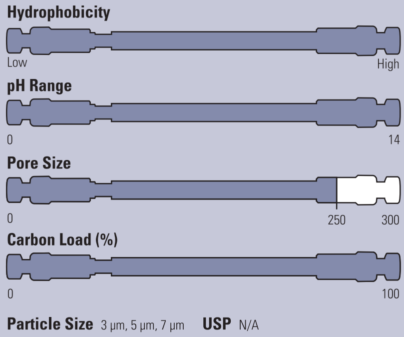
Hypercarb Columns

100% porous graphitic carbon for extended separation capabilities



- **Exceptional retention of very polar analytes**
- **Separates structurally related substances**
- **pH stable from 0 to 14**
- **Ideal for high temperature applications**

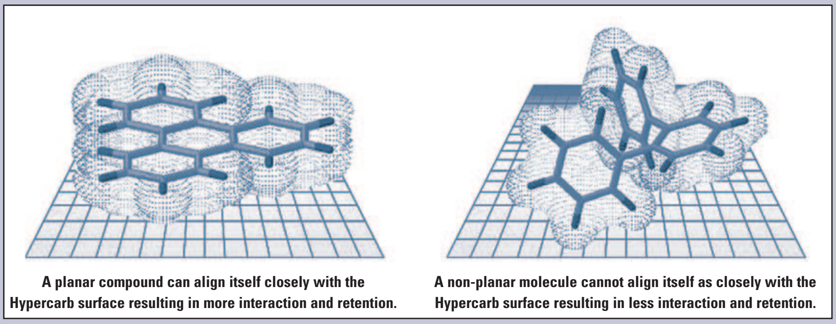
Porous Graphitic Carbon (PGC) is a unique stationary phase composed of flat sheets of hexagonally arranged carbon atoms with a satisfied valence, as in a very large polynuclear aromatic molecule. Thermo Scientific Hypercarb is unlike traditional silica bonded phases in both its structure and retentive properties, allowing for total pH stability and the retention and separation of highly polar species. Hypercarb™ columns are ideally suited to solve "problem" separations, in both reversed phase and normal phase HPLC and LC/MS applications.

Retention and Resolution

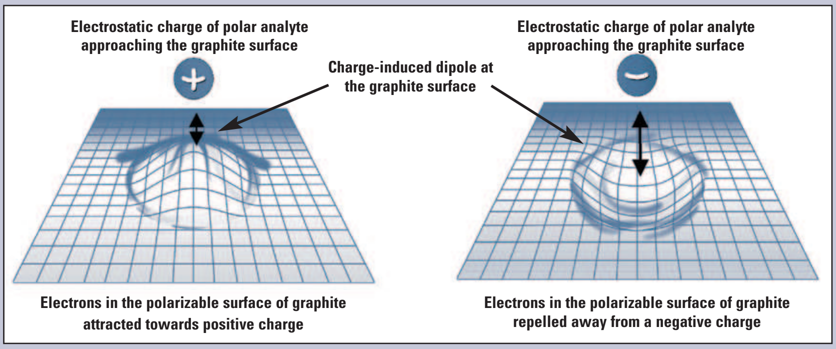
Hypercarb's mechanism of interaction is very dependent upon both the polarity and planarity (shape) of the solute. These specific interaction mechanisms allow the successful retention and resolution of analytes that cannot be separated by typical reversed phase HPLC. Removal of complex buffering systems or ion-pair reagents, and use of increased organic modifier concentration for polar analytes allows greater compatibility with detection techniques such as MS.

The overall retention on Hypercarb columns is a combination of two mechanisms:

1) Adsorption: The strength of analyte interactions with Hypercarb is largely dependent on the molecular area in contact with the graphite surface, and also on the type and positioning of the functional groups in relation to the graphite surface at the points of contact. The approach of a planar and a non-planar molecule to the Hypercarb surface is shown. The strength of the interaction depends upon the size and orientation of the molecular area that is able to come in contact with the flat graphite surface. More planar molecules will show more retention than rigid molecules with a 3-dimensional spatial arrangement.



Schematic representation of molecular area of a planar and non-planar molecule interacting with the Hypercarb surface



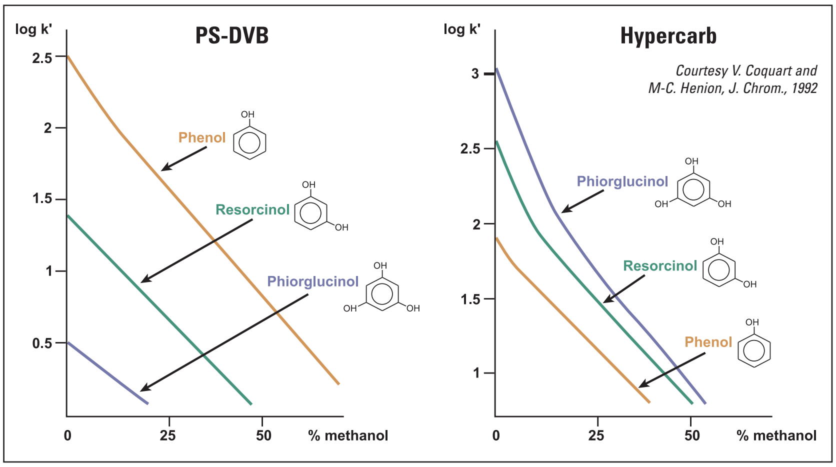
Schematic representation of a point charge approaching the Hypercarb surface

2) Charge induced interactions of a polar analyte with the polarizable surface of graphite: The second mechanism, charge-induced dipole, is illustrated above and accounts for the strong retention exhibited by polar analytes. As the polar group with a permanent dipole approaches the surface, an induced dipole is formed, increasing the attraction between the analyte and graphite surface. These charges should not be confused with the overall ionic charge of the molecule, such as a basic compound ionized in acidic pH conditions. The charge-induced dipole mechanism is strictly due to the interaction of the electrostatic charge of the polar molecule with the graphite surface.

The strong mechanisms of interaction with Hypercarb usually allow for shorter columns to be used during the method development process. In most cases, 100 mm length columns or shorter are sufficient for a separation.

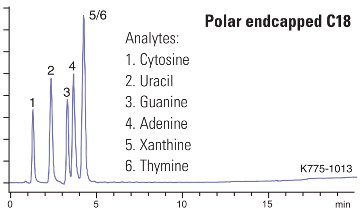
Increased Retention of Polar Analytes

In typical reversed phase chromatography, the retention of an analyte is directly related to its hydrophobicity: the more hydrophobic the analyte, the longer its retention. Conversely, as the polarity of the analyte increases, analyte-solvent interactions begin to dominate and retention is reduced. This observation holds true for the majority of reversed phase systems. An exception to this rule is Hypercarb™, for which retention may in some cases increase as the polarity of the analyte increases, illustrated to the right. This phenomenon is referred to as the "polar retention effect on graphite" (PREG). This property makes Hypercarb columns particularly useful for the separation of highly polar compounds (with logP as low as -4) that are normally difficult to retain and resolve on silica-based alkyl chain phases. The retention of very polar solutes on Hypercarb can be achieved without ion pair reagents or complex mobile phase conditions, as illustrated in the chromatogram below.

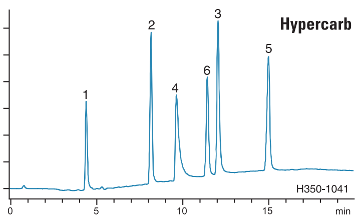


Retention on Hypercarb increases as polarity of the analyte increases, which is the opposite of typical reversed phase materials such as PS-DVB

Mobile Phase: A: H_2O + 0.1% formic acid
B: ACN + 0.1% formic acid
Gradient: 0 to 25% B in 15 minutes
Flow Rate: 8 µL/min
Temperature: 25 °C
Detection: UV at 254 nm



Hypercarb, 5 µm, 100 x 0.32 mm
Part Number: 35005-100365



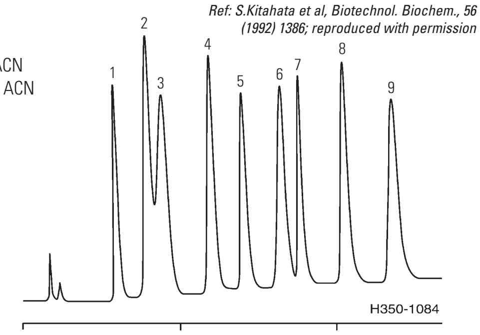
Additional retention is achieved for polar compounds using a Hypercarb column compared to a polar endcapped C18. Note also the change in elution order.

Extended pH Range

One of the other key benefits of Hypercarb columns is the extreme stability of the phase to chemical or physical attack. Due to the unique characteristics of the media, it can withstand chemical attack across the entire pH range of 0 to 14, allowing applications to be run at pH levels that are incompatible with typical silica-based columns. Hypercarb columns offer more choice in buffer selection while handling both high temperature and high pressure.

Hypercarb, 7 µm, 100 x 4.6 mm
Part Number: 35007-104630
Mobile Phase: A: 1mM NaOH containing 5% ACN
B: 1mM NaOH containing 1.5% ACN
Gradient: 30 to 100% A in 15 min
Flow rate: 1.0 mL/min
Detection: PAD 2

Analytes
1. Trehalose
2. Nigerose
3. Isomaltose
4. Maltose
5. Kojibiose
6. Laminaribiose
7. Gentiobiose
8. Cellobiose
9. Sophorose

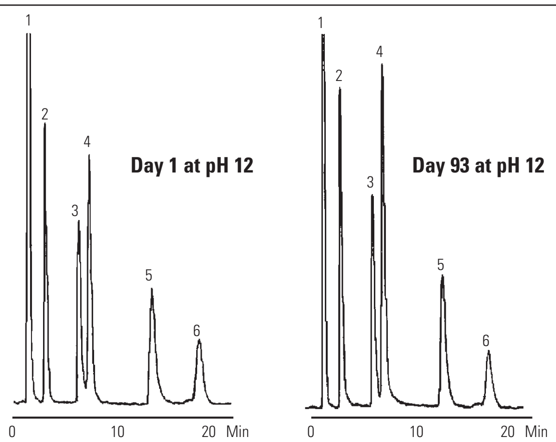


Glucobioses analysis with a mobile phase of NaOH at pH 11

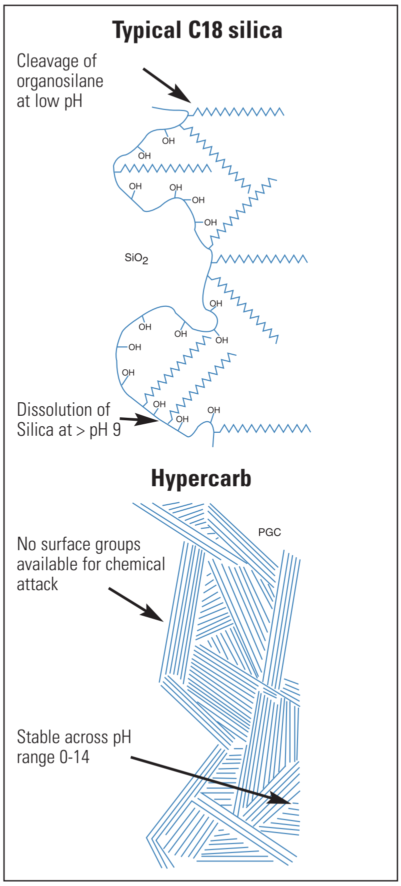
Hypercarb, 5 µm, 100 x 4.6 mm
Part Number: 35005-104630
Mobile Phase: MeOH:H_2O
Isocratic: 70:30
Flow Rate: 0.7 mL/min
Detection: UV at 254 nm

Sample:
1. Acetone
2. Phenol
3. p-Cresol
4. Anisol
5. Phenetole
6. 3,5 -Xylenol

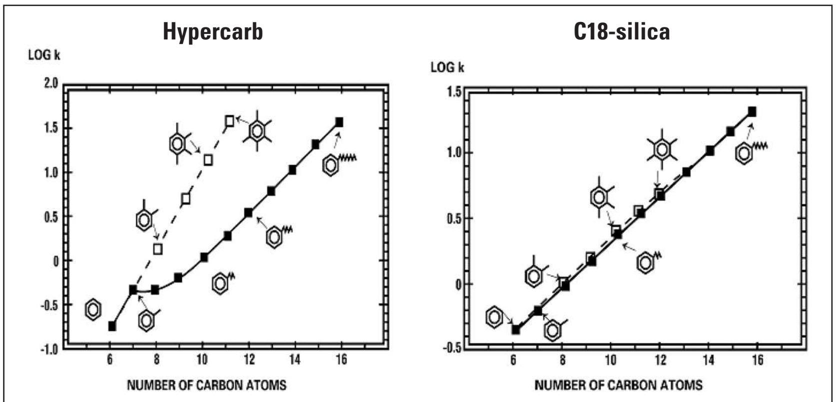
PGCpH12



Hypercarb stability at pH 12: retention and selectivity do not change even after 93 days of storage in 0.1 M NaOH/MeOH



Surface comparison between C18 bonded silica and Hypercarb porous graphitic carbon

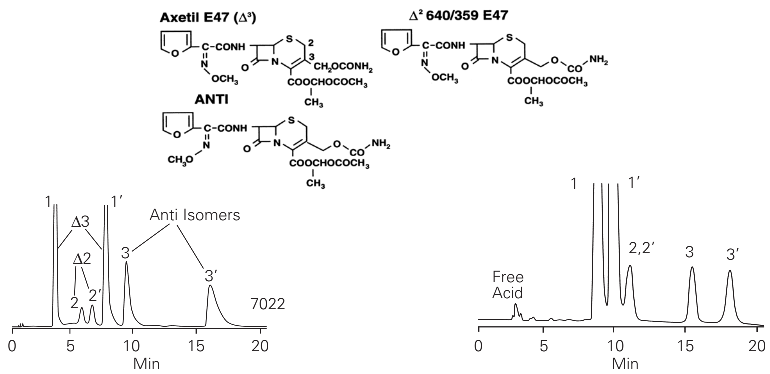


Courtesy of Josef Kriz et al, 1994

Comparison of methyl and methylene group selectivity on C18 and Hypercarb columns

Hypercarb, 5 µm, 100 x 4.6 mm
Part Number: 35005-104630
Mobile Phase: ACN:H_2O:MeOH:Dioxan
Isocratic: 38:20:35:10
Flow Rate: 1 mL/min
Detector: UV at 254 nm

Hypersil SAS, 5 µm, 200 x 4.6 mm
Part Number: 30505-204630
Mobile Phase: MeOH:0.05M $NH_4H_2PO_4$
Isocratic: 38:62
Flow Rate: 1 mL/min
Detector: UV at 254 nm



Courtesy of Norman Smith, Glaxo Group Research, Greenford, 1988

Separation of geometric isomers of Axetil: comparison of a Hypercarb and bonded silica column

Resolution of Structurally Related Compounds

By virtue of the nature of the surface and the way solute shape affects retention, Hypercarb™ columns can differentiate between closely related analytes such as isomers and homologous series. Where no discrimination between methylene and methyl groups is observed using a traditional C18 column, considerable resolving power is observed with Hypercarb columns, as shown to the right. The differentiation of analytes is based on their fit to the graphite surface, allowing for the chromatographic resolution of compounds that are very similar in structure as shown with the resolution of diastereomers of the antibiotic Axetil. The Hypercarb column provides both a significant improvement in separation over the silica-based column originally used, as well as a change in elution order.

Ideal for Reversed Phase LC/MS of Polar Compounds

Reversed phase-LC/MS analysis of very polar compounds is challenging because the typical hydrophobic stationary phases when combined with the most suitable mobile phases for MS detection do not provide the necessary retention to resolve and quantify these compounds.

Hypercarb overcomes these challenges because it:

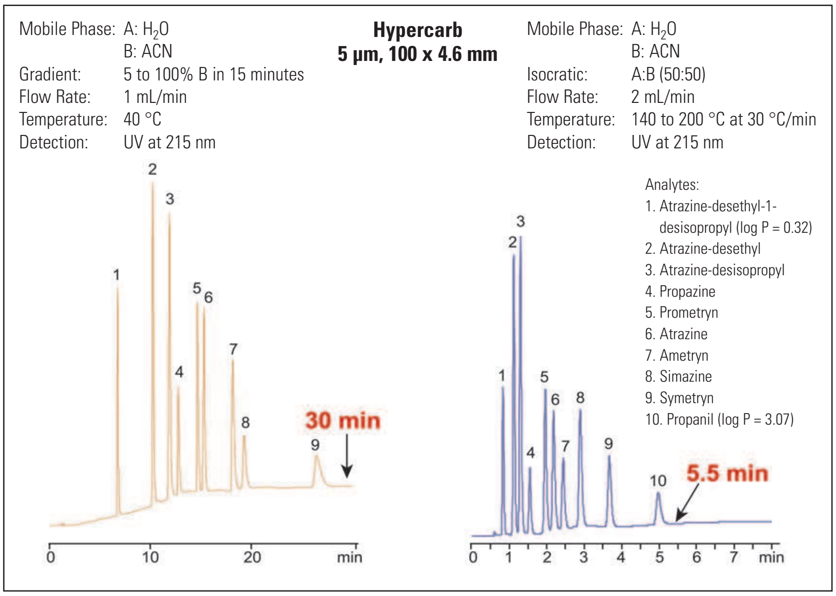
- Retains and separates very polar compounds using “MS friendly” mobile phases such as 0.1% formic or acetic acid and low concentrations of volatile buffers such as ammonium acetate or ammonium formate;
- Can be used with high concentrations of organic modifiers in the mobile phase, which improves nebulization in atmospheric pressure ionization techniques, improving the sensitivity of the analysis;
- Allows shorter column lengths and smaller diameters to be used without compromising peak capacity, often with increased sensitivity. The flow rates used with narrowbore and capillary columns are more compatible with MS techniques;
- Is stable with any mobile phase and produces no phase bleed issues because Hypercarb’s porous graphitic surface is not modified.

Hypercarb™ for Ultra High Temperature HPLC

The use of very high temperatures (up to 200 °C) in liquid chromatography (UHT-LC), generally reduces retention and has the following advantages:

- **Higher speed:**
  In addition to the decrease in capacity factor with temperature, mobile phase viscosity is reduced at high temperatures and therefore higher flow rates can be utilized for fast separations, without compromising efficiency or exceeding the pressure limits of the HPLC system.
- **Higher peak capacity:**
  The reduction of mobile phase viscosity at higher temperatures enhances mass transfer of the solute between mobile and stationary phase, resulting in more efficient and sharper peaks and increased peak capacity
- **Higher sensitivity:**
  Sharper chromatographic peaks improve signal-to-noise ratios. Additionally, when UHT-LC is used in combination with ESI and APCI detection, the mobile phase reaches the ion source at elevated temperature which aids the vaporization and desolvation processes, increasing the ionization efficiency and consequently the sensitivity of the analysis.

Temperature may also affect selectivity. This effect may be small for neutral species, but more significant for ionized compounds, since mobile phase pH and solute pK_a may change with changes in temperature.



Separation of herbicides and metabolites, showing over seven-fold reduction in analysis time with a high temperature gradient

Column Stability at Elevated Temperatures

UHT-LC has specific requirements in terms of column stability. Columns packed with modified silica supports, which are generally used in reversed phase HPLC, should not be used above 60 to 80 °C. At these extreme temperatures, hydrolysis of the organosilane bond or dissolution of the silica may occur. Hypercarb is the ideal stationary phase for UHT-LC, since it is not affected by physical or chemical degradation at high temperature regardless of mobile phase used. Moreover, when used in UHT-LC/MS, there is no phase bleed.

The column hardware used for high temperature applications should not contain PEEK™ components as these are not stable above 120 °C. To avoid column leaking, it is important to use 100% stainless steel columns. A list of Hypercarb columns specifically for use at elevated temperatures is given at the end of the product ordering section on page 105.

Ordering Information

Hypercarb Columns

Particle Size	Length (mm)	4.6 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
3 µm	30	35003-034630	35003-033030	35003-032130	35003-031030
	50	35003-054630	35003-053030	35003-052130	35003-051030
	100	35003-104630	35003-103030	35003-102130	35003-101030
	150	35003-154630	35003-153030	35003-152130	–
5 µm	30	35005-034630	35005-033030	35005-032130	35005-031030
	50	35005-054630	35005-053030	35005-052130	35005-051030
	100	35005-104630	35005-103030	35005-102130	35005-101030
	150	35005-154630	35005-153030	35005-152130	35005-151030
7 µm	50	35007-054630	35007-053030	inquire	–
	100	35007-104630	35007-103030	inquire	–

Other column dimensions are also available. Please call Customer Service for more information. For high temperature applications please refer to columns listed below.

Hypercarb™ Drop-in Guard Cartridges (pk/2)

Particle Size	Length (mm)	4.6 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
3 µm	10	35003-014001	35003-013001	35003-012101	35003-011001
5 µm	10	35005-014001	35005-013001	35005-012101	35005-011001
7 µm	10	35007-014001	35007-013001	35007-012101	35007-011001
UNIGUARD™ Direct-Connect Drop-in Guard Cartridge Holder 		850-00	852-00	852-00	851-00

Hypercarb KAPPA™ Capillary Columns

Particle Size	Length (mm)	500 µm ID	320 µm ID	180 µm ID	100 µm ID	75 µm ID
5 µm	50	35005-050565	35005-050365	35005-050265	35005-050165	35005-050065
	100	35005-100565	35005-100365	35005-100265	35005-100165	35005-100065

Hypercarb Nanobore Columns

Particle Size	Length (mm)	IntegraFrit 75 µm ID	IntegraFrit Multipack 75 µm ID	IntegraFrit 150 µm ID	IntegraFrit Multipack 150 µm ID
5 µm	10	35005-017563	35005-017564 (4/pk)	35005-011563	35005-011564 (4/pk)
	50	35005-057563	35005-057564 (3/pk)	35005-051563	35005-051564 (3/pk)

		PicoFrit 75 µm ID x 15 µm Tip	PicoFrit Multipack 75 µm ID x 15 µm Tip
5 µm	10	35005-017581	35005-017583 (4/pk)
	50	35005-057581	35005-057582 (3/pk)

Hypercarb Specialized Column Hardware for High Throughput


Particle Size	Quantity	DASH™ HTS 20 x 2.1 mm	Javelin™ HTS 20 x 4.0 mm	Javelin HTS 20 x 2.1 mm	Javelin HTS 20 x 1.0 mm
5 µm	3	35005-022151	35005-024035	35005-022135	35005-021035

Hypercarb Preparative Columns

Particle Size	Length (mm)	10 mm ID	21.2 mm ID	30 mm ID	50 mm ID
5 µm	50	35005-059070	35005-059270	35005-059370	35005-059570
	100	35005-109070	35005-109270	35005-109370	35005-109570
	150	35005-159070	35005-159270	inquire	inquire
7 µm	50	35007-059070	35007-059270	35007-059370	35007-059570
	100	35007-109070	35007-109270	35007-109370	35007-109570
	150	35007-159070	35007-159270	35007-159370	35007-159570

Hypercarb High Temperature Columns

Particle Size	Length (mm)	4.6 mm ID	3.0 mm ID	2.1 mm ID	1.0 mm ID
3 µm	30	35003-034646	35003-033046	35003-032146	35003-031046
	50	35003-054646	35003-053046	35003-052146	35003-051046
	100	35003-104646	35003-103046	35003-102146	35003-101046
5 µm	30	35005-034646	35005-033046	35005-032146	35005-031046
	50	35005-054646	35005-053046	35005-052146	35005-051046
	100	35005-104646	35005-103046	35005-102146	35005-101046

Please note that these columns are for use with elevated temperatures. For other dimensions, please inquire.