Practical HPLC Method Development- 3 days

The practical part of the course is run on an instrument in the training room. During the course we run a sample mixture using different columns, we run a 0-100% gradient to establish approximate k' conditions, and then optimise the eluent composition for methanol, acetonitrile and THF using the Snyder triangle. We use diode array detection to monitor changes in peak elution order, and method development software to optimise for temperature, pH, buffer concentration and %B.

DAY 1

0900-1100

HPLC Theory in terms of:
- Selectivity, Efficiency and Resolution
- Relative Retention Time
- Asymmetry
- Solvophobic Theory

1115-1300

Requirements of an HPLC method
- Stationary Phase, Mobile Phase-Column, Detector, Data system, Sample preparation, Injector.

Method Development- Definition of Separation Objectives
- Resolution, Run Time, back pressure, LOD, LOQ, Accuracy, Precision and Robustness

1400-1700

Method Development – Initial Assessment
- Objectives of method development
- Sample properties
- Quantitation
- Literature search
- Columns and Detectors

DAY 2

0900-1100

Establishing a starting point for method development
Choice of Column- packing material and size, optimisation of throughput and selectivity
Choice of solvent
- Objectives of method development
- Sample properties
- Quantitation
- Literature search
- Columns and Detectors
- Solvent properties, optimisation – binary, ternary or quaternary mixtures and ion suppression

1115-1300

Running an initial gradient - What can be learned from the result
Temperature Optimisation
Choice of buffer
Optimising buffer concentration
Setting up integration conditions
Redeveloping a method

1400-1700

Gradient Elution
Effect of changing gradient profile and run time
Choice of Isocratic or gradient elution
%B Optimisation

DAY 3

0900-1100

Setting up an appropriate calibration
- Area %
- Internal Standards
- External Standards
- Calibration Range
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)

Analysis of errors and how to minimise them
Changing detector to increase sensitivity

1115-1300

Establishing confidence in peak identification
Sample preparation
Chromatographic data handling
IQ/OQ/PQ
Standard operating procedures

1400-1700

Method Validation
Risk assessment
Case Studies
Discussion and Course assessment