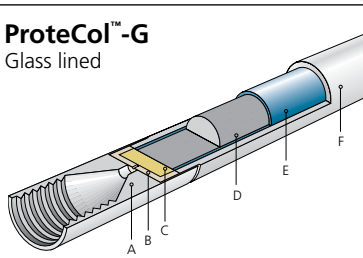
ProteCol™ LC Columns - Exceed the limit

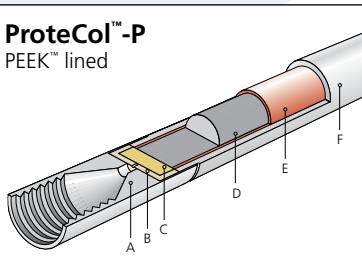
A premium inert range of LC columns delivering optimal peak shape.

ProteCol™-G
Glass lined



A Column end cap
B PEEK™ frit housing
C Porous PEEK™ frit
D Stationary phase
E Glass lining
F Stainless steel column body

ProteCol™-P
PEEK™ lined



A Column end cap
B PEEK™ frit housing
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F Stainless steel column body

50 SGE years
1960 - 2010

SGE Analytical Science
www.sge.com

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The ProteCol™ range of LC columns

The ProteCol™ range of LC columns feature proprietary column designs, incorporating inert materials throughout the flow path and the highest quality stationary phases. The combination of these factors delivers unparalleled separation performance.

Benefits of an inert flow path are:

- Optimized analyte recovery.
- Superior peak shape and reproducibility.
- Less artifacts due to reduced carry over.

ProteCol™-P C18 HQ105 – Premium phase for optimal performance

SGE's inert LC column range enables you to focus on your application rather than the column's performance.

The NIST SRM870 method is designed to provide an even bench mark for C18 column performance. As shown in Figure 1, ProteCol™-P C18 HQ105 approaches the 'ideal' performance of a column with no non-specific interactions.

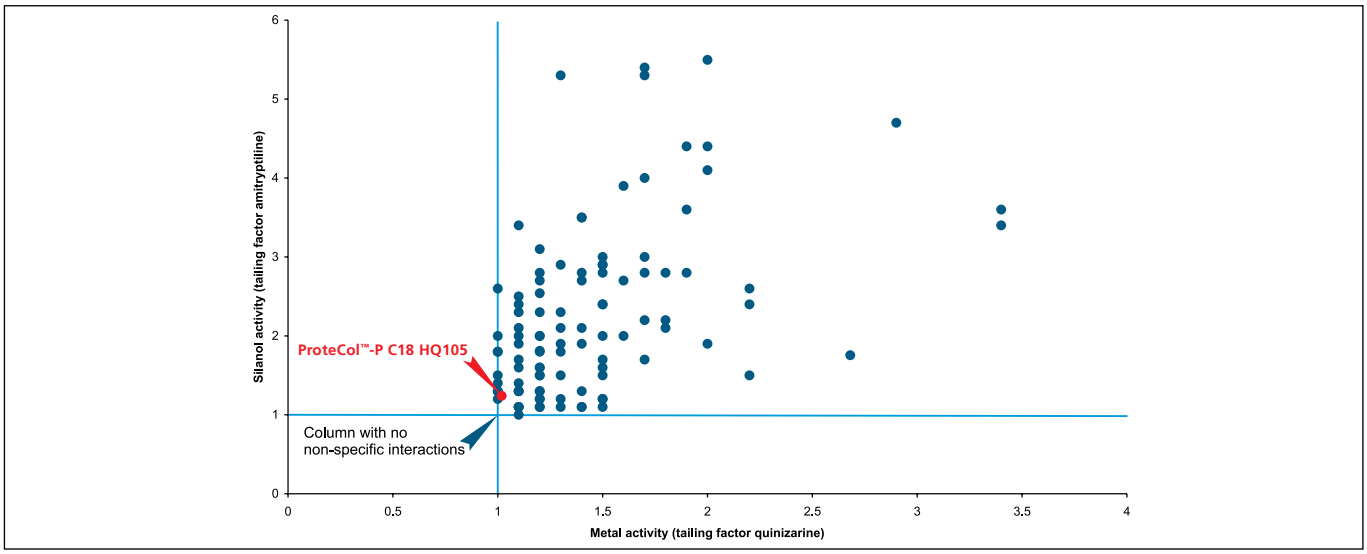

Figure 1: Non-specific interactions of the NIST SRM870 probe molecules on commercially available C18 columns. (Comparison data available at: www.usp.org/USPNF/columns.html)

Ideally, you have symmetrical peaks of the NIST SRM870 test mix – deviations indicate non-specific binding.

In Figure 2 the overlay of chromatograms illustrates clearly the superior peak performance of the ProteCol™-P C18 HQ105 phase compared to a Type I silica and an average C18 competitor's product.

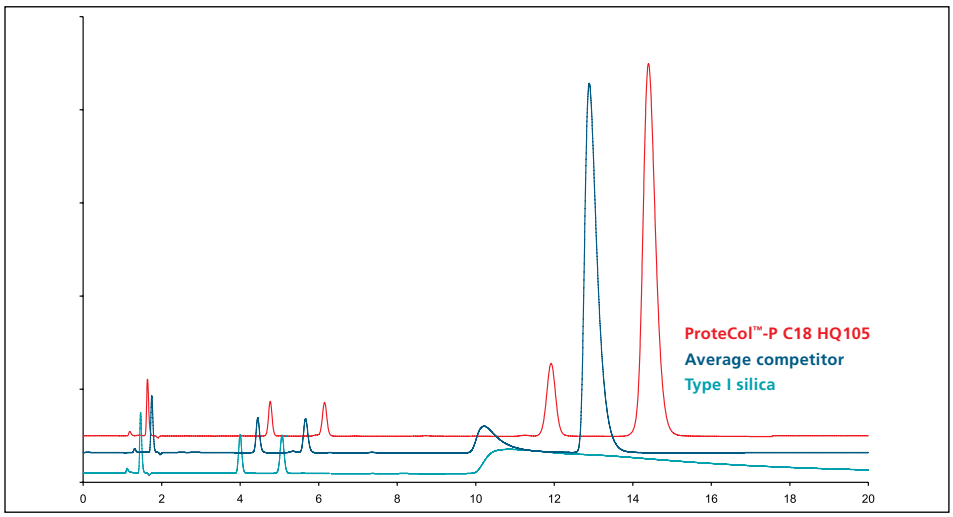

Figure 2. Chromatograms of the NIST SRM870 mix on different types of columns

Why metal-free chromatography?

Many pharmaceutical active substances are rich in oxygen and can therefore interact with metal (Fe). Our tests have shown the N-hydroxypyridine-2-on – the chelating part of the anti-fungal cyclopirox molecule – is a powerful probe for metal activity. In one experiment, a PEEK™ lined ProteCol™-P C18 HQ105 coated column was compared to a stainless steel ProteCol™ C18 HQ105 column where the connection tubing also varied between stainless steel and PEEKsil™ (PEEK™ coated fused silica tubing). As demonstrated in Figure 3, peak tailing was minimized with the reduction of metal in the flow path resulting in increased peak height – hence, sensitivity.

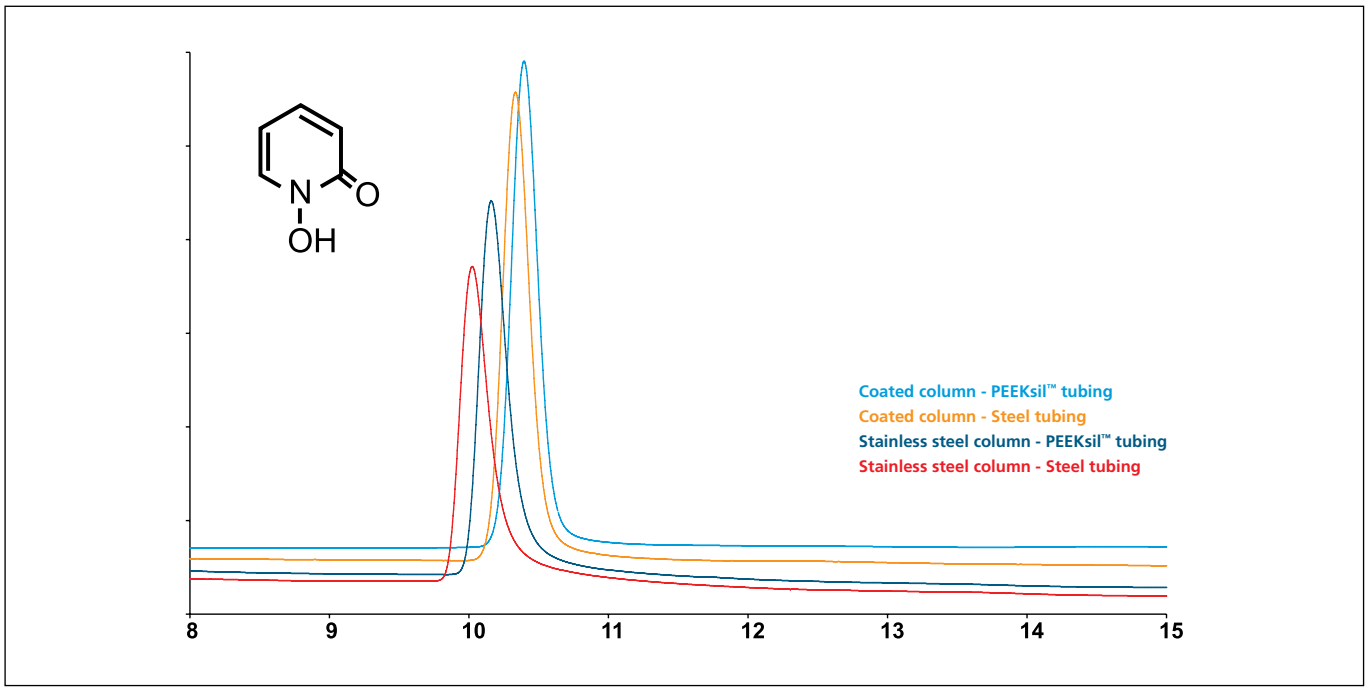

Figure 3: N-hydroxypyridine-2-on peak with various amounts of metal surfaces present in the flow path.

A second experiment was conducted with tetracycline antibiotics. Tetracycline and related drugs have a number of potential chelating groups aligned on one side of the molecule and are known to form metal complexes (dietary calcium and iron render the antibiotic ineffective). Figure 4 demonstrates the improved peak sensitivity using the PEEK™ lined ProteCol™-P C18 HQ105 column format.

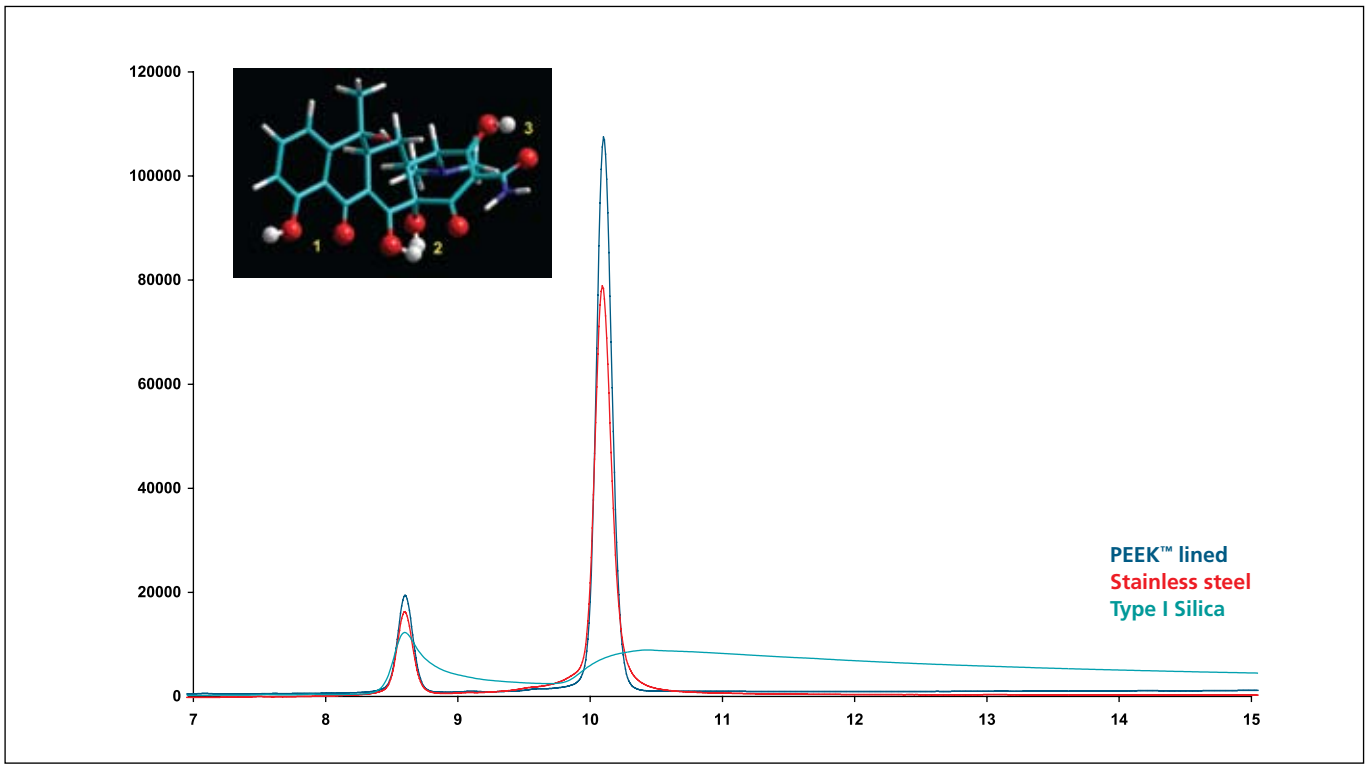

Figure 4: Chromatogram of tetracycline and its major degradation product. Note the peak broadening on the base of the peak run through the stainless steel column. Inset: the tetracycline molecule depicting the three potential chelating groups.

ProteCol™-P C18 HPH125 – Special phase for enhanced durability

The ProteCol™-P C18 HPH125 column has a specially modified stationary phase which allows it to be used outside commonly recommended pH ranges. While the silica matrix material of any silica based LC column has a stability limit of ~pH8, the special C18 modification of the support in combination with the PEEK™ lined column hardware, enables the column to be run at pH9 and above without deterioration of performance.

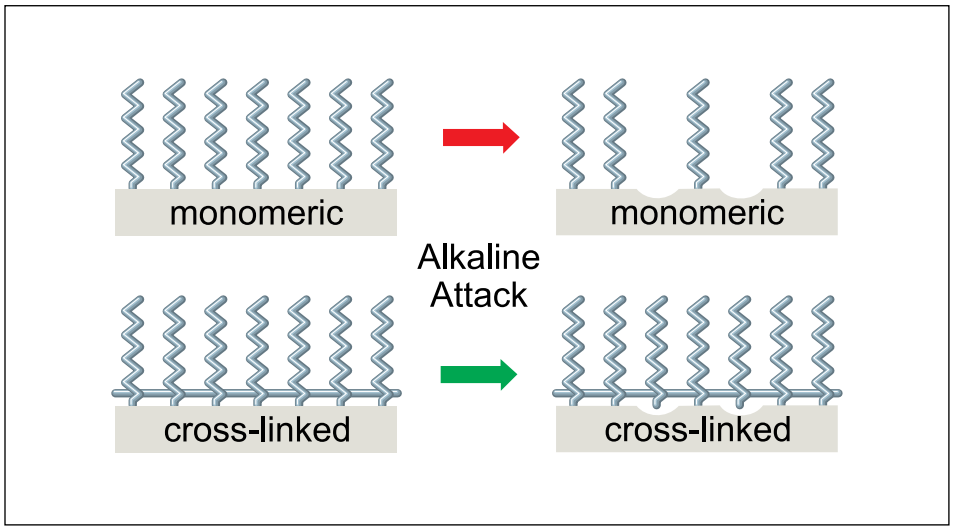

Figure 5: Difference between the pH stability of monomeric modified stationary phases and crosslinked stationary phases.

In our tests on the effects of elevated pH on ProteCol™-P C18 HPH125 chromatographic performance, we have observed that peak symmetry is the most sensitive parameter to measure column performance compared to the often quoted retention time or the k'-value. Figure 6 illustrates that peak symmetry on the ProteCol™-P C18 HPH125 column remains unchanged at pH 9.15 and over 3000 column volumes at 40°C. When compared to monomeric silica, peak symmetry deteriorated at less than 1000 column volumes whereas the retention time remained unchanged (see inset).

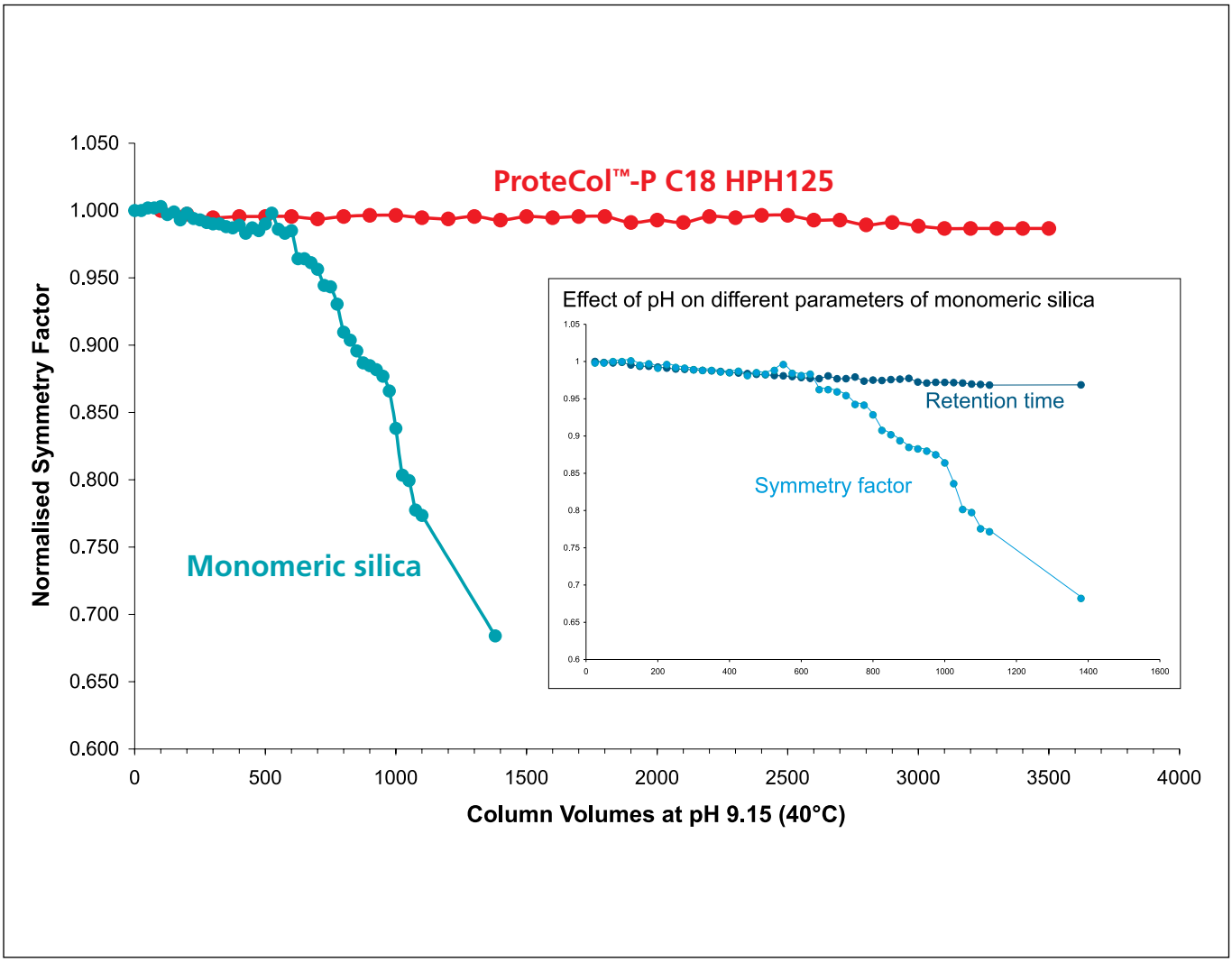

Figure 6: Effect of pH 9.15 on the performance of conventional monomeric silica and ProteCol™-P C18 HPH125.

Chemical properties of the ProteCol™ range

Stability at pH 1:

All columns in the ProteCol™ range show no deterioration when exposed to pH 1.0 buffers.

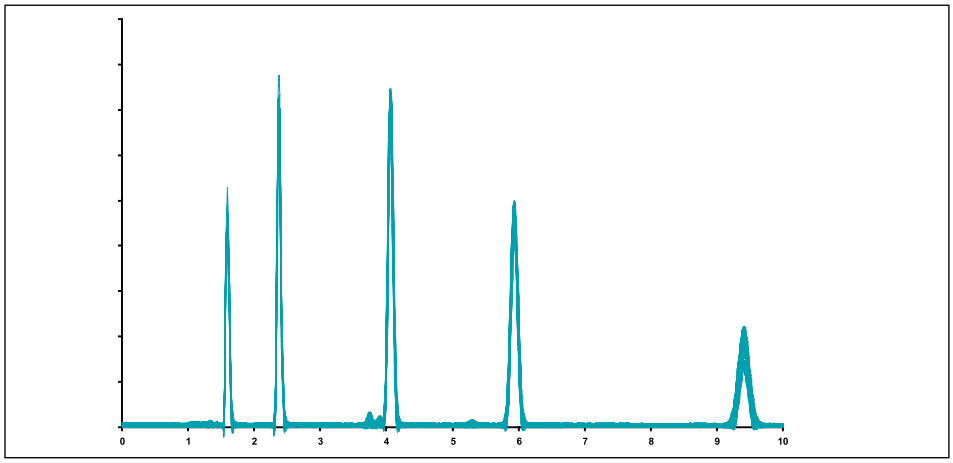

Figure 7: Overlay of 40 chromatograms run at pH 1.0 spanning 1200 column volumes

Long-term reproducibility

All columns in the ProteCol™ range show a remarkable reproducibility of thousands of injections (subject to sample purity and mobile phase conditions).

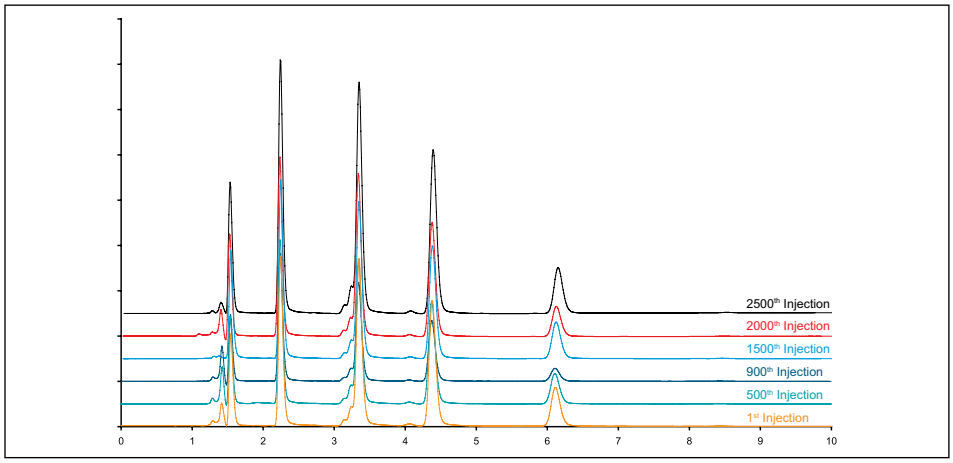

Figure 8: Chromatograms of a test mix over a period of 2500 injections

Analysis of larger molecules

When analyzing samples containing large molecules (peptides, proteins, polymers with MW>3000) the size of the molecule and the size of the pore structure play an important role in the quality of the separation. As the analyte increases in size (relative to the pore size) the diffusion rate inside the pore becomes smaller and mass transfer in and out of the pore system becomes slow leading to band broadening. Obviously, when the analyte size is equal to or bigger than the pore size there can be no pore diffusion. A mathematical description of this relationship was published by Renkin (E.M. Renkin, J.Gen.Physio., 38 (1954) 225.) and helps to illustrate the phenomenon.

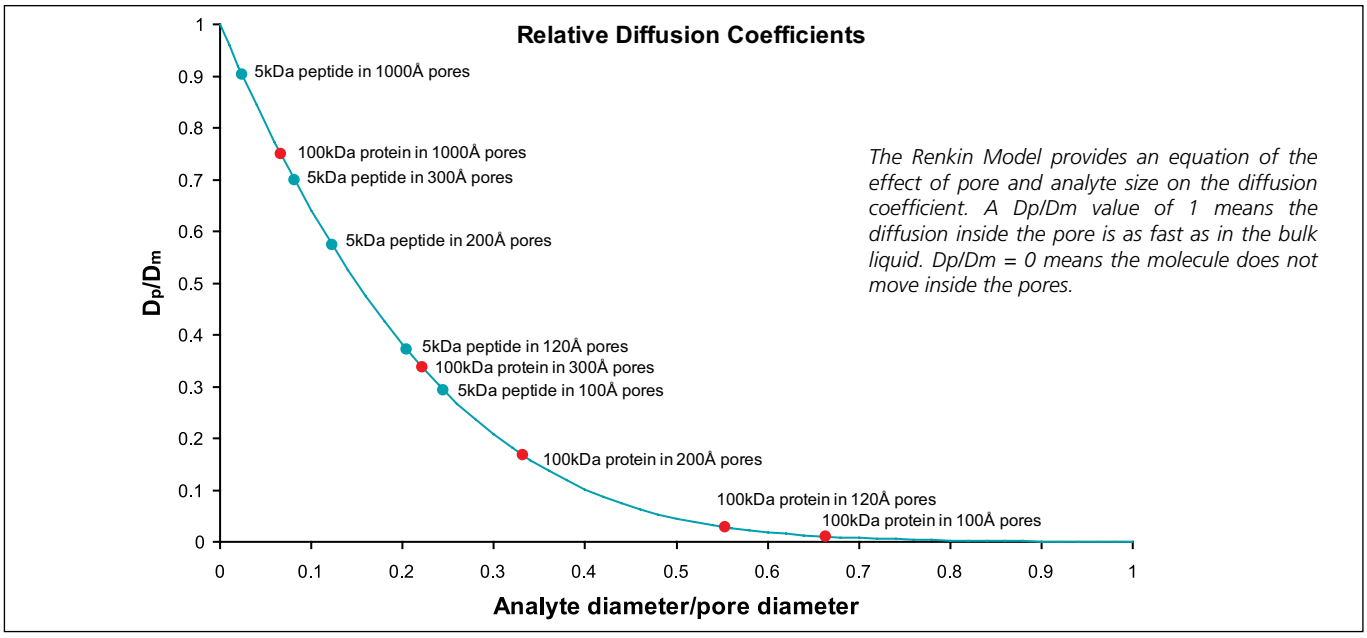

Figure 9: An illustration of the relative diffusion rate of a 5kDa peptide and a 100kDa protein in a number of pore systems

ProteCol™-G C18 HQ203 and HQ303 – Columns for peptides

ProteCol™-G C18 HQ203 and HQ 303 are columns packed with stationary phases of 200Å and 300Å pores, respectively. They both show reasonable fast diffusion rates for a typical peptide (MW~5000) or any other molecule of a similar size.

The difference between them is the specific surface area (surface area are per unit of weight) which in case of the 200Å pore size material is twice as high as for the 300Å pore size particles (200 and 100 m²/g respectively). The surface area is directly linked to the column capacity. Figure 10 shows that in terms of resolution and peak capacity both columns provide very similar results.

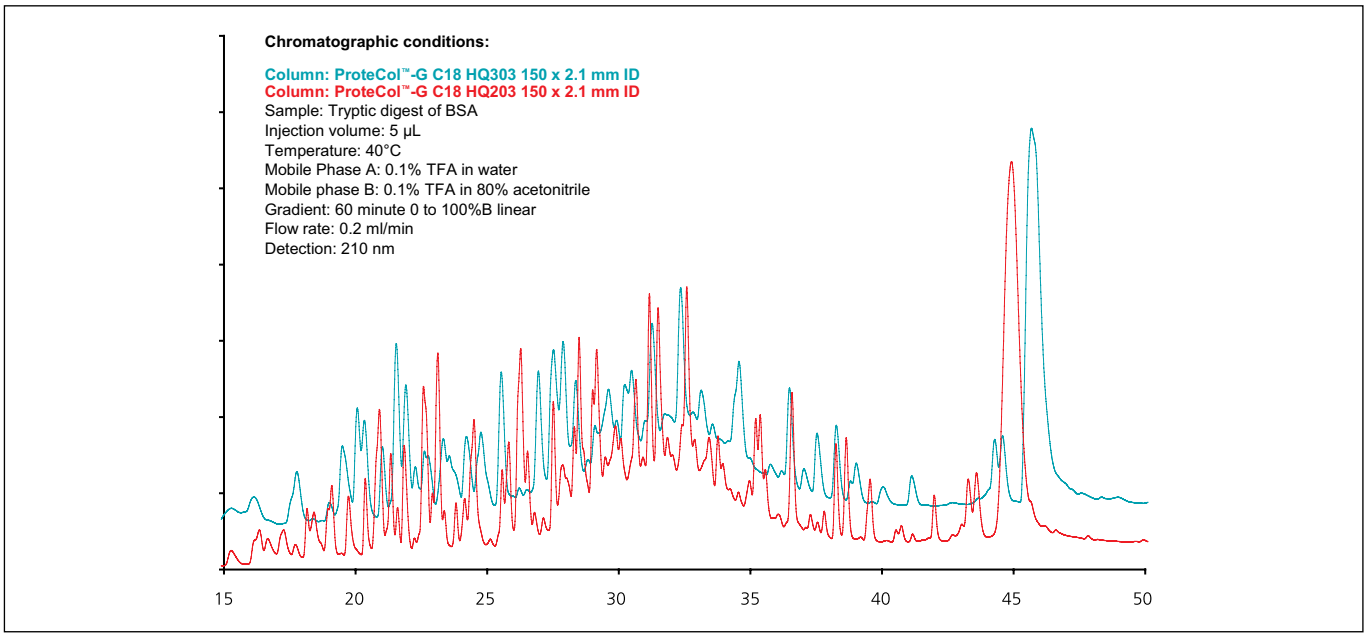

Figure 10: Separation of a BSA digest on a ProteCol™-G C18 HQ203 and ProteCol™-G C18 HQ303 column

ProteCol™-G C8 HQ1003 – The protein separation column

Figure 9 shows clearly that a 300Å pore size is not ideal for separating larger proteins. Our ProteCol™-G C8 HQ1003 column therefore uses a 1000Å pore size packing material.

Most proteins have a very hydrophobic core and they tend to unfold when coming in contact with a reversed phase surface. This can lead to very strong binding and sample loss. A C8 stationary phase gives much higher recoveries. The following example is the separation of the AOHUPO Membrane Protein Initiative mouse liver membrane proteins standard.

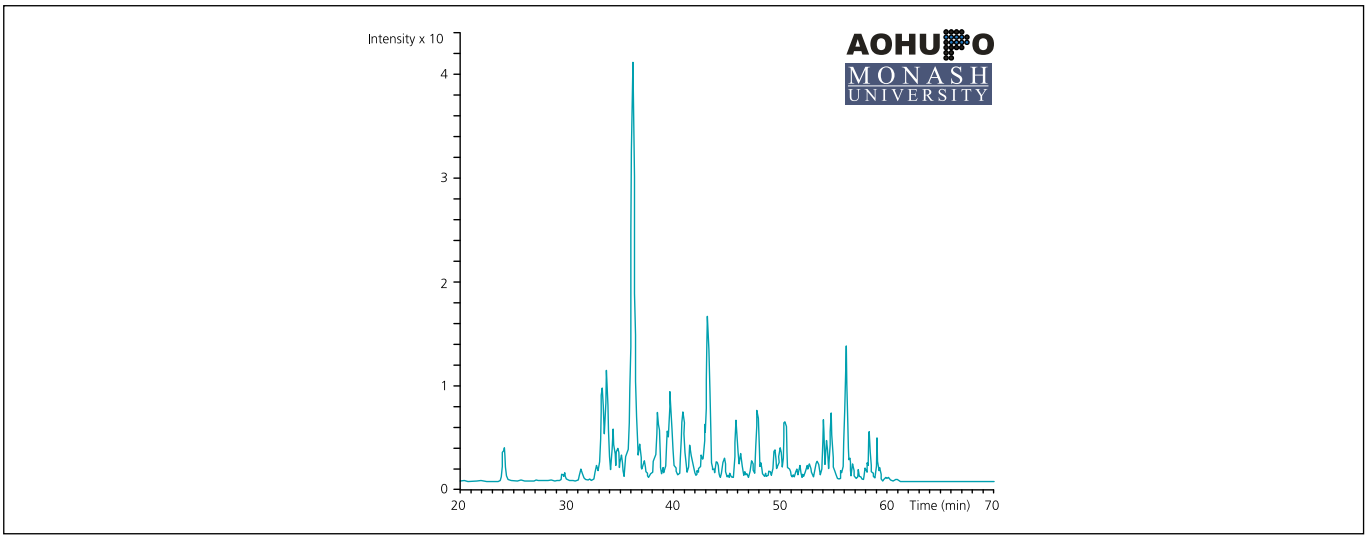

Figure 11: Base peak chromatogram of mouse liver membrane proteins separated on a ProteCol™-G C8 HQ1003 300 µm x 100mm column.

ProteCol™ – Product range and part numbers

Naming convention: **SGE ProteCol™-P C18 HQ105**

Lining
Stationary phase
Pore size with particle number
Phase description: HQ = High Quality, GP = General Purpose, HPH = High PH

Description	Stationary Phase	Pore Size	Particle Size	Lining	I.D.	Length	Pack Size	Part no.
Analytical Columns								
ProteCol™-P C18 HQ105	C18 HQ	100 Å	5 µm	PEEK™ lined	4.6 mm	250 mm	1	250100
ProteCol™-P C18 HQ105	C18 HQ	100 Å	5 µm	PEEK™ lined	4.6 mm	150 mm	1	250102
ProteCol™-P C18 HQ105	C18 HQ	100 Å	5 µm	PEEK™ lined	2.1 mm	250 mm	1	250105
ProteCol™-P C18 HQ105	C18 HQ	100 Å	5 µm	PEEK™ lined	2.1 mm	150 mm	1	250107
ProteCol™-P C18 HQ103	C18 HQ	100 Å	3 µm	PEEK™ lined	2.1 mm	150 mm	1	250200
ProteCol™-P C18 HQ103	C18 HQ	100 Å	3 µm	PEEK™ lined	2.1 mm	100 mm	1	250202
ProteCol™-P C18 HPH125	C18 HPH	120 Å	5 µm	PEEK™ lined	4.6 mm	250 mm	1	250110
ProteCol™-P C18 HPH125	C18 HPH	120 Å	5 µm	PEEK™ lined	4.6 mm	150 mm	1	250112
ProteCol™-P C18 HPH125	C18 HPH	120 Å	5 µm	PEEK™ lined	2.1 mm	250 mm	1	250115
ProteCol™-P C18 HPH125	C18 HPH	120 Å	5 µm	PEEK™ lined	2.1 mm	150 mm	1	250117
ProteCol™ C18 GP125	C18 GP	120 Å	5 µm	SS (PEEK™ frit)	4.6 mm	250 mm	1	250210
ProteCol™ C18 GP125	C18 GP	120 Å	5 µm	SS (PEEK™ frit)	4.6 mm	150 mm	1	250212
ProteCol™ C18 GP125	C18 GP	120 Å	5 µm	SS (PEEK™ frit)	2.1 mm	250 mm	1	250215
ProteCol™ C18 GP125	C18 GP	120 Å	5 µm	SS (PEEK™ frit)	2.1 mm	150 mm	1	250217
ProteCol™-G C18 HQ305	C18 HQ	300 Å	5 µm	GLT™	4.6 mm	250 mm	1	250120
ProteCol™-G C18 HQ305	C18 HQ	300 Å	5 µm	GLT™	4.6 mm	150 mm	1	250122
ProteCol™-G C18 HQ305	C18 HQ	300 Å	5 µm	GLT™	2.1 mm	250 mm	1	250125
ProteCol™-G C18 HQ305	C18 HQ	300 Å	5 µm	GLT™	2.1 mm	150 mm	1	250127
ProteCol™-G C18 HQ303	C18 HQ	300 Å	3 µm	GLT™	2.1 mm	150 mm	1	250130
ProteCol™-G C18 HQ303	C18 HQ	300 Å	3 µm	GLT™	2.1 mm	100 mm	1	250132
ProteCol™-G C18 HQ303	C18 HQ	300 Å	3 µm	PEEKsil™	300 µm	150 mm	1	250135
ProteCol™-G C18 HQ303	C18 HQ	300 Å	3 µm	PEEKsil™	300 µm	100 mm	1	250137
ProteCol™-G C18 HQ303	C18 HQ	300 Å	3 µm	PEEKsil™	150 µm	150 mm	1	250140
ProteCol™-G C18 HQ303	C18 HQ	300 Å	3 µm	PEEKsil™	150 µm	100 mm	1	250142
ProteCol™-G C18 HQ203	C18 HQ	200 Å	3 µm	GLT™	2.1mm	150 mm	1	250150
ProteCol™-G C18 HQ203	C18 HQ	200 Å	3 µm	GLT™	2.1mm	100 mm	1	250152
ProteCol™-G C18 HQ203	C18 HQ	200 Å	3 µm	PEEKsil™	300 µm	150 mm	1	250155
ProteCol™-G C18 HQ203	C18 HQ	200 Å	3 µm	PEEKsil™	300 µm	100 mm	1	250157
ProteCol™-G C18 HQ203	C18 HQ	200 Å	3 µm	PEEKsil™	150 µm	150 mm	1	250160
ProteCol™-G C18 HQ203	C18 HQ	200 Å	3 µm	PEEKsil™	150 µm	100 mm	1	250162
ProteCol™-G C8 HQ125	C8 HQ	120 Å	5 µm	GLT™	4.6 mm	250 mm	1	250190
ProteCol™-G C8 HQ125	C8 HQ	120 Å	5 µm	GLT™	4.6 mm	150 mm	1	250192
ProteCol™-G C8 HQ125	C8 HQ	120 Å	5 µm	GLT™	2.1 mm	250 mm	1	250195
ProteCol™-G C8 HQ125	C8 HQ	120 Å	5 µm	GLT™	2.1 mm	150 mm	1	250197
ProteCol™-G C8 HQ1003	C8 HQ	1000 Å	3 µm	GLT™	2.1mm	150 mm	1	250170
ProteCol™-G C8 HQ1003	C8 HQ	1000 Å	3 µm	GLT™	2.1mm	100mm	1	250172
ProteCol™-G C8 HQ1003	C8 HQ	1000 Å	3 µm	PEEKsil™	300 µm	150 mm	1	250175
ProteCol™-G C8 HQ1003	C8 HQ	1000 Å	3 µm	PEEKsil™	300 µm	100mm	1	250177
ProteCol™-G C8 HQ1003	C8 HQ	1000 Å	3 µm	PEEKsil™	150 µm	150 mm	1	250180
ProteCol™-G C8 HQ1003	C8 HQ	1000 Å	3 µm	PEEKsil™	150 µm	100mm	1	250182

Description	Stationary Phase	Pore Size	Particle Size	Frit	I.D.	Length	Pack Size	Part no.
Guard Columns								
ProteCol™ C18 GP125	C18 GP	120 Å	5 µm	PEEK™	2.1 mm	10 mm	3	250032
ProteCol™ C18 GP125	C18 GP	120 Å	5 µm	PEEK™	4 mm	10 mm	3	250033
ProteCol™ C8 HQ125	C8 HQ	120 Å	5 µm	PEEK™	2.1 mm	10 mm	3	250003
ProteCol™ C8 HQ125	C8 HQ	120 Å	5 µm	PEEK™	4 mm	10 mm	3	250005
ProteCol™ C18 HQ105	C18 HQ	100 Å	5 µm	PEEK™	2.1 mm	10 mm	3	250007
ProteCol™ C18 HQ105	C18 HQ	100 Å	5 µm	PEEK™	4 mm	10 mm	3	250009
ProteCol™ C18 HPH125	C18 HPH	120 Å	5 µm	PEEK™	2.1 mm	10 mm	3	250013
ProteCol™ C18 HPH125	C18 HPH	120 Å	5 µm	PEEK™	4 mm	10 mm	3	250015
ProteCol™ C8 HQ1003	C8 HQ	1000 Å	3 µm	PEEK™	2.1 mm	10 mm	3	250019
ProteCol™ C8 HQ1003	C8 HQ	1000 Å	3 µm	PEEK™	4 mm	10 mm	3	250017
ProteCol™ C18 HQ203	C18 HQ	200 Å	3 µm	PEEK™	2.1 mm	10 mm	3	250021
ProteCol™ C18 HQ203	C18 HQ	200 Å	3 µm	PEEK™	4 mm	10 mm	3	250023
ProteCol™ C18 HQ305	C18 HQ	300 Å	5 µm	PEEK™	2.1 mm	10 mm	3	250025
ProteCol™ C18 HQ305	C18 HQ	300 Å	5 µm	PEEK™	4 mm	10 mm	3	250027
ProteCol™ C18 HQ303	C18 HQ	300 Å	3 µm	PEEK™	2.1 mm	10 mm	3	250029
ProteCol™ C18 HQ303	C18 HQ	300 Å	3 µm	PEEK™	4 mm	10 mm	3	250031

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