TRAINING COURSE

# How to Develop HPLC Methods

Learn how to select appropriate method conditions and perform suitable investigative experiments to obtain a set of method parameters which enables the desired separation for mixtures of analytes. This one day course is ideal for those who have experience of running HPLC methods and now want to learn how to develop new methods.

The course will enable you to take a strategic approach to developing HPLC methods with an understanding of the factors which can be adjusted to manipulate the retention time of analytes. In addition you will be able to:

- Define the objectives for the development of a HPLC analytical method.
- Effectively assess all the available relevant information relating to the desired method, e.g. pKa of the analyte.
- Select and prepare a suitable sample or samples to be used for the method development.
- Select suitable scouting conditions to find a suitable column and mobile phase system.
- Optimise the chromatographic conditions to result in the best possible separation.

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

**Developing an HPLC method using a 5-step strategy**
Common strategies for method development are compared and the benefits of using a strategic approach are discussed.

**Step 1: Setting suitable objectives for method development**

- Defining the requirement for a HPLC method: the analyte; the sample to be tested; the type of test required; and the purpose of the test.
- Setting appropriate criteria for the method: Goals for the separation in terms of resolution (R), efficiency (N) and capacity factor (k'); consideration of restrictions and preferences for the method, e.g. available equipment, run time, etc.



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**Step 2: Assessing all available information**

- Identifying potential sources of information which may be useful during method development.
- Assessing the effects of the structure of the analyte and in particular: how molecular weight and polarity of analytes are related to the most suitable type of HPLC; how pKa affects the choice of mobile phase; how the properties of the analyte impacts on the choice of detector; the effect of analyte solubility.
- Assessing available information relating to interferences which are likely to be encountered for the method.
- Using previous methods or methods used for similar compounds.

**Step 3: Selecting suitable samples**

- Selecting suitable samples for HPLC method development.
- Obtaining a source of the samples selected.
- Preparation of the test sample(s) to be used for method development.

**Step 4: Performing scouting experiments to select suitable initial conditions**

- Separation theory for reversed phase HPLC including discussion of the parameters which affect selectivity, e.g. mobile phase composition, %B, gradient time and steepness, temperature, pH etc.
- The effects of HPLC method chromatographic parameters: e.g. column attributes, mobile phase composition, temperature, flow rate, injection volume, etc.
- Selecting initial conditions for HPLC method development: stationary phase and mobile phase.
- Designing scouting experiments: consideration of the requirements of the method, selection of suitable stationary phase and mobile phase combinations and set-up of the scouting experiments.
- Interpretation of scouting experiments: how to identify promising potential conditions for the method, e.g. measurement of resolution, peak shape, etc.

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

- Adjusting method parameters to achieve the desired separation, i.e. optimising the separation.
- Using computer modelling to optimise the separation.
- Assessing peak purity for analyte peaks.
- Setting up a suitable calibration method.
- Assessing the robustness of the method.
- Designing an appropriate system suitability test.