

## **HPLC Channel (46 hours)**

### **The Theory Of HPLC (29.5 hours)**

#### **Introduction (1.5 Hours)**

1. Aims and Objectives
2. Origins of Liquid Chromatography
3. Why Choose Liquid Chromatography
4. Suitable Samples for HPLC
5. Comparison with Gas Chromatography
6. Typical HPLC Data
7. Chromatography Separation Mechanisms
8. The Liquid Chromatograph
9. The Liquid Chromatographic Process
10. The Chromatogram
11. Modes of Chromatography
12. Assessment

#### **Chromatographic Parameters (3.0 hours)**

1. Aims and Objectives
2. Resolution
3. The Resolution Equation
4. Retention (Capacity) Factor
5. How to change Retention (Capacity) Factor
6. Effect of Retention Factor on Resolution
7. Selectivity (Separation Factor)
8. How to change Selectivity (Separation Factor)
9. Effect of Selectivity on Resolution
10. Efficiency
11. How to change Efficiency
12. Effect of Efficiency on Resolution
13. Resolution - Real Life Examples
14. Peak Asymmetry
15. Assessment

#### **Band Broadening (3.0 hours)**

1. Aims and Objectives
2. Band Broadening - the Van Deemter Equation
3. Band Broadening - Eddy Diffusion
4. Longitudinal Diffusion
5. Band Broadening - Mass Transfer
6. Optimising Flow Rate
7. Optimising Particle Size
8. Minimising System Volume
9. Assessment

**Column chemistry (4.0 hours)**

1. Aims and Objectives
2. Silica as a Packing Material
3. Chemically Bonded Phases
4. Surface Treatment - End capping
5. Reversed Phase Stationary Phases
6. Silanol and Separation
7. Water Wettable Phases
8. Aq Type Stationary Phases
9. Polar Embedded Phases
10. Working at Low pH
11. Working at High pH
12. Other Stationary Phase Types
13. Column Test Probes
14. Column Characterisation
15. Assessment

**Reverse phase (partition) chromatography (6.0 hours)**

1. Aims and Objectives
2. Mechanism of Reversed Phase HPLC
3. Applications of Reversed Phase HPLC
4. Analyte Retention in Reversed Phase HPLC
5. Retention Order in Reversed Phase HPLC
6. Reversed Phase Mobile phase Solvents (a)
7. Reversed Phase Mobile phase Solvents (b)
8. Mobile phase strength and retention
9. Changing the Organic Modifier
10. Eluotropic Series
11. Selecting Reverse Phase Columns
12. Reverse Phase HPLC of Ionisable Samples
13. Analyte Ionisation
14. Controlling Extent of Ionisation
15. pH vs Retention in Reverse Phase HPLC
16. Basic Analytes & Ion Suppression
17. Buffers for Reverse Phase HPLC
18. Buffer Concentration
19. Getting Started with Ionisable Compounds
20. Assessment

**Ion-Pair Chromatography (3.0 hours)**

1. Aims and Objectives
2. Ion Pair Chromatography - Fundamental Mechanism
3. Ion Pair Chromatography - Reagents
4. Ion Pair Chromatography - Retention & Selectivity
5. Important Parameters in Ion Pair Chromatography
6. Optimising Ion Pair Concentration
7. Applications and Ion Pairing for LC-MS
8. Practical Ion Pair Chromatography
9. Getting Started with Ion Pair HPLC

10. Assessment

**Normal phase (absorption) chromatography (3.0 hours)**

1. Aims and Objectives
2. Mechanism of Normal Phase Chromatography
3. Applications of Normal Phase Chromatography
4. Retention and Selectivity Stationary Phases
5. Stationary Phases for Normal Phase HPLC
6. Typical Mobile Phases
7. Controlling Retention
8. Mobile Phase Optimisation
9. Problems with Water in the Mobile Phase
10. Getting Started with Normal Phase HPLC
11. Assessment

**Gradient HPLC (3.0 hours)**

1. Aims and Objectives
2. Isocratic HPLC Analysis
3. Gradient HPLC Analysis
4. Gradient Elution Parameters
5. Gradient Elution Principles
6. Peak Shape in Gradient HPLC
7. Scouting Gradients
8. Gradient Steepness
9. Optimising Gradient Analyses
10. Practical Gradient HPLC
11. Estimating Gradient Parameters
12. Assessment

**Quantitative and Qualitative HPLC (3.0 hours)**

1. Aims and Objectives
2. Qualitative Analysis Overview
3. Peak Identification and Assignment
4. Sample Spiking
5. Spectral Peak Identification
6. Quantitative Analysis Overview
7. Chromatographic Requirements
8. Peak Integration
9. Peak Height or Peak Area
10. Principles of Quantitative Analysis
11. Area / Height %
12. External Standard Quantitation
13. Calibration Curve
14. External Standard Multi-level Calibration
15. Internal Standard Analysis
16. Assessment

## **Instrumentation of HPLC (16.5 hours)**

### **Mobile Phase Considerations (3.5 hours)**

1. Aims and Objectives
2. The Mobile Phase - Introduction
3. The Liquid Chromatographic Process
4. Analyte Retention Processes
5. The Partition Coefficient
6. Retention and Selectivity
7. Solvent Type and Selectivity
8. Optimising Selectivity using Retention
9. Gradient Elution
10. Mobile Phase Delivery
11. Solvent Miscibility
12. UV Cut Off
13. Other Solvent Considerations
14. Mobile Phase Preparation
15. Degassing
16. Outlet Degassing
17. Degassing / Column Damage
18. Methods of Degassing
19. Helium Sparging
20. Vacuum Degassing
21. Assessment

### **HPLC Solvent Pumping Systems (4.0 hours)**

1. Aims and Objectives
2. Pumps - Introduction
3. Simple Pumping Systems
4. Disadvantages of Single Piston Pumps
5. Dual Piston Reciprocating Pumps
6. Mixing Solvents, Binary Pumps
7. Mixing Solvents, Quaternary Pumps
8. Mixing Solvents, Ternary Pumps
9. Check Valves
10. Pistons
11. Pulse Dampers
12. Purge Valves
13. Flushing, Gradients and Gradient Dwell Time
14. Experimental Determination of System Dwell
15. Troubleshooting - High Back Pressure
16. Troubleshooting - Check Valves and Pistons
17. Troubleshooting - Solvent Mixing Issues
18. Preparative Pumps
19. Capillary (Low Volume) Pumps
20. Calibration and Testing
21. Assessment

**Autosamplers (4.5 hours)**

1. Aims and Objectives
2. Introduction
3. Injection Valves
4. Injection Valve Anatomy
5. Manual Injection Systems
6. Manual Injection Complete and Partial Loop Filling
7. Autosamplers
8. Pull to Fill Auto Samplers
9. Push to Fill Auto Samplers
10. Integral Loop Auto Samplers
11. Autosampler Contamination
12. Autosampler Contamination
13. Assessment

**Detectors (4.5 hours)**

1. Aims and Objectives
2. Detectors for HPLC - Introduction
3. General Terms and Concepts
4. Limit of Detection / Quantification (LOD / LOQ)
5. UV - Vis Detectors, The Flow Cell
6. UV - Vis Detectors, Quantitation
7. External Standard Quantitation
8. Calibration Curve
9. UV - Vis Detectors, UV absorbance
10. Variable Wavelength UV - Vis Detectors
11. UV Detectors - Diode Array Detectors
12. UV Detectors - DAD Spectra
13. DAD Detectors - Bandwidth
14. DAD Detectors - Slit Width
15. DAD Detectors - Response time
16. DAD Detectors - Reference Wavelength
17. Choosing Sample and Reference Settings
18. DAD - Peak Suppression
19. Fluorescence Detectors
20. Fluorescence Detectors - Principles
21. Fluorescence Detectors - Excitation & Emission
22. Refractive Index Detectors - Instrumentation
23. Refractive Index Detectors - Principles
24. Assessment

## **GC Channel (30 hours)**

### **Theory and Instrumentation of GC (30 hours)**

#### **Introduction (1.5 hours)**

1. Aims and Objectives
2. Origins of Gas Chromatography
3. Why choose Gas Chromatography (a)
4. Why choose Gas Chromatography (b)
5. Gas Chromatography Separation Mechanism
6. The Gas Chromatograph
7. The Chromatogram
8. GC Advantages and Disadvantages
9. Typical GC Applications
10. Assessment

#### **Chromatographic Parameters (3.0 hours)**

1. Aims and Objectives
2. Chromatographic Resolution ( $R_s$ )
3. The Resolution Equation
4. Retention Factor ( $k$ )
5. How to change Retention Factor ( $k$ )
6. Effect of Retention Factor on Resolution
7. Selectivity (Separation) Factor
8. How to Change Selectivity
9. Effect of Separation Factor on Resolution
10. Efficiency
11. How to change Efficiency
12. Effect of Efficiency on Resolution
13. Resolution - Real Life Examples
14. Resolution - Real Life Examples [Q1]
15. Resolution - Real Life Examples [Q2]
16. Resolution - Real Life Examples [Q3]
17. Peak Asymmetry
18. Assessment

#### **Band Broadening (3.0 hours)**

1. Aims and Objectives
2. Importance of Efficiency in GC Separations
3. Van Deemter and Golay Equations
4. Eddy Diffusion
5. Longitudinal Diffusion
6. Stationary Phase Mass Transfer
7. Mobile Phase Mass Transfer
8. Stationary Phase Film Thickness Effects
9. Column Internal Diameter Effects
10. Carrier Gas Flow Rate Effects
11. Effect of Carrier Gas Type on Efficiency
12. Assessment

**Gas Supply and Pressure Control (2.0 hours)**

1. Aims and Objectives
2. Gases required for GC
3. Gas Supply Management
4. Quality of Gas Supply
5. Gas Generators - Using Hydrogen in the Lab
6. Manual Pressure Control
7. Electronic Pressure Control
8. Pressure / Flow Programming
9. Assessment

**Sampling Techniques (4.5 hours)**

1. Aims and Objectives
2. Sampling Techniques Overview
3. Manual Injection
4. Cold Needle Technique
5. Hot Needle Technique
6. Air Gap Techniques
7. Solvent Flush Technique
8. Automatic Liquid Sampling (Autosamplers)
9. Gas Sampling Devices
10. Purge and Trap Autosamplers (a)
11. Purge and Trap Adsorbents (b)
12. Thermal Desorption Autosamplers (TD)
13. Two Stage Thermal Desorption
14. Two Stage Desorption with Cold Trapping
15. Thermal Desorption - Important Parameters
16. TD Sorbent Selection and Applications
17. Solid Phase Microextraction (SPME)
18. Important SPME Parameters
19. Headspace Sampling (HS)
20. Headspace Autosamplers
21. Headspace Calibration and Quantitation
22. Assessment

**Sample Introduction (5.0 hours)**

1. Aims and Objectives
2. GC Inlet Systems
3. Spilt / Splitless Inlet
4. Spilt Injection
5. Setting the Split Ratio
6. Sample Discrimination
7. Injection Volume
8. Optimising Injection Volume
9. Split Injection - Experiments [Q1]
10. Split Injection - Experiments [Q2]
11. Split Injection - Experiments [Q3]
12. Split Injection - Experiments [Q4]

13. Splitless Injection
14. Optimising Splitless Injection - Purging the inlet
15. Optimising Splitless Injection - Analyte Focussing
16. Optimising Splitless Injection - Solvent Choice
17. Splitless Injection - Experiments [Q1]
18. Splitless Injection - Experiments [Q2]
19. Splitless Injection - Experiments [Q3]
20. Choosing an Inlet Temperature
21. Liners for Split / Splitless injection
22. Septa for Split / Splitless injection
23. Septa Problems
24. Cool-on column (COC) Inlet
25. Optimising COC injection
26. Use of Retention Gaps for Cool-on-Column injection
27. Programmed Thermal Vaporising (PTV) Inlets
28. PTV Sample Flow Rate
29. PTV Liner Type and Packing
30. PTV Flow and Temperature
31. PTV Solvent Elimination
32. Direct (Packed Column) Inlets
33. Assessment

### **GC Columns (5.5 hours)**

1. Aims and Objectives
2. Open Tubular Capillary Columns
3. Comparison of Packed and Capillary GC columns
4. Chemistry Review - Analyte & Stationary Phase Polarity
5. Electronegativity
6. Dispersive Interactions
7. Dipole Interactions
8. Hydrogen Bonding
9. Stationary Phases - Polysiloxanes
10. Polysiloxane Classifications
11. Stationary Phases Polyethylene Glycols
12. Stationary Phase Selection
13. Phase selection Dispersive Phases
14. Dispersive Interactions and Polarity
15. Dipole Interactions and Hydrogen Bonding
16. Stationary Phase Selection PLOT Columns
17. Stationary Phase Selection Summary
18. Stationary Phases for Packed Column GC
19. Column Dimensions - Length
20. Column Dimensions - Internal Diameter
21. Column Dimensions - Film Thickness (df)
22. Phase Ratio ( $\beta$ )
23. Carrier Gas Flow Rate
24. Column Bleed
25. Column Installation & Conditioning
26. Exercises in Column Selection [Q1]

27. Exercises in Column Selection [Q2]
28. Assessment

**GC Temperature Programming (3.0 hours)**

1. Aims and Objectives
2. The Role of Temperature in GC Separations
3. Isothermal and Gradient Temperature GC
4. Theory of Temperature Programmed GC
5. Using and Developing Temperature Programs
6. Scouting Gradients
7. Predicting Isothermal Conditions
8. Optimising Isothermal Conditions
9. Initial Temperature and Hold Time
10. Adjusting the Ramp Rate
11. Final Temperature and Time
12. Assessment

**GC Detectors (2.5 hours)**

1. Aims and Objectives
2. GC Detectors Overview
3. GC Detectors - Characteristics (a)
4. GC Detectors - Characteristics (b)
5. The Flame Ionisation Detector
6. FID - Operating and Optimising
7. FID - Uses and Performance
8. The Nitrogen Phosphorous Detector (NPD)
9. NPD - Operating and Optimising
10. NPD - Uses and Performance
11. The Electron Capture Detector (ECD)
12. ECD - Operating and Optimising
13. ECD - Uses and Performance
14. The Thermal Conductivity Detector (TCD)
15. TCD - Operating and Optimising
16. TCD - Uses and Performance
17. Other GC Detectors (a)
18. Other GC Detectors (b)
19. Assessment

## **MS Channel (57.5 hours)**

### **Fundamental LC-MS (37.5 hours)**

#### **Introduction (1.5 hours)**

1. Aims and Objectives
2. Definitions
3. Instrument Fundamentals
4. Process
5. Why and when to use LC/MS
6. Ionisation Overview
7. Ionisation Atmospheric Pressure Ionisation (API)
8. Ionisation Electrospray Ionisation (ESI)
9. Ionisation Atmospheric Pressure Chemical Ionisation (APCI)
10. Ionisation Atmospheric Pressure Photo Ionisation (APPI)
11. Mass analysers
12. Quadrupole
13. Mass analysers Time-of-flight
14. Mass analysers Ion Trap Mass Analyser
15. Tandem mass spectrometry (MS/MS)
16. Detectors
17. Applications
18. Assessment

#### **Electrospray Ionisation Theory (6.5 hours)**

1. Aims and Objectives
2. Introduction
3. Suitable analytes for ES
4. Production of charged droplets at the capillary tip.
5. Formation of the Taylor cone
6. Nebulisation Overview
7. Nebulisation Applied Potential Difference
8. Spray Breakdown and Discharge
9. Electrospray Production - Pneumatic Assistance
10. Electrospray Production - Eluent Flow Rate
11. Electrospray Production - Surface Tension
12. Electrospray Production - Ionic Strength
13. Electrospray Ionisation Droplet Desolvation
14. Droplet desolvation and Jet fission
15. Gas Phase Ions The Dole Model
16. Gas Phase Ions Iribarne / Thompson Model
17. Gas Phase Ions Experimental Factors
18. Gas Phase Ions Charge to Droplet Radius Ratio
19. Gas Phase Ions Energy Barriers
20. Gas Phase Ions Mass / Charge Ratio
21. Quantitative aspects Sensitivity constants
22. Sensitivity constants - Droplet Radius
23. Droplet Surface Activity

24. Ion Suppression Principles
25. Ion Suppression - Competing Ions
26. Ion Suppression in Practice
27. Mass Dependence of the Ion Signal
28. Optimising the Sprayer Position
29. Solute Changes in Evaporating Droplets
30. pH vs Ion Intensity
31. Assessment

### **Electrospray Ionisation – Instrumentation (4.0 hours)**

1. Aims and Objectives
2. Electrospray source design
3. Electrospray capillary design
4. Source Heating (Drying Gas)
5. T-Piece designs
6. Optimising Sprayer / Sampling Plate Configuration
7. Orthogonal Spray Source Designs
8. Cluster Ion Sampling
9. Curtain Gas Systems to Prevent Cluster Ions
10. Dielectric Capillaries to Prevent Cluster Ions
11. Source Cleaning
12. Ion Optics Ring Electrodes
13. Ion Optics Ion Bridges
14. Collision Induced Dissociation
15. Effect of Nozzle Skimmer Voltages
16. Collision Induced Dissociation Probes
17. E-Lab
18. Assessment

### **Mass Analyzers (9.5 hours)**

1. Aims and Objectives
2. Introduction to Mass Analysis
3. Topic Overview
4. Terms and Definitions
5. Quadrupole Mass Analyser Introduction
6. Quadrupole Rods
7. Quadrupole Voltages
8. Quadrupole Electrostatic Fields
9. Quadrupole Ion Trajectories
10. Equations of Ion Motion
11. Quadrupole Mass Analysers - Mass Gain & Offset
12. Quadrupole - Resolution and Sensitivity
13. Quadrupole Data Acquisition Modes
14. Quadrupole Scan vs. Selected Ion Modes
15. RF only Ion Bridges
16. Quadrupole - Mass Accuracy
17. Quadrupole - Performance Limitations
18. Quadrupole - Scanning Speeds
19. Time-of-Flight (TOF) Mass Analysers

20. TOF - Equations of Motion
21. TOF - Resolution
22. TOF - Issues with High Mass Resolution
23. TOF - The Reflectron
24. TOF - Increased Resolution using Reflectrons
25. TOF - Performance Limitations
26. Orthogonally Accelerated TOF Instruments
27. oaTOF Pusher Electrode
28. oaTOF Pusher Pulse Rate
29. oaTOF Deflecting Voltage
30. oaTOF Interfacing Details
31. oaTOF Mass Resolution and Accuracy
32. oaTOF Dynamic Range
33. Ion Trap Mass Analysers - Introduction
34. Ion Trap Equations of Ion Motion
35. Ion Trap Stability Diagram
36. Ion Trap Space Charge Effects
37. Ion Trap Ion Manipulation
38. Ion Trap Scanning Experiments
39. Ion Trap Other Ion Experiments
40. Ion Trap Mass Resolution and Accuracy
41. Magnetic Sector Mass Analysers - Introduction
42. Magnetic Sector Equations of Ion Motion
43. Magnetic Sector Data Acquisition Modes
44. Electrostatic Analysers
45. Double Focussing Instruments
46. Magnetic Sector Ion Optics
47. Magnetic Sector Performance Limitations
48. Magnetic Sector Mass Resolution and Accuracy
49. Magnetic Sector Performance figures
50. Mass analysers Selection
51. Introduction to Tandem Mass Spectrometry
52. Assessment

### **Atmospheric Pressure Chemical Ionisation (APCI) (3.5 hours)**

1. Aims and Objectives
2. Introduction
3. Interface Overview
4. Suitable Samples for APCI
5. APCI Interfacing Details
6. APCI Nebuliser Types APCI Nebuliser Types
7. APCI Nebuliser Gas Flow
8. APCI Interfacing Details
9. APCI Analyte Ion Declustering
10. APCI Ionisation - Mechanisms
11. APCI Proton Affinity
12. APCI Ionisation - Negative Ion Mode
13. APCI Ionisation – Positive Ion Mode
14. APCI Gas Phase Reactant Ions

15. APCI Ionisation – Negative Ion Mode
16. APCI Reagent Gas Formation
17. Eluent Additives in APCI
18. Signal Suppression by Additives in APCI
19. Alternative APCI Charging Mechanisms
20. APCI Source Parameter Optimisation
21. Comparison of ESI/APCI ionisation techniques
22. E-Lab
23. Assessment

#### **Atmospheric Pressure Photoionisation (APPI) (3.0 hours)**

1. Aims and Objectives
2. APPI Introduction
3. APPI Instrumentation
4. Suitable Samples for APPI
5. APPI Interfacing Details
6. APPI Eluent flow rates
7. APPI Dual mode of operation
8. APPI Ionisation Mechanisms
9. APPI Positive ion mode
10. APPI Negative ion mode
11. Ion sampling and transfer in APPI interfaces
12. APPI - Analyte Ion Declustering
13. APPI Source Parameter Optimisation
14. E-Lab
15. Assessment

#### **Solvents, Buffers and Additives (3.5 hours)**

1. Aims and Objectives
2. Introduction
3. LC-API Compatibility
4. ESI Solution Chemistry
5. ESI Eluent Solvent
6. Solvent Viscosity in ESI
7. Organic Modifiers in ESI
8. Eluent Solvent – Positive ESI
9. Eluent pH – Positive ESI
10. Eluent pH – Negative ESI
11. ESI Positive/Negative Ion Mode
12. ESI Reagents for pH Control
13. Sources for non-Volatile Systems
14. Z Spray and non-Volatile Systems
15. ESI Test Compound Infusion
16. ESI Buffer Choice and Concentration
17. ESI Buffer Systems
18. ESI Ion Pair Reagents – Overview
19. ESI Ion Pair Reagents – Considerations
20. Cationisation vs. Anionisation in ESI
21. Pre and Post Column Addition in ESI

22. Post Column Addition in ESI
23. ESI Adduct Formation
24. ESI Considerations
25. APCI Solvent Choice – Overview
26. APCI Solvent Choice –Mechanisms
27. Buffers for APCI – Overview
28. Buffers for APCI – Concentration
29. Ion pairing reagents in APCI
30. APCI Considerations
31. E-Lab
32. Assessment

### **Vacuum Systems (3.0 hours)**

1. Aims and Objectives
2. Introduction
3. Vacuum Systems
4. Mean Free Path
5. Rotary Pumps
6. Foreline Pumps
7. Turbomolecular Pumps
8. Diffusion Pumps
9. Vacuum and Flow Rate Incompatibility
10. Establishing Vacuum and Transmission
11. The Sampling Orifice Plate
12. Nozzle Skimmer Region – Analyte Enrichment
13. Second Pumping Stage
14. Molecular Beam Theory
15. Practical Implication of the Skimmer Position
16. Vacuum System Troubleshooting & Maintenance
17. Vacuum Leaks
18. Foreline Pumps – Gas Ballasting
19. Foreline Pumps – Exhaust Filters
20. Foreline Pumps – Rotary Pump Oil
21. High Vacuum Pumps
22. Assessment

### **Flow Rates and Flow Splitting (3.0 hours)**

1. Aims and Objectives
2. Introduction
3. Interface Similarities
4. The ESI Interface
5. The APCI Interface
6. Flow Rates for APCI
7. Flow Rates for ESI
8. Flow Rates for Pneumatically Assisted ESI
9. Micro and Nanoflow ESI
10. Flow Rate Incompatibility – Reasons
11. Flow Rate Incompatibility – Solutions
12. Flow Splitting – Overview

13. Flow Splitting – Adjustable Flow Splitters
14. Flow Splitting – Practicalities
15. MS Detector
16. Columns – Internal Diameter
17. Columns – Low Flow Rates
18. Capillary LC systems – Overview
19. Capillary LC Systems – Practicalities
20. Column Selection
21. E-lab
22. Assessment

## **MS Interpretation (11.5 hours)**

### **General Interpretation Strategies (9.5 hours)**

1. Aims and Objectives
2. Introduction
3. Mass to Charge Ratio
4. Mass Resolution
5. High Mass Resolution
6. Mass Accuracy
7. High Mass Accuracy
8. Mass Range
9. Multiply Charged Ions
10. Spectral Features
11. Isotopic abundances
12. High Mass Region – Brominated Sample
13. High Mass Region – Chlorinated Sample
14. High Mass Region – Dichlorinated Sample
15. The Nitrogen Rule
16. Interpretation Strategy
17. Fragmentation in API
18. Rings and Unsaturation
19. Number of Carbons
20. Postulating a Molecular Formula
21. Cleavages – Ion Abundance
22. Cleavages – Simple Mechanisms
23. Electrospray Ionisation
24. ESI Considerations
25. APCI Considerations
26. APPI Considerations
27. LC-MS Structural Information Modes
28. MS/MS Overview
29. MS/MS Experiments
30. Product Ion Scanning - Overview
31. Product Ion Scanning – Application
32. Precursor Ion Scanning - Overview
33. Precursor Ion Scanning – Application
34. Constant Neutral Loss Scanning - Overview

35. Constant Neutral Loss Scanning – Application
36. Single/Multiple Reaction Monitoring - Overview
37. Single/Multiple Reaction Monitoring - Application
38. MS/MS Quantitative Considerations
39. E-Lab Part 1 - Preliminary considerations
40. E-Lab Part 1 - Molecular Weight
41. Assessment

## **Fundamental GC-MS (8.5 hours)**

### **Introduction (1.5 hours)**

1. Aims and Objectives
2. Definitions
3. Instrument Fundamentals GC
4. Instrument Fundamentals MS
5. GC-MS Process
6. Why and when to use GC-MS
7. Coupling GC to MS systems
8. Ionisation Overview
9. Ionisation - Electron Impact (EI)
10. Ionisation - Chemical Ionisation (CI)
11. Ionisation - Suitable samples for GC-MS
12. Mass analysers – Overview
13. Mass analysers – Quadrupole
14. Mass analysers – Time of flight (TOF)
15. Mass analysers – Ion trap
16. Mass analysers – Magnetic sector
17. Tandem mass Spectrometry (MS-MS)
18. Detectors
19. Applications
20. Assessment

### **GC Considerations (4.5 hours)**

1. Aims and Objectives
2. Introduction
3. Carrier gas
4. Sample introduction
5. Split Injection – Overview
6. Split Injection – Setting Split Ratio
7. Split Injection – Sample Discrimination
8. Split Injection – Injection Volume
9. Splitless Injection – Overview
10. Splitless Injection – Purging the Inlet
11. Split Injection – Analyte Focusing
12. Split Injection – Solvent Choice
13. PTV inlets
14. Headspace sampling
15. Headspace Autosamplers

16. Headspace Analysis Important Parameters
17. Columns
18. Stationary phases
19. GC-MS column selection
20. Fittings
21. Guard columns (Retention Gap)
22. Air leaks
23. Ferrules
24. Ferrules – Practicalities
25. Septum Overview
26. Septum – Selection
27. Septum Considerations
28. Contamination
29. Assessment

#### **GC -MS Interfaces (2.5 hours)**

1. Aims and Objectives
2. Introduction
3. Coupling GC to MS Detectors - Jet Separator
4. Coupling GC to MS Detectors - Direct Interface
5. Column Diameter
6. Interface-column coupling I
7. Interface-column coupling II
8. Interface-column coupling III
9. Interface-column coupling IV
10. Assessment

### **Sample Preparation Channel (22 hours)**

#### **Solid Phase Extraction (22 hours)**

#### **Molecular Properties (4.0 hours)**

1. Aims and Objectives
2. Functional groups
3. Molecular Properties
4. Functional group interactions
5. Hydrophobic or Non-Polar Groups
6. Hydrophobic Interactions - Solubility
7. Hydrophobic Interactions - Sorbents
8. Polar Groups
9. Polar Interactions - Solubility
10. Polar Interactions - Sorbents
11. Ionic groups
12. Ionic groups - pH
13. Ionic groups -  $K_a$
14. Ionic groups - pKa
15. Ionic groups - Ionic strength
16. Ionic Interactions - Solubility

17. Ionic Interactions - Sorbents
18. Chelating Groups
19. Chelating Groups - Chelating Interactions - Solubility
20. Chelating Groups - Chelating Interactions - Sorbents
21. Protein Binding
22. Assessment

### **SPE Overview (3.5 hours)**

1. Aims and Objectives
2. Solid Phase Extraction - Overview
3. SPE Terminology
4. SPE Sorbent Physical Properties I
5. SPE Sorbent Substrates
6. SPE Sorbent Surface Chemical Nature
7. SPE Sorbent Surface Chemical Nature
8. Choosing Sorbent Mass
9. Protocol Steps in SPE
10. SPE Sample Pretreatment
11. SPE Column Conditioning
12. SPE Column Equilibration
13. Sample Loading
14. Column Washing
15. Analyte Elution
16. Pharmaceutical SPE
17. Assessment

### **SPE Mechanisms (4.5 hours)**

1. Aims and Objectives
2. Non-Polar SPE
3. Non-Polar Sample Pretreatment
4. Equilibration and Sample Loading
5. Non-Polar Sorbent Washing and Analyte Elution
6. Polar SPE
7. Polar Sample Pretreatment
8. Polar Sorbent Conditioning, Equilibration and Sample Loading
9. Polar Sorbent Washing and Analyte Elution
10. Cation-Exchange SPE
11. Cation-Exchange SPE Sorbents
12. Cation-Exchange Sample Pretreatment
13. Sorbent Conditioning, Equilibration and Sample Loading
14. Cation-Exchange Washing and Analyte Elution
15. Anion-Exchange SPE
16. Anion-Exchange SPE Sorbents
17. Anion-Exchange Sample Pretreatment
18. Anion-Exchange Sorbent Conditioning, Equilibration and Sample Loading
19. Anion-Exchange Washing and Analyte Elution
20. Minimum pKa Differential
21. Mixed-Mode SPE
22. Mixed-Mode SPE Sorbents

23. Mixed-Mode Sample Pretreatment
24. Mixed-Mode Sorbent Conditioning Equilibration and Sample Loading
25. Mixed-Mode Washing and Analyte Elution
26. Assessment

**Method Development (5.0 hours)**

1. Aims and Objectives
2. Sorbent Column Processing
3. Centrifugal Column Processing
4. Vacuum Column Processing
5. Disadvantages of Vacuum Column Processing
6. Sorbent Column Processing
7. Sorbent Bed Geometry
8. Disk Products for SPE
9. Disk Based Sorbents
10. Fines
11. Fines - Channelling
12. SPE Method Development - Overview
13. Analyte Assessment I
14. Analyte Assessment II
15. Analyte Assessment III
16. Mechanism Selection I
17. Mechanism Selection II
18. Sorbent Screening - Alternative Sorbents I
19. Sorbent Screening - Alternative Sorbents II
20. Sorbent Screening Process
21. Procedure Optimization I
22. Procedure Optimization II
23. Procedure Optimization III
24. Soak Steps
25. Drying Steps
26. Elution Optimisation
27. Elution Optimisation - Results
28. SPE Method Troubleshooting Overview
29. Recovery Problems I
30. Recovery Problems II
31. Recovery Problems III
32. Reproducibility Problems I
33. Reproducibility Problems II
34. Cleanliness Problems
35. Cleanliness Problems - Alternative Solvents
36. Cleanliness Problems - Poor Sorbent Selectivity
37. Inadequate Throughput
38. Generic Methods in SPE - Overview
39. Mixed Mode Sorbents
40. Generic Methods - Overview
41. Generic Methods - Basic Protocol
42. Generic Methods - Further Optimization
43. Assessment

**Primary Sample Preparation Techniques (2.0 hours)**

1. Aims and Objectives
2. Primary Sample Prep Techniques
3. Sample Dilution in Pharmaceuticals
4. Sample Filtration and Ultrafiltration
5. Centrifugation and Ultracentrifugation
6. Protein Precipitation
7. Extraction Approaches to Sample Preparation
8. Selectivity
9. The Partition Coefficient
10. Liquid / liquid Extraction
11. Support-Assisted Liquid/Liquid Extraction
12. Solid Phase Extraction
13. Assessment

**Liquid / Liquid Extraction Techniques (1.5 hours)**

1. Aims and Objectives
2. Liquid / liquid Extraction II
3. Liquid / liquid Extraction III
4. Drawbacks of Liquid/liquid Extraction
5. Emulsions
6. Support-Assisted Liquid / liquid Extraction II
7. Support-Assisted Liquid / liquid Extraction III
8. Assessment

**Approaches to Automation for SPE (1.5 hours)**

1. Aims and Objectives
2. Automation via On-line Solid Phase Extraction
3. Mechanisms for On-line SPE
4. Turbulent Flow Chromatography
5. Molecular Imprinted Polymer Sorbents (MIPS)
6. Selecting a Sample Prep Technique
7. Summary of Sample Prep Technique Features
8. Automation of Sample Preparation I
9. Automation of Sample Preparation II
10. Assessment