The Power of Stationary Phase Selectivity

APEX SCIENTIFIC

Introduction

Method development is a process to select the chromatographic conditions best suited to obtain adequate separation of an analyte mixture. One of the key parameters in the method developers toolkit is stationary phase selectivity. Stationary phase chemistry can influence the mechanisms of interactions with analytes, thereby offering potential selectivity differences. This ACE Knowledge Note will briefly look at what selectivity means and the different reversed-phase interactions.

Selectivity

Retention (R_t) is described as the time taken for an analyte to elute from the column. Retention factor (k) is a value that describes the analyte elution from the column taking into account the column void volume (t_0). Selectivity (α) is the ratio of retention factors of two adjacent analytes and is described by Equation 1.

$$\propto = \frac{k_2}{k_1} \qquad \textbf{Equation 1}$$

A selectivity value of 1 indicates coelution of the two analytes. The combination of the column and elution conditions prohibits the separation of those peaks, regardless of the column efficiency. This therefore indicates further work must be performed to improve separation. Many method and instrument parameters can affect the separation as described in Figure 1[1]. Column stationary phase (and the various mechanisms of interaction) is a useful, controllable parameter to explore when developing methods.

Selectivity is important, but other parameters must also be considered when developing methods to provide suitable resolution between analytes. Resolution can be described using Equation 2.

$$R_s = \frac{2(R_{t2} - R_{t1})}{W_1 + W_2} \qquad \textbf{Equation 2}$$

A minimum resolution value of 1.5 for the critical pair (ie closest eluting pair in the chromatogram) is optimal for most chromatographic separations. The resolution equation can also be re-written as Equation 3. This new equation brings together the influence on resolution of efficiency, selectivity and retention.

From a practical perspective it is important to understand how changes to efficiency, selectivity and retention affect resolution.

Efficiency Selectivity Retention

$$R_s = \boxed{\frac{\sqrt{N}}{4}} \ \boxed{\frac{(\propto - 1)}{\propto}} \ \boxed{\frac{k}{(1+k)}} \qquad \textbf{Equation 3}$$

From this equation, the resolution can be improved by varying either k, α or N. However, as can be seen from the graph in Figure 2, the selectivity parameter is the most powerful parameter to alter in order to influence resolution. Thus exploring column chemistries as a key factor to influence selectivity can be helpful.

Most influential	Isocratic separation	Gradient separations
↑	Column stationary phase	Same as isocratic separation, plus…
	Organic modifier choice	Gradient steepness
	pH (ionisable species only)	$k^* = \dfrac{t_G F}{\Delta \Phi V_M S}$
	% organic modifier	Dwell volume
	Column temperature	Column dimensions
Least Influential	Buffer concentration	

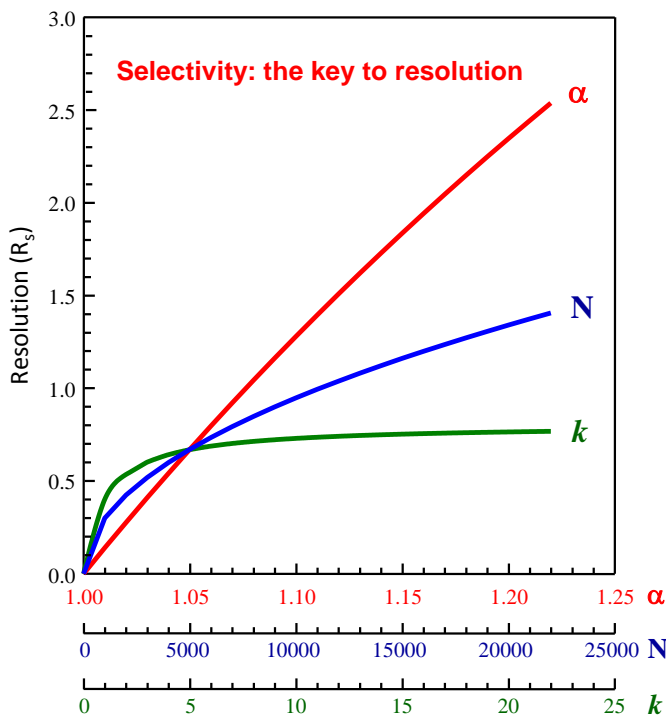**Figure 1:** Important factors which influence selectivity in reversed-phase liquid chromatography

Figure 2: Effect of k, N and α on resolution [2]

Common Reversed-Phase Interactions

The ACE portfolio is based on modern type B silica, to minimise contaminants which increases batch to batch reproducibility. The bonding procedure is such that residual silanols are reduced which improves peak shape by removing secondary interactions.

The ACE reversed phase portfolio (Figure 3), was designed and engineered with the aim to produce a broad array of functionalities to offer orthogonal selectivity – the key to resolution. The complementary phases cover a range of retention mechanism, namely hydrophobic, π-π interactions, dipole-dipole, hydrogen bonding and shape selectivity.

The primary retention mechanism for alkyl chain ligands, such as C18, C8 and C4 is hydrophobicity. The C18 ligand is the gold standard of reversed phase LC, however, method development is somewhat limited when other ligands are not considered.

The ACE C18-AR contains an electron rich ring attached to an extended alkyl chain, increasing hydrophobicity and introducing aromatic selectivity. The chromatograms in Figure 4 compare the selectivity of the ACE C18, ACE Phenyl and ACE C18-AR with toluene, trinitrobenzene, dinitrobenzene and nitrobenzene. When using the ACE C18-AR, there is a different elution order and full resolution of the analytes observed due to the combination of π-π and hydrophobic interactions.

ACE Stationary Phase	Retention Mechanisms
ACE C18	Mainly hydrophobic
ACE C18-AR	Hydrophobic, shape selectivity and π-π donor interactions
ACE C18-PFP	Hydrophobic, π-π acceptor interactions, dipole-dipole, hydrogen bonding and shape selectivity
ACE C18-Amide	Hydrophobic, hydrogen bonding, shape selectivity
ACE CN-ES	Hydrophobic, Electrostatic, and dipole interactions

Figure 3: ACE stationary phase ligands and the dominant retention mechanisms

The ACE C18-PFP mechanism combines hydrophobicity from the alkyl chain, with shape selectivity, dipole-dipole and π-π interactions provided by the electron deficient ring moiety.

The ACE C18-Amide contains an alkyl tail to increase hydrophobic retention, whilst the embedded amide moiety is ideal for hydrogen bonding analytes, such as acidic, phenolic and amino analytes.

The terminal polar CN group on the ACE CN-ES utilises a polar and dipole mechanism whilst, again, the alkyl chain enhances hydrophobicity, which can be of advantage, as seen in Figure 5.
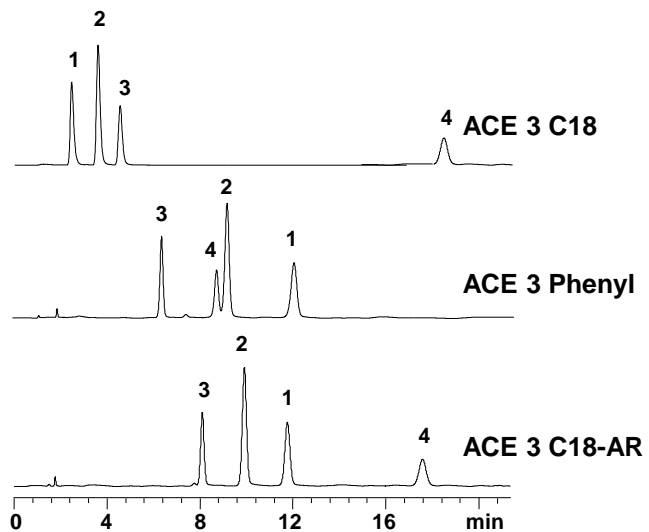
Figure 4: Separation of substituted aromatic analytes,
1. TNB, 2. DNB, 3. NB, 4. Tol,
150 x 4.6 mm, MeOH/H$_2$O 1:1 v/v, 1 mL/min, 40 °C, 210 nm
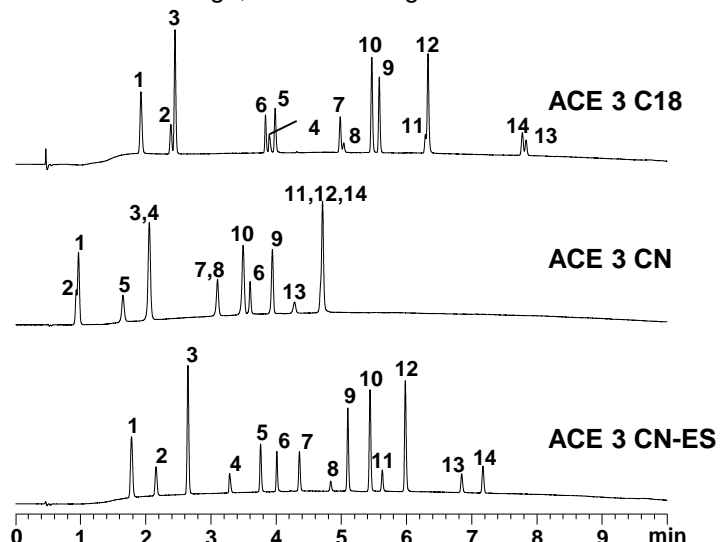
Figure 5: Various compounds, 100 x 2.1 mm,
A 0.1% formic acid in H$_2$O, B 0.1% formic acid in MeCN,
3-100%B in 10 mins. 0.6 mL/min, 40 °C, 210 nm
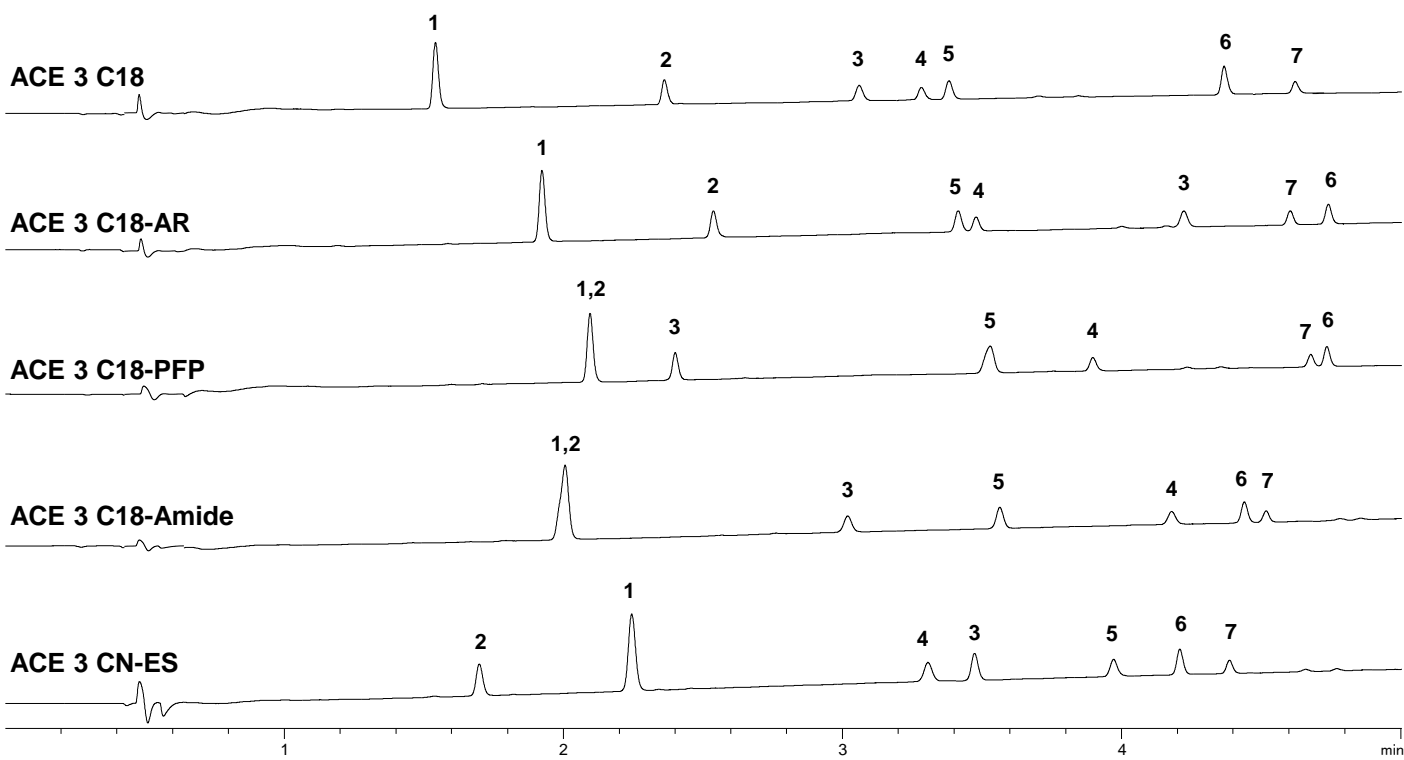
Figure 6: Different eltuion orders and retention of acidic, neutral and basic compounds using the power of phase selectivity.

How To Understand Selectivity Differences Between Columns

Column characterisation, such as the protocols reported by Tanaka or Synder and Dolan, determines the distinctive attributes of a column, such as hydrogen bonding capacity, ion exchange capacity and hydrophobicity. There are various databases available which have characterised columns from different vendors, which can aid the process in finding orthogonal stationary phase selectivities.

Alternatively, selectivity screens are particularly useful in demonstrating elution differences. A screen consists of a standard gradient run at a particular pH with two different organic modifiers and a statistically relevant sized group of diverse analytes (including acidic, basic, neutral, phenolic) with differing physico-chemical properties. The retention time of the analytes of one column can be plotted against another, or one solvent against another, like Figure 7.
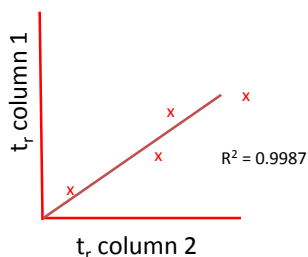
An R^2 correlation can be calculated and inputted into Equation 4[3]. The S value signifies the diversity of the column combination. A large value suggests significant selectivity differences, ideal for method development, whilst a small number would suggest a lack of selectivity differences.

$$S = 100 \times \sqrt{(1 - R^2)}$$ **Equation 4**

The S values were calculated between the ACE portfolio in both acetonitrile and methanol based solvents at pH 3.0, as seen in Figure 8. The ideal method development kit requires S values greater than ~8, which the ACE range offers in abundance. The lower S values for the C18-AR and C18-PFP in MeCN are due to the supression of pi-pi interactions by MeCN – a known and reported phenomenon fro these phase types. It is therefore advisable to use these phases to their full potential in MeOH.

Which Phase Combinations Should Be Chosen?

A 3 phase, 2 solvent selection is pragmatic using the different mechanisms of interaction and organic solvent to fully explore the selectivity space. The 3 phases to be chosen depend upon your application. The 3 columns in Figure 8a represent a good method development platform. Figure 8b would be superb for more polar based analyte mixtures and also include the ACE SuperC18 should a wider pH range be desired.
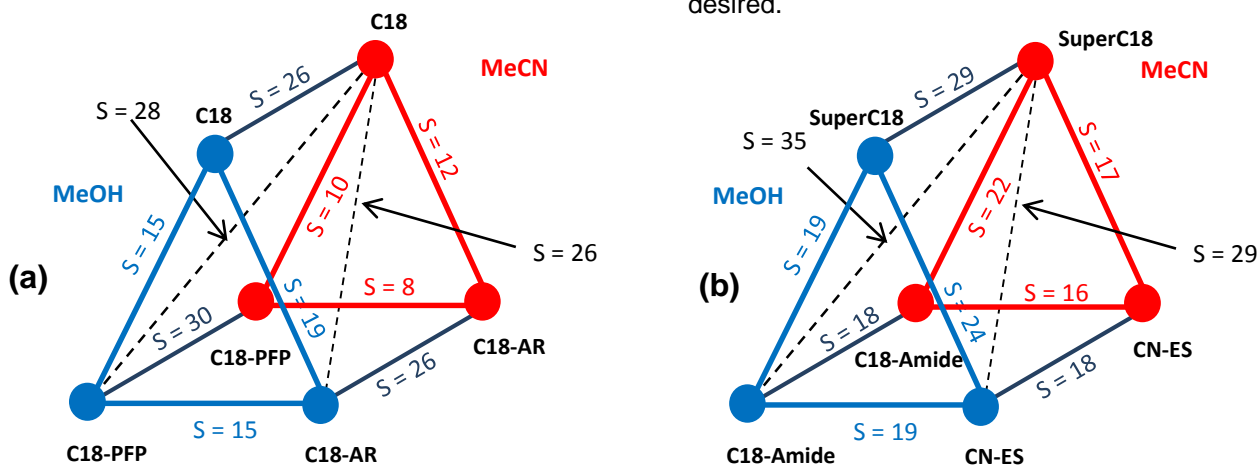

Figure 7

Figure 8: Comparison of S values between the different ACE phases in both acetonitrile and methanol at pH 3.

Conclusion

This ACE Knowledge Note demonstrated the importance in selectivity as a tool to improve resolution, with the ACE range offering orthogonal retention mechanisms. It has also provided methods to determine selectivity differences between phases, which are important for any method development tool kit.

References

[1] Adapted from 'Introduction to Modern Liquid Chromatography", 3rd Edition, Snyder, Kirkland, Dolan, 2010, p.29, Wiley & sons

[2] Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632

[3] Neue, O'Gara, Méndez, J. Chromatogr. A 1127 (2006), 161-174