

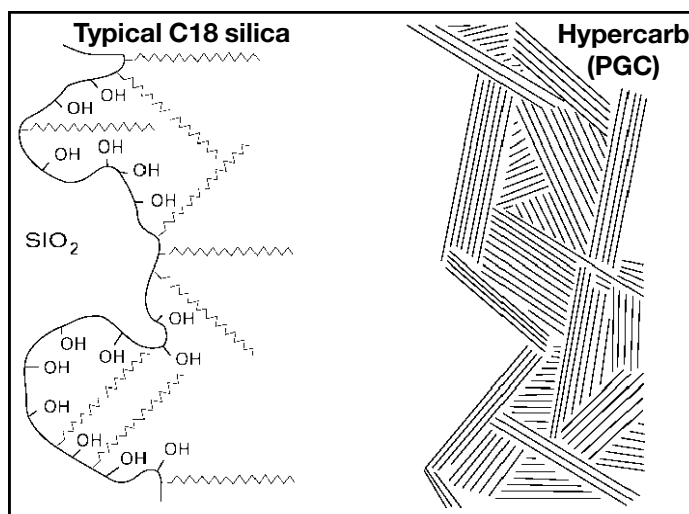
Hypercarb®: A Unique HPLC Column Ideal for the Retention of Polar Compounds

By Paul Ross

Hypercarb (Porous Graphitic Carbon or PGC) has unique properties as a stationary phase. Many chromatographers have made use of these properties to address a wide range of 'problem separations'. Typically, Hypercarb columns have been shown to:

- Retain very polar analytes not normally retained on silica-based C18 columns
- Separate structurally similar compounds such as diastereoisomers that are not separated on silica-based C18
- Provide stability across the pH range 1-14
- Allow rapid equilibration times
- Work in either aqueous-based mobile phase systems (reversed phase) or organic solvent systems (normal phase)

In this short report we focus on how the unique properties of Hypercarb columns have been used to retain both polar compounds and relatively non polar compounds in the same analysis. We will also demonstrate the retention and separation of very polar compounds, which typically cannot be achieved using traditional reversed phase methods.



Retention of polar analytes in reversed phase systems

In any reversed phase system, analyte retention increases as its hydrophobic properties increase. This is due to the strong interactions that take place between the hydrophobic stationary phase and the analyte and repulsive interactions that take place between the analyte and the solvent. Conversely, as the polarity of the analyte increases, affinity for the aqueous phase increases. Analyte-solvent interactions begin to dominate and retention is reduced. This simple observation holds true for all reversed phase systems with the exception of Hypercarb columns, where retention increases as the analyte becomes more polar. We have called this effect "the polar retention effect on graphite". This property makes Hypercarb columns particularly useful for the separation of highly polar compounds which would otherwise be difficult to retain on typical C18 packings. Hypercarb columns are often a workable alternative to ion exchange chromatography for compounds that are ionizable or highly polar, including carbohydrates and compounds containing numerous -OH, -COOH, -NH groups, etc.

Retention of Polar Analytes on Hypercarb Columns

Coquart and Hennion^{1 2 3} have carried out in depth studies into the retention of polar hydroxy-substituted benzenes. Their work demonstrated how differently Hypercarb columns retain polar compounds compared to silica-based C18 and SDVB Hamilton PRP-1™, a polymer-based reversed phase material. An example from their paper is given in Table 1. By measuring log k' values for a range of compounds in their mono-, di- and tri-substituted forms at different methanol:water compositions, it was possible to extrapolate these values to give log k'_w data in pure water, i.e. log k'_w .

The results show that the retention of monosubstituted benzenes is similar for C18-silica, PRP-1, and Hypercarb® columns. Increasing the polarity by di- or tri-hydroxyl group substitution to the benzene ring significantly increases retention on the Hypercarb column, while decreasing retention on C18-silica and PRP-1 columns. In the case of C18-silica, the di- and tri-hydroxybenzenes are not retained at all.

The unique retention properties of Hypercarb columns for the retention of polar compounds are put to good use in the following applications.

Retention of Morphine and its Metabolites⁴

Barrett et al report methods that give complete baseline resolution for the analysis of morphine based opiates when run between pH 8.5 and 11.6. Analytes include morphine, codeine, and related metabolites, such as normorphine, norcodeine, morphine-3-glucuronide and morphine-3-O-sulphate. When using silica-C18, a separate isocratic method is required for complete retention and separation of metabolites. Conditions that allow adequate retention of the parent drug cause metabolites such as the glucuronide or sulphate to elute at the void volume. As an alternative, the Hypercarb column is shown to separate both the parent drug and its conjugated metabolites in the same chromatographic run. In this separation the author makes use of the 'polar retention effect on graphite' and the ability of the Hypercarb column to separate compounds closely related in structure. A typical chromatogram from their study is given in Figure 1.

Table 2

Values of log k' _w for Polyhydroxybenzenes on Reversed Phase Media			
	C18-Silica Column	Hamilton PRP-1 Column	Hypercarb Column
Phenol	1.55	2.40	1.80
1,3-dihydroxybenzene	-	1.35	2.35
1,3,5-trihydroxybenzene	-	0.5	2.70

LC/ESI-MS of Ribonucleotides using Hypercarb⁵

Ribonucleotides are ubiquitous in living cells and have major biochemical functions. They are the monomer unit precursors of RNA and they serve regulatory functions. Cyclic nucleotides play a vital role in biochemical signal transduction as the intracellular mediators of a hormone or neurotransmitter. Thus their isolation, identification and quantification is of major importance in biochemical analysis and potentially in clinical diagnosis. Ribonucleotides are usually separated by ion exchange chromatography or reversed phase chromatography with an ion pairing reagent. These non-volatile mobile phases are generally incompatible with mass spectrometric detection. The unique polar retention properties of graphite in this example were exploited to allow the separation of several cyclic nucleotides and respective isomeric mononucleotides using a MS compatible mobile phase (Figure 2).

Figure 1

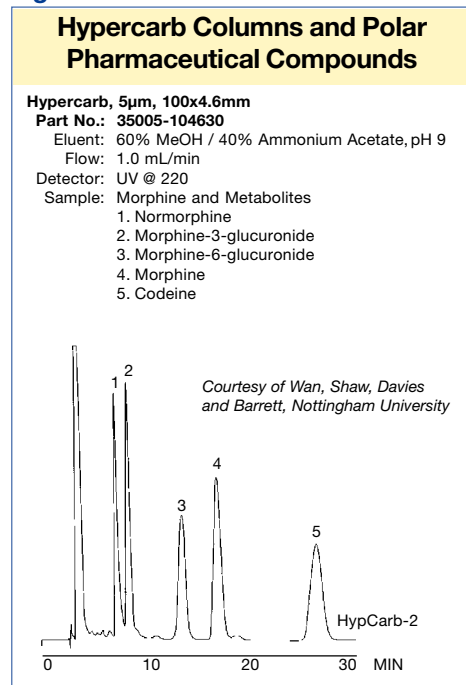
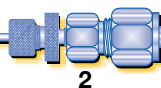
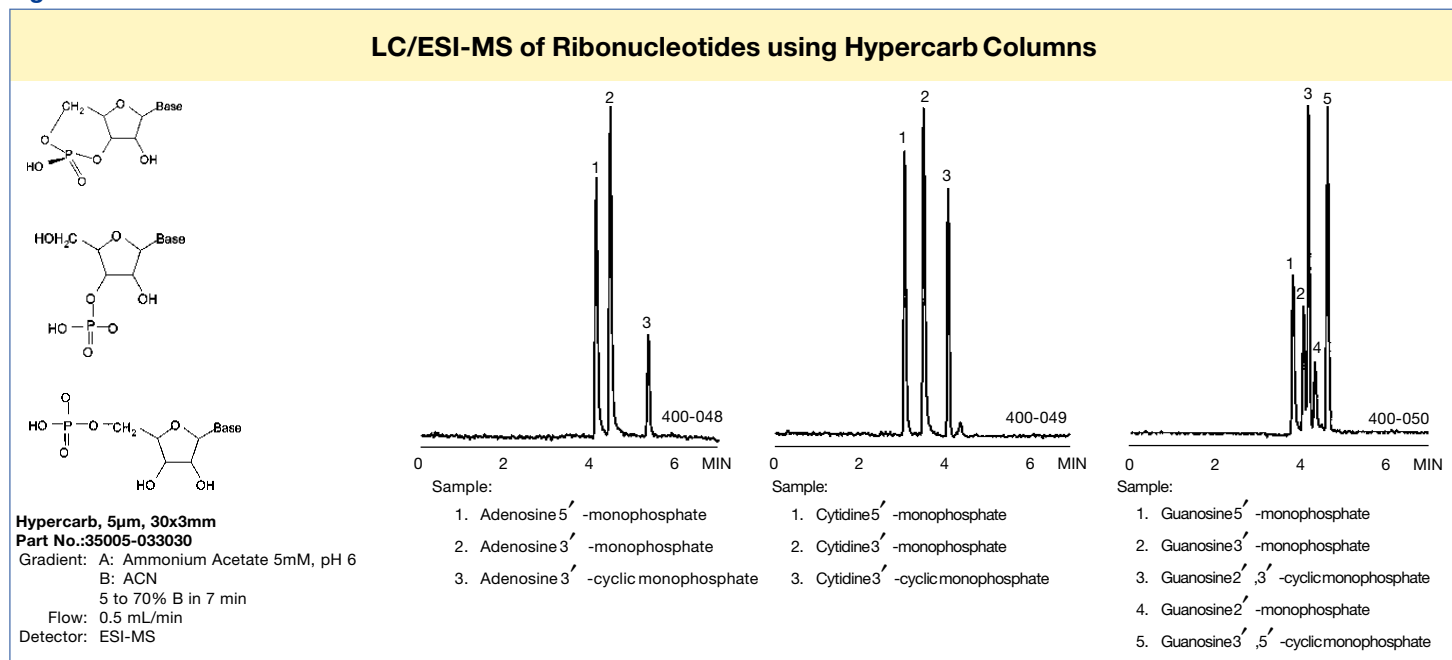


Figure 2



Polyethylene Glycols

Polyethoxylated alcohols (PEAs) and polyethoxylated alkylphenols (PEAPs) are widely used as non-ionic surfactants. Polyethyleneglycols (PEGs) are often present in surfactant mixtures as by-products and contain no surfactant properties. Their identification and quantification are therefore of considerable interest.

In reversed phase HPLC these residual compounds are eluted close to the void volume, while in normal phase chromatography they are strongly retained on the polar support. Their characterization is difficult due to the complexity of the mixture and the lack of chromophores present in PEGs and PEAs. Using a Hypercarb column to analyze the mixture of PEGs and PEAPs, a gradient running from aqueous acetonitrile to 100% acetonitrile then 100% acetonitrile to 80% dichloromethane, enables excellent resolution (two nice fingerprints) for all the compounds that make up this mixture (Figure 3).

¹ Coquart, V. F., Ph.D. thesis, University of Paris, (1993)

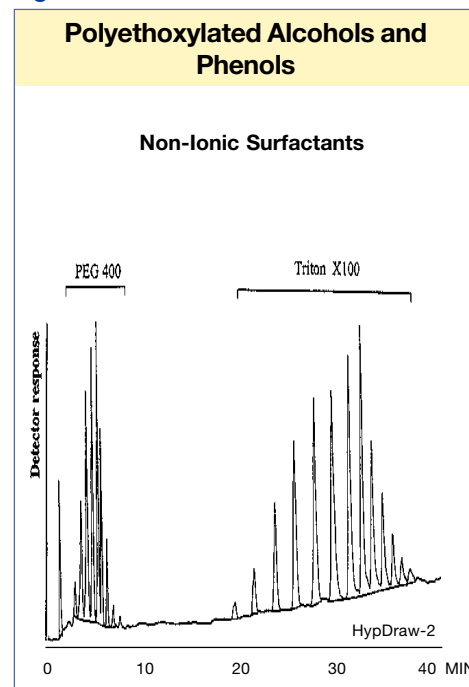
² Coquart, V. F., and Hennion, M.-C., *J. Chromatogr.*, 600, 195 (1992)

³ Hennion, M.-C. and Coquart, V. F., *J. Chromatogr.*, 642, 211 (1993)

⁴ Barrett, D.A et al., *Chromatographia* Vol. 47 No 11/12, June 1998

⁵ Luisa Pereira, Thermo Hypersil Applications Laboratory, UK, 2000

Figure 3



Hypercarb®, 5µm, 100x4.6mm

Part No.: 35005-104630

Gradient: A: H₂O

B: ACN

C: Dichloromethane

From 20% A - 100% B during 15 min then 0% C

to 80% C in 25 min

Courtesy P.Chambault et al. *J.Chromatogr A* 797 (1998)

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