PRODUCT INFORMATION

Chromalite® resins for reverse-phase chromatography, adsorption and SPE

Presents an overview of the advantages of Purolite's Chromalite® polymeric resins over silica for the purification of biomolecules in reverse-phase chromatography.

Purolite®

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Chromalite® product line

Mechanical robustness, inertness, pH stability, compatibility with both polar and non-polar organic solvents and even water are the most desirable properties of modern chromatographic resins.

Purolite provides a comprehensive line of products for chromatographic applications, ranging from ion exchange resins to non-functionalized resins for reverse-phase chromatography (RPC), with particle size ranges from 10 micron to up to > 200 micron. Most products are characterized by narrow particle size distribution. Smaller particle sizes (3, 4 and 5 micron) are available on request for analytical HPLC applications.

Table 1 shows the complete Chromalite product portfolio, including ion exchange resins (CGA, CGC, SBG, SBM products) and RPC resins.

Chromalite ion exchange resins are described in detail in a separate technical brochure, "Chromalite CGA and CGC Ion Exchange Resins," which can be found on www.purolite.com. This information guide looks specifically at Chromalite polymeric resins for reverse-phase chromatography (RPC).

Table 1 – Overview of Chromalite products by particle size and functional group

TYPE	MEAN PARTICLE SIZE*						
	10 µm	15 µm	35 µm	50 µm	75 µm	125 µm	> 200 µm
CATION EXCHANGE				CGC50X2 CGC50X4 CGC50X8		CGC100X2 CGC100X4 CGC100X8	CGC200X2 CGC200X4 CGC200X8
ANION EXCHANGE		15SBG		CGA50X2 CGA50X4 CGA50X8 50SBM		CGA100X2 CGA100X4 CGC100X8	CGA200X2 CGA200X4 CGA200X8
REVERSE PHASE	10AD1 10AD2 10MN	15AD1 15AD2 15MN PCG1200F15	PCG600F PCG900F PCG1200F		70MN PCG600M PCG900M PCG1200M	PCG600C PCG900C PCG1200C	

* Smaller particle sizes are available by request.

Table 2 shows the range of applications of Chromalite RPC products depending on particle size.

Table 2 –Chromalite applications depending on resin particle size

CHROMALITE SIZE RANGE (µm)	APPLICATION
10, 15	Preparative RP-HPLC Process/polishing of small molecules and synthetic biomolecules
35	Process/polishing of synthetic biomolecules
50, 75	Low-pressure chromatography, front-end capture, desalting, and purification applications of small organic molecules and synthetic biomolecules (peptides and oligonucleotides) Solid phase extraction
> 75	Large-scale purification of small organic molecules and synthetic biomolecules

Reverse-phase chromatography

Reverse-phase chromatography is a proven technique for the purification of biomolecules and results from the adsorption of molecules onto a hydrophobic solid support in a polar mobile phase. Decreasing the mobile phase polarity by adding more organic solvent reduces the hydrophobic interaction between the solute and the solid support resulting in desorption. The more hydrophobic the molecule, the more time it will spend on the solid support and the higher the concentration of organic solvent required to promote desorption.

- RPC applies to a very wide range of molecules including charged and polar molecules
- Variables such as organic solvent type and concentration, pH and temperature are used to optimize and control separation in RPC
- Once the stationary phase is packed in a column, RPC columns are robust, efficient and stable
- RPC media are robust and tolerate a wide range of pH, use of harsh chemicals, wide temperature range and are easily regenerated and cleaned

At process scale, RPC is applied to organic compounds like APIs, biomolecules like peptides and oligonucleotides. RPC is rarely used for purification of larger biomolecules since the presence of the organic solvent can cause denaturation of proteins and destroy their biological activity. For purification of proteins, more hydrophilic matrices like agarose or acrylates are preferred. There are exceptions, such as the successful industrial purification by reverse phase of insulin, which will be described later. Reverse-phase chromatography is the most common analytical HPLC technique. There are many different stationary phases available for method optimization, like silica functionalized with hydrophobic groups (C18, C8 silica) or the more robust polymeric phases like Chromalite.

Advantages of synthetic polymeric material over silica

Polymeric resins are becoming used more frequently in modern liquid chromatography applications thanks to great improvements in resin design and manufacturing technology and the discovery of new applications.

Modern polymers have overcome earlier problems associated with their use such as swelling. High rigidity and strength of new polymers now allow their use at high eluent flow rates resulting in faster analysis and increased column life. Improvements in synthetic resins have also increased efficiency, particularly in comparison to even the best silica materials. At times, use of polymeric materials can achieve efficiencies exceeding 100,000 plates/m.

In addition, polymers offer distinct advantages over silica packing for particular applications. Silica media are sensitive to more extreme pH conditions, additives such as metals and buffer salts.

In comparison, polymeric resins are particularly useful for purification and analysis of amino acids, peptides, small proteins such as insulin, organic acids, carbohydrates, and inorganic cations and anions.

Chromalite polymers for reverse-phase chromatography

Purolite offers a wide range of Chromalite products for reverse- phase chromatography with particle size ranging from 10 micron to greater than 200 micron.

All these products are manufactured with the highest industry standard to achieve:

- high mechanical stability
- uniform particle size
- control of porosity
- high chemical stability
- More accurate separations

Packaging options:

- Slurry: 20% ethanol for particle size lower than 45 micron (See Table 3)
- Wet: for all products with particle size higher than 45 micron (See Table 4)
- Dry: specific for resins used for solid phase extraction by reverse-phase mode (MN products) for easy cartridge filling and packing

Table 3 – Chromalite products with particle size < 45 micron (supplied as 20% EtOH slurry)

PRODUCT	PARTICLE SIZE * (90% in range) (µm)	SURFACE AREA (m^2/g)	PORE DIAMETER (Å)	BULK DENSITY (g_{dry}/mL of bed volume)
10AD1[†]	9 – 11	> 400	100	0.28 – 0.33
10AD2[†]	9 – 11	> 400	300 – 700	0.28 – 0.33
15AD1[†]	9 – 11	> 400	100	0.28 – 0.33
15AD2[†]	13.5 – 16.5	> 400	300 – 700	0.28 – 0.33
10MN	9 – 11	> 1400	30	0.28 – 0.33
15MN	13.5 – 16.5	> 1400	30	0.28 – 0.33
PCG600F	20 – 55	> 700	100	0.20 – 0.24
PCG900F	20 – 55	> 600	200 – 300	0.17 – 0.20
PCG1200F15	12 – 18	> 600	300 – 700	0.16 – 0.19
PCG1200F	20 – 55	> 600	300 – 700	0.16 – 0.19

† Smaller particle sizes are available by request.
* 90% in the range

Table 4 –Purolite Chromalite resins with particle size >45 micron (supplied as wet)

PRODUCT	PARTICLE SIZE * (µm)	SURFACE AREA (m^2/g)	PORE DIAMETER (Å)	BULK DENSITY (g_{dry}/mL of bed volume)	TOTAL MOISTURE AS SHIPPED (%)
70MN[††]	65 – 75	> 1400	30	0.28 – 0.33	<5
PCG600M	50 – 100	> 700	100	0.20 – 0.24	55 – 60
PCG600C	85 – 155	> 700	100	0.20 – 0.24	55 – 60
PCG900M	50 – 100	> 600	200 – 300	0.17 – 0.20	65 – 70
PCG900C	85 – 155	> 600	200 – 300	0.17 – 0.20	65 – 70
PCG1200M	50 – 100	> 600	300 – 700	0.16 – 0.19	65 – 70
PCG1200C	85 – 155	> 600	300 – 700	0.16 – 0.19	65 – 70

†† Supplied in dry form for easy packing of SPE cartridges. Available in wet form by request.
* 90% in the range

Different resins for different applications

Chromalite AD and Chromalite PCG rigid polymeric reverse-phase resins are used extensively for analysis and purification in reverse-phase chromatography. The range of particle sizes provides scalability from an analytical platform through to industrial high-performance purification. Purolite offers three different resins featuring a

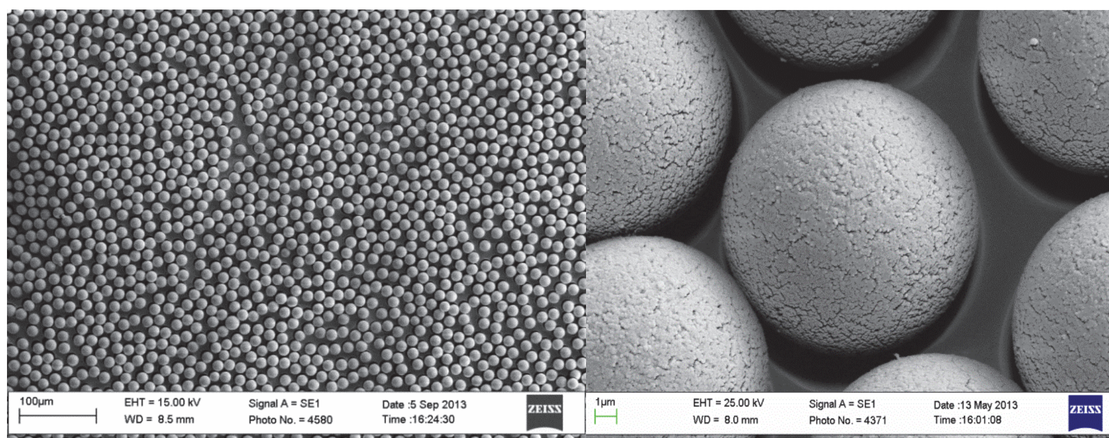
selection of particle sizes, porosities and surface areas, all producing stable packed columns with excellent flow rates, chemical stability and capacity.

Chromalite AD

Chromalite AD1 and AD2 are hydrophobic polystyrene porous adsorbents of high divinylbenzene content with outstanding chemical and physical stability. Chromalite AD matrices are designed for preparative reverse-phase chromatography with particle size of 10 or 15 micron.

Chromalite AD1 and AD2 HPLC resins are inherently hydrophobic and an excellent alternative to C18 or C8 silica matrices. Chromalite AD1 has a medium porosity (100Å), ideal for separation of medium/small molecules with MW < 1000Da, and can substitute C8/C18 silica with porosity of 50 – 100Å. Chromalite AD2 has a higher porosity (300 – 700Å), which is ideal for separation of larger molecules (> 1000Da) such as synthetic oligomers, biomolecules, peptides, small proteins and oligonucleotides.

Figure 1 – Electron microscopy of Chromalite AD1 (15 micron)



a) Uniform particle size distribution **b) Controlled porosity**

Chromalite PCG

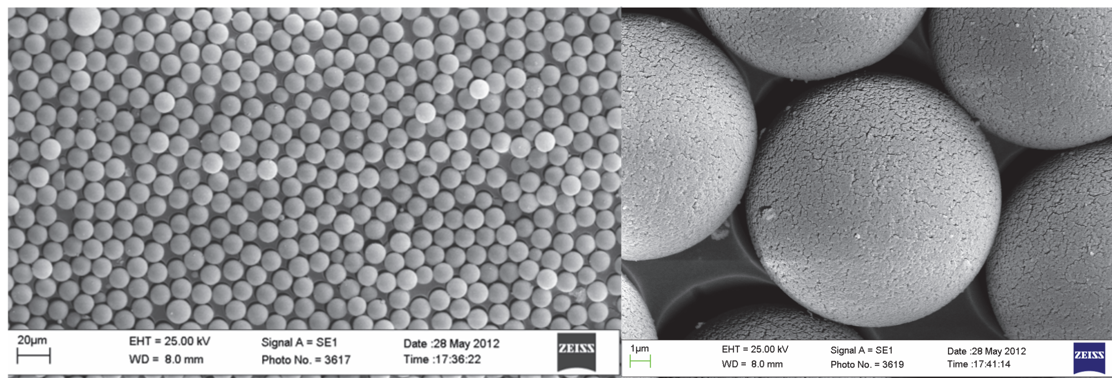
Chromalite PCG resins are macroporous hydrophobic polystyrene adsorbents intended for use in high-, medium- and low-pressure reverse-phase chromatography. Three different porosities enable Chromalite PCG resins to accommodate a variety of molecule sizes.

- High porosity: Chromalite PCG1200 with a porosity of 300 – 700 Å
- Medium porosity: Chromalite PCG900 with a porosity of 200 – 300 Å
- Low porosity: Chromalite PCG600 with a porosity of 100 Å

Chromalite PCG resins can be used for RP-HPLC or medium- and low-pressure RP-LC due to the particle sizes offered:

- 12 – 18 micron (Chromalite PCG1200F15)
- 20 – 55 micron (F grade)
- 50 – 100 micron (M grade)
- 85 – 150 micron (C grade)

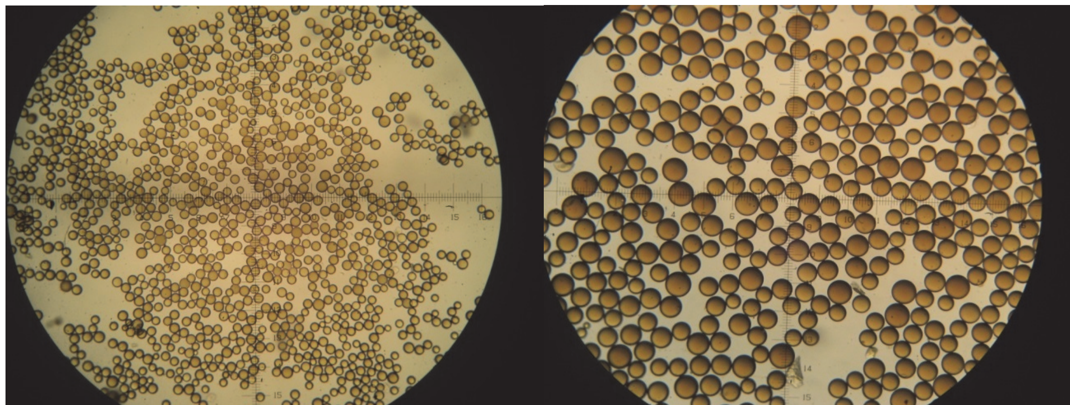
Figure 2 –Electron microscopy of Chromalite PCG1200F15 (12 – 18 micron)



a) Uniform particle size distribution

b) Controlled porosity

Figure 3 – Optical photographs of PCG1200F (20 – 55 micron) and PCG1200M (50 – 100 micron)



a) F grade resin

b) M grade resin

Chromalite MN

Principally differing from conventional styrene-divinylbenzene copolymers like Chromalite PCG and AD, Chromalite MN, hypercrosslinked polystyrene is obtained by an extensive post-crosslinking of long polystyrene chains. The resulting structure of hypercrosslinked polystyrene is an expanded, rigid three-dimensional network.

Chromalite MN takes advantage of Purolite proprietary technology.[1]

The open-work-type hypercrosslinked network displays an extremely high apparent inner surface area (up to 1000-1500 m^2/g) and no change in solvent uptake in both polar and non-polar resins, which enables good compatibility of the material with all mobile phases—from hexane to methanol and water. The whole interior of the hypercrosslinked polystyrene bead is well accessible to small/medium size analytes thanks to the network composition formed by small micro pores of about 2.0 to 4.0 nm in diameter.

Solid phase extraction (SPE) is an increasingly useful sample preparation technique. With SPE, many of the problems associated with liquid/liquid extraction can be prevented, such as incomplete phase separations, less-than-quantitative recoveries, use of expensive, breakable specialty glassware and disposal of large quantities of organic solvents. SPE is more efficient than liquid/liquid extraction, yields quantitative extractions that are easy to perform, is rapid and can be automated. It also reduces solvent use and lab time.

Chromalite MN matrices are ideal for solid-phase extraction (SPE) of small and medium organic compounds with aromatic functions.

The robustness of Chromalite MN resins also makes this hypercrosslinked non-modified polystyrene resin ideal for HPLC applications. Due to compatibility with any type of organic solvents and water, Chromalite MN can operate throughout a wide range of eluent polarity. Combining the non-polar Chromalite MN with an aqueous or polar aqueous-organic mobile phase corresponds to conventional reverse-phase mode of chromatography.

While hypercrosslinked polystyrene is chemically stable and easily withstands temperatures of 200°C and higher, the target application of Chromalite MN is modern high-temperature HPLC. Enhanced temperatures, through facilitating mass transfer, substantially increase column efficiency and reduce analysis time.

Chromalite MN ensures the following:

- Exceptional lot-to-lot reproducibility
- High recoveries and yields resulting from exceptional high surface area/binding sites
- Exceptional pH stability (1 to 14)
- Excellent packing and storage qualities due to the dry supply of the resin

Chromalite MN is manufactured with very narrow particle distribution and it is offered in three different particle sizes:

- 9 – 11 micron: Chromalite 10MN
- 13.5 – 16.5 micron: Chromalite 15MN
- 65 – 75 micron: Chromalite 70MN

Small particle sizes (5 micron) are also available by request.

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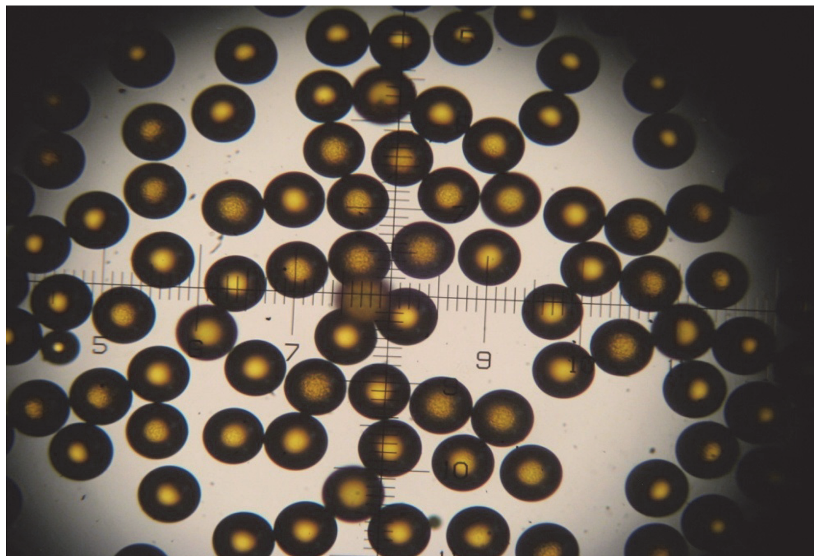
[1] [1] C.S. Sychov, M.M. Ilyin, V.A. Davankov, K.O. Sochilina. Elucidation of retention mechanisms on hypercrosslinked polystyrene used as column packing material for high performance liquid chromatography. / *J. Chromatogr. A* 1030 (2004) 17-24

[2] V.A. Davankov, C.S. Sychov, M.M. Ilyin, K.O. Sochilina. Hypercrosslinked polystyrene as a novel type of high-performance liquid chromatography column packing material. Mechanisms of retention. / *J. Chromatogr. A* 987 (2003) 67-75

[3] V. Davankov, M. Tsyurupa, M. Ilyin, L. Pavlova. Hypercross-linked polystyrene and its potentials for liquid chromatography: a mini-review, / *J. Chromatogr. A* 965 (2002) 65

Resins are offered in dry form for the analysis of biological fluids to make packing of SPE cartridges easy and reproducible.

Figure 4 – Optical photographs of Chromalite 70MN



pH and chemical stability

Chromalite AD, PCG and MN are poly(styrene/divinylbenzene) copolymers with excellent chemical stability. This gives flexibility in the choice of elution conditions and cleaning regimes, including the use of sodium hydroxide, which increases sample throughput and the number of cycles achieved per column. All resins are stable from pH 1 to 14.

Column efficiency testing

The preparation and qualification of packed columns are important steps to ensure robustness and safety for both the purification process and the final product. Column efficiency testing plays a central role in the qualification and monitoring of packed bed performance.

Column packing efficiency tests were performed using sodium chloride as a tracer. 0.4 M sodium chloride was added to the eluent to suppress any possible charge interaction effects between the resin and a tracer. Column packing efficiency test was evaluated based on HETP and asymmetry factor. Evaluation tests were carried out as follows:

Table 5 – Column efficiency testing for Chromalite PCG600, Chromalite PCG900 and Chromalite PCG1200 with NaCl tracer

GRADE	PARTICLE SIZE (µm)	PLATES (per meter)	PLATE HEIGHT (cm)	ASYMMETRY*
F	20 – 55	> 3000	0.03	1.4
M	50 – 100	> 1800	0.04	1.8
C	85 – 155	> 900	0.08	0.9

* An asymmetry factor close to AS = 1 is ideal. A typical acceptable range would be 0.8 – 1.8.

Experimental conditions:

Column type: HiScale™ 16/20
Bed height: 20 cm
Bed volume: 40 mL
Flow rate: 200 cm/h
Equilibration: 2.5 CV 0.4 M NaCl
Tracer: 0.01 CV 2 M NaCl
Detection: Conductivity, mS/cm
Elution: 1.5 CV 0.4 M NaCl
System: ÄKTA avant 25

Table 6 – Column efficiency testing for Chromalite PCG600, Chromalite PCG900 and Chromalite PCG1200 with Lysozyme tracer

GRADE	PARTICLE SIZE (µm)	PLATES (per meter)	PLATE HEIGHT (cm)	ASYMMETRY*
F	20 – 55	> 1800	0.05	2.5
M	50 – 100	> 500	0.16	1.6
C	85 – 155	> 400	0.23	1.2

* An asymmetry factor close to *AS* = 1 is ideal. A typical acceptable range would be 0.8 – 1.8.

Experimental conditions:

Column type: HiScale™ 16/20
Bed height: 20 cm
Bed volume: 40 mL
Flow rate: 300 cm/h
Equilibration: 1 CV 20% ethanol in water
Tracer: 0.01 CV of 1% acetone in 20% ethanol solution
Detection: UV1, 280 nm
Elution: 2.5 CV of 20% ethanol in water
System: ÄKTA avant 25

Quality, reproducibility and scalability

Chromalite AD, PCG and MN resins are produced with the highest level of quality. The flexibility of particle size allows for applications to range from analytical/prep to process development.

Packing procedures

- Drain the resin of water/ethanol (EtOH) and replace with EtOH or methanol (MeOH). Avoid drying the resins. In case of supplied dried resins, these can be used directly to fill cartridges.
- Suspension slurry should have a concentration of 5 – 20% by volume of adsorbent. It should be homogeneous with no aggregates. Mild treatment with ultrasound can be used to prevent aggregation.
- To determine quantity for packing, see bulk densities within Tables 3 and 4.
- Use 10 – 15% excesses of resin to fill columns and to allow some resin bed compression.
- Pack preparative column at 50 bars pressure for the best performance. Analytical columns can be packed at 100 – 200 bar. Chromalite AD resins do not get damaged at pressures up to 300 bar, but can develop pore compression, which can deteriorate resin performance.

Always keep packed column wet in alcohol/water eluent. To regenerate the resins, use 1 – 2 N caustic and 6 M thiourea, if needed. Also hydrochloric and acetic acids in any concentration could be applied to Chromalite. Avoid using pure toluene, chlorinated hydrocarbons, acetonitrile and acetone for column washing as well as any oxidizing substances.

Mechanical stability/back pressure

The pressure of a chromatography system is measured after the system pumps. This pressure is called system back pressure. If a column is attached to the system, the additional pressure is called column back pressure. Chromalite PCG and AD are fully stable to typical back pressures generated in chromatographic applications due to high cross-linking.

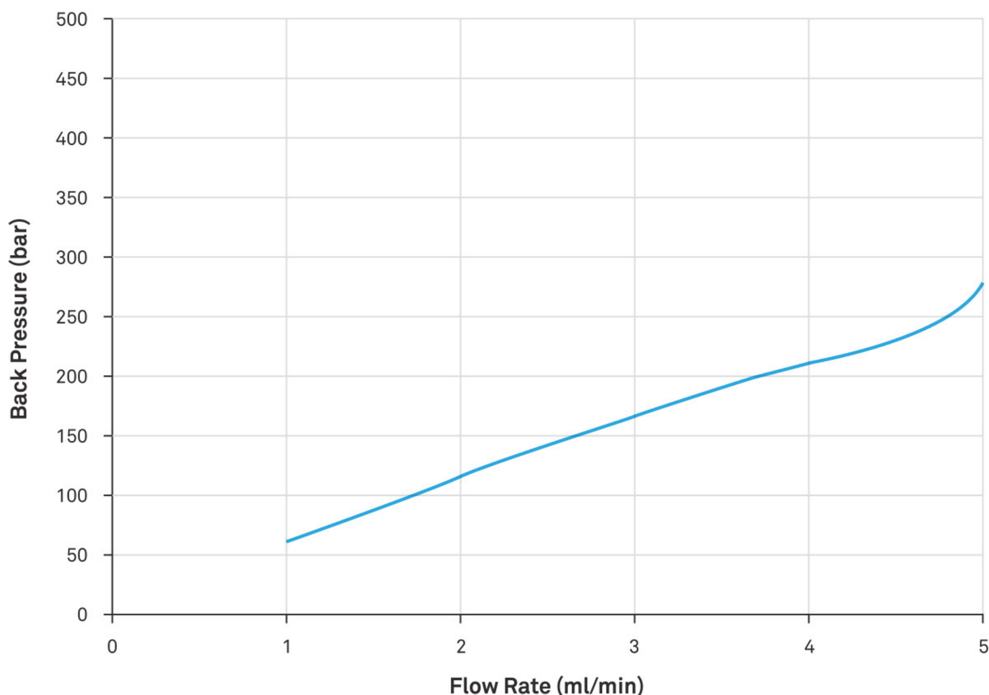
Table 7 – Typical pressure limits for Chromalite resins

GRADE	MAX BACK PRESSURE (bar)
F15*	200
F	50
M	10
C	10

* 12 – 18µm particle size

Figure 5 shows the excellent mechanical stability of Chromalite PCG1200F15 with particle size 12 – 18 micron under pressure. The resin is fully stable up to 200 bar before starting to show the typical profile of irreversible deformation. Compared to other commercially available resins with similar particle size, Chromalite PCG1200F15 shows outstanding performance.

Figure 5 – Pressure flow rate curve for Chromalite PCG1200 15 micron in a 4.0 mL (250 mm × 4.6 mm) column



The column was pre-equilibrated with water/i-propanol and back pressure was registered by increasing flow rate using i-propanol as eluent. Back pressure linearly increases up to a flow rate of 5 mL/min (1800 cm/hour) linear velocity.

Applications

Insulin

Insulin is a widely used peptide hormone for the treatment of diabetes mellitus. It is composed of two chains of 21 amino acids and 30 amino acids held together by two disulfide bonds. An additional disulfide bond is formed within the short chain. Insulin has a molecular weight of about 5,800 Daltons.

Nowadays, insulin is mainly produced using fermentation of recombinant micro organisms (*Escherichia coli*).The *E. coli* have had a human gene spliced into their DNA compelling them to produce human proinsulin. The pro-insulin is harvested by lysing the bacteria followed by clarification of the fermentation broth by centrifugation and filtration. The proinsulin has then to be refolded into its active tertiary structure by treatment in a refolding vessel with buffers of various concentrations. After enzymatic cleavage of proinsulin to obtain insulin, industrial purification of crude insulin proceeds via 1) ion exchange chromatography for the removal of impurities from fermentation and 2) reverse-phase chromatography for the removal of analogues of insulin.

Figure 6 – Insulin structure showing short and long chains

The reverse-phase chromatography step

Insulin-like components (desamido-, carbamoyl-, formyl-insulin and other forms) represent 6 to 20% of the fermentation mixture and are the most difficult molecules to be separated. Desamido-insulin is a key impurity in insulin purification. Its purification is particularly difficult for separation as it differs from insulin only in 1 amino acid moiety. This purification is perfomed using reversed-phase chromatography.

Desamido-insulin can be efficiently separated from insulin with Chromalite PCG1200F15 (15 micron).

Insulin resolution

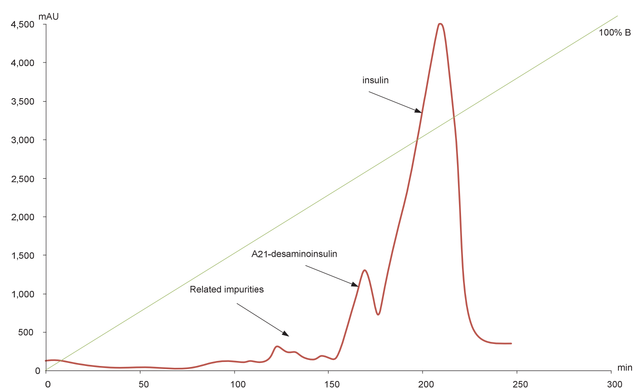
Mixtures of 85% insulin/10% of desamido-insulin/5% of other insulin-like impurities can be efficiently purified using Chromalite PCG1200F15 obtaining yields higher than 87% and purity higher than 98.4%.

Scale-up purification

Chromalite PCG1200 resins also allow efficient separation on the preparative scale. By keeping the same linear flow rate, same load per column volume and bed height, scale-up is easy and highly predictable. Chromalite PCG1200 resins allow the packing of columns in dimensions that suit optimal resolution and capacity needs.

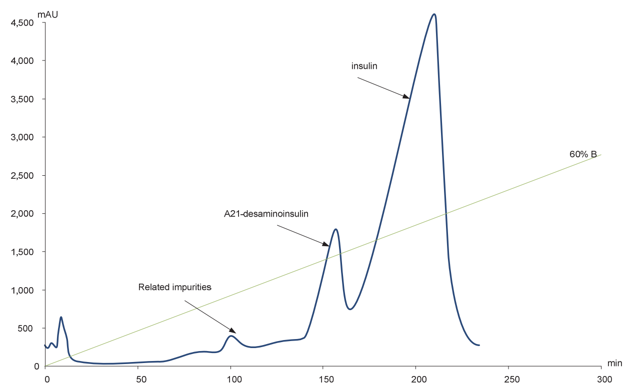
Figure 7 – Performance comparison between PCG1200F15 15 µm and C8 Silica (15 µm)

PCG1200F15



Column:	25x250 mm column
Adsorbent:	PCG1200F15 (15 µm)
Sample:	10 mg mixture of 85% insulin / 10% of desamido-insulin / 5% of other insulin-like impurities insulin per 1 mL of resin
Buffer A:	0.1 M acetic acid / 10% ethanol in water
Buffer B:	50% ethanol, water
Flow rate:	5.5 mL/min (70 cm/hour linear velocity)
Elution:	Gradient elution
System:	Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection:	UV-1 monitor, 280 nm

C8 Silica



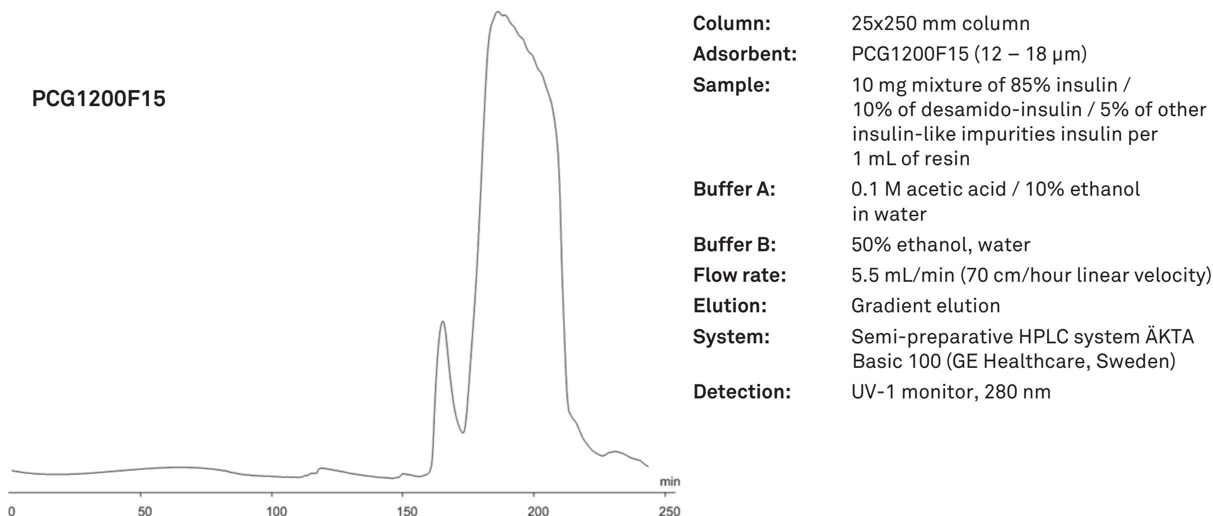
Column:	25x250 mm column
Adsorbent:	C8 Silica (15 µm)
Sample:	10 mg mixture of 85% insulin / 10% of desamido-insulin / 5% of other insulin-like impurities insulin per 1 mL of resin
Buffer A:	0.1 M acetic acid / 10% ethanol in water
Buffer B:	50% ethanol, water
Flow rate:	5.5 mL/min (70 cm/hour linear velocity)
Elution:	Gradient elution
System:	Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection:	UV-1 monitor, 280 nm

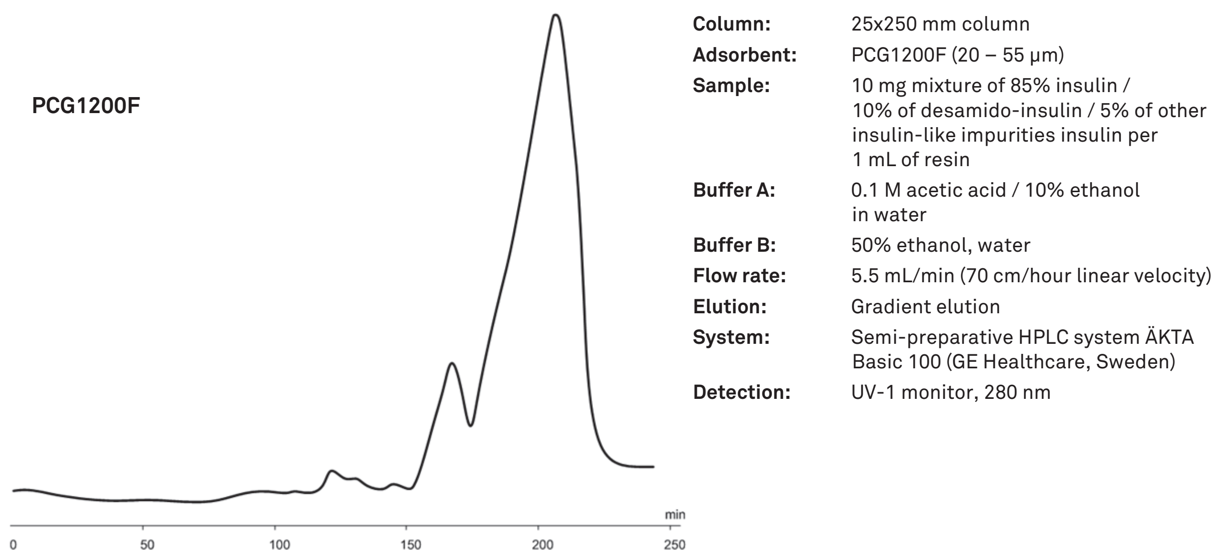
Advantages of Chromalite resins:

PCG1200F15 offers the same resolutions as silica material with the advantages of synthetic materials such as mechanical robustness and the ability to use in all range of pH conditions.

Figure 8 shows retained resolution upon scale-up with PCG1200 with different particle sizes.[2]

Figure 8 - Retained resolution upon scale-up with PCG1200F15 and PCG1200F





Advantages of Chromalite resins:

Scaling up from PCG1200F15 to PCG1200F guarantees the same separation profile. Scaling up from laboratory to production scale is reproducible and predictable.

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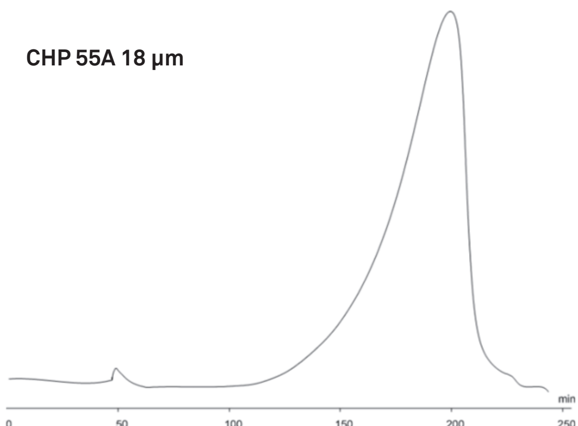
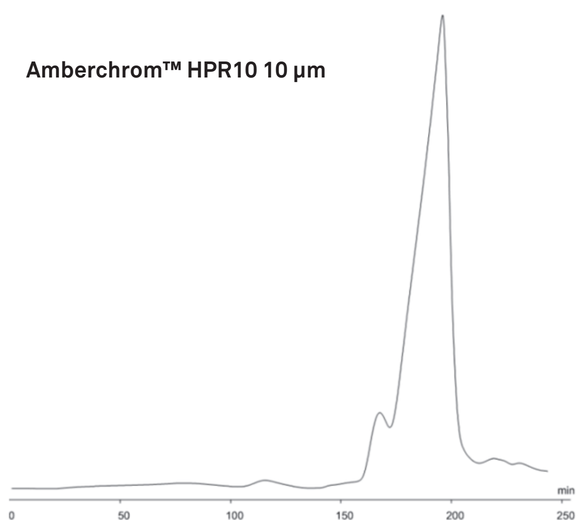
[2] Tested by Institute of Bioorganic Chemistry, Experimental Biotech Plant, Russian Academy of Sciences, Laboratory of Proteins Isolation and Purification, Moscow.

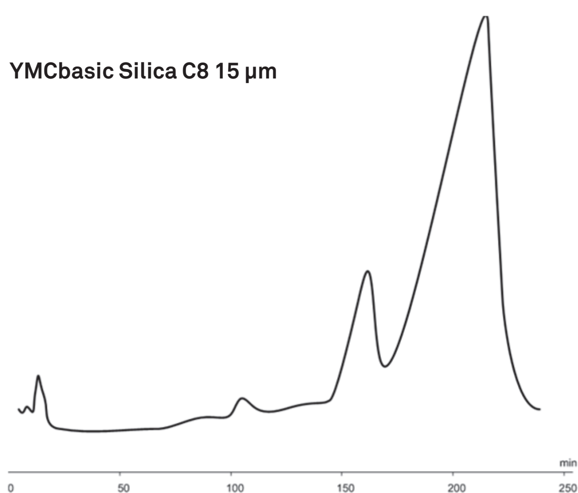
Figure 9 - Comparison with other synthetic resin



Column: 25x250 mm column
Adsorbent: CHP 55A (18 µm) (Mitsubishi)
Sample: 10 mg mixture of 85% insulin / 10% of desamido-insulin / 5% of other insulin-like impurities insulin per 1 mL of resin
Buffer A: 0.1 M acetic acid / 10% ethanol in water
Buffer B: 50% ethanol, water
Flow rate: 5.5 mL/min
Elution: Gradient elution
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 280 nm



Column: 25x250 mm column
Adsorbent: Amberchrom™ HPR10 (10 µm) (Dow)
Sample: 10 mg mixture of 85% insulin / 10% of desamido-insulin / 5% of other insulin-like impurities insulin per 1 mL of resin
Buffer A: 0.1 M acetic acid / 10% ethanol in water
Buffer B: 50% ethanol, water
Flow rate: 5.5 mL/min
Elution: Gradient elution
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 280 nm



Column: 25x250 mm column
Adsorbent: YMCbasic Silica C8 (15 µm) (YMC Co.)
Sample: 10 mg mixture of 85% insulin / 10% of desamido-insulin / 5% of other insulin-like impurities insulin per 1 mL of resin
Buffer A: 0.1 M acetic acid / 10% ethanol in water
Buffer B: 50% ethanol, water
Flow rate: 5.5 mL/min
Elution: Gradient elution
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 280 nm

Advantages of Chromalite resins:

Chromalite PCG1200F15 (see Figure 8) provides better resolution and performance than other commercially available polymeric resins or silica materials. Resolution obtained with PCG1200F15 is even better than using small-particle polymeric materials (10 micron) such as Amberchrom™.

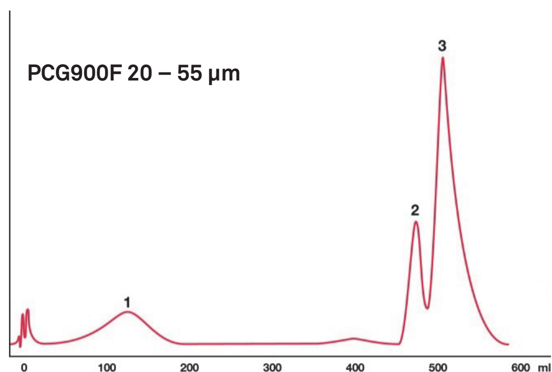
APIs: Peptides obtained by fermentation

Bradykinin is a 9 amino acid peptide chain with a MW of 1059 that is used to decrease blood pressure.

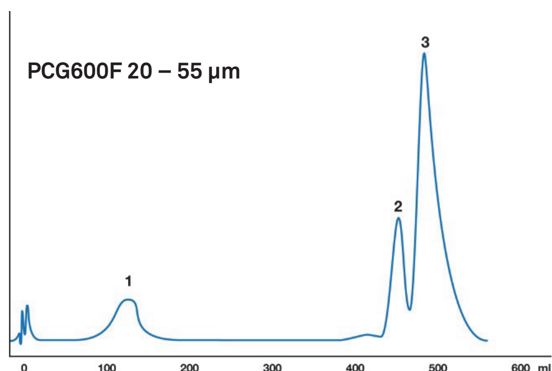
Bacitracin is a mixture of related cyclic oligopeptides (MW 1422) produced by microorganisms of the *Bacillus* species. Bacitracin is an effective topical antibacterial.

Gramicidin is a heterogeneous mixture of six antibiotic compounds, gramicidins A, B and C, making up 80%, 6%, and 14% respectively. Gramicidins are oligopeptides containing 15 amino acids with MW of about 1880. Its therapeutic use is limited to topical antibacterial applications.

Figure 10 – Separation of a bradykinin (1), bacitracin (2) and gramicidin C (3) mixture using Chromalite PCG900F and Chromalite PCG600F



Column:	25x250 mm column
Adsorbent:	PCG900F (20 – 55 µm)
Sample:	Mixture of brandykinin, bacitracin and gramicidin C
Buffer A:	10 mM citric acid / 10% ethanol / water for injections
Buffer B:	50% ethanol; water
Flow rate:	7.5 mL/min
Elution:	Gradient elution
System:	Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection:	UV-1 monitor, 220 nm



Column:	25x250 mm column
Adsorbent:	PCG600F (20 – 55 µm)
Sample:	Mixture of brandykinin, bacitracin and gramicidin C
Buffer A:	10 mM citric acid / 10% ethanol / water for injections
Buffer B:	50% ethanol; water
Flow rate:	7.5 mL/min
Elution:	Gradient elution
System:	Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection:	UV-1 monitor, 220 nm

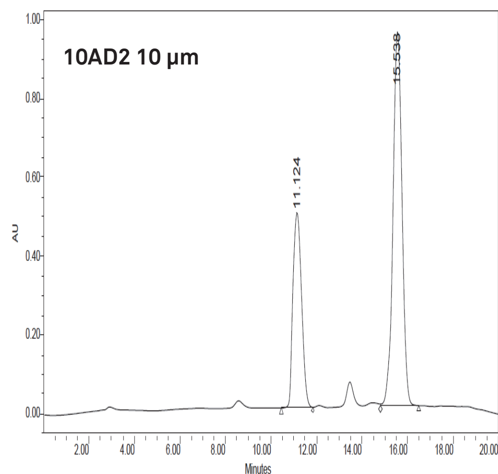
Advantages of Chromalite resins:

Chromalite PCG600F and Chromalite PCG900F are ideal for separation of small biomolecules such as small peptides as the polymeric structure of PCG resins allows for perfect separation of peptides.

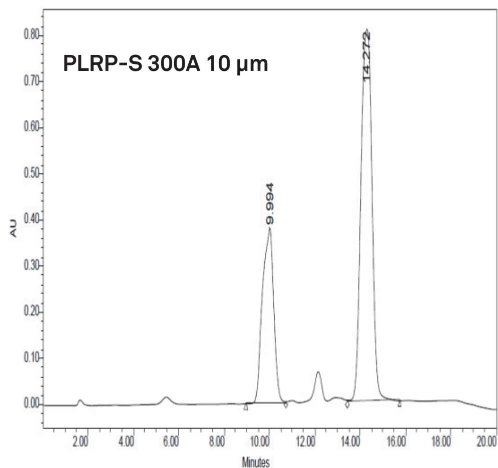
Peptides

The capability of Chromalite resins is demonstrated in the resolution of peptide mixtures.

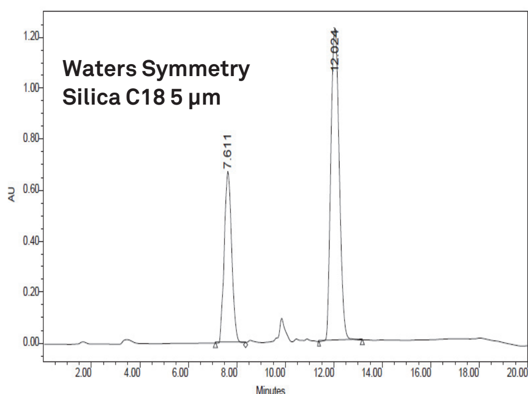
Figure 11 – Peptide separation using Chromalite resins and comparison with silica media



Column:	4.6x150 mm column
Adsorbent:	10AD2 (10 µm)
Sample:	Mixture of octapeptide (main peak at 16 minutes) and impurity (heptapeptide, minor peak at 11.5 minutes)
Buffer A:	0.5% TFA in water
Buffer B:	Acetonitrile
Flow rate:	1 mL/min
Elution:	Linear gradient 10 – 40%
System:	Waters Alliance® HPLC
Detection:	UV-1 monitor, 220 nm
Rs:	2.8



Column:	4.6x150 mm column
Adsorbent:	PLRP-S 300A (10 µm)
Sample:	Mixture of octapeptide (main peak at 16 minutes) and impurity (heptapeptide, minor peak at 11.5 minutes)
Buffer A:	0.5% TFA in water
Buffer B:	Acetonitrile
Flow rate:	1 mL/min
Elution:	Linear gradient 10 – 40%
System:	Waters Alliance® HPLC
Detection:	UV-1 monitor, 220 nm
Rs	2.1

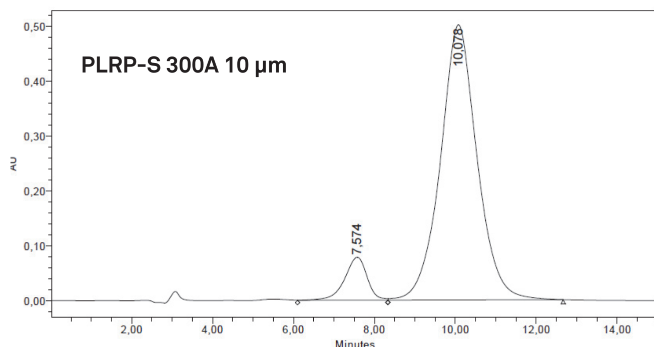


Column:	4.6x150 mm column
Adsorbent:	Waters Symmetry® Silica C18 (5 µm)
Sample:	Mixture of octapeptide (main peak at 16 minutes) and impurity (heptapeptide, minor peak at 11.5 minutes)
Buffer A:	0.5% TFA in water
Buffer B:	Acetonitrile
Flow rate:	1 mL/min
Elution:	Linear gradient 10 – 40%
System:	Waters Alliance® HPLC
Detection:	UV-1 monitor, 220 nm
Rs:	2.9

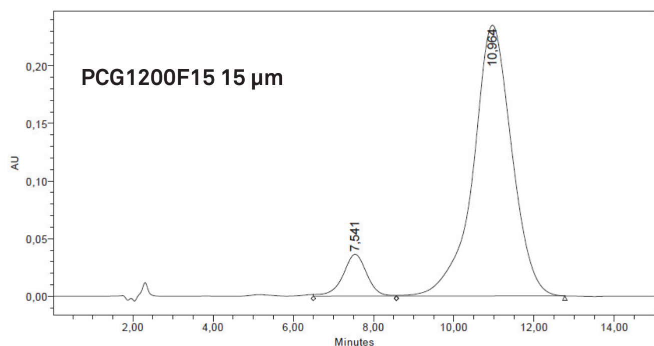
Advantages of Chromalite resins:

Figures 11 and 12 show better separation efficiency (resolution Rs) of Chromalite resins compared to other commercial polymers and silica materials.

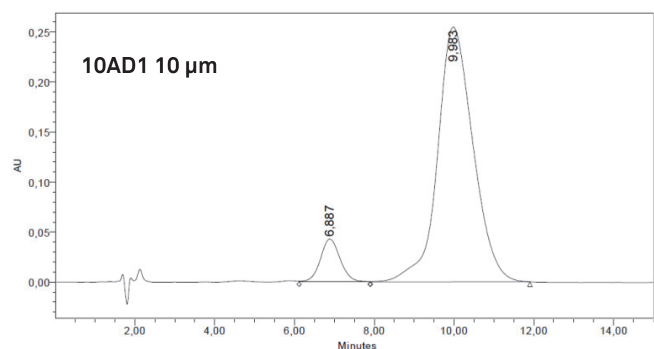
Figure 12 – Peptide separation comparison using Chromalite resins and a synthetic commercial resin



Column: 4.6x150 mm column
Adsorbent: PLRP-S 300A (10 µm)
Sample: Mixture of octapeptide (main peak) and impurity (heptapeptide, minor peak)
Buffer A: 0.5% TFA in water
Buffer B: 0.5% TFA in 80% acetonitrile
Flow rate: 1 mL/min
Elution: Isocratic at 27% B
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 220 nm



Column: 4.6x150 mm column
Adsorbent: PCG1200F15 (12 – 18 µm)
Sample: Mixture of octapeptide (main peak) and impurity (heptapeptide, minor peak)
Buffer A: 0.5% TFA in water
Buffer B: 0.5% TFA in 80% acetonitrile
Flow rate: 1 mL/min
Elution: Isocratic at 27% B
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 220 nm



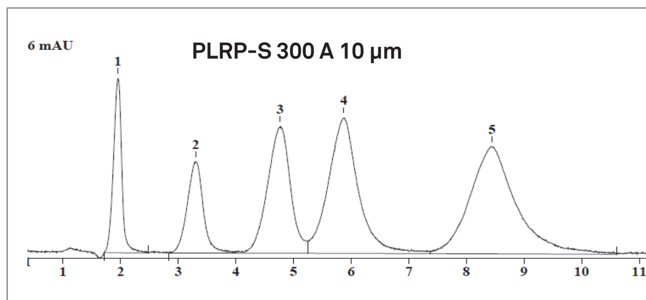
Column: 4.6x150 mm column
Adsorbent: 10AD1 (10 µm)
Sample: Mixture of octapeptide (main peak) and impurity (heptapeptide, minor peak)
Buffer A: 0.5% TFA in water
Buffer B: 0.5% TFA in 80% acetonitrile
Flow rate: 1 mL/min
Elution: Isocratic at 27% B
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 220 nm

Advantages of Chromalite resins:

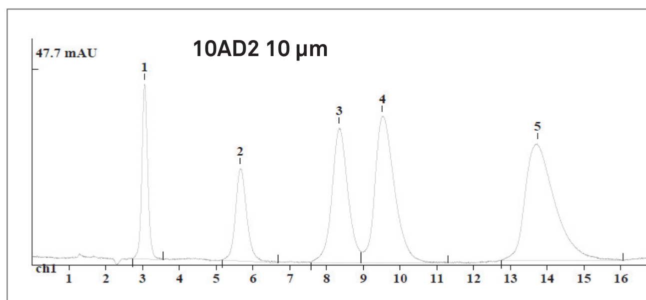
Figure 12 highlights that Chromalite 10AD1 can resolve as good as silica materials at 5 µm, which can present significant advantages in terms of scale-up and back pressure limitations.

Aromatic compounds

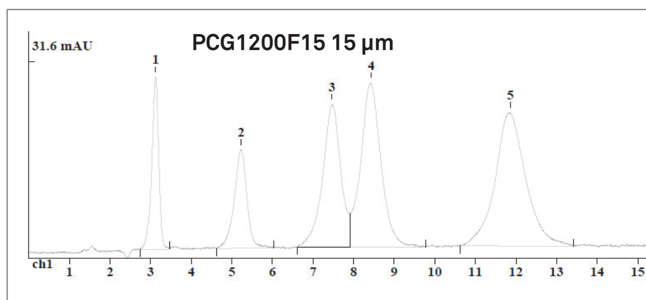
Figure 13 – Separation of aromatic compounds using Chromalite resins and a commercial synthetic resin



Column: 4.6x150 mm column
Adsorbent: PLRP-S 300 A (10 µm)
Sample: Mixture of phenol (1)./.acetophenol (2) / nitrobenzene (3) / benzene (4) / toluene (5)
Buffer A: Water
Buffer B: Acetonitrile
Flow rate: 1 mL/min
Elution: Isocratic, 60% B
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 220 nm



Column: 4.6x150 mm column
Adsorbent: 10AD2 (10 µm)
Sample: Mixture of phenol (1) / acetophenol (2) / nitrobenzene (3) / benzene (4) / toluene (5)
Buffer A: Water
Buffer B: Acetonitrile
Flow rate: 1 mL/min
Elution: Isocratic, 60% B
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 220 nm

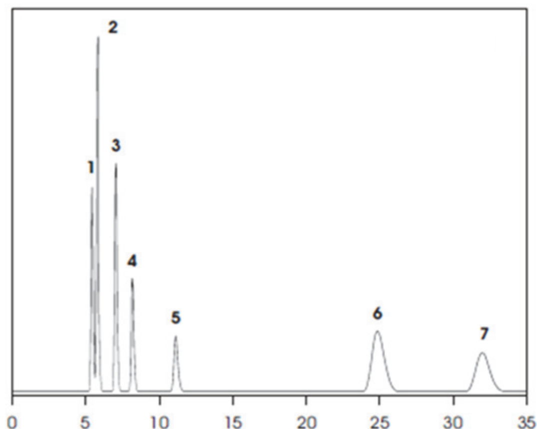


Column: 4.6x150 mm column
Adsorbent: PCG1200F15 (15 µm)
Sample: Mixture of phenol (1) / acetophenol (2) / nitrobenzene (3) / benzene (4) / toluene (5)
Buffer A: Water
Buffer B: Acetonitrile
Flow rate: 1 mL/min
Elution: Isocratic at 60% B
System: Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection: UV-1 monitor, 220 nm

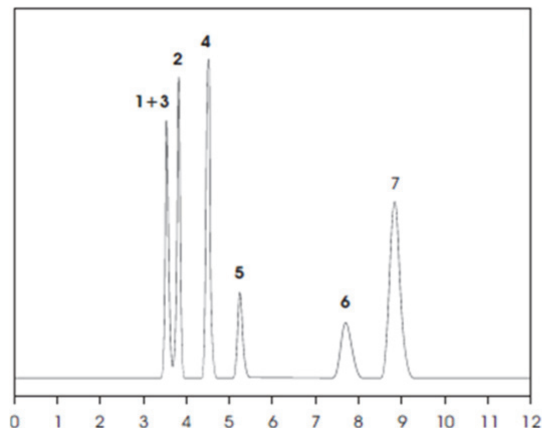
Advantages of Chromalite resins:

The hydrophobic matrix of Chromalite resins for RPC optimizes separation of aromatic compounds and obtains the same efficiency as other commercially available resins.

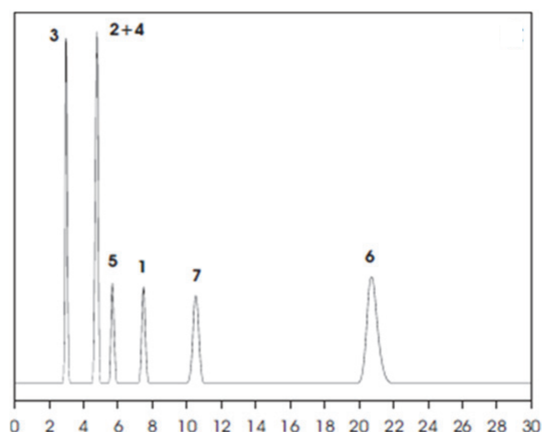
Figure 14 – Separation of aromatic compounds using Chromalite 10MN 10µm and different elution conditions



Column:	4.6x250 mm column
Adsorbent:	10MN (10 µm)
Sample:	Model mix of benzaldehyde (1) / anisole (2) / cumene (3) / bromobenzene (4) / naphthalene (5) / anthraquinone (6) / anthracene (7)
Buffer A:	Water
Buffer B:	ACN/iPrOH 85/15
Flow rate:	1 mL/min
Elution:	Isocratic, 5% A
System:	Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection:	UV-1 monitor, 254 nm



Column:	4.6x250 mm column
Adsorbent:	10MN (10 µm)
Sample:	Model mix of benzaldehyde (1) / anisole (2) / cumene (3) / bromobenzene (4) / naphthalene (5) / anthraquinone (6) / anthracene (7)
Buffer A:	Chloroform
Buffer B:	Methanol
Flow rate:	1 mL/min
Elution:	Isocratic, 50% A
System:	Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection:	UV-1 monitor, 254 nm



Column:	4.6x250 mm column
Adsorbent:	10MN (10 µm)
Sample:	Model mix of benzaldehyde (1) / anisole (2) / cumene (3) / bromobenzene (4) / naphthalene (5) / anthraquinone (6) / anthracene (7)
Buffer A:	Hexane
Buffer B:	Chloroform
Flow rate:	1 mL/min
Elution:	Isocratic, 80% A
System:	Semi-preparative HPLC system ÄKTA Basic 100 (GE Healthcare, Sweden)
Detection:	UV-1 monitor, 254 nm

Advantages of Chromalite resins:

The polymer backbone of Chromalite RPC resin is stable to many organic solvents and offers incredible flexibility in separation conditions. It is possible to use organic solvents ranging from methanol, i-PrOH, ACN, hexane, chloroform, etc. The elution profile of the organic solvent changes the significantly, providing many possibilities for optimal resolution of target molecules.

Chromalite products

Table 8 – Chromalite resins for reverse-phase chromatography, adsorption and SPE

PRODUCT CODE	PRODUCT†	PARTICLE SIZE (90% in range) (μm)	PACKING	SUPPLIED AS
06845-190 06845-187 06845-193	10AD1	9 – 11	10 g dry 50 g dry 100 g dry	Supplied as 20% EtOH slurry
06840-190 06840-187 06840-193	10AD2	9 – 11	10 g dry 50 g dry 100 g dry	Supplied as 20% EtOH slurry
06895-190 06895-187 06895-193	15AD1	13.5 – 16.5	10 g dry 50 g dry 100 g dry	Supplied as 20% EtOH slurry
06890-190 06890-187 06890-193	15AD2	13.5 – 16.5	10 g dry 50 g dry 100 g dry	Supplied as 20% EtOH slurry
04685-190 04685-187 04685-193	10MN	9 – 11	10 g dry 100 g dry 250 g dry	Dry
04690-190 04690-187 04690-193	15MN	13.5 – 16.5	10 g dry 100 g dry 250 g dry	Dry
06910-190 06910-187 06910-193	70MN	65 – 75	10 g dry 100 g dry 250 g dry	Dry
04740-190 04740-187 04740-193	PCG600F	20 – 55	10 g dry 50 g dry 100 g dry	Supplied as 20% EtOH slurry
04750-186 04750-192 04750-195	PCG600M	58 – 92	50 g wet 250 g wet 500 g wet	Wet
04760-186 04760-192 04760-195	PCG600C	100 – 140	50 g wet 250 g wet 500 g wet	Wet
04770-190 04770-187 04770-193	PCG900F	20 – 55	10 g dry 50 g dry 100 g dry	Supplied as 20% EtOH slurry
04770-186 04770-192 04770-195	PCG900M	58 – 92	50 g wet 250 g wet 500 g wet	Wet
04790-186 04790-192 04790-195	PCG900C	100 – 140	50 g wet 250 g wet 500 g wet	Wet
06715-190 06715-187 06715-193	PCG1200F15	12 – 18	10 g dry 50 g dry 100 g dry	Supplied as 20% EtOH slurry
06720-190 06720-187 06720-193	PCG1200F	20 – 55	10 g dry 50 g dry 100 g dry	Supplied as 20% EtOH slurry
06725-186 06725-192 06725-195	PCG1200M	58 – 92	50 g wet 250 g wet 500 g wet	Wet
06730-186 06730-192 06730-195	PCG1200C	85 – 155	50 g wet 250 g wet 500 g wet	Wet

HPLC columns

HPLC columns are essential for optimal resolution in laboratory analysis. Purolite offers 150x4.6 mm pre-packed columns filled with Chromalite resins for reverse-phase chromatography. Columns are available with particle sizes averaging 10 micron and 15 micron, as shown in Table 9.

Table 9 – Analytical HPLC columns

PRODUCT CODE	CHROMALITE	PARTICLE SIZE (µm)	COLUMN SIZE
06845-601	10AD1	9 – 11	150 x 4.6
06840-601	10AD2	9 – 11	150 x 4.6
06895-601	15AD1	13.5 – 16.5	150 x 4.6
06890-601	15AD2	13.5 – 16.5	150 x 4.6
06715-601	PCG1200F15	13.5 – 16.5	150 x 4.6

The correct use of an HPLC column is critical for the life of the column and the accuracy of HPLC analysis. HPLC columns packed with Chromalite resins can be cleaned, regenerated and used following the same precautions used for silica-based HPLC columns, taking into consideration that a pH of 1 to 14 is acceptable.

Figure 15 – HPLC Column pre-packed with Chromalite resin

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