ACE Step-by-Step HILIC Method Development Protocol

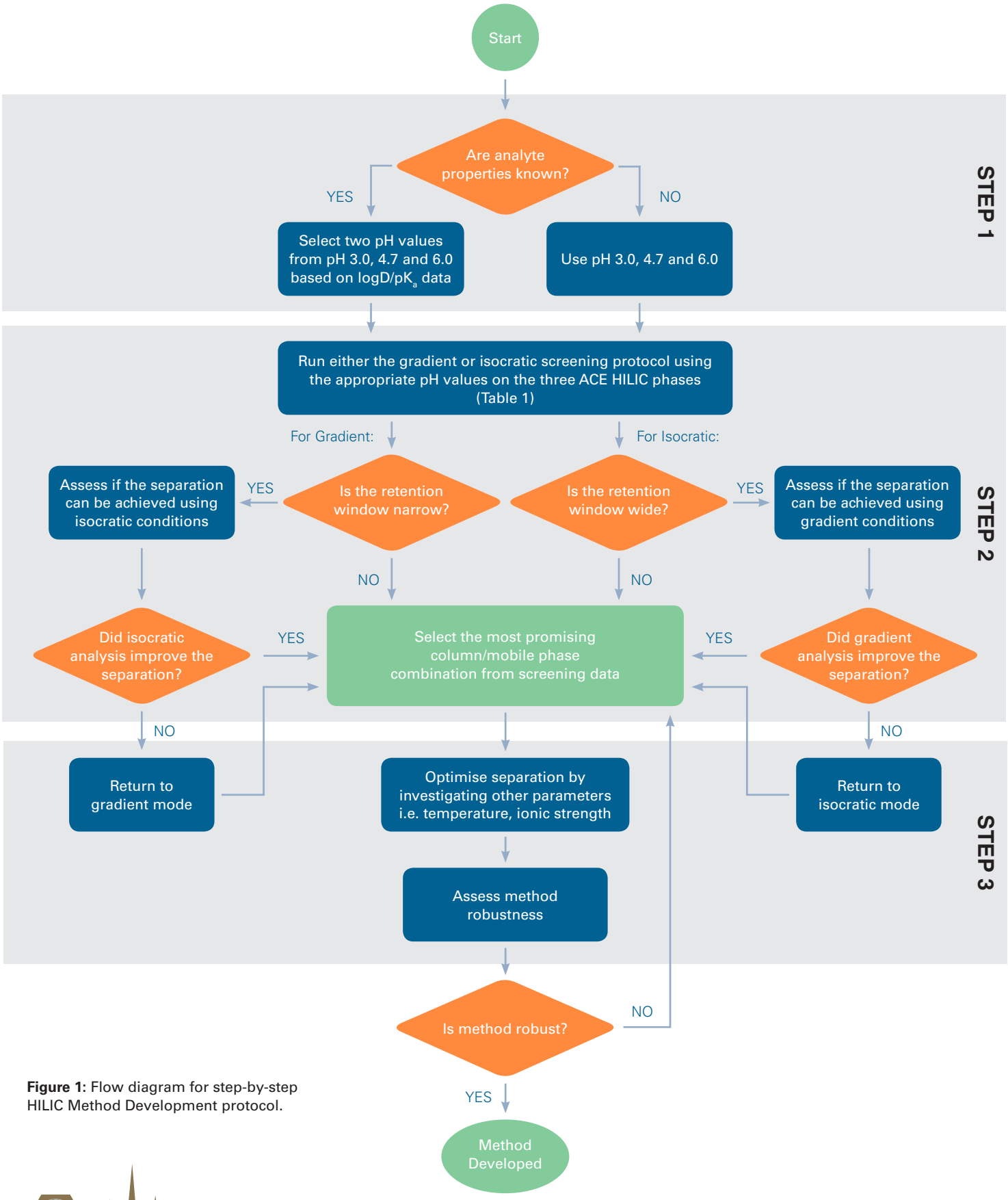

Figure 1: Flow diagram for step-by-step HILIC Method Development protocol.

ACE Step-by-Step HILIC Method Development Protocol

Step-by-Step Rational Method Development

Introduction: 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 overleaf 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.

Table 1: Suggested conditions for HILIC screening

Parameter	Comments
Column	ACE HILIC-A, ACE HILIC-B and ACE HILIC-N, 150 x 4.6 mm, 5 µm
Isocratic screening	10 mM ammonium formate in MeCN/H_2O (90:10 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. Gradient: **Time (mins.)** / **%B**: 0 / 0; 15 / 100; 20 / 100; 21 / 0; 41 / 0
Flow rate	1.5 mL/min
Temperature	25 °C
Detection	Dependent on sample

Contact us for further support and technical information on HILIC method development. An ACE HILIC Method Development Guide and ACE HILIC Method Development Wall Chart are also available on request. For your free copies contact us today on: info@ace-hplc.com