

3. How to Develop HPLC Methods for Challenging Separations

Summary

Learn how to implement strategies to achieve satisfactory separation for 'complex' samples and use computer modelling to develop robust and fit for purpose HPLC methods. This one day course is ideal for those who have experience of developing HPLC methods but want to increase their knowledge to deal with more challenging separations. Although designed as a follow-up to the course '*How to Develop HPLC Methods – Part 1*', it is not a prerequisite. This course is suitable for learners who feel confident that they are familiar with all the content detailed for the course '*How to Develop HPLC Methods – Part 1*'.

Course Outline

Implementing a 5-step strategy for method development of complex samples

Review of a 5-step strategy for HPLC method development with particular attention to reasons why some separations are 'challenging':

Step 1: Setting suitable objectives for method development

Step 2: Assessing all available information

Step 3: Selecting suitable samples

Step 4: Performing scouting experiments to select suitable initial conditions

Step 5: Optimising the method to define method parameters which achieve the desired separation

Separation Theory

Method development objectives: Strategies for maximising resolution in HPLC using retention factor, k' , selectivity, α , and efficiency, N .

Case Study Using Computer Modelling

A case study is used to demonstrate how a computer model may be used to find the best conditions for the separation.

Strategy 1: Sample Preparation

A general approach to sample preparation is provided with considerations regarding the most suitable technique.

Strategy 2: Detection Techniques

How to choose a suitable detector when analytes are not UV active: the different types of detectors used for HPLC, derivatisation to improve detectability.

Strategy 3: Retaining Very Polar Analytes

Techniques which enable analysis of very polar molecules by reversed phase HPLC: High pH, aqua columns, mixed mode columns, HILIC, Ion-pairing chromatography.

Strategy 4: Optimising Gradient Methods

Understanding gradient analysis; using multi-segment gradients for complex mixtures of analytes.

Strategy 5: Selecting Columns

Selecting columns which give different selectivity – tools for column comparison.

Strategy 6: Scouting/Screening Experiments

Setting up multiple scouting experiments for complex sample mixtures.

Practical Skills Acquired

This course will enable you to find solutions for difficult HPLC separations. In addition you will be able to:

- Understand why some separations can be challenging and identify potential problem separations.
- Apply strategies to achieve satisfactory separations for 'complex' samples with respect to:
 1. Sample preparation,
 2. Detection methods,
 3. Retaining very polar analytes,
 4. Optimising gradient methods,
 5. Selecting columns,
 6. Setting up scouting/screening experiments,

This course focuses on reversed phase mode separations.