

TRAINING COURSE

How to Develop HPLC Methods for Challenging Separations

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*', 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*'.

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. Obtaining impurity samples using forced degradation studies,
 5. Selecting columns,
 6. Setting up scouting/screening experiments,
 7. Optimising gradient methods (and use of computer modelling).

This course is available in two options: You can attend one of our open enrolment training courses at an external location (dates of upcoming events are available on the MTS website); or we can deliver the course at your site. On-site training allows customisation to meet your specific requirements, this may include customer method development projects.

Comprehensive course handout, access to training resources via e-MTS, certificate of training and post training support are all included in the course fees.

This course focuses on reversed phase mode separations.

Course Outline

Reasons why some separations are 'challenging'

Introduction looks at problem separations, e.g. samples containing large numbers of analytes and/or complex matrix, and problematic molecules and mixtures.

Implementing a 5-step strategy for method development of complex samples (see '*How to Develop HPLC Methods*')

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Review of the 5-step strategy for HPLC method development.

Step 1: Setting suitable objectives for method development

- Identifying potentially challenging separations.
- Defining a desired 'design space' for an HPLC method following Quality by Design (QbD) principles.

Step 2: Assessing all available information

- Choosing a suitable detector when analytes are not UV active.
- Analysing very polar molecules by reversed phase HPLC.

Step 3: Selecting suitable samples

- Sourcing and preparing sample component mixtures, e.g. performing forced degradation studies for stability indicating methods.
- Sample preparation procedures for complex matrices.

Step 4: Performing scouting experiments to select suitable initial conditions

- Selecting columns which give different selectivity – tools for column comparison.
- Setting up multiple scouting experiments for complex sample mixtures.

Step 5: Optimising the method to define method parameters which achieve the desired separation - Using computer simulation to develop fit for purpose HPLC methods

- Setting up optimisation experiments based on promising scouting runs which can be used to create a computer model of the separation.
- Creating a computer model for a separation and using the model to find the best conditions for the separation.
- Using multi-segment gradients for complex mixtures of analytes.
- Investigating robustness using QbD principles.

Computer simulation is demonstrated using LC Simulator by ACD/Labs.