

## TRAINING COURSE

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# How to Develop HPLC Methods – Part 2 (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 – 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*’.



This course will enable you to find solutions for difficult HPLC separations. 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:
  - ✓ Optimising gradient methods,
  - ✓ Sample preparation,
  - ✓ Detection methods,
  - ✓ Retaining very polar analytes,
  - ✓ Selecting columns,
  - ✓ Setting up scouting/screening experiments,

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.

## 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,  $\alpha$ , and efficiency,  $N$ .

### **Optimising Gradient Methods**

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

### **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.

### **Sample Preparation**

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

### **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.

### **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.

### **Selecting Columns**

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

### **Scouting/Screening Experiments**

Setting up multiple scouting experiments for complex sample mixtures.

This course focuses on reversed phase mode separations.