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On your instruments.
At a time that suits you.

Onsite training from Crawford Scientific in partnership with APEX Scientific

# Onsite Training Course Portfolio

- » HPLC Fundamentals
- » HPLC Troubleshooting & Maintenance
- » HPLC Method Development
- » GC Fundamentals
- » GC Troubleshooting & Maintenance
- » GC Method Development
- » LC-MS For the Chromatographer
- » GC-MS For the Chromatographer
- » Introductory Statistics for Analytical Chemists

Crawford Scientific are a recognised provider of high quality training in analytical science. Our onsite courses are designed to provide maximum impact on student knowledge and effectiveness in the laboratory.





# **HPLC Fundamentals**

This one-day course introduces the fundamentally important concepts associated with HPLC analysis including hardware basics, modes of analysis, basic troubleshooting, column chemistry, principles of ionisation and more.

Suitable as a refresher for the more experienced chemist or as an invaluable introduction to the technique for those with limited experience, this course provides an invaluable insight into HPLC principles and practice.

# **Course Contents**

#### **Basics of the Chromatographic Process**

- Main retention mechanisms in HPLC
- Distribution constant
- Retention theory
- Model of the chromatographic process

#### **Sample Preparation Protocols**

- Principles
- Matrix elimination
- Liquid and Solid Phase Extraction

#### **Separation Mode / Retention Mechanisms**

- Absorption (normal phase)
- Reverse phase
- Principals of ionisation (Ion Suppression Chromatography)
- lon pairing
- Ion exchange

#### **Quantitation**

- Integration parameters
- System suitability testing

#### **Injectors and Columns**

- Sample introduction
- Rheodyne injectors / auto-samplers
- Silica as a solid support
- Column & packing geometry
- Efficiency the van Deemter & Knox equations

#### **Detectors**

- Choosing the right detector
- Operating principles
- Optimisation
- Typical operating conditions
- UV (Diode Array) / RI / Fluorescence

# Measuring & Optimising Chromatographic Parameters

- Efficiency
- · Capacity factor
- Selectivity
- Resolution
- Interdependence via the resolution equation



# **HPLC Troubleshooting & Maintenance**

A logical approach to troubleshooting is explored using both the component (hardware based) and Symptomatic (chromatogram based) perspectives.

Best practice for instrument maintenance and column handling are discussed to aid the user in prolonging the intervals between essential system maintenance.

In-depth treatment of the causes of peak shape and baseline anomalies are fully covered, this course is invaluable to anyone who wishes to gain further insight into the problems associated with HPLC analysis.

# **Course Contents**

### **Approaches to Logical Troubleshooting**

- System overview
- Component perspective
- Symptomatic perspective
- System maintenance records
- Symptoms / Causes / Diagnosis & Solution

# Component Perspective What to look for / what can go wrong with:

- Autosamplers
- Detectors: UV / RI / Fluorescence
- Solvent delivery systems & mobile phase

#### **Columns**

- Installation and conditioning
- Column chemistry
- Efficiency loss
- pH operating range / bleed
- Proper column management
- Loss of sensitivity

### **Symptomatic Perspective - Baselines**

- Baseline spikes
- Noisy baselines
- Cycling baselines
- Rising / falling baselines

#### **Symptomatic Perspective - Peaks**

- No peaks
- Fronting / tailing peaks
- Split peaks / shoulders
- Broad peaks
- Ghost peaks
- Retention stability
- Loss of sensitivity
- Correct integration methods

#### Maintenance

- Maintenance schedules
- Correct maintenance procedures for all system components
- Column maintenance



# **HPLC Method Development**

For the experienced chromatographer, this course provides a step-by-step explanation of logical HPLC method development.

The course includes detailed discussion of the crucial aspects of method development with relevant examples used to demonstrate theoretical principles and software based exercises to give a deeper understanding.

# **Course Contents**

### **Objectives**

- Establishing method objectives
- Literature searching
- What is known?
- What needs to be known?

#### **Sample Preparation**

- Sample clean up
- Analyte extraction
- SPE explained
- Mobile phase selection
- Optimising for sample type / application

#### **System Choices**

- How to choose the appropriate injector/detector
- Typical operating conditions
- Developing and optimising injection conditions
- Mobile phase flow & band broadening (Van Deemter)
- Modes of chromatography

### **Choosing a Column & Mobile Phase**

- Choosing the correct phase
- Computer based tools for column choice
- Effects of column geometry
- Review of modern stationary phases
- Isocratic vs. Gradient operation
- Theory & development of eluent gradients

#### **Optimisation Strategies**

- Capacity factor, Efficiency, Resolution, Selectivity
- Resolution Equation
- Step-by-step guide for logical method development
- Example method developments

#### **Quantitation & System Characterisation**

- Single and multi-level calibration
- Internal standards
- System suitability testing
- Introduction to validation



# **GC** Fundamentals

For the less experienced chromatographer or those wishing to update their skills, this course covers the fundamentally important concepts in modern GC analysis.

Basics of the chromatographic process, sample preparation, inlet systems, column and detector selection are important topics covered to give the participant a thorough grounding in the technique. Instrument hardware is also covered with basic troubleshooting and maintenance tips as well as an introduction to chromatographic optimisation.

## **Course Contents**

#### **Basics of the Chromatographic Process**

- Retention mechanisms in GC
- Temperature/retention relationships
- Column theory
- Stationary phase chemistries

#### **Sample Preparation Protocols**

- Principles
- Matrix elimination
- Solvent considerations
- Liquid and Solid Phase Extraction

#### **Sample Introduction**

- Operating principles
- Typical operating conditions
- Optimisation
- Split / splitless
- Cool on-column
- Headspace (on request)

### **Columns and Temperature programming**

- Choosing the right phase
- Column geometries explained
- Phase types
- Temperature effects
- Band Broadening (van Deemter & Golay treatment)
- Isothermal vs. gradient operation

#### **Detectors**

- Choosing the right detector
- Operating principles and Optimisation
- Typical operating conditions
- FID / ECD / GC-MSe

# Measuring & Optimising Chromatographic Parameters

- Efficiency
- Capacity factor
- Selectivity
- Resolution
- Interdependence via the resolution equation



# **GC Troubleshooting & Maintenance**

A logical approach to troubleshooting is explored using both the component (hardware based) and symptomatic (chromatogram based) perspectives.

Best practice for instrument maintenance and column handling, as well as, routines for cleaning and deactivating inlet and detection systems are discussed.

The causes of peak shape and baseline anomalies are fully covered, this course is invaluable to anyone who wishes to gain further insight into the problems associated with GC analysis.

# **Course Contents**

## **Approaches to Logical Troubleshooting**

- Logical troubleshooting
- System overview
- Component perspective
- Symptomatic perspective
- System maintenance records
- Symptom / Causes / Diagnosis & Solution

## **Component Perspective**

What to look for / what can go wrong with:

- Injectors: on-column / split splitless / large volume
- Detectors: FID / ECD / NPD / FPD
- Temperature and pressure control

#### **Columns**

- Installation and conditioning
- Operating principles
- Optimisation
- Operating range / bleed
- Band broadening

### **Symptomatic Perspective - Baselines**

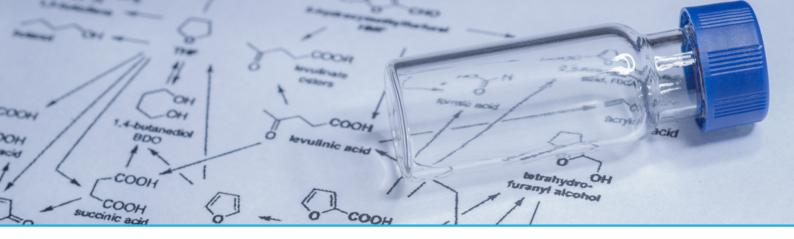
- Baseline spikes
- Noisy baselines
- Cycling baselines
- Rising / falling baselines

#### **Symptomatic Perspective - Peaks**

- No peaks
- Fronting / tailing peaks
- Split peaks / shoulders
- Broad shoulders
- Ghost shoulders
- Retention stability
- Solvent incompatibility
- Loss of sensitivity

#### Maintenance

- Maintenance schedules
- Correct maintenance procedures injectors and detectors



# **GC Method Development**

For the experienced chromatographer, this course provides a step-by-step approach to method development. The course includes all of the crucial aspects of method development including; Column dimensions, phase type, inlet type and operating conditions, detector settings and optimisation along with sample preparation regimes.

Each aspect is discussed in detail supplemented by a host of real world separation examples and tutorial exercises to aid understanding.

# **Course Contents**

#### **Objectives**

- Establishing method objectives
- Literature searching
- What is known?
- What needs to be known?

#### **Sample Preparation**

- Sample clean up
- Analyte extraction
- Solvent selection
- Optimising for sample type / application

### Inlet, and Flow Rate Parameters

- The effect of split ratio of peak shape and quantitative Accuracy
- Investigating oven initial temperature
- Conversion into a splitless method
- Optimising purge on time
- Carrier gas choice and flow rate optimisation (van Deemter & Golay treatment)

### **Choosing a Column & Temperature**

- Choosing the correct phase
- Effects of column geometry
- Solute stationary phase interactions
- Isothermal vs. Gradient operation
- Theory and development of Temperature gradients

#### **Optimisation Strategies**

- Measuring and Optimising
- Capacity factor, Efficiency, Resolution, Selectivity
- Resolution equation
- Developing effective methods
- Example method developments

#### Putting it all together!

 Developing a method for the separation of a complex mixture of compounds from scratch.



# LC-MS For the Chromatographer

The Atmospheric Pressure Interface (API) is the core element to the course with the principles of operation, limitations and applicability fully explored.

The course covers ion suppression, the use of Electrospray or APCI and MS-MS data acquisition modes. Optimisation of interface and mass filter settings and how to best utilise reduced dimension LC to speed up sample throughput will be discussed.

All popular interface types and mass analysing equipment (Quadrupole, TOF, Ion Trap etc.) will be comprehensively covered.

## **Course Contents**

#### Introduction – Fundamentals Review

- Commonly used terms and concepts
- Atmospheric Pressure Ionsation mechanisms of ESI / APCI / APPI
- API source design
- LC-MS Eluent design solvents buffers and additives
- API (ESI) interface optimisation

#### **Mass Analysers**

- Quadrupole mass analysers
- Time of flight mass analysers
- Ion trap mass analysers

#### **Mass Accuracy and Resolution**

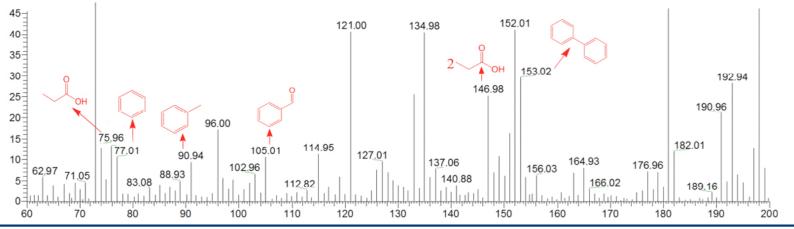
- Calibration of mass axis
- Mass accuracy / resolution
- Advantages of various analyser types
- Tuning the mass analyser (sensitivity vs resolution)

#### **Scan Functions**

- LC-MS data acquisition modes (sensitivity vs specificity)
- Scanning vs SIM
- Singly & multiply charged species
- Cone voltage fragmentation
- Up-front CID

#### LC-MS/MS Data Acquisition Modes

- Product ion scanning precursor
- Ion scanning
- Constant neutral loss
- Data dependant scanning
- Introduction to MS interpretation
- Product ion scanning
- Choosing precursor ions
- Establishing MRM method parameters
- Constant neutral loss experiments of ionisable compounds from scratch



# **GC-MS For the Chromatographer**

A course designed to highlight the powerful possibilities of GC analysis with spectral detection. Tuning and tune reports will be explained and instruction given in the use of tune reports as a powerful diagnostic tool.

The functionality of the MS will be discussed in detail including principles of the quadrupole mass filter. Ionisiation will be thoroughly investigated and practically optimised along with cleaning principles and regimes being explained and demonstrated.

# **Course Contents**

#### **Chromatographic Considerations**

- Sample preparation
- Column configurations for GC-MS
- Sample loading and stationary phase choice
- Flow rate considerations

#### Sample Introduction

- The Transfer Line
- Flow splitting
- The ion source explained
- Modes of ionisation
- Electron impact / chemically induced ionisation examples and fragmentation

#### **MS Hardware**

- Why use vacuum?
- Controlling and monitoring vacuum
- Quadrupole mass analysis explained
- Ion traps explained

#### **Detector Systems**

- Electron multipliers and detector electronics
- Matthieu stability diagrams
- X-ray lens and high energy dynodes
- AMU gain and offset
- Spectral resolution

#### **Tuning and Calibration**

- Purpose of tuning
- Tuning compounds
- Explanation of auto-tune voltages
- Troubleshooting from the auto-tune
- User tuning and voltage ramping

#### Quantitation

- Scan & SIM modes
- High sensitivity data acquisition



# **Introductory Statistics for Analytical Chemists**

This is a one day introductory course to the fundamental of statistics, relevant to all professionals in the analytical sciences, life sciences and related fields.

# **Course Contents**

#### Introduction

- Analytical problems
- Errors in quantitative analysis
- Random and systematic errors
- Accuracy, repeatability, reproducibility
- Standard reference materials

#### **Statistical Measures**

- Mean and standard deviation
- Variance and coefficient of variation

#### Normal (Gaussian) Distribution

- Sampling distributions
- Confidence limits
- Significant figures
- Propagation of errors

### Significance Tests

- Null hypothesis, type I and type II errors
- Comparison of x and μ / Comparison of x1 and x2
- t-tests / F-tests
- One-sided and two-sided tests
- Outliers
- Analysis of variance (ANOVA)
- Fixed effects and random effects
- Comparison of several means

#### **Regression and Correlation**

- Calibration graphs / correlation coefficient
- Regression of y on x / errors in the regression line
- Calculation of a concentration
- Limit of detection
- Standard additions
- Outliers in regression
- Weighted regression lines





# Meet the Training Team



**Claire Paterson** Training Manager

HPLC method development, validation, troubleshooting, documentation and laboratory processes.



**Colin Towers**Training and Technical Consultant

Method development, validation, routine analysis and troubleshooting in LC, LC-MS/MS, GC, GC-MS and SPE.



**Philip Aston**Training and Technical Consultant

NMR Spectroscopy, LC-MS, Spectroscopy, and Protein Purification.



**Gilles Beck** Training and Technical Consultant

HPLC, GC, LC-MS and sample preparation.

# Contact Us



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