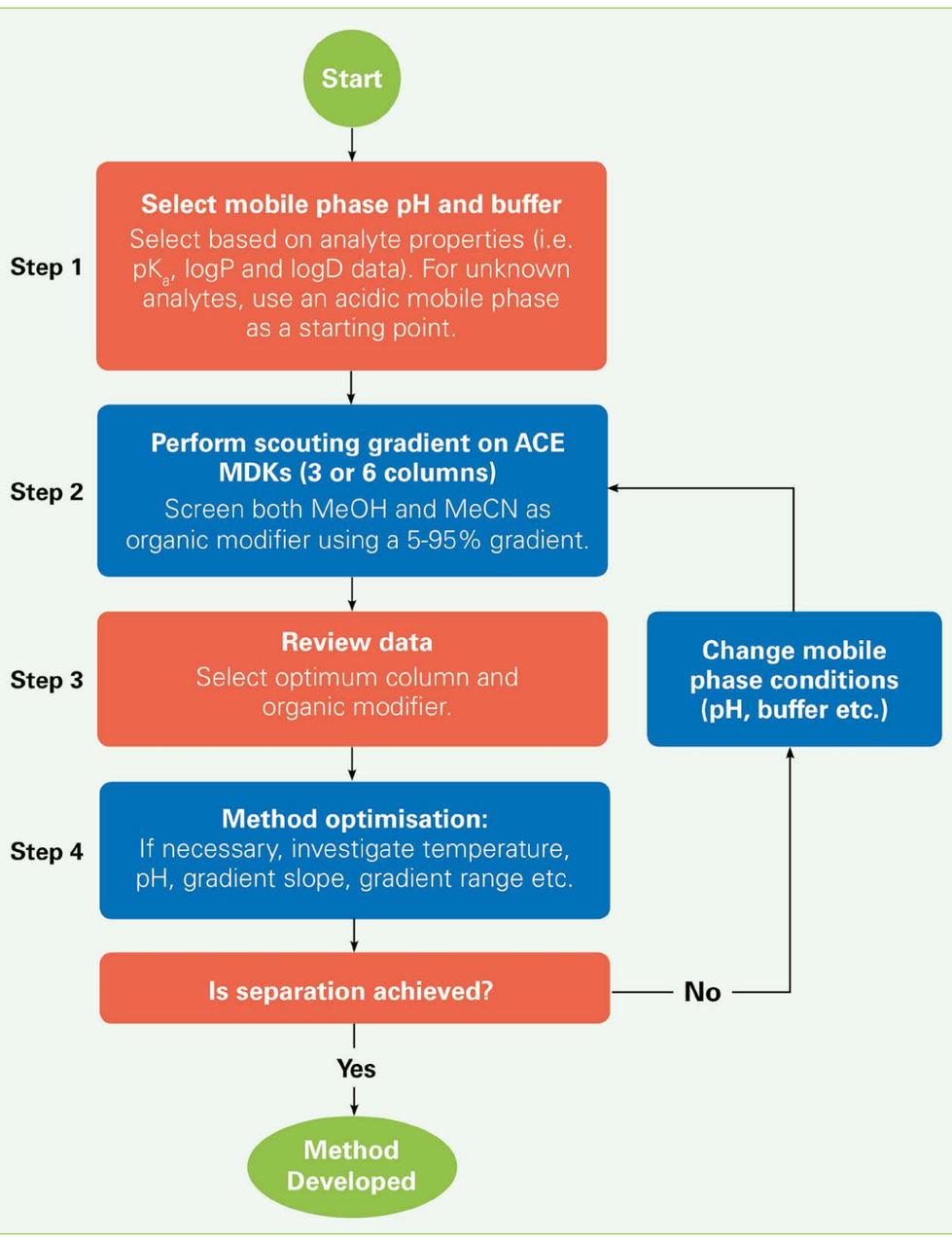
Avantor® ACE® reversed-phase systematic method development protocol

COLUMN SCREENING WITH ACE METHOD DEVELOPMENT KITS (MDKS)

Four Steps for Streamlined Method Development

- Avantor® ACE® MDKs include 3 columns designed with complementary selectivity
- Column screening is a simple yet powerful approach, allowing a suitable column to be quickly identified
- The approach can be made more comprehensive by screening 2 different mobile phase organic modifiers
- The flow chart summarises how a method development screen can be carried out in 4 simple steps



SELECTING COLUMN DIMENSIONS AND PARTICLE SIZE

Defined by the LC system and user preference. For 400 bar HPLC systems, 5 µm 150 x 4.6 mm is a good choice. For 600 bar optimised HPLC systems, 2 and 3 µm particles in shorter columns (e.g. 100 mm) can be used. 1.7 µm particles in short column lengths (e.g. 50 mm) are suitable for UHPLC systems.

HOW TO DETERMINE AN APPROPRIATE SCREENING GRADIENT TIME

The gradient time can be selected using equation 1.
V_M can be estimated using equation 2.

$$t_G = \frac{k^* \times \Delta\Phi \times V_M \times S}{0.87 \times F} \quad (1)$$

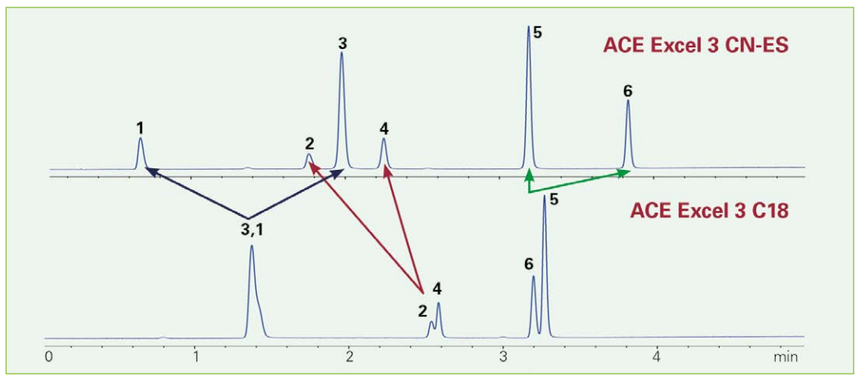
$$V_M = \frac{0.5 \times L \times d_c^2}{1000} \quad (2)$$

tG	Gradient time (min)
k*	Gradient retention factor (typically set to approx. 5)
ΔΦ	Gradient range (i.e. for a 5 - 95%B gradient, ΔΦ = 0.9)
VM	Column internal volume (ml)
S	5 for small molecules (<1000 Da)
F	Flow rate (ml/min)
L	Column length (mm)
dc	Column internal diameter (mm)

Always remember to include a post-gradient isocratic re-equilibration of at least 10 x V_M before the next injection.

WHY USE COLUMN SCREENING?

- Changing the column stationary phase can have a dramatic impact on selectivity (Figure 1)
- Screening different columns with the same mobile phase conditions can help achieve your desired separation quicker with better resolution



Columns	50 x 2.1 mm
Mobile phase	A = 0.1% formic acid in H_2O B = 0.1% formic acid in MeOH:H_2O (9:1 v/v)
Gradient	3 to 100% B in 5 mins
Detection	UV, 254 nm
Flow rate	0.60 mL/min
Temperature	40 °C
Sample	1) Metronidazole, 2) Benzyl alcohol, 3) Hydrochlorothiazide, 4) Vanillin, 5) Methyl paraben, 6) 1,2-Dinitrobenzene

FIGURE 1: The effect of changing column stationary phase.

- ACE MDKs group columns with different mechanisms of interaction to maximise selectivity and increase the likelihood of separating challenging mixtures
- The two most popular ACE reversed-phase (RP) MDKs (see table below) include unique phases engineered to exploit different retention mechanisms and maximise selectivity
- All six phases can be used with standard RP conditions and are as robust as a C18 phase
- Other ACE MDKs available include HILIC, Bioanalytical 300 Å and UltraCore

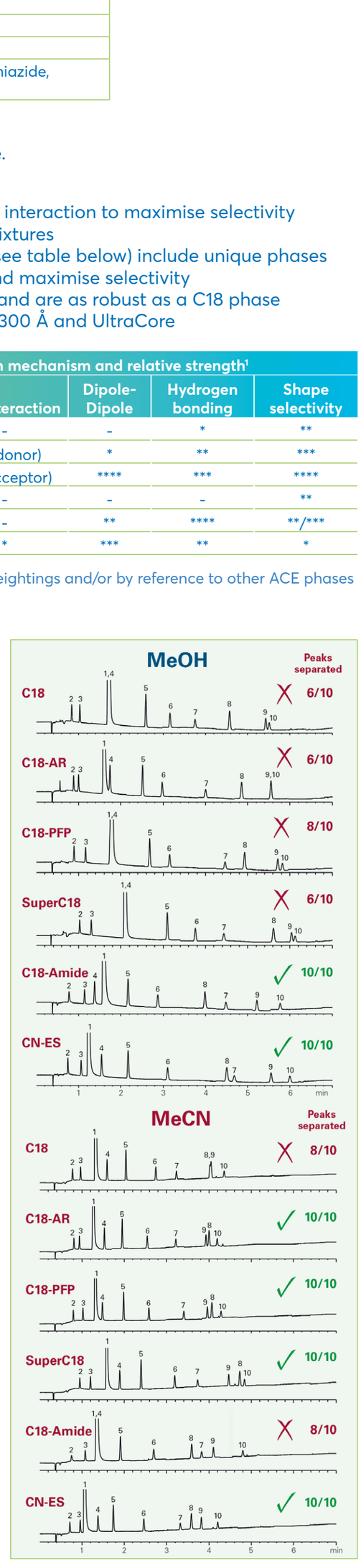
		Separation mechanism and relative strength[1]				
	Bonded Phases	**Hydrophobic binding**	**π -π Interaction**	**Dipole-Dipole**	**Hydrogen bonding**	**Shape selectivity**
ACE Advanced Method Development Kit	ACE C18	****	-	-	*	**
	ACE C18-AR	****	*** (donor)	*	**	***
	ACE C18-PFP	****	*** (acceptor)	****	***	****
ACE Extended Method Development Kit	ACE SuperC18	****	-	-	-	**
	ACE C18-Amide	****	-	**	****	**/***
	ACE CN-ES	***	*	***	**	*

[1] Approximate value – determined by semi-quantitative mechanism weightings and/or by reference to other ACE phases using > 100 characterising analytes

WORKED EXAMPLE

- Acetaminophen and Related Substances
- Mobile phase pH selection based on analyte pKa and logD
- The most common starting point for method development (C18) did not separate all analytes using either MeOH or MeCN
- Further method development would be required
- Using a 6 column/2 mobile phase screen, 6 solutions were immediately identified
- No further method development required!

Columns	2 µm 100 x 3.0 mm
Mobile phase	A = 20 mM NH_4OAc pH 6.0 B = 20 mM NH_4OAc pH 6.0 in Organic: H_2O (9:1 v/v)
Gradient	5 to 95%B in 10 min
Flow rate	1.2 ml/min
Temperature	40 °C
Injection volume	2 µl
Sample	1) Acetaminophen (paracetamol) 2) 4-Aminophenol 3) Hydroquinone 4) 2-Aminophenol 5) 2-Acetamidophenol 6) Phenol 7) 4-Nitrophenol 8) 2-Nitrophenol 9) 4-Chloroacetanilide 10) 4-Chlorophenol



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