



HPLC Technical Training

Module Name: **Developing the HPLC Method**

Time Allocated: **1.5 - 2 hours**

Intended Audience: **This module assumes no previous liquid chromatography knowledge**

Content Covers:

- Optimizing the separation by the correct column choice
- Mobile phase selection, especially for reversed phase HPLC
- Selection of optimum instrument conditions
- Setting up isocratic conditions
- Optimizing gradient elution
- Selection of buffers
- Relationship of particle size to the separation
- Chemistry of the stationary phases
- Practical issues in mobile phase selection e.g. UV absorbance and viscosity

At the completion of this seminar, attendees should be able to:

- Know the effect of carbon loading on the separation
- Understand the effect on mobile phase strength on retention
- Choose a good starting point of mobile phase binary composition based on analyte types
- Understand the benefit of optimizing gradient curves
- Understand pH and pKa of an analyte
- Understand how buffers works and why they are used
- Record accurately all instrument conditions for method documentation