

Practical Masterclass in Modern HPLC

Day 1

0900-1300

- 1.1 History of HPLC and Introduction to Health & Safety
- 1.2 Introduction to HPLC Equipment, including lab tour
- 1.3 Introduction to Peak Integration

1400-1700

Practical A – Setting up External Standard Calibration and running standards

Day 2

0900-1300

- 2.1 External Standard Calibration and Internal Standard Calibration
- 2.2 Introduction to HPLC Columns
- 2.3 Principles of HPLC - Isocratic Elution

1400-1700

Practical B – Running unknown samples using the external standard calibration

Day 3

0900-1300

- 3.1 Importance of the Void Volume Peak
- 3.2 Injection Valves and Autosamplers
- 3.3 Scaling down to microbore and scaling up to prep.

1400-1700

Practical C – Setting up an Internal Standard Calibration and running standards

Day 4

0900-1300

- 4.1 Principles of HPLC – The Resolution Equation
- 4.2 HPLC Solvents
- 4.3 Ionisable samples – Use of pH, Ion Suppression and Ion Pair

1400-1700

Practical Series 1-8 – eight practicals taken in rotation, working in pairs. See below.

Day 5

0900-1300

- 5.1 Calibration using Area %, Response factor and Standard Addition
- 5.2 Set up and install an HPLC System – demonstration.
- 5.3 Selectivity – Illustration of the effect of changing solvent or pH

1400-1700

Practical Series 1-8 – eight practicals taken in rotation, working in pairs. See below.

Day 6

0900-1300

- 6.1 Troubleshooting an HPLC System
- 6.2 Making up pH buffers – calculate and discussion
- 6.3 Reversed Phase Columns

1400-1700

Practical Series 1-8 – eight practicals taken in rotation, working in pairs. See below.

Day 7

0900-1300

- 7.1 HPLC Pumps
- 7.2 Principles of Gradient Elution, High and Low Pressure mixing
- 7.3 Importance of Degassing

1400-1700

Practical Series 1-8 – eight practicals taken in rotation, working in pairs. See below.

Day 8

0900-1300

- 8.1 UV Detection
- 8.2 Normal Phase HPLC and HILIC
- 8.3 Diode Array Detection

1400-1700

Practical Series 1-8 – eight practicals taken in rotation, working in pairs. See below.

Day 9

0900-1300

- 9.1 Electrochemical and Conductivity Detection
- 9.2 Ion Exchange and ion Chromatography
- 9.3 Refractive Index and Light Scattering Detectors

1400-1700

Practical Series 1-8 – eight practicals taken in rotation, working in pairs. See below.

Day 10

0900-1300

- 10.1 Fluorescence Detection and Derivatisation
- 10.2 HPLC Column Troubleshooting, the use of Guard Columns
- 10.3 Size Exclusion Chromatography – GPC and GFC

1400-1700

Practical Series 1-8 – eight practicals taken in rotation, working in pairs. See below.

Day 11

0900-1300

- 11.1 LC-MS
- 11.2 uHPLC and Fused Core columns
- 11.3 HPLC Method Development

1400-1700

Practical Series 1-8 – eight practicals taken in rotation, working in pairs. See below.

Day 12

0900-1600

Practical Assessment: Take an HPLC method and using the SOP, set up the HPLC system, select the column and equilibrate, prepare the standards, construct the calibration, extract the sample and run to identify and quantify the active ingredient.

1600-1700

Discussion and comparison of the results and presentation of course certificates.

Practical Series 1-8

Practical 1

Analysis of very polar compounds. Use of AQ and other columns with pure water as eluent. What to do if compounds won't retain on a C18 column.

Practical 2

Diode Array Detection. Analysis of Polyaromatic hydrocarbons using reversed phase with diode array detection. Identification of peaks from their UV spectrum. Determination of peak purity. Discussion of solvent and pH effects on UV spectra.

Practical 3

Analysis of basic compounds, eg basic drugs. The use of high pH. Use of a pre-column. The use of new wide pH columns, and the use of polymer-based columns.

Practical 4

Determination of Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Practical 5

Ion Chromatography. Analysis of anions and cations by ion chromatography with conductivity detection.

Practical 6

The use of Area% to calculate drug purity.

Practical 7

The analysis of sugars and syrups, using w/w dilutions and refractive index detection, comparing ligand exchange columns with an amino column.

Practical 8

Analysis of amino acids using fluorescence detection with post column derivatisation.