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Hypercrosslinked Gel-type Polystyrene Resin

Applications Guide

CHROMALITE SHGN

STRUCTURE AND DISTINCTIVE FEATURES  
OF HYPERCROSSLINKED NON-MODIFIED  
POLYSTYRENE RESIN CHROMALITE 5HGN



Hypercrosslinked Gel-type Polystyrene Resin

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Applications Guide

CHROMALITE 5HGN



## CHROMALITE 5HGN

Mechanical robustness, inertness, pH stability, compatibility with both polar and non-polar organic solvents and even water are in fact among the most desirable properties of modern HPLC packings. With the increased demand for such packings, a number of different polymeric phases has emerged in recent years.

The above-mentioned requirements are best met by the new generation of polymeric adsorbent materials, hypercrosslinked polystyrene resins. Principally differing from conventional styrene-divinylbenzene copolymers, hypercrosslinked polystyrene is obtained by an extensive post-crosslinking of long polystyrene chains in the presence of an excess of good solvent, preferably by introducing numerous methylene bridges between phenyl groups. The structure of hypercrosslinked polystyrene is an expanded, rigid three-dimensional network. The main structural element of this network is a spatially non-planar cycle formed by crosslinking bridges and very short chain segments confined between the branching points. The open-work-type hypercrosslinked network displays an extremely high apparent inner surface area (up to 1000-1500 m<sup>2</sup>/g) and almost identical solvent uptake in both polar and non-polar media, which explains good compatibility of the material with all mobile phases, from hexane to methanol and water. The whole interior of the hypercrosslinked polystyrene bead is accessible to analytes as if the rather homogeneous network was composed of small «pores» of about 2.0 to 4.0 nm in diameter.

Hypercrosslinked polystyrene materials have already found wide applications for large-scale adsorption technologies (Macronet Hypersol, Purolite, UK) and for solid phase extraction (Purosep, Purolite, UK; Isolute-ENV+, IST, UK; LiChrolut EN, Merck).

Chromalite 5HGN is a hypercrosslinked non-modified polystyrene resin packing designed especially for HPLC applications.

Due to compatibility with any type of organic solvents and water, Chromalite 5HGN can operate throughout a wide range of eluent polarity. Combining the non-polar Chromalite 5HGN with an aqueous or polar aqueous-organic mobile phase corresponds to conventional reversed phase mode of chromatography. Experiments that involve a non-polar eluent can be named quasi-normal phase chromatography. The word «quasi» emphasizes its difference from conventional normal phase mode where stationary phase (usually, bare silica) is polar.

When the eluent is composed of water and polar organic modifier, the retention mechanism on Chromalite 5HGN appears to be based on typical reversed phase partitioning. However, when a non-polar organic solvent as hexane makes up 50% or more of the organic eluent, retention and separations appear to be based on charge transfer interactions, so only unsaturated compounds with pi-electron donating or accepting groups can be retained.

One of the distinctive features of Chromalite 5HGN is the limitation on molecule size for compounds to be separated; packings possessing such a property are called restricted access ones. The molecule of an analyte must not be too large to fit into the «micro-pores» of hypercrosslinked polystyrene, otherwise the compound would not be retained. When the size of analyte molecules is not large, the separation efficiency offered by Chromalite 5HGN does not yield significantly to the one for silica-based packings. Therefore, the restricted access property makes Chromalite 5HGN excellent HPLC packing for the determination of small analytes in «dirty» samples where the main contaminants are proteins, hydrophilic natural polymers such as humic acids, various glycosides and lipids.

## CHROMALITE 5HGN

Another, barely examined area of application for the microporous packing material is size exclusion (gel permeation) chromatography.

While hypercrosslinked polystyrene is chemically stable and easily withstands temperatures of 200 °C and higher, the perspective area of application of Chromalite 5HGN is the modern high-temperature HPLC. Enhanced temperatures, through facilitating mass transfer, substantially increase column efficiency, and reduce the analysis time.

### References

- [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

CHROMALITE 5HGN FOR MULTI-MODE  
HPLC APPLICATIONS



Hypercrosslinked Gel-type Polystyrene Resin

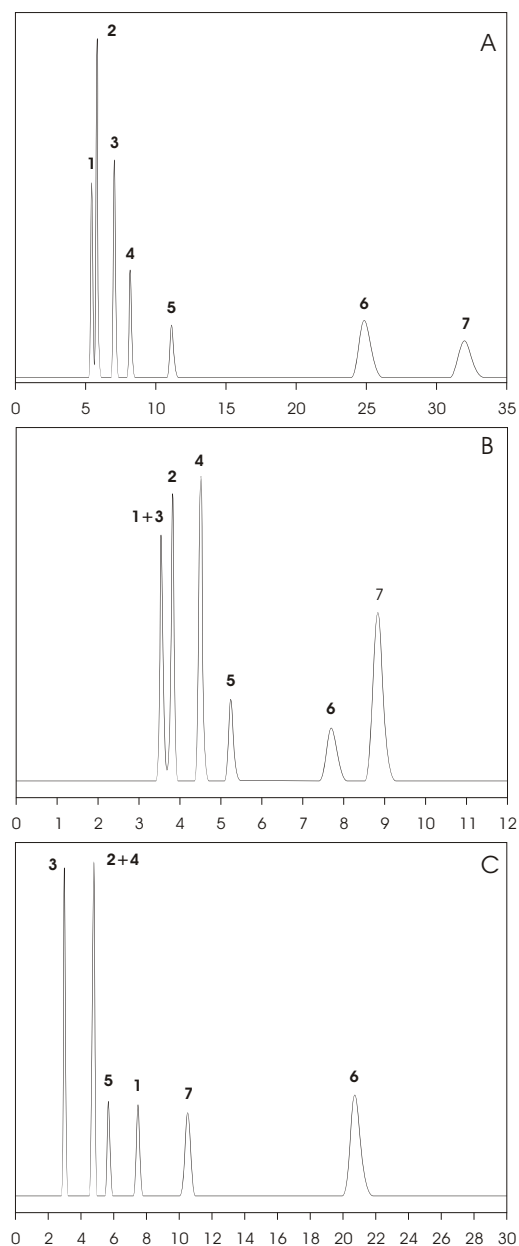
Applications Guide

CHROMALITE 5HGN



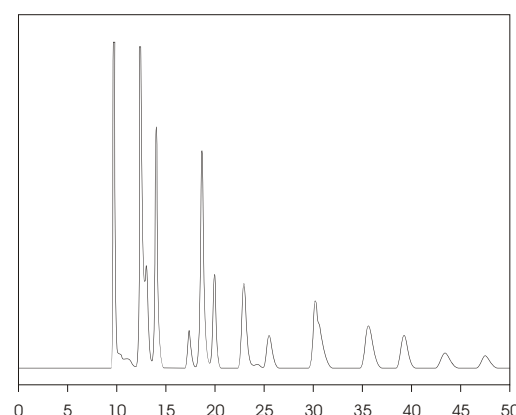
# CHROMALITE 5HGN

Although usable as a general purpose reversed phase or quasi-normal phase packing, Chromalite 5HGN is also suitable for mixed mode separations that involve eluents composed of medium polarity organic solvents. An easy mode changeover provides unsurpassed method development flexibility necessary for fine tuning the required column selectivity (Fig. 1).

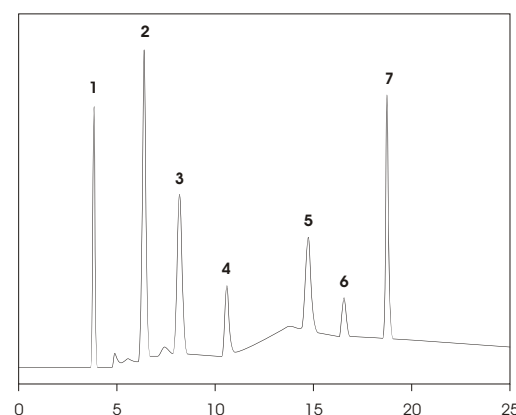


**Figure 1.**  
**Sample:** model mix, 1 - benzaldehyde, 2 - anisole, 3 - cumene, 4 - bromobenzene, 5 - naphthalene, 6 - anthraquinone, 7 - anthracene.  
**Conditions:** 250x4.6 Chromalite 5HGN. A) Reversed phase mode, 80:20 AcN-water; B) mixed mode, 80:15:5 AcN-2-propanol-water; C) quasi-normal phase mode, 80:20 hexane-chloroform. Isocratic. 1 ml/min. UV 254 nm.

While the mixed mode provides the least retention of various analytes as compared to reversed phase and quasi-normal phase modes, two distinct types of gradient elution are possible. The starting eluent may be of water-rich (reversed phase, Fig. 2) or hexane-rich (quasi-normal phase, Fig. 3) type, but the finishing eluent must correspond to the mixed mode one. Fast column equilibration under all conditions provides high reproducibility of such gradient analysis. Yet, the limitation of the method is that one column may be used only for one of the two possible gradient modes (reversed phase-to-mixed or for quasi-normal phase-to-mixed), thus ensuring safety of the polymeric packing.



**Figure 2.**  
**Sample:** 16 priority PAHs standard mix.  
**Conditions:** 250x4.6 Chromalite 5HGN. A: 80:20 AcN-water; B: 80:20 AcN-chloroform. Linear Gradient 0-100% B in 50 min. 1 ml/min. UV 254 nm.

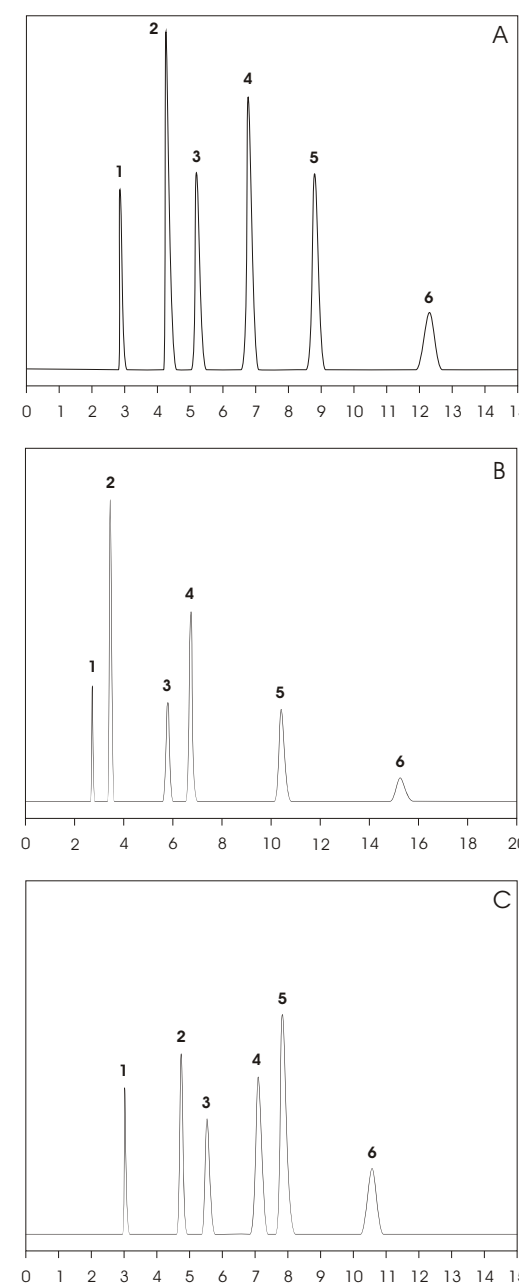


**Figure 3.**  
**Sample:** model mix, 1 - cumene, 2 - anisole, 3 - naphthalene, 4 - benzaldehyde, 5 - anthracene, 6 - anthraquinone, 7 - o-nitroaniline.  
**Conditions:** 250x4.6 Chromalite 5HGN. A: 80:20 hexane-chloroform; B: 2:1 chloroform-methanol. Linear gradient 0-100% B in 25 min. 1 ml/min. UV 254 nm.

## Reversed phase mode

Ability to operate throughout a wide pH range and high hydrophobicity of the material are two main advantages of Chromalite 5HGN over common silica-based packings under reversed phase conditions.

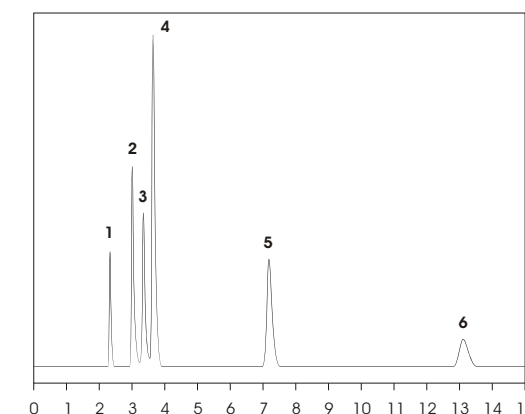
Stability over a wide pH range provides longer column life when using eluents based on aggressive buffers.



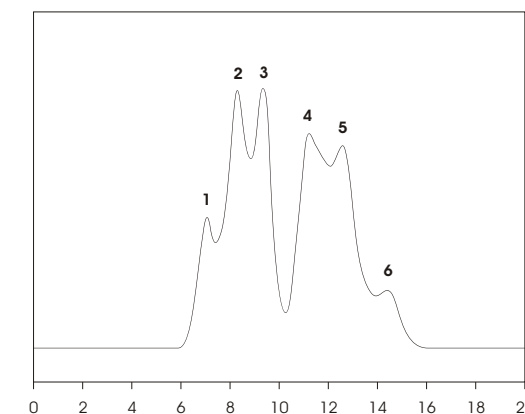
**Figure 4.**  
**Sample:** RP test mix, 1 - acetone, 2 - pyridine, 3 - p-chlorophenol, 4 - benzaldehyde, 5 - toluene, 6 - cumene.  
**Conditions:** 250x4.6 Chromalite 5HGN. A) 90:10 AcN-water; B) 70:10:20 AcN-THF-water; C) 45:50:5 AcN-methanol-water. Isocratic. 1 ml/min. UV 254 nm.

Since hydrophobicity of hypercrosslinked polystyrene resin is the same or even greater than that of most modern polymeric adsorption materials for solid phase extraction, Chromalite 5HGN is the HPLC packing of choice for on-line SPE applications.

When compared to silica-based columns for analysis of different classes of aromatic compounds (Fig. 5), Chromalite 5HGN offers comparable efficiency, yet provides unique selectivities and greater method development flexibility (Fig. 4). Besides, most conventional PS-DVB packings can hardly be applied for such separations since, when used with low water content eluents, they swell and lose their efficiency (Fig. 6).



**Figure 5.**  
**Sample:** RP test mix, 1 - acetone, 2 - pyridine, 3 - p-chlorophenol, 4 - benzaldehyde, 5 - toluene, 6 - cumene.  
**Conditions:** 250x4.6 Zorbax SB-C18. 60:40 AcN-water. Isocratic. 1 ml/min. UV 254 nm.



**Figure 6.**  
**Sample:** RP test mix, 1 - acetone, 2 - pyridine, 3 - p-chlorophenol, 4 - benzaldehyde, 5 - toluene, 6 - cumene.  
**Conditions:** 150x4.6 conventional PS-DVB column. 90:10 AcN-water. Isocratic. 0.3 ml/min. UV 254 nm.



# CHROMALITE 5HGN

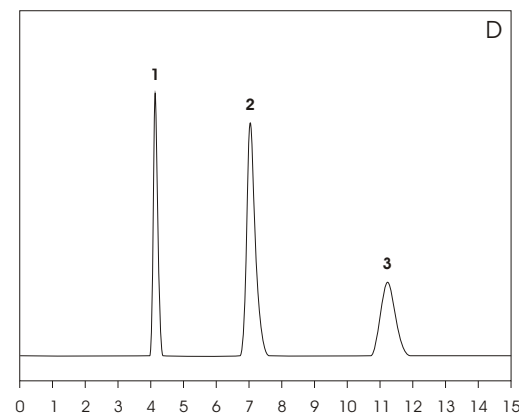
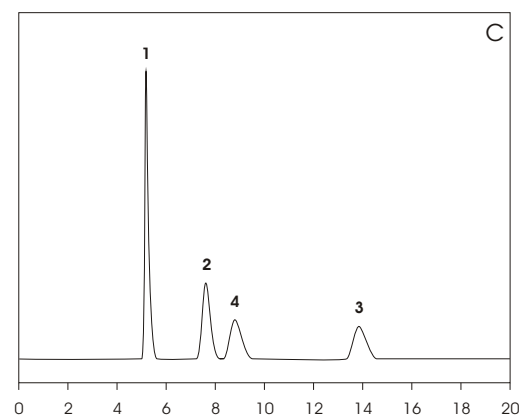
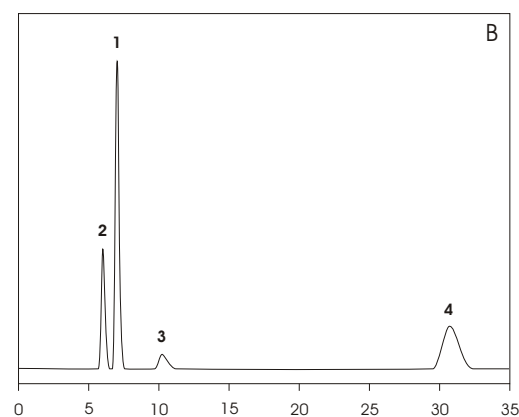
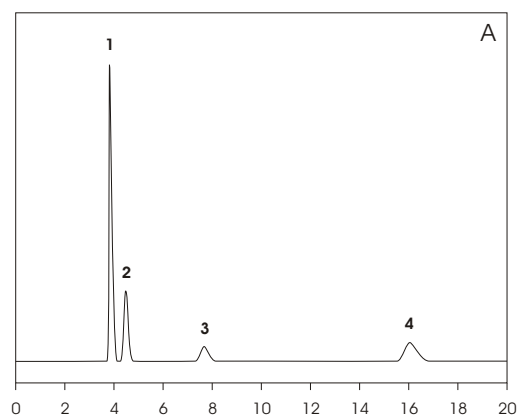
## Quasi-normal phase mode

While the nature of primary retention mechanism under quasi-normal phase conditions is the charge-transfer interaction, Chromalite 5HGN is able to perform many separations that are usually impossible on common silica-based polar as well as non-polar HPLC columns, except some chiral packings (Fig. 8). Quasi-normal phase mode is best suited for the separation of aromatic compounds, particularly when determining such analytes in various non-polar matrices. Both direct LC analysis or preliminary fractionation of aromatics for the further GC analysis are possible.

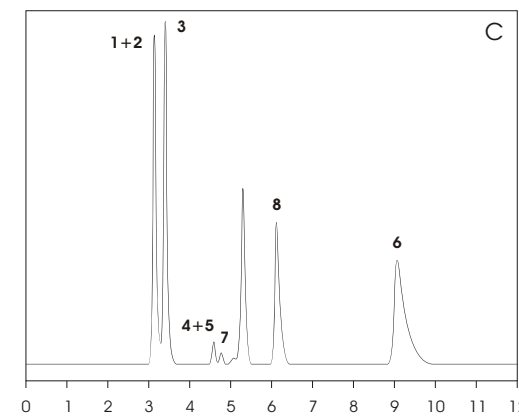
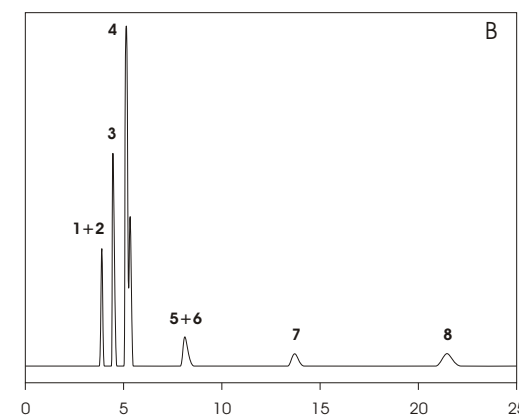
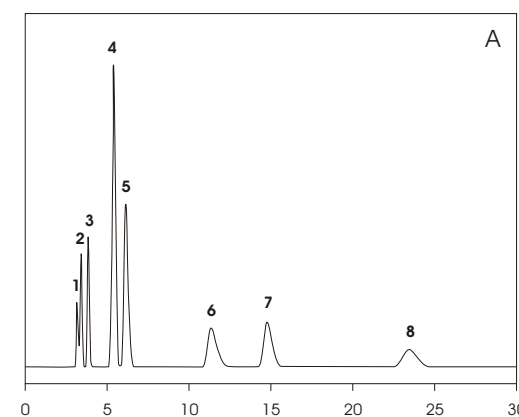
The eluting strength of organic solvents in the case of quasi-normal conditions is different from the one appearing under normal phase conditions on conventional polar stationary phases. The average elution strength under quasi-normal phase conditions increases in the following order: hexane < 2-propanol << toluene < THF < ethylacetate < acetone < dichloromethane. Besides, each of the solvents used as the component of the eluent provide special separation selectivity (Fig. 7).

Under quasi-normal phase conditions, homologues elute in the opposite order as compared with the reversed phase conditions, thus indicating a significant contribution of size exclusion mechanism in the separation.

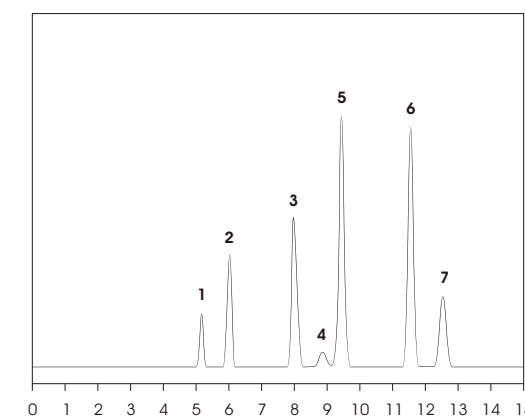
Gradient elution is also possible under quasi-normal phase conditions, with the fast column equilibration providing high retention time reproducibility (Fig. 9).



**Figure 7.**  
**Sample:** model mix,  
 1 - nitrobenzene,  
 2 - phenanthrene,  
 3 - anthraquinone,  
 4 - carbazole.  
**Conditions:** 250x4.6 Chromalite 5HGN.  
 A) 60:40 hexane-dichloromethane;  
 B) 60:40 hexane-toluene;  
 C) 60:40 hexane-THF;  
 D) 60:40 hexane-acetone.  
 Isocratic.  
 1.5 ml/min.  
 A, C) UV 254 nm,  
 B) UV 280 nm,  
 D) UV 330 nm.



**Figure 8.**  
**Sample:** model mix,  
 1 - benzene,  
 2 - styrene,  
 3 - anisole,  
 4 - nitrobenzene,  
 5 - phenanthrene,  
 6 - anthraquinone,  
 7 - indole,  
 8 - carbazole.  
**Conditions:** A) 250x4.6 Chromalite 5HGN.  
 60:40 hexane-dichloromethane.  
 B) 250x4.6 Chiralcel OD-H.  
 90:10 hexane-2-propanol.  
 C) 250x4.6 Whelk-1,O.  
 80:20 hexane-2-propanol.  
 Isocratic.  
 1 ml/min.  
 UV 254 nm.

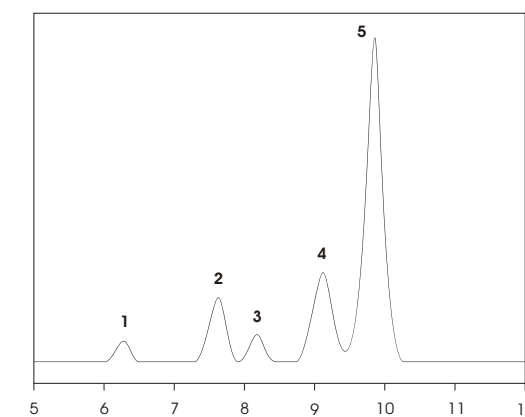


**Figure 9.**  
**Sample:** model mix, 1 - benzene, 2 - styrene,  
 3 - anisole, 4 - naphthalene, 5 - phenylisothiocyanate,  
 6 - nitrobenzene, 7 - phenanthrene.  
**Conditions:** 250x4.6 Chromalite 5HGN. A: 95:5 hexane-dichloromethane; B: 60:40 hexane-dichloromethane.  
 Linear Gradient 0-100% B in 15 min. 1 ml/min. UV 254 nm.

## Size exclusion mode

Since the average pore size of a hyper-crosslinked polystyrene resin is relatively small, Chromalite 5HGN is best suited to determine molecular weight distribution and perform analysis of low molecular weight materials in polymers.

The calibration curve for Chromalite 5HGN is composed of two linear parts which intersect at about MW 1000, hence MW 1,000 is the first molecular weight exclusion limit. The presence of the second molecular weight exclusion limit MW 1,000,000 is due to interstitial voids of the packed particles in the column, which present «pores» in the range of 300-3,000 nm.



**Figure 10.**  
**Sample:** polystyrene standards, 1 - MW 330,000, 2 - MW 10,100,  
 3 - MW 680, 4 - styrene dimer, 5 - styrene.  
**Conditions:** 250x4.6 Chromalite 5HGN.  
 Dichloromethane. Isocratic. 0.3 ml/min. UV 254 nm.



### Mixed mode

Mixed mode is characterized by the contribution of virtually all retention mechanisms possible for hypercrosslinked polystyrene including the reversed phase mechanism, size exclusion and charge transfer.

Under appropriate mixed mode conditions, the contributions of size exclusion and dispersive interaction of methylene groups are roughly equal, which yield many homologues eluting in one peak (Fig. 11).

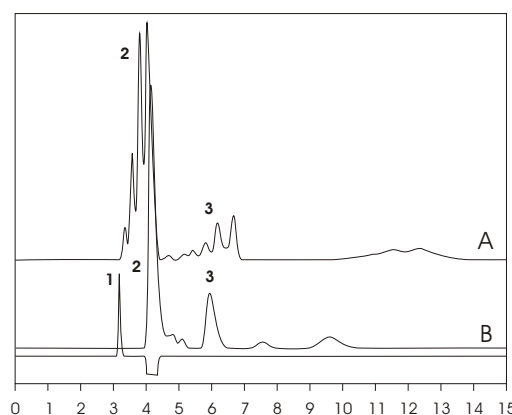
### Restricted access properties

Chromalite 5HGN is the restricted access packing opening up new possibilities for the selective determination of analytes on low detection level in extremely complex and «dirty» samples with the use of the on-line SPE pre-concentration.

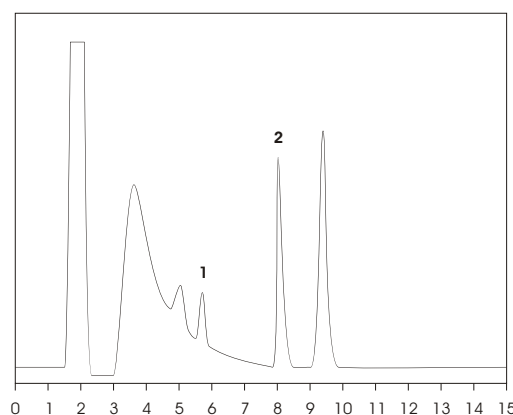
Besides, Chromalite 5HGN can also be used for the separating of target analytes from various matrix contaminants in order to obtain extremely clean samples.

### Testing methods

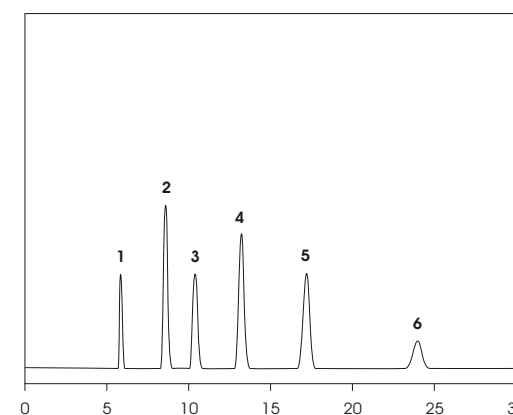
To verify the performance of Chromalite 5HGN in reversed phase and quasi-normal phase modes two test mixtures are used. The reversed phase test mix includes acetone, pyridine, p-chlorophenol, benzaldehyde, toluene and cumene; the optimum efficiency corresponds with the peak of toluene. The quasi-normal phase test mix includes bromobenzene, 2,7-dimethylnaphthalene, naphthalene, nitrobenzene, phenanthrene and anthraquinone; the optimum efficiency corresponds with the peak of nitrobenzene. Chromalite 5HGN offers separation efficiency up to 50,000 plates/m under reversed phase mode and up to 25,000 plates/m under quasi-normal phase mode.



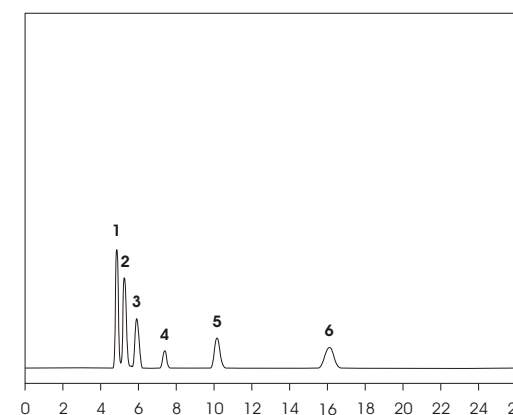
**Figure 11.**  
**Sample:** gasoline A,  
1 - saturates,  
2 - monoaromatics,  
3 - diaromatics.  
**Conditions:** 250x4.6 Chromalite 5HGN.  
A) Quasi-normal phase mode, 80:20 hexane-chloroform;  
B) mixed mode, 1:1 chloroform-methanol.  
Isocratic.  
1 ml/min.  
A) UV 254 nm,  
B) UV 254 nm & Refractive Index.



**Figure 12.**  
**Sample:** untreated soil extract spiked with p-chlorophenol & phenanthrene,  
1 - p-chlorophenol,  
2 - phenanthrene.  
**Conditions:** 250x4.6 Chromalite 5HGN. 45:50:5 AcN-methanol-water.  
Isocratic.  
1 ml/min.  
UV 280 nm.



**Figure 13.**  
**Sample:** RP test mix,  
1 - acetone,  
2 - pyridine,  
3 - p-chlorophenol,  
4 - benzaldehyde,  
5 - toluene,  
6 - cumene.  
**Conditions:** 250x4.6 Chromalite 5HGN. 90:10 AcN-water.  
Isocratic.  
0.5 ml/min.  
UV 254 nm.



**Figure 14.**  
**Sample:** QNP test mix,  
1 - bromobenzene,  
2 - 2,7-dimethylnaphthalene,  
3 - naphthalene,  
4 - nitrobenzene,  
5 - phenanthrene,  
6 - anthraquinone.  
**Conditions:** 250x4.6 Chromalite 5HGN. 70:20:10 hexane-dichloromethane-2-propanol.  
Isocratic.  
1 ml/min.  
60 °C.  
UV 254 nm.

### Service conditions

Chromalite 5HGN may be used only (i) in the reversed and mixed modes (i.e. when eluents are composed of water, water miscible organic solvents with the possible addition of dichloromethane, ethylacetate or diethyl ether) or (ii) in the quasi-normal and mixed modes (i.e. when eluents are based on hexane or another non-polar organic solvent), in order to ensure stability of the column packing.

On receiving a column subjected to the quasi-normal phase separations, before testing, it must be conditioned with dichloromethane or chloroform for an hour, then with the testing eluent for an hour, and kept overnight with that eluent.

Chromalite 5HGN may be used with aqueous-organic eluents with the content of water not exceeding 85%, otherwise polymeric beads shrink, and the packing loses its efficiency. Shrinked polymeric beads may be regenerated once by purging the column with THF at a flow rate 2 ml/min for an hour, and then changing the inlet and outlet of the column.

APPLICATIONS



Hypercrosslinked Gel-type Polystyrene Resin

Applications Guide





## CHROMALITE 5HGN

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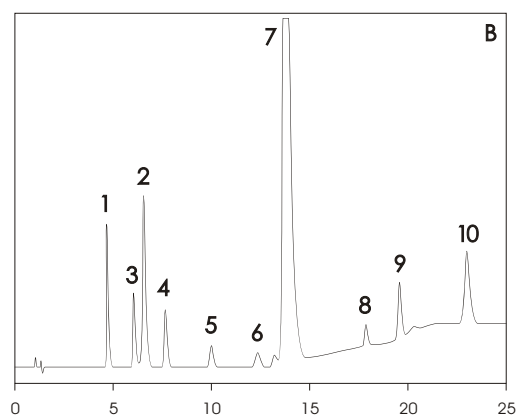
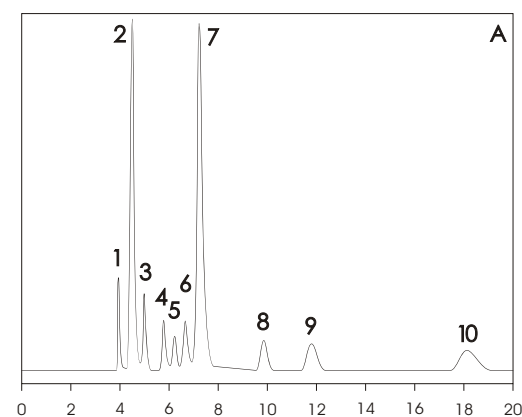


Figure 15.

**Sample:** 10 priority phenols standard mix,

- 1 - phenol,
- 2 - 4-nitrophenol,
- 3 - 2-methylphenol,
- 4 - 2-chlorophenol,
- 5 - 3-methyl-4-chlorophenol,
- 6 - 2,4-dichlorophenol,
- 7 - 2,6-dinitrophenol,
- 8 - 2,4,6-trichlorophenol,
- 9 - 2,3,4,5-tetrachlorophenol,
- 10 - pentachlorophenol.

**Conditions:** 250x4.6 Chromalite 5HGN.

A) 80:10:10:1.5 AcN-THF-water-acetic acid. Isocratic.

B) 45:15:40:2 AcN-THF-water-acetic acid;

B: 85:15:2 AcN-THF-acetic acid.

Linear Gradient

0-20% B in 15 min,

20-100% B in 10 min.

1.5 ml/min.

UV 254 nm.

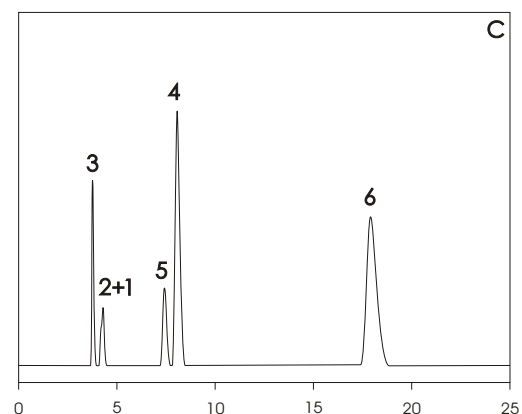
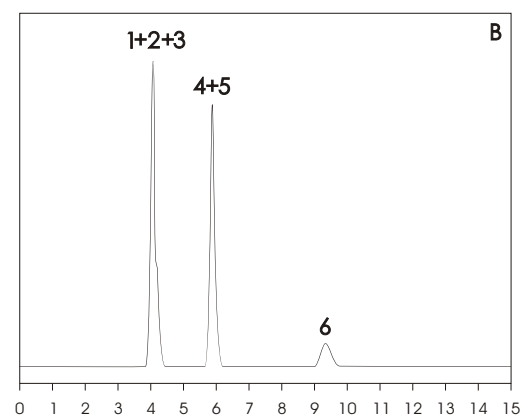
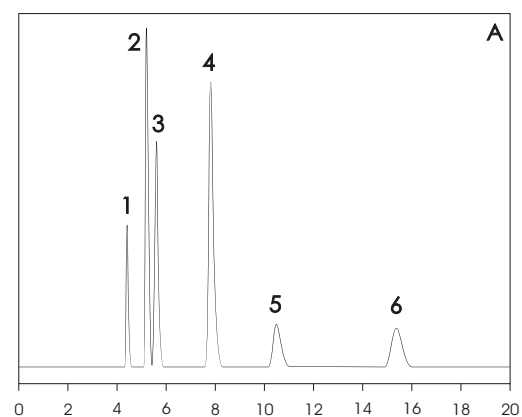


Figure 16.

**Sample:** model mix,

- 1 - benzene,
- 2 - toluene,
- 3 - cumene,
- 4 - naphthalene,
- 5 - 2,7-dimethylnaphthalene,
- 6 - phenanthrene.

**Conditions:** 250x4.6 Chromalite 5HGN.

A) 60:30:10 AcN-THF-water.

B) 1:1 methanol-dichloromethane.

C) 90:10 hexane-dichloromethane.

Isocratic.

1 ml/min.

UV 254 nm.

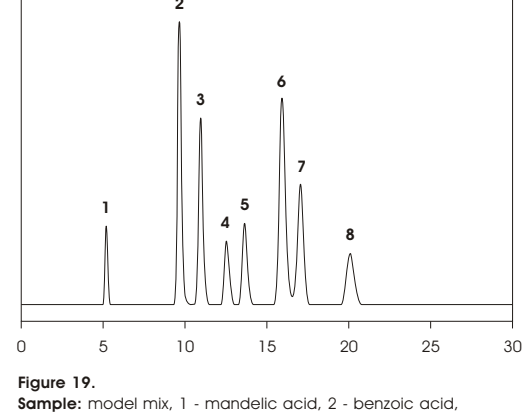
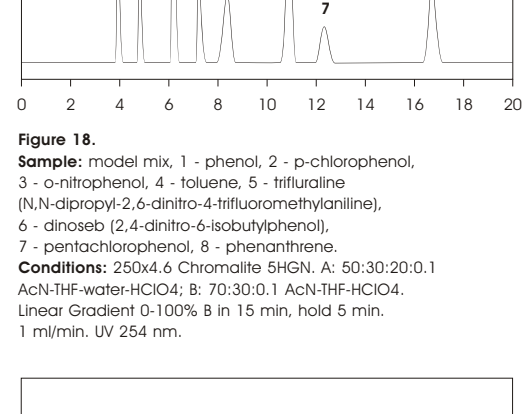
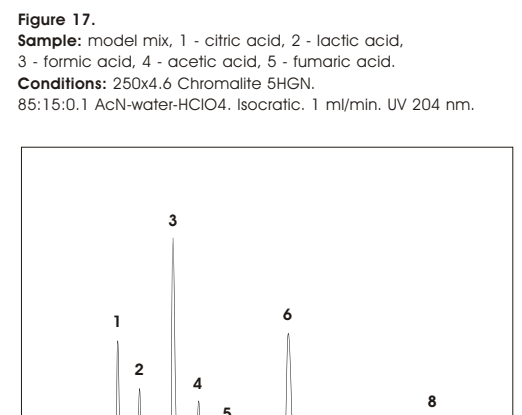
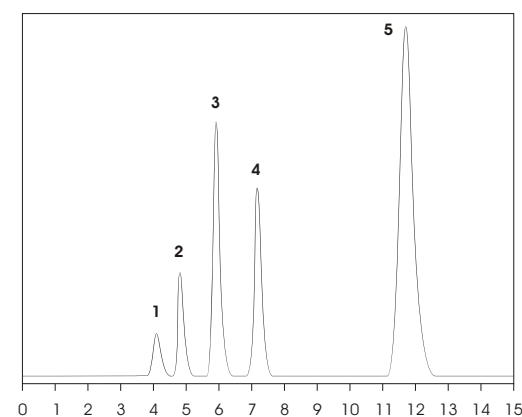


Figure 19.

**Sample:** model mix, 1 - mandelic acid, 2 - benzoic acid, 3 - phenol, 4 - p-toluic acid, 5 - p-nitrophenol, 6 - p-chlorobenzoic acid, 7 - p-chlorophenol, 8 - p-bromobenzoic acid.

**Conditions:** 250x4.6 Chromalite 5HGN. 70:30:0.1 AcN-water-HClO<sub>4</sub>. Isocratic. 0.5 ml/min. UV 254 nm.

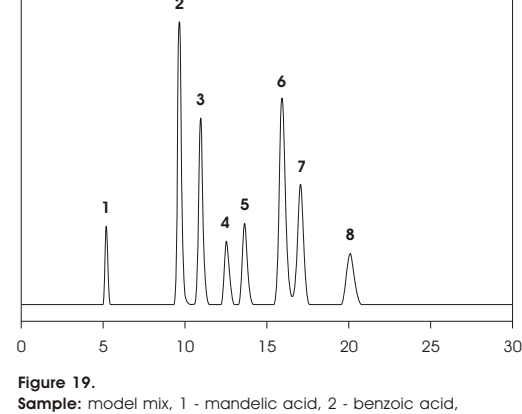
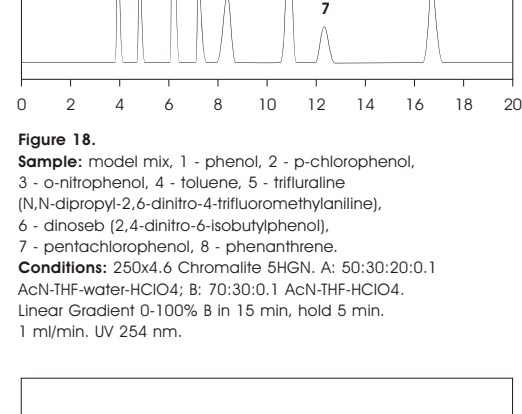
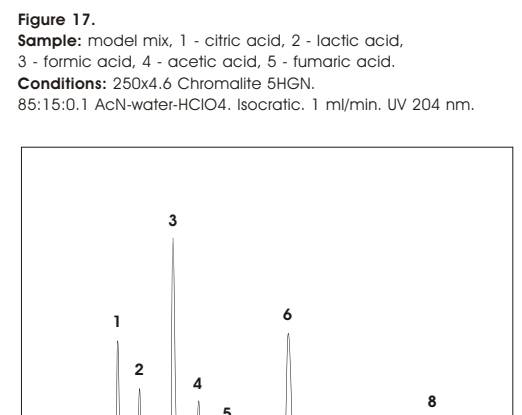
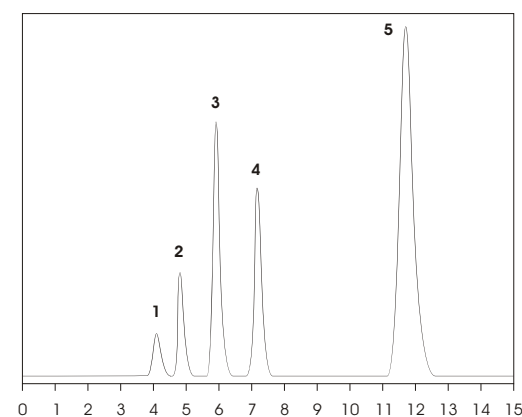


Figure 18.

**Sample:** model mix, 1 - phenol, 2 - p-chlorophenol, 3 - o-nitrophenol, 4 - toluene, 5 - trifluoromethylaniline (N,N-dipropyl-2,6-dinitro-4-trifluoromethyl-aniline), 6 - dinoseb (2,4-dinitro-6-isobutylphenol), 7 - pentachlorophenol, 8 - phenanthrene.

**Conditions:** 250x4.6 Chromalite 5HGN. A: 50:30:20:0.1 AcN-THF-water-HClO<sub>4</sub>; B: 70:30:0.1 AcN-THF-HClO<sub>4</sub>. Linear Gradient 0-100% B in 15 min, hold 5 min. 1 ml/min. UV 254 nm.

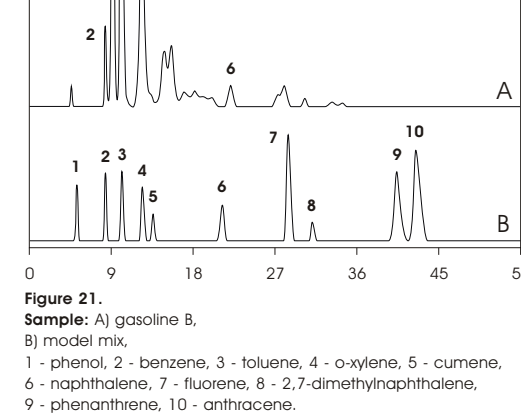
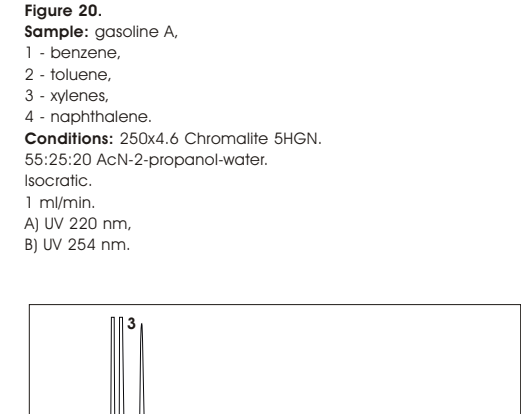
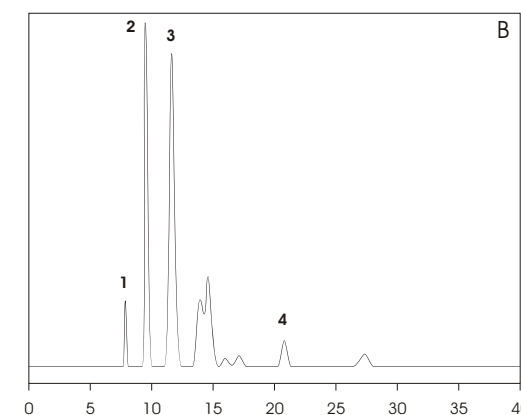
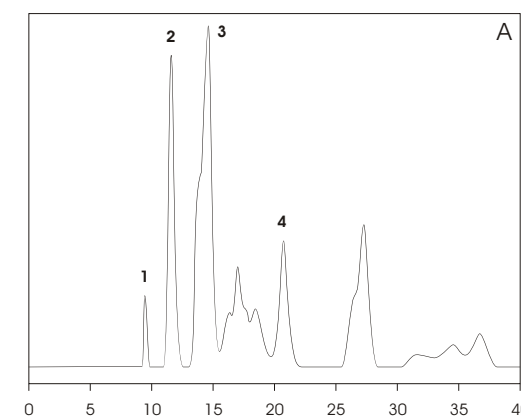


Figure 21.

**Sample:** A) gasoline B,

B) model mix,

- 1 - phenol, 2 - benzene, 3 - toluene, 4 - o-xylene, 5 - cumene,
- 6 - naphthalene, 7 - fluorene, 8 - 2,7-dimethylnaphthalene,
- 9 - phenanthrene, 10 - anthracene.

**Conditions:** 250x4.6 Chromalite 5HGN.

A: 55:25:20 AcN-2-propanol-water; B: 70:30 AcN-2-propanol.

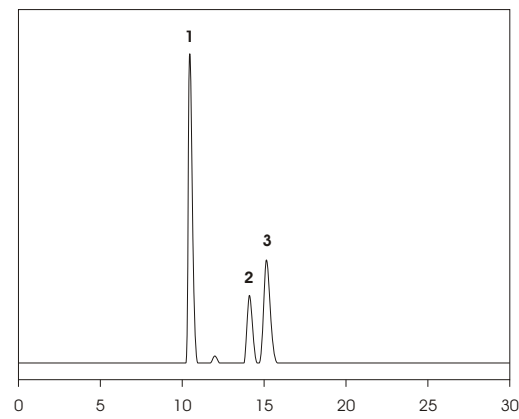
Hold A 20 min, linear gradient 0-100% B in 35 min.

1 ml/min.

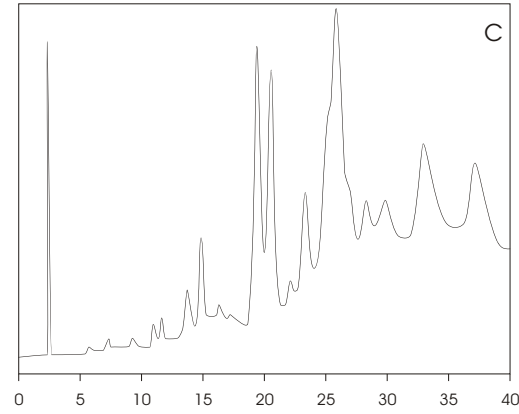
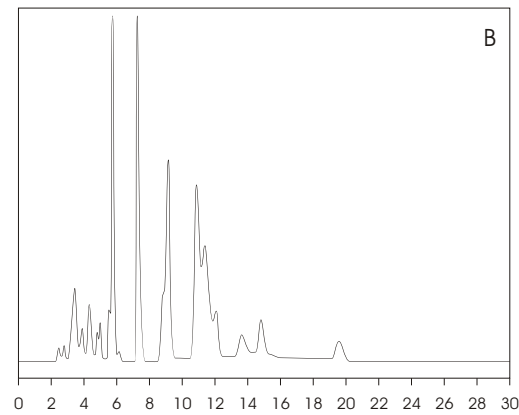
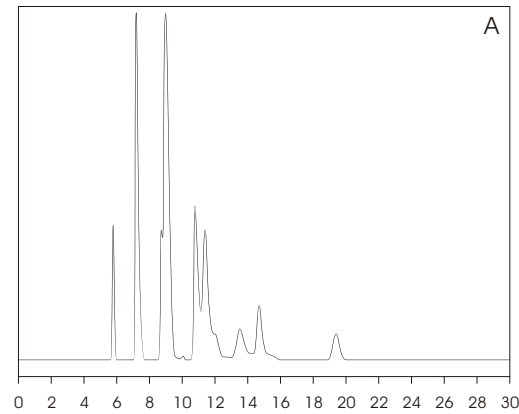
UV 254 nm.



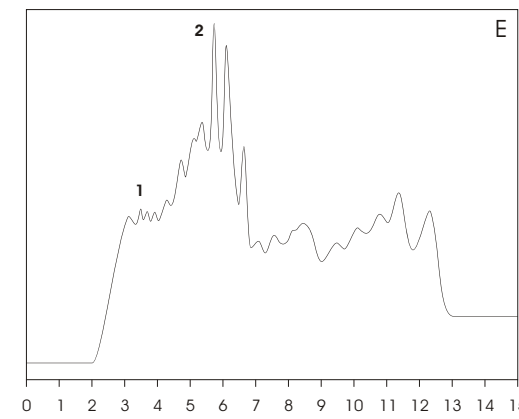
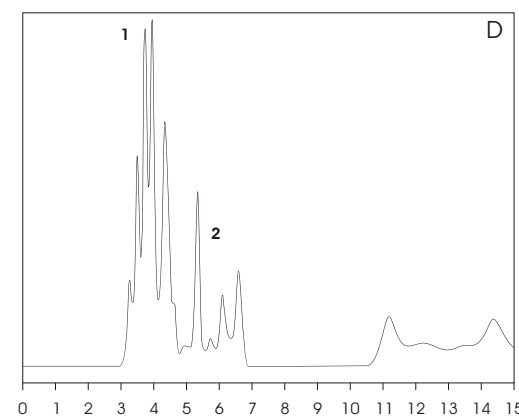
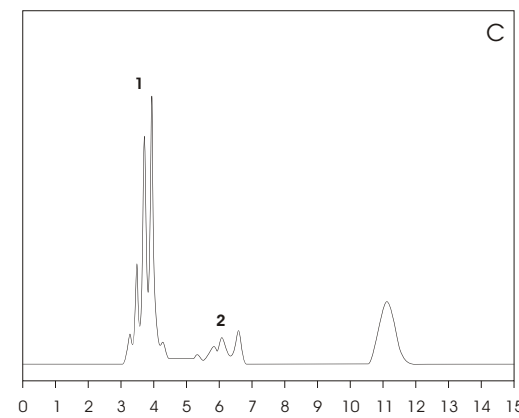
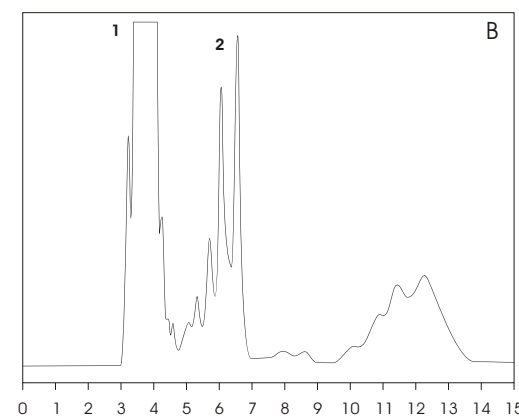
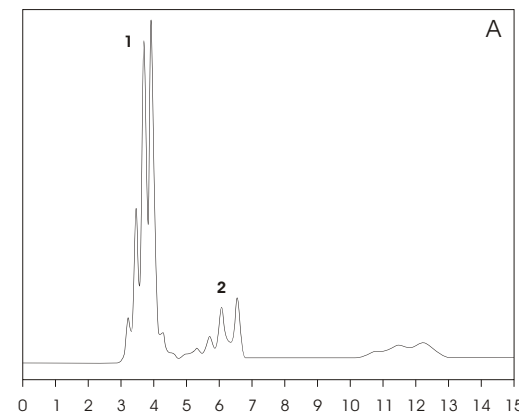
## CHROMALITE 5HGN



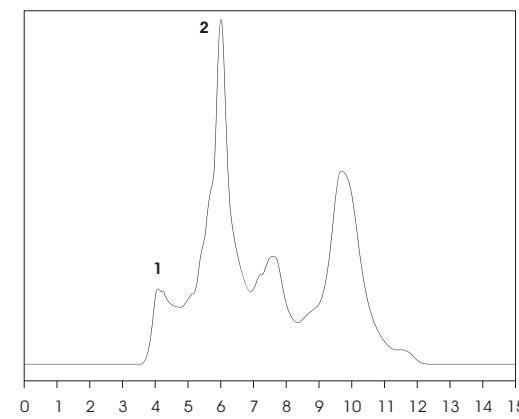
**Figure 22.** Sample: model mix. 1 - naphthalene, 2 - 2,7-dimethylnaphthalene, 3 - 1,8-dimethylaphthalene.  
Conditions: 250x4.6 Chromalite 5HGN. 60:30:10 AcN-THF-water. Isocratic. 1 ml/min. UV 254 nm.



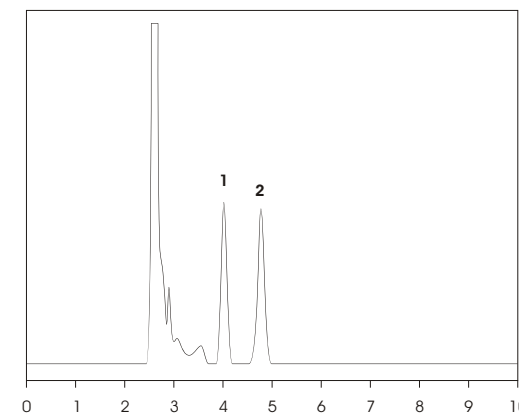
**Figure 23.** Sample: A) gasoline A, B) gasoline C, C) diesel fuel.  
Conditions: 250x4.6 Chromalite 5HGN. 90:10 AcN-water. Isocratic. 1 ml/min. UV 254 nm.



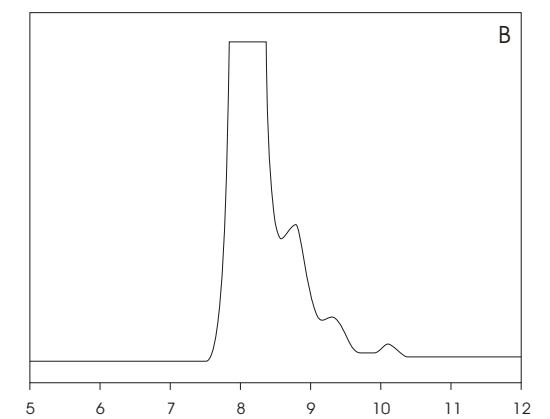
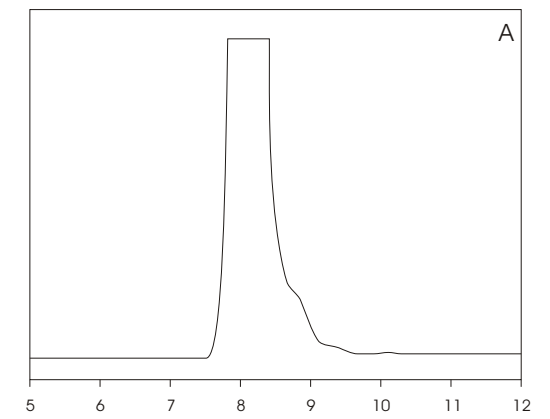
**Figure 24.** Sample: A, B) gasoline A, C) gasoline C, B, D) gasoline C, E) diesel fuel, 1 - monoaromatics, 2 - diaromatics.  
Conditions: 250x4.6 Chromalite 5HGN. 80:20 hexane-dichloromethane. Isocratic. 1 ml/min. UV 254 nm.



**Figure 25.** Sample: diesel fuel, 1 - monoaromatics, 2 - diaromatics.  
Conditions: 250x4.6 Chromalite 5HGN. 1:1 methanol-dichloromethane. Isocratic. 1 ml/min. UV 254 nm.



**Figure 26.** Sample: vegetable oil spiked with hexachlorobenzene & phenanthrene, 1 - hexachlorobenzene, 2 - phenanthrene.  
Conditions: 250x4.6 Chromalite 5HGN. 60:40 hexane-dichloromethane. Isocratic. 1 ml/min. UV 280 nm.

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**Figure 27.** Sample: A) fresh vegetable oil, B) vegetable oil exposed to light for 2 months.  
Conditions: 250x4.6 Chromalite 5HGN. Dichloromethane. Isocratic. 0.3 ml/min. UV 280 nm.



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