quick adoption. With the increasing customer base, more and more applications were developed on the new particles. Some of these applications meet analyst's needs for faster separations, and some meet the requirement to resolve challenging samples.

Thus, Poroshell 120 and other superficially porous columns such as HALO/Ascentis Express and Kinetex have revolutionised the HPLC column market by providing high resolution with relatively low back pressure, in many cases under 400 bar. Superficially porous particles for small molecules have average pore sizes between 90 and 120 Å.

Making superficially porous particles

Poroshell 120 particles have a 1.7 µm solid silica core with a 0.5µm porous outer layer (Figure 1). This unique configuration gives all the performance advantages of sub 2 µm particles with the backpressure of a sub 3 µm particle.

The cores of superficially porous particles should have a very smooth surface and a uniform particle size (see Figure 2a), which contributes to a tight overall particle size distribution. As a result, the column bed is

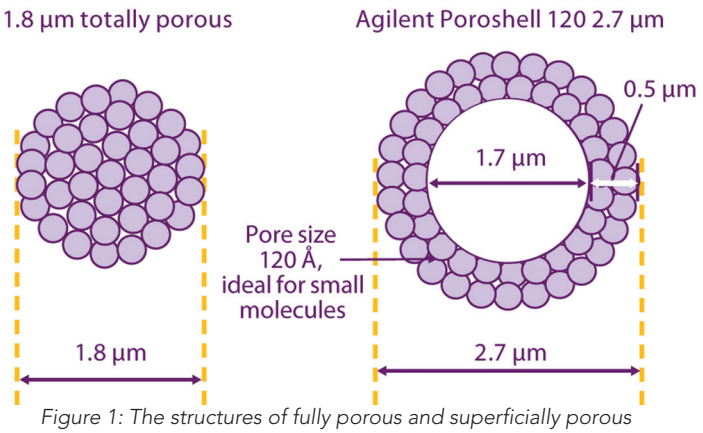


Figure 1: The structures of fully porous and superficially porous particles

more uniformly packed to provide higher efficiency than that found in totally porous particles.

Some manufacturers create the porous shell by applying layer after layer of particles. However, it is preferable to minimise the manufacturing steps involved, in order to provide maximum particle reproducibility for the best chromatography from batch to batch. Therefore, a better alternative is to apply the porous shell in one single step – similar to the coacervation technique used to make traditional ZORBAX columns (see Figure 2b). This unique single-step process delivers higher yields and excellent column-to-column reproducibility.

The range of superficially porous particle bonded phases is expanding to align with those provided by traditional columns for method development flexibility and assured scalability. For example, Agilent has recently released Phenyl-Hexyl and SB-Aq on Poroshell 120 and will be releasing SB-C8 and Bonus-RP later this summer.

Reproducibility

As we have discussed, the simpler the manufacturing process, the more consistent the column. A single-step application of the outer shell creates a highly reproducible column, as is evident in the lot-to-lot comparison shown in Figure 3. Here, batches from near the time of initial release of Poroshell 120 in 2009 up to 2012 were analysed for batch-to-batch retention time stability. The sample was a challenging beverage additive, which was very sensitive to changes between batches.

Performance advantages

Fast LC from a standard HPLC

With some superficially porous particles, it is possible to achieve 80-90% or more of the efficiency that would be expected from a sub 2 µm Fast-LC/UHPLC column, and now at HPLC pressures below 400 bar. This ability to perform fast separations at low pressures can dramatically enhance productivity by allowing more samples to be run in less time, using the laboratory's existing HPLC systems. In addition, method transfer would be seamless, for even more productivity, because Poroshell 120 phases are aligned with phases available in the ZORBAX family.

In this sample of neutral alkylphenones (Figure 4), the SPP column delivered >90% of

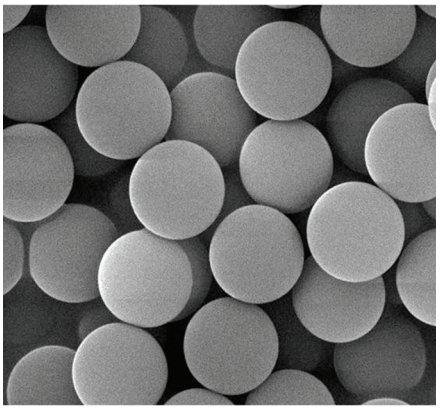


Figure 2a: Solid Cores – the first step of the manufacturing process

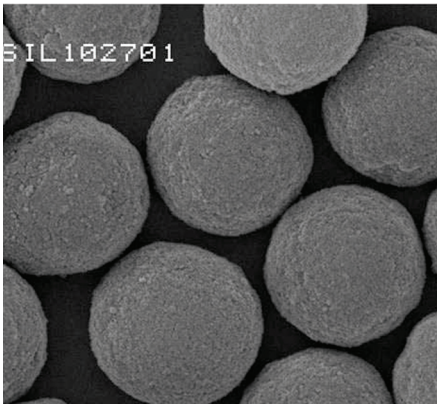


Figure 2b: Poroshell 120 particles after the superficially porous outer shell has been applied in one step

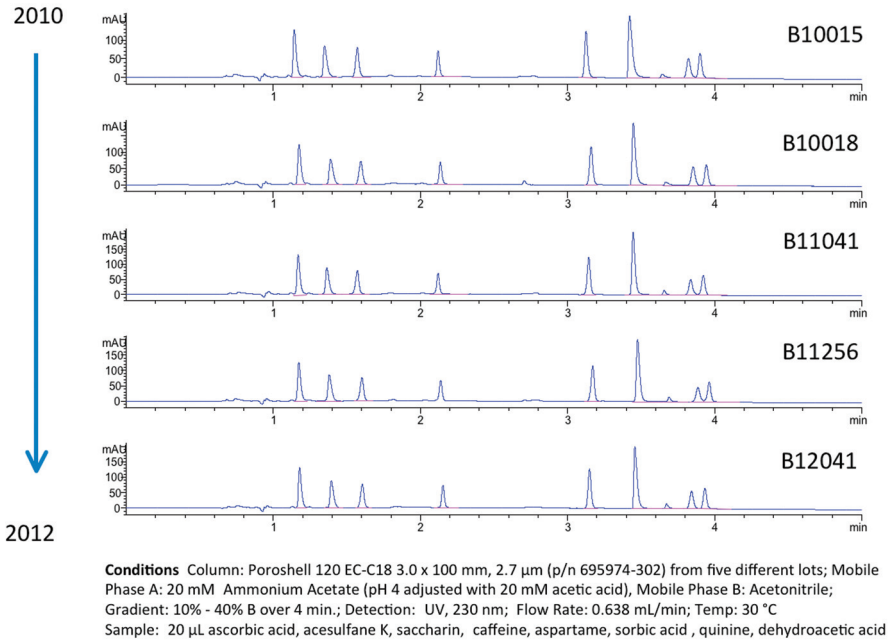


Figure 3: Separation of a mix of beverage additives shows minimal variation from different batches.

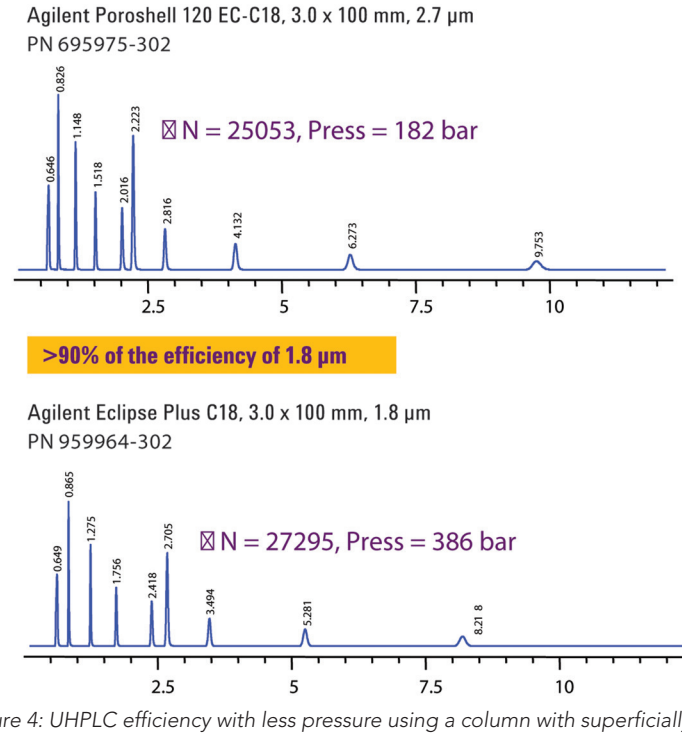


Figure 4: UHPLC efficiency with less pressure using a column with superficially porous particles.

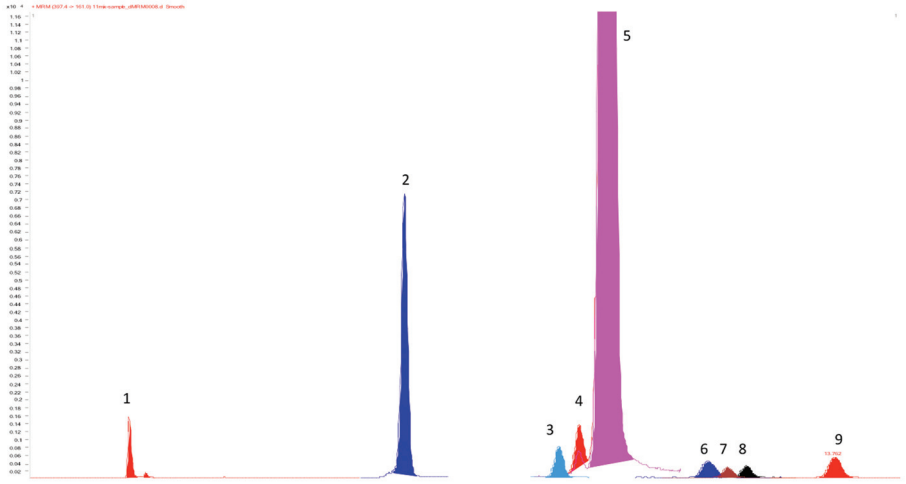


Figure 5: Separation of cholesterol and other sterols using a superficially porous particle column with LC/MS/MS. Note that adequate resolution was obtained, even at the 2000:1 ratio for cholesterol:lathosterol. This is critical for effective quantification, because the two compounds have the same molecular weight.

the efficiency attained by the Fast-LC 1.8 µm column. Note, too, that the pressure on the SPP column was about 50% of the pressure on the 1.8 µm column.

Conditions

Flow Rate: 0.58mL/min

Mobile Phase: 60% acetonitrile: 40% water

Injection Volume: 4 µL

TCC: 26°C

Detection: DAD Sig = 254,4nm, Ref = 360,100nm

Sample: RRLC Checkout Sample spiked with 50 µL 2mg/mL thiourea in water:acetonitrile (65:35)

Ruggedness

As well as providing opportunities for Fast LC, superficially porous particles can deliver speed and resolution advantages with the added confidence of a rugged method. For example, when using LC/MS and LC/MS/MS, a porous outer layer and solid core limit diffusion distance and improve separation speed, while the narrow particle size distribution improves efficiency and resolution (Figure 5).

Conditions

Columns: Poroshell 120 EC-C18, 3.0 x 100mm, 2.7 µm

Mobile Phase: 80% ACN:20% methanol

Flow Rate: 0.6mL/min

Temperature: 20°C

Injection Volume: 2 µL

Detection: APCI, Positive Ion

Another demonstration of ruggedness is repeatability and longevity. Table 1 summarises changes in retention time over 3,000 injections, expressed in the consistency of the retention times (%RSD). This test confirms the outstanding longevity of superficially porous particles.

Analyte	%RSD (RT)	Analyte	%RSD (RT)	Analyte	%RSD(RT)
Morphine	0.7	Meperidine	0.4	Triazolam	0.0
Codeine	0.4	Zolpidem	0.3	Naltrexone	0.1
hydrocodone	0.4	Fentanyl	0.1	Chlordiazepoxide	0.1
MDMA	0.3	EDDP	0.1	Desmethyl diazepam	0.1
norFentanyl	0.2	Nitrazepam	0.1	Cocaethylene	0.3
Heroin	0.2	Propoxephine	0.1	Buprenorphine	0.2
Methyl phenidate	0.2	Buprenorphine	0.3	11-nor-9-Carboxy-delta9-thc	0.0

Table 1: Consistency of retention times in the analysis of some drugs on a Poroshell 120 column after 3,000 injections (%RSD).

Method transfer

Many methods developed on longer 5 µm C18 columns can be moved to shorter superficially porous particles quickly and easily. For example, changes to the USP regulations are making it easier to transfer conventional methods to newer technologies. This enables chromatographers to significantly increase throughput and reduce costs. An example is shown in Figure 5 using the USP method for naproxen tablets. Using a column with superficially porous particles delivers a 4.5x faster analysis at HPLC pressures.

This naproxen separation (Figure 6) demonstrates how easy it can be to convert a method without changing the flow rate or mobile phase. The top chromatogram shows a USP analysis on an Agilent Eclipse Plus C18 column, which delivers sharp peaks, three times the needed efficiency, and a resolution of 15. In the middle chromatogram, the Poroshell 120 EC-C18 column (100mm length) provides greater efficiency and resolution at twice the speed of the original method. As the pressure is only 238 bar, this isocratic method offers an alternative HPLC option. The Poroshell 120 EC-C18 column (50mm length) on the bottom chromatogram still meets the requirements for efficiency and resolution, but is 4.5 times faster than the 5 µm column. Furthermore, the pressure is only 133 bar, which is compatible with HPLC instrumentation.

Conditions

Columns: as indicated:

Mobile phase: 50:49:1 MeCN:H2O acetic acid

Flow rate: 1.2mL/min

Sample:

1. Naproxen

2. Butyrophenone

Using new bonded phases to improve selectivity

As discussed, superficially porous columns are an option for achieving high efficiency, high resolution and fast analysis at HPLC pressures. But what happens when the optimal separation with a superficially porous C18 or C8 is not available? Alternate selectivities could provide an opportunity to refine a separation.

Selectivity and resolution

A method development scheme cannot overlook the basics of chromatography selectivity. This is absolutely critical no matter the particle size or composition. It is worth remembering that selectivity is a function of the mobile phase as well as bonded phase. Temperature can also play a role in selectivity; however, mobile phase and stationary phase tend to play a greater role in analyte retention.

The first and easiest option is to change the mobile phase, either the organic modifier (acetontrile, methanol, etc.) or the pH over a wide pH range (we recommend pH 2 to pH 7). Another option is to change the bonded phase, which provides additional optimisation potential.

Aromatic groups

With an alternative selectivity to alkyl bonded phases, Poroshell 120 Phenyl-Hexyl is recommended for aromatic groups, particularly pi active compounds in methanol. This phase is compatible with highly aqueous mobile phases for the separation of polar compounds such as azo dyes. Figure 8 is a comparison of four phases for the separation of nine azo dyes using a simple generic gradient. The dyes contain from one to three aromatic rings. The separation on this phenyl-hexyl column was compared to two C18 columns and a polar embedded column.

Phenyl-hexyl phases have unique reversed-phase selectivity, especially for polar aromatics and heterocyclic compounds, arising from analyte interaction with the aromatic ring and its delocalised electrons. More retention and selectivity will often be observed for compounds with aromatic electron-withdrawing groups such as fluorine, or nitro, groups. Phenyl-hexyl phases have greater selectivity compared to standard alkyl phases for aromatics, due to pi-pi interactions. These interactions are enhanced in methanol, though acetonitrile can also be used.

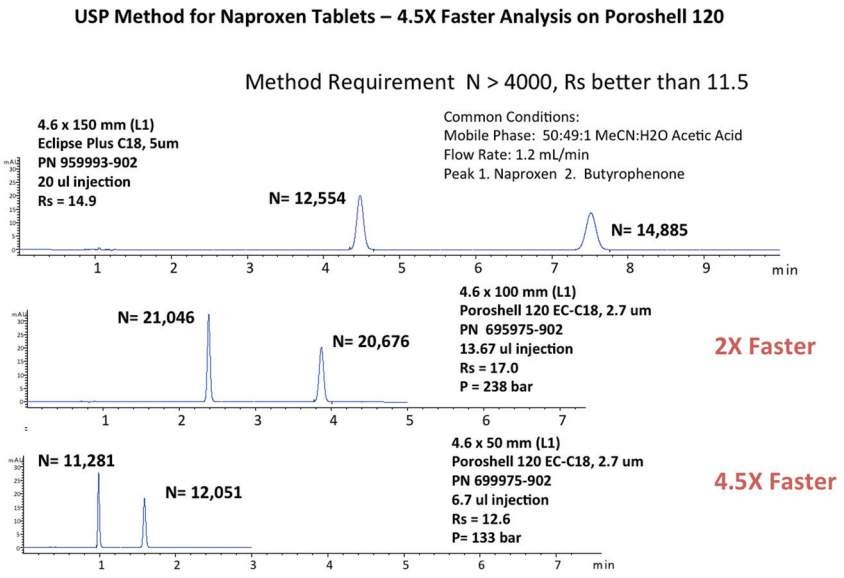
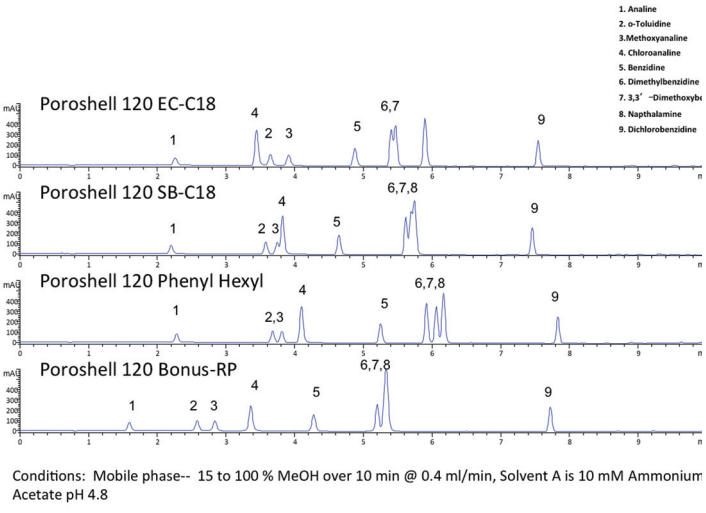


Figure 6: Using a column with superficially porous particles for faster methods at HPLC pressures. The method requirement was N > 4,000, Rs better than 11.5.

Hydrogen-bond donors

The polar embedded amine phase shows preferential retention of hydrogen-bond donors, as well as decreased retention for hydrogen-bond acceptors or ionised bases. This phase is generally less hydrophobic than alkyl or phenyl columns, and provides an improved peak shape for bases. Figure 8 compares the same four SPP columns in the separation of seven beta blockers.

Selecting the right bonded phase can often



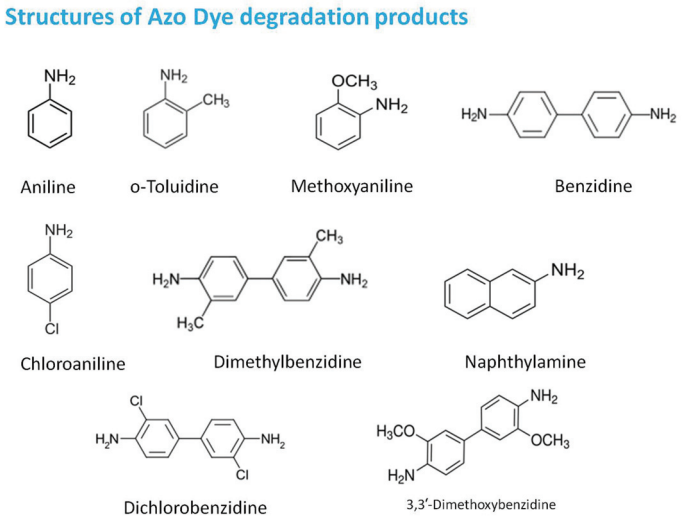


Figure 7: Separation of azo dye degredants. The Phenyl-Hexyl column with superficially porous particles shows the best separation of these polar compounds, with no co-elution.

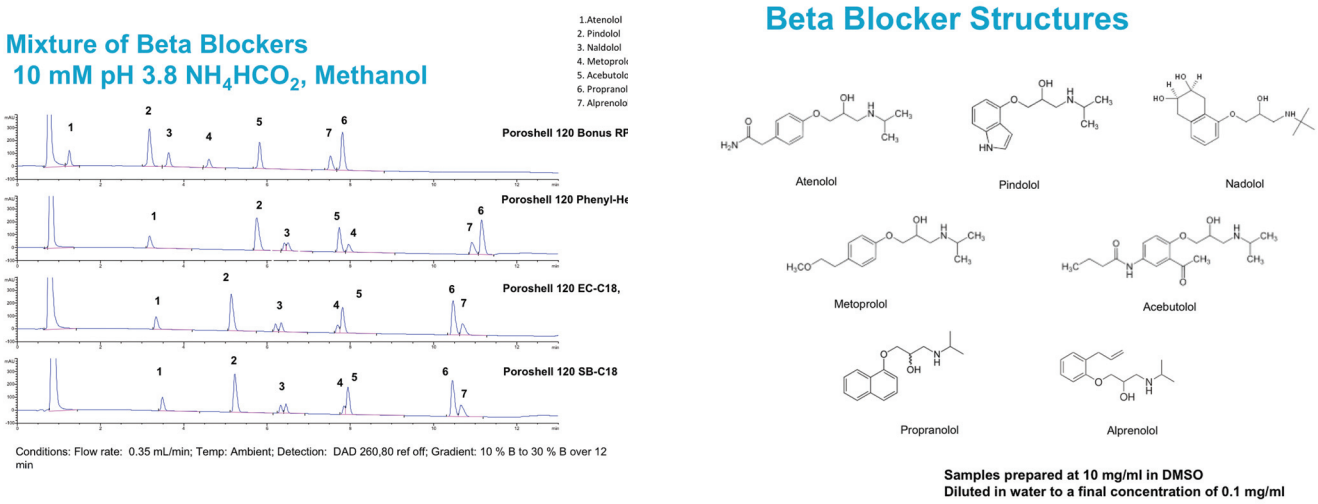


Figure 8: Separation of beta blockers. Poroshell 120 Bonus RP showing the best separation of these polar compounds and the earliest elution. Note the change in elution order

decrease the total run time and can change peak elution order for the analysis of samples that contain non-polar and polar compounds. Earlier eluting polar compounds, however, generally show smaller retention differences on these different columns - so that a savings in analysis time does not result in lost resolution for early eluting analytes. These selectivity differences can be used to develop faster separations with good resolution.

Conclusions

The impressive chromatographic performance of modern superficially porous particles has stimulated a great deal of research activity in the past few years. Applications with such particles continue to be developed and used in many industries on both HPLC and UHPLC systems. It is likely that quality control or contract research laboratories will receive methods with superficially porous particles from R&D laboratories in the future.

References

1. C.G. Horvath, B.A. Preiss, S.R. Lipsky. Anal. Chem., 39, 1422 (1967).

2. R. Majors. LC–GC North Am., 29, 218 (2011).