A Practical, Selectivity Based Hydrophilic Interaction Liquid Chromatography (HILIC) Method Development Protocol

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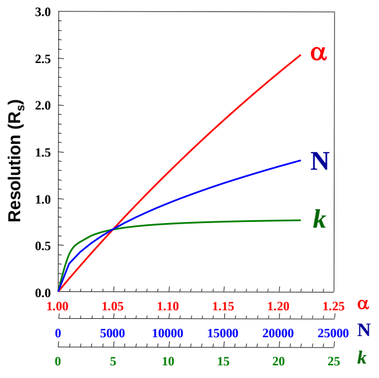
1. Introduction

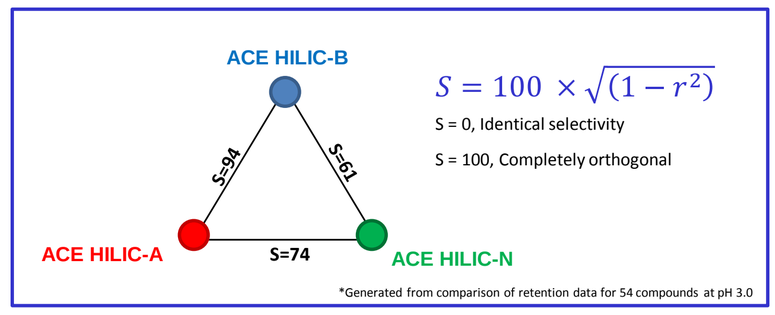
- Exploring chromatographic selectivity is a powerful approach for LC method development.
- Efficient method development requires a logical exploration of the key chromatographic parameters affecting selectivity.
- Rationally designed method development strategies assess key parameters and allow well informed decision making leading to robust stationary phase / mobile phase selection.
- Method development strategies based on column / mobile phase screening and optimisation are commonly utilised for reversed phase.
- For HILIC, such strategies are less common / less well defined.
- This poster demonstrates a simple, step-by-step approach to HILIC method development, based on the concept of exploring column selectivity.



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

2. Selectivity in HILIC

- In HILIC, the column stationary phase has a significant effect on chromatographic selectivity.
- The ACE HILIC range consists of three complementary phases specifically designed to offer maximum selectivity differences – ideal for method development:
  - **ACE HILIC-A**
  - **ACE HILIC-B**
  - **ACE HILIC-N**



- Mobile phase pH is also a powerful parameter and can affect ionisation of analytes and the stationary phase itself.
- Method development strategies based on screening different stationary phases and mobile phase pH are therefore the optimum choice.
- Buffer concentration and temperature are less influential, however can be used to fine-tune methods.

3. ACE MD Protocol

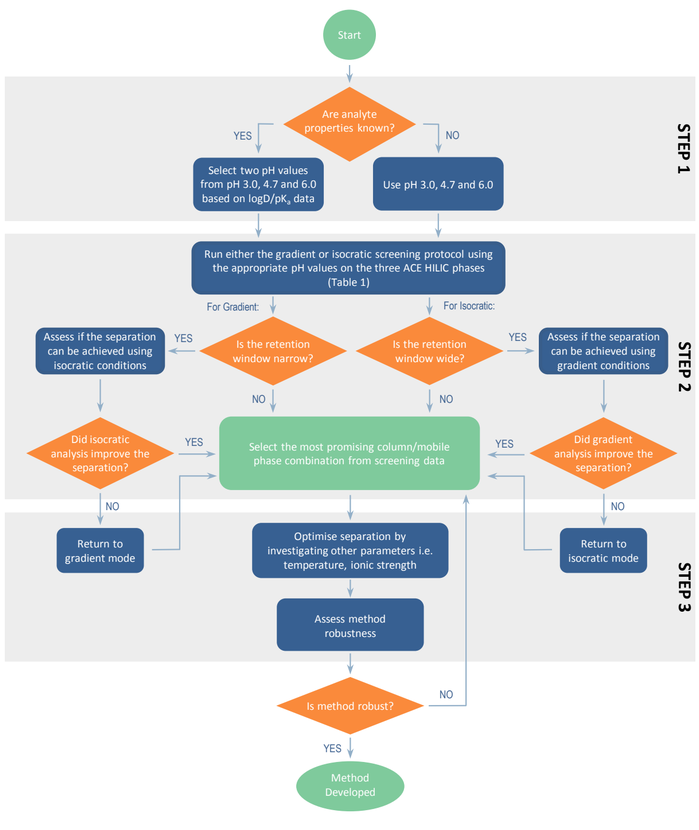
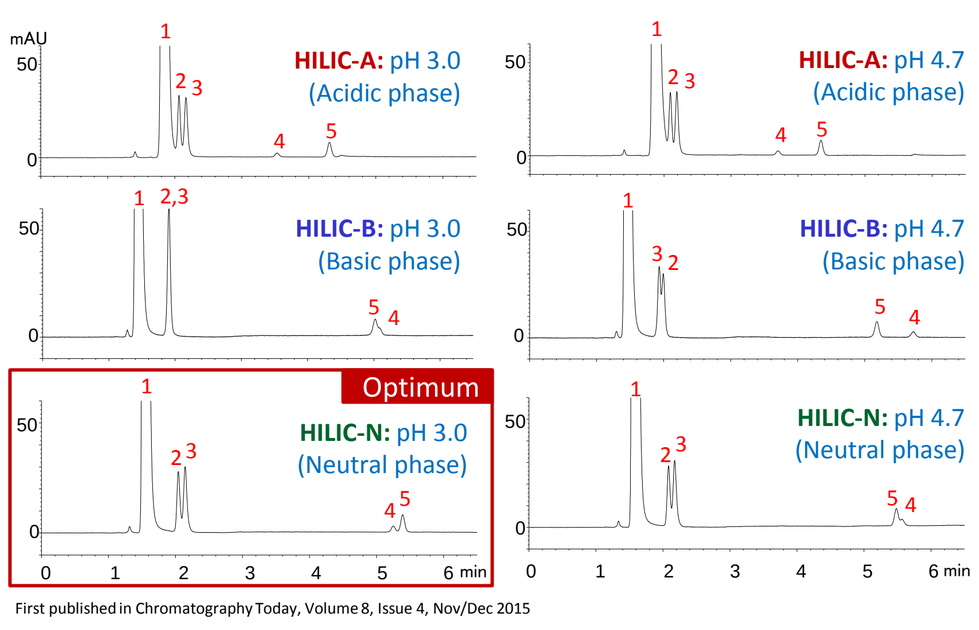


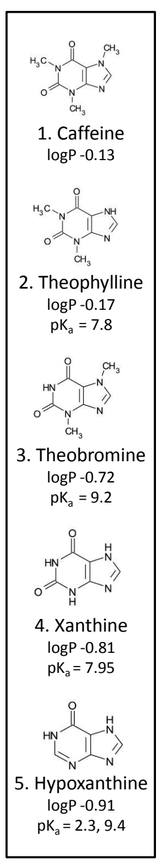
Table 1: Suggested conditions for HILIC screening

Columns:	ACE HILIC-A, ACE HILIC-B and ACE HILIC-N, 150 x 4.6 mm
Isocratic screening:	10 mM NH_4COOH in MeCN/H_2O (90:10 v/v) pH 3.0, 4.7 and 6.0 60 column volume initial equilibration
Gradient screening:	A: 10 mM NH_4COOH in MeCN/H2O (94:6 v/v) B: 10 mM NH_4COOH in MeCN/H2O (50:50 v/v) pH 3.0, 4.7 and 6.0
Gradient:	Time (mins.) / %B: 0/0, 15/100, 20/100, 21/0, 41/0
Column re-equilibration:	20 min. (20 column volumes)
Flow rate:	1.5 mL/min
Temperature:	25 °C
Detection:	Dependent on sample

4. Example: Caffeine and Related Substances

- A caffeine and related substances HILIC method was developed.
- All analytes are polar neutral with negative logP values.
- The ACE HILIC-A, HILIC-B and HILIC-N phases were screened using gradient conditions at pH 3.0 and 4.7 (Table 1).





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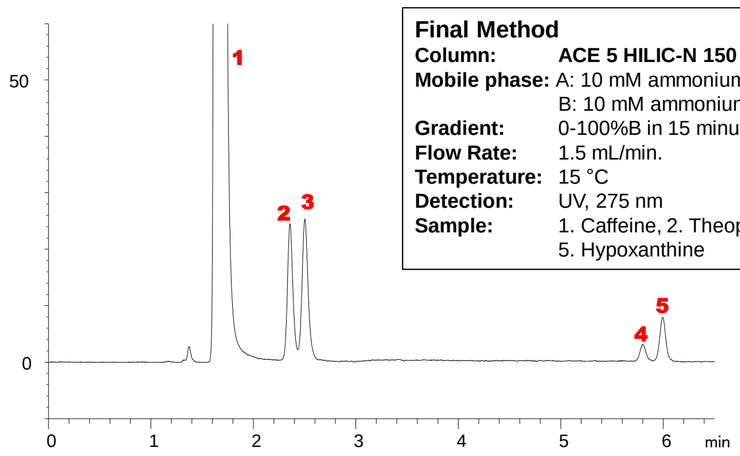
5. Caffeine and Related Substances: Optimisation

- Isocratic conditions were assessed. Retention of later eluting compounds increased but resolution of the critical pairs was not improved.
- Reducing the temperature improved the resolution of both impurity peak pairs.
- Lower temperature was used to achieve separation on the ACE 5 HILIC-N at pH 3.0.
- A small increase in acetonitrile in the gradient starting conditions was also found to be beneficial



Final Method

Column:	**ACE 5 HILIC-N 150 x 4.6 mm**
Mobile phase:	A: 10 mM ammonium formate pH 3.0 in MeCN/H_2O (96:4 v/v), B: 10 mM ammonium formate pH 3.0 in MeCN/H_2O (50:50 v/v)
Gradient:	0-100%B in 15 minutes
Flow Rate:	1.5 mL/min.
Temperature:	15 °C
Detection:	UV, 275 nm
Sample:	1. Caffeine, 2. Theophylline, 3. Theobromine, 4. Xanthine, 5. Hypoxanthine

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6. Summary

- A systematic and rationally designed method development strategy can aid in streamlining the method development process.
- In HILIC, column stationary phase and mobile phase pH are the most critical parameters affecting selectivity.
- The step-by-step method development strategy proposed in this poster therefore provides a powerful means by which to probe selectivity of a new application.
- The ACE HILIC-A, HILIC-B and HILIC-N phases provide complimentary selectivity, ideal for method development.
- Screening an analyte mixture on these three phases has been demonstrated as an effective method development strategy for selecting an appropriate stationary phase/mobile phase combination.
- Optimisation can be achieved by altering parameters such as ionic strength, % organic and temperature.