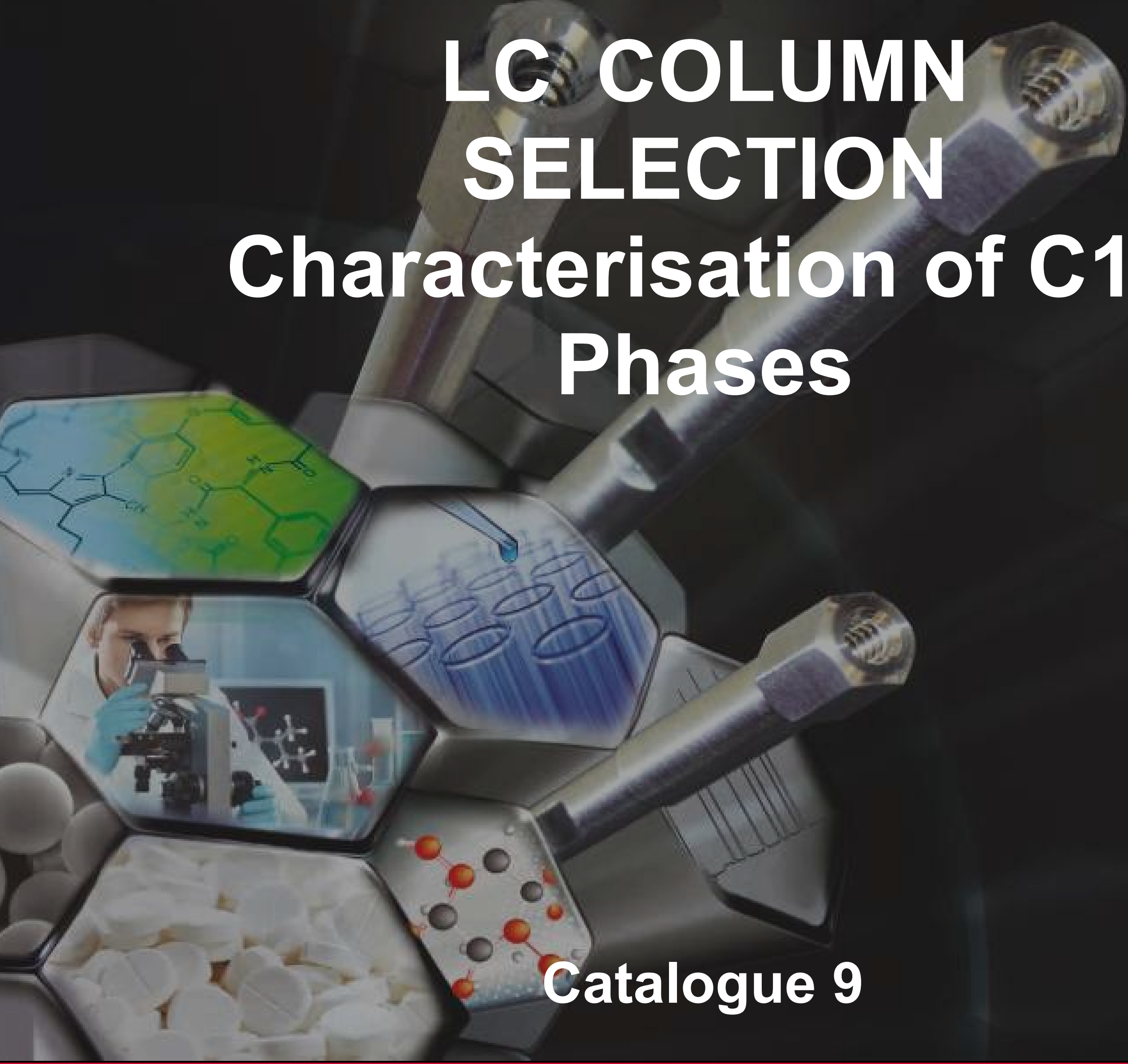
# HICHROM

Chromatography Columns and Supplies

# LC COLUMN SELECTION

## Characterisation of C18 Phases



Catalogue 9

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# CHARACTERISATION OF C18 PHASES



Hydrophobicity is the primary mechanism of analyte interaction with C18 and other alkyl-bonded stationary phases (see page 34). In addition, the polarity of the phase will also contribute to the overall selectivity observed.

## Hydrophobicity

The strength of hydrophobic interaction can be measured by the retention of neutral (non-polar) molecules. The k values (retention factors) for a neutral species, for a given C18 phase, will give an indication of the surface area and surface coverage (ligand density) of the silica.

The percentage of carbon in the material is a simplistic but useful guide to the hydrophobic retention characteristics of a column. In Figure 1 this loose correlation is demonstrated by the increase in retention observed when alkyl chain length (i.e. carbon load) is increased. This increase results from an increase in hydrophobicity of the stationary phase. Similarly an increase in retention would be expected in going from a C18 phase with low carbon load to one of high carbon load.

Hydrophobic selectivity can be determined from the retention factor ratio between two neutral species. This is a better measure of surface coverage than carbon content, as surface area and porosity may vary from silica to silica.

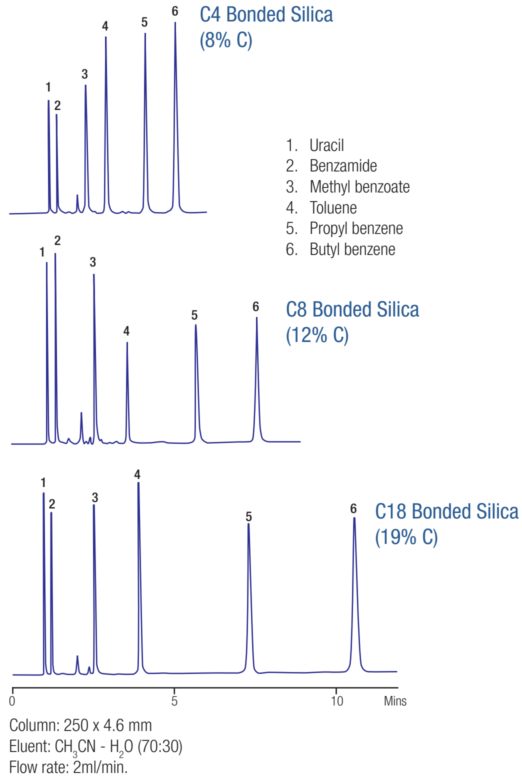



Figure 1. Increase in retention with alkyl chain length

## Polarity

The second key property of C18 materials is their silanol activity, often discussed in terms of polarity. This can be determined by measuring the retention factor ratio between a basic and an acidic compound. At pH >7 the total ion-exchange capacity will correspond to a measure of the total silanol activity. At acidic pH (e.g. pH 2.7) an indication of the acidic activity of the silanol groups can be obtained. The presence of metal ions in the base silica increases the level of silanol activity. Older generation silicas have higher and less tightly controlled levels of metal ions, and hence higher silanol activity compared to newer generation alkyl bonded phases. For this and other reasons, it is strongly recommended that new method development should be approached using newer generation higher purity silicas.

## High Purity Base Deactivated Phases

Modern alkyl bonded phases have very low cumulative metal ion levels within the base silica (<10ppm), resulting in the number of isolated silanol groups, and hence the polarity of the silica surface, also being reduced. Combined with more effective and reproducible bonding processes, these newer generation reversed-phase materials lead to significantly improved chromatography for the more basic polar solute molecules. Use of bonded alkyl groups containing hydrophilic substituents (i.e. polar embedded) can either enhance this effect and/or offer alternative selectivity.

## Optimising Selectivity

Figure 2 illustrates the relationship between the change in polarity and hydrophobicity for typical C18, C8 and C4 materials, showing a decrease in hydrophobicity on reducing alkyl chain length. Greater ligand density, and hence lower polarity, is also seen as the length of the alkyl chain is reduced. However, changing the alkyl chain length may reduce analysis time but will not significantly affect selectivity. Changing the chemistry to an alternative bonded phase is a more powerful tool to achieve this.

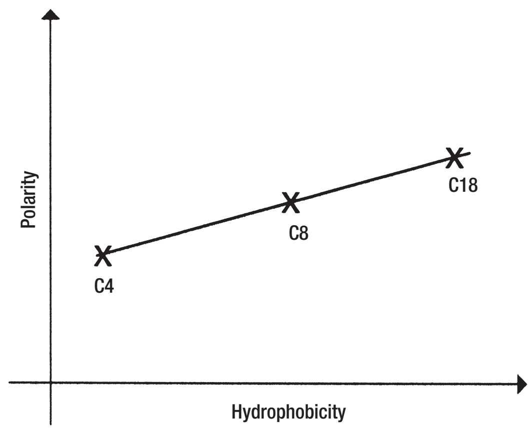


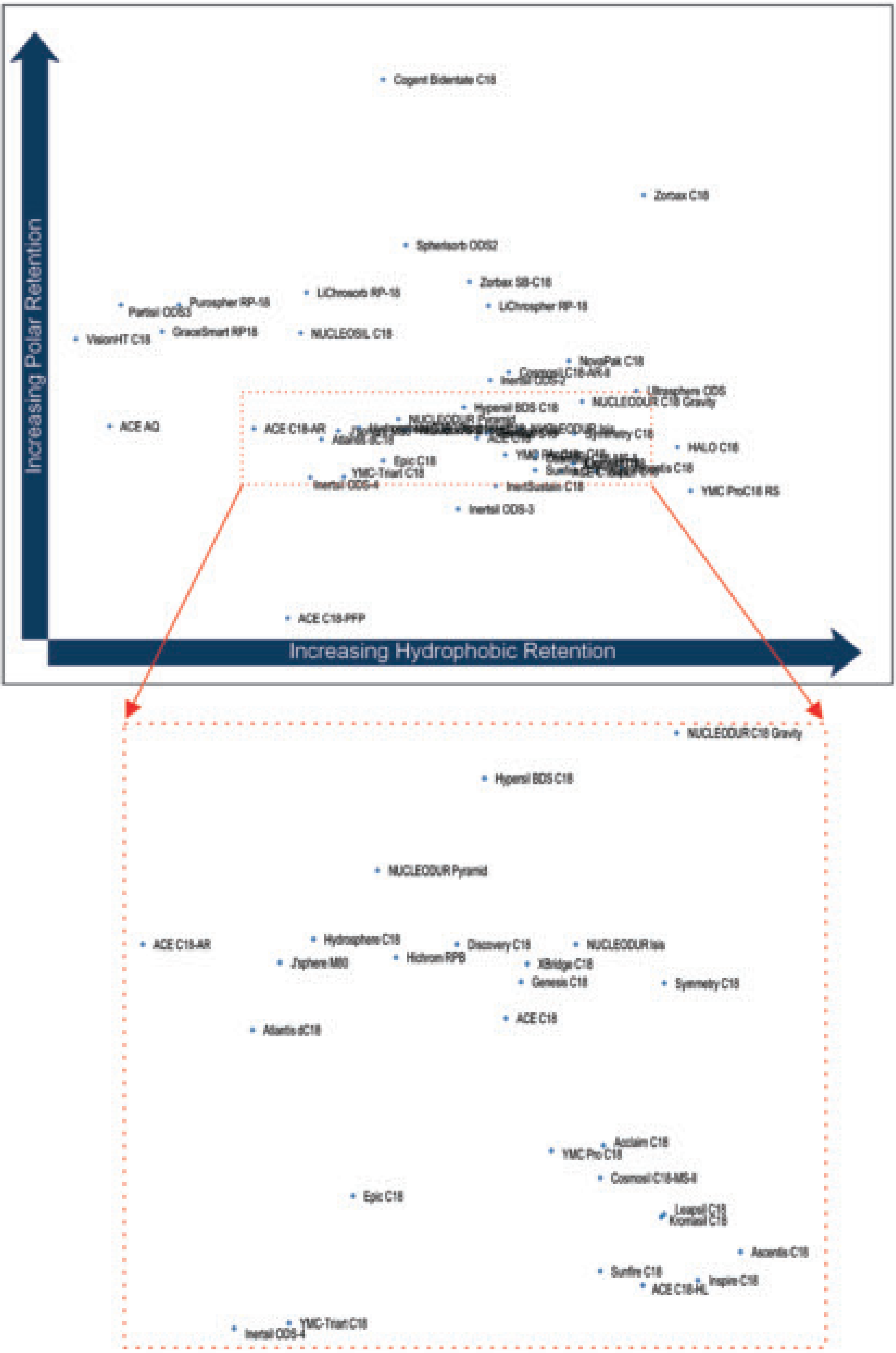

Figure 2. Variation of phase polarity with change in hydrophobicity

## Older Generation 'Traditional' Phases

The older 'traditional' C18 phases are hydrophobic and have a high polarity due to the lower purity silica containing a higher level of acidic silanol groups on which they are based. Use of the newer high purity silicas reduces the resultant phases' silanol activity and improves reproducibility. Employing a polar embedded functionality may also result in a reduced polarity material. For basic solutes that will interact strongly with surface silanols, lower polarity phases are generally recommended. However, for certain analyses, the additional interactions provided by the surface silanols of a 'traditional' C18 material may be beneficial to the overall separation.

## Characterisation of C18 Phases (continued)

Figure 3 is a plot showing the relative selectivity comparison for a number of C18 bonded columns, in which the hydrophobicity is plotted versus increasing polar retention.




**Based on the PQRI database (http://www.usp.org/app/USPNF/columnsDB.html)*
*Plot of hydrophobicity (H) and cation exchange capacity at pH 7 (C @ pH 7.0)*

Figure 3*