Avantor® ACE® step-by-step HILIC method development protocol

HILIC stationary phase and mobile phase pH are two of the most powerful parameters for altering HILIC selectivity. Assessing these two critical parameters is therefore the optimum starting point for method development. The recommended approach uses stationary and mobile phase screening data to identify a column/mobile phase combination that is most promising for the sample. Once selected, the method can then be fine-tuned using other parameters such as buffer strength and temperature.

Figure 1 shows a flow diagram summarising the following step-by-step HILIC method development protocol.

Step 1: If analyte properties are known, select two appropriate mobile phase pH's for screening. If unknown, use pH 3.0, 4.7 and 6.0 (these pH values are designed to maximise selectivity differences).

Step 2: The sample is screened on the three ACE HILIC phases at the specified pH values using either isocratic or gradient conditions as specified in Table 1. If retention times are too short or too long in isocratic mode, the percentage of strong solvent (water) may require adjustment. If the retention window is wide leading to excessive resolution (i.e. some analytes show much stronger retention than others) in isocratic mode, a gradient screen should be attempted to assess whether this provides a better option. Likewise, if analyte peaks are clustered too closely in gradient mode, an isocratic separation may be required. From this data, the stationary phase/mobile phase combination that gives the most promising result is selected for further development.

Step 3: The effects of other parameters such as temperature and buffer concentration can be used to fine-tune the method. Once development is complete, the method robustness can be assessed as required.

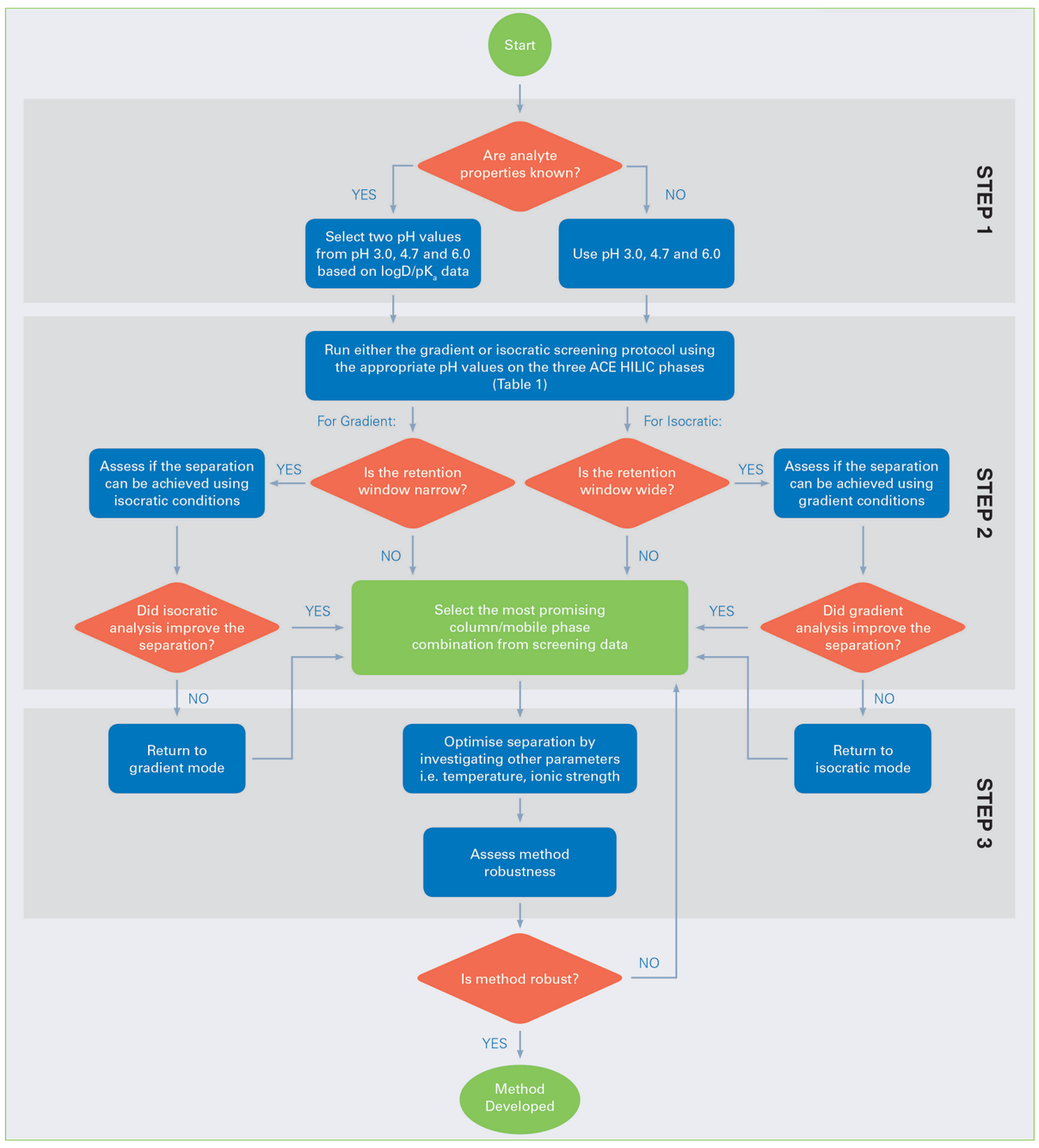

FIGURE 1: HILIC method development flow chart.

WHAT IS HILIC?

- Hydrophilic Interaction Liquid Chromatography (HILIC) was first described by Alpert*
- HILIC is ideal for the separation and retention of polar species including polar neutral and polar ionised analytes
- HILIC separations typically utilise a polar stationary phase with high organic solvent containing mobile phases
- Mechanistically HILIC is complex (Figure 2) and provides multiple modes of interaction between the analyte, stationary phase, eluent and water enriched layer at the stationary phase particle-eluent interface**

* A. J. Alpert, J. Chromatogr., 499 (1990) 177.
** See the FREE ACE guide to reproducible HILIC method development for more information – order your copy now.

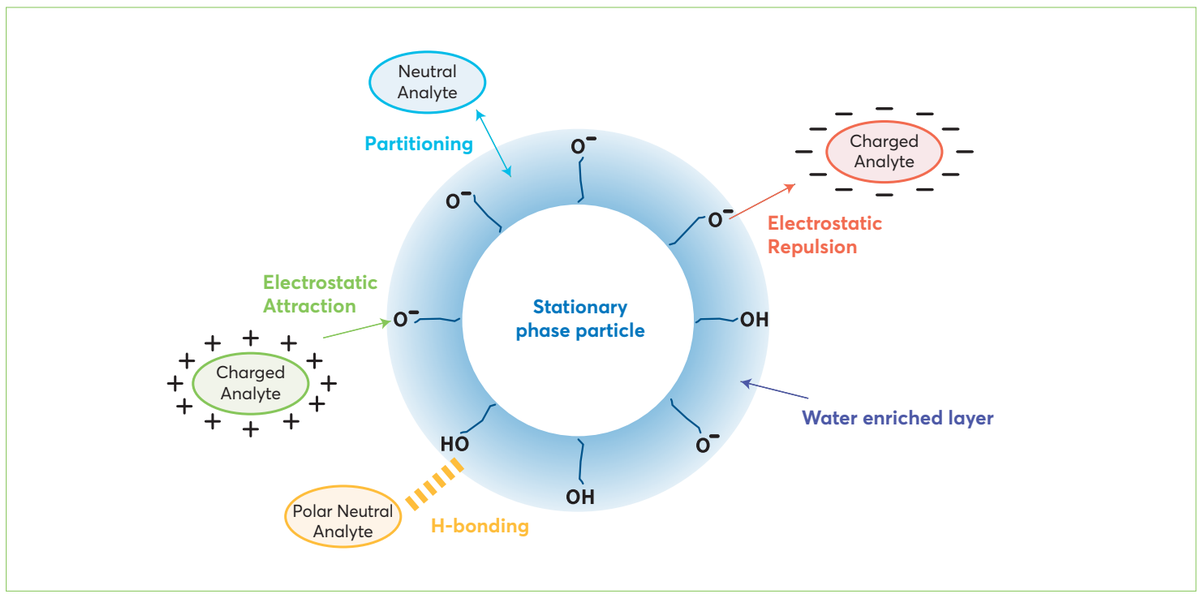

FIGURE 2: Interactions in HILIC mode.

WHEN SHOULD YOU CONSIDER HILIC?

- HILIC provides the retention and separation of hydrophilic or polar to very polar analytes not well retained in RPLC
- Hydrophilic or polar to very polar analytes have log P values (measure of lipophilicity) of around zero or less
- Generally, polar analytes are suitable for HILIC if they elute before caffeine in gradient RPLC (Figure 3)

Column	ACE HILIC-A, ACE HILIC-B and ACE HILIC-N, 150 x 4.6, 5 µm
Isocratic screening	10 mM ammonium formate in MeCN/H_2O (9:1 v/v) ammonium formate at pH 3.0, 4.7 or 6.0
Gradient screening	Line A: 10 mM ammonium formate in MeCN/H_2O (94:6 v/v) Line B: 10 mM ammonium formate in MeCN/H_2O (50:50 v/v) Ammonium formate at pH 3.0, 4.7 or 6.0
Flow rate	1.5 ml/min
Temperature	25 °C
Detection	Dependent on sample

Time (min)	%B
0	0
15	100
20	100
21	0
41	0

TABLE 1: Recommended isocratic and gradient HILIC screening conditions.

Column	ACE Excel C18, 100 x 3.0, 2 µm
Cat. No.	EXL -101-1003U
Mobile phase	A = 10 mM ammonium formate, pH 3.0 (aq) B = 10 mM ammonium formate, pH 3.0 in 90:10 v/v MeCN:H_2O
Gradient	5 - 100 %B in 10 min
Detection	ELSD
Flow rate	0.4 ml/min
Temperature	30 °C
Injection	10 µl

Analysed using VWR-Hitachi Chromaster with VWR ELSD90.

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FIGURE 3: Analyte suitability for HILIC from gradient RPLC and from Log P.