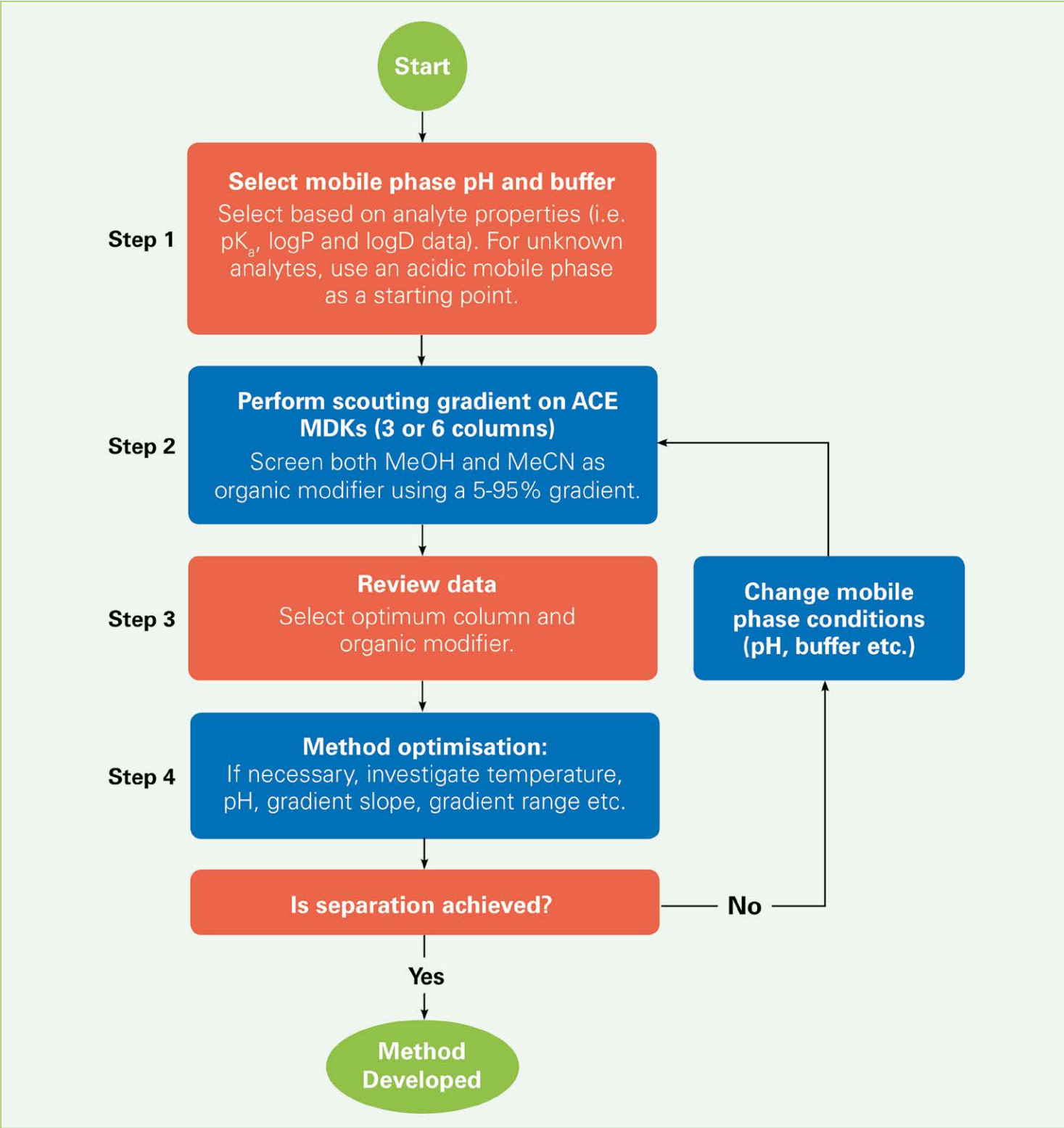


Avantor® ACE® reversed-phase systematic method development protocol

COLUMN SCREENING WITH ACE METHOD DEVELOPMENT KITS (MDKS)

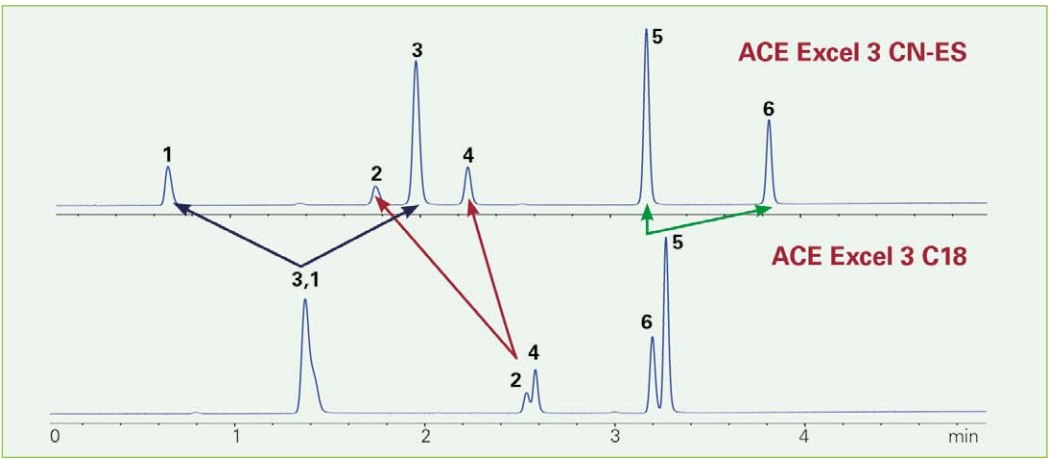
Four Steps for Streamlined Method Development

- 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



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 ₂ O B = 0.1% formic acid in MeOH:H ₂ O (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

	Bonded Phases	Separation mechanism and relative strength ¹				
		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

SELECTING COLUMN DIMENSIONS AND PARTICLE SIZE

Defined by the LC system and user preference. For 400 bar HPLC systems, 5 μ m 150 x 4.6 mm is a good choice. For 600 bar optimised HPLC systems, 2 and 3 μ m particles in shorter columns (e.g. 100 mm) can be used. 1.7 μ m 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.

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

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

t _G	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)
V _M	Column internal volume (ml)
S	5 for small molecules (<1000 Da)
F	Flow rate (ml/min)
L	Column length (mm)
d _c	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.

WORKED EXAMPLE

- Acetaminophen and Related Substances
- Mobile phase pH selection based on analyte pK_a 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 μ m 100 x 3.0 mm
Mobile phase	A = 20 mM NH ₄ OAc pH 6.0 B = 20 mM NH ₄ OAc pH 6.0 in Organic: H ₂ O (9:1 v/v)
Gradient	5 to 95%B in 10 min
Flow rate	1.2 ml/min
Temperature	40 °C
Injection volume	2 μ l
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

