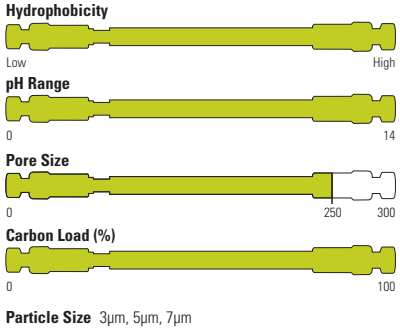
Thermo Scientific Hypercarb HPLC Columns

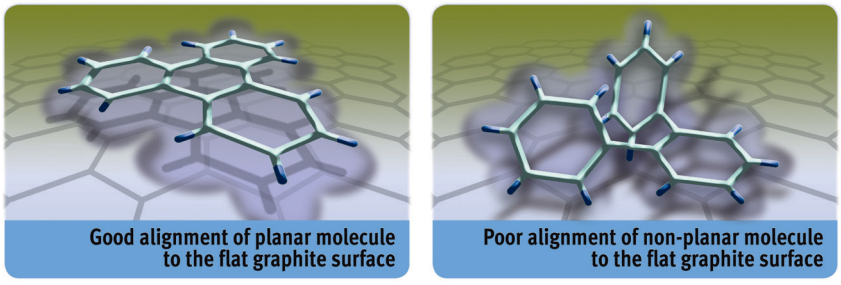
100% porous graphitic carbon for extended separation capabilities

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

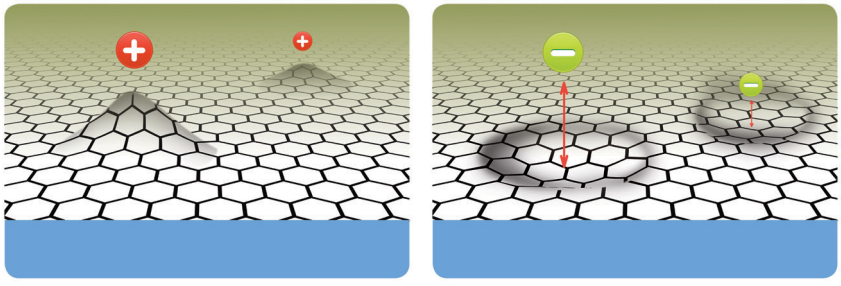


Particle Size 3µm, 5µm, 7µm

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™ columns are 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.



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

Retention and Resolution

The 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:

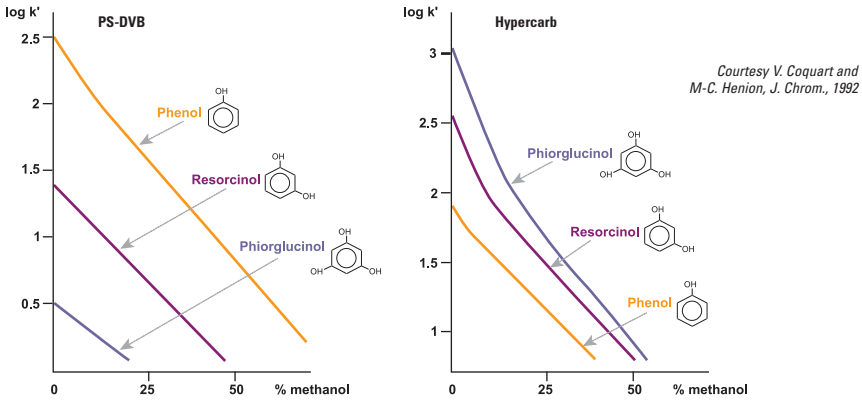
1) Adsorption: The strength of analyte interactions with Hypercarb columns is largely dependent on the molecular area in contact with the graphite surface, and also on the type and positioning of the functional groups in relation to the graphite surface at the points of contact. The approach of a planar and a non-planar molecule to the Hypercarb surface is shown in the diagrams above. 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.

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 columns usually allows for shorter columns to be used during the method development process. In most cases, 100mm length columns or shorter are sufficient for a separation.

Increased Retention of Polar Analytes

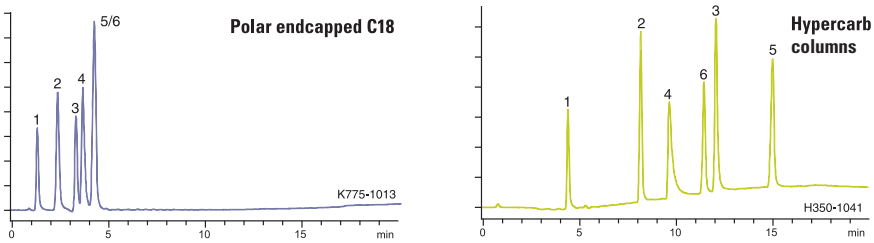
In typical reversed phase chromatography, the retention of an analyte is directly related to its hydrophobicity: the more hydrophobic the analyte, the longer its retention. Conversely, as the polarity of the analyte increases, analyte-solvent interactions begin to dominate and retention is reduced. This observation holds true for the majority of reversed phase systems. An exception to this rule is Hypercarb columns, 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 columns can be achieved without ion pair reagents or complex mobile phase conditions, as illustrated in the chromatogram below.



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

Extended pH Range

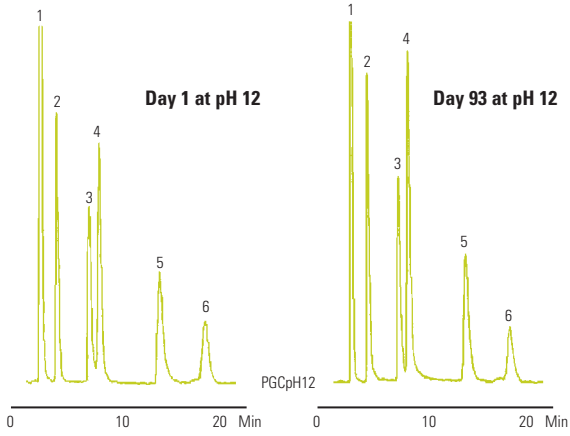
One of the other key benefits of Hypercarb columns is the extreme stability of the phase to chemical or physical attack. Due to the unique characteristics of the media, it can withstand chemical attack across the entire pH range of 0 to 14, allowing applications to be run at pH levels that are incompatible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



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

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

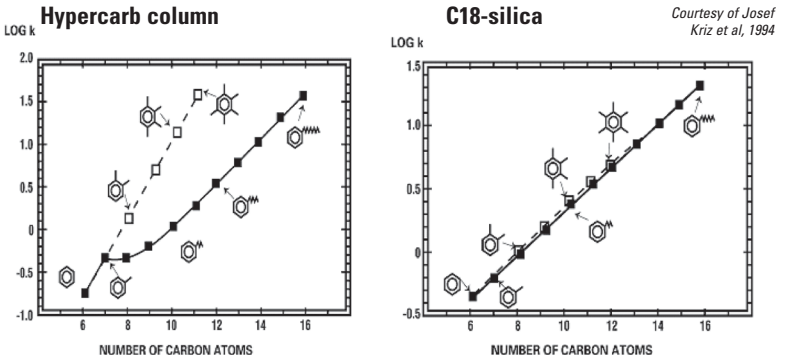
Mobile Phase:	A: H_2O + 0.1% formic acid
	B: ACN + 0.1% formic acid
Gradient:	0 to 25% B in 15 minutes
Flow Rate:	8µL/min
Temperature:	25°C
Detection:	UV at 254nm
Analytes:	1. Cytosine 2. Uracil 3. Guanine 4. Adenine 5. Xanthine 6. Thymine



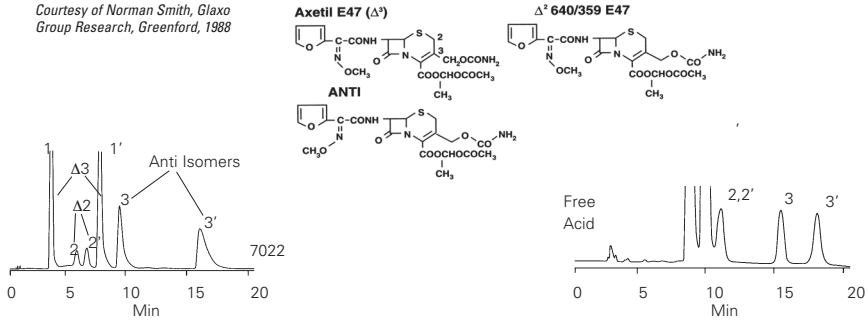
Hypercarb column, 5µm, 100 x 4.6mm

Part Number:	35005-104630
Mobile Phase:	MeOH:H_2O
Isocratic:	70:30
Flow Rate:	0.7mL/min
Detection:	UV at 254nm
Sample:	1. Acetone 2. Phenol 3. p-Cresol 4. Anisol 5. Phenetole 6. 3,5 -Xylenol

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



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



Hypercarb column, 5µm, 100 x 4.6mm

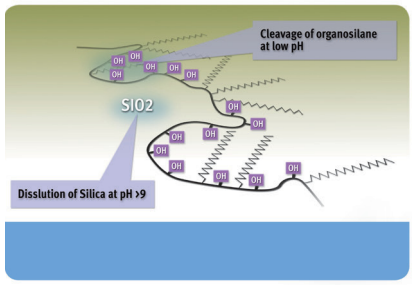
Part Number:	35005-104630
Mobile Phase:	ACN:H_2O:MeOH:Dioxan
Isocratic:	38:20:35:10
Flow Rate:	1mL/min
Detector:	UV at 254nm

Hypersil SAS, 5µm, 200 x 4.6mm

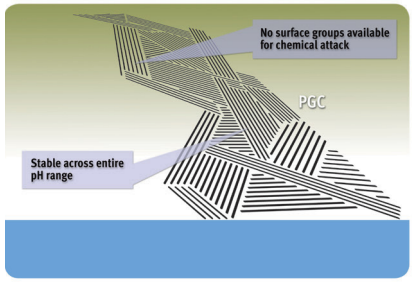
Part Number:	30505-204630
Mobile Phase:	MeOH:0.05M $NH_4H_2PO_4$
Isocratic:	38:62
Flow Rate:	1mL/min
Detector:	UV at 254nm

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

Typical C18 silica



Hypercarb



Surface comparison between C18 bonded silica and Hypercarb porous graphitic carbon

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 above. 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 above 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.

A Hypercarb column 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 the Hypercarb column's porous graphitic surface is not modified.