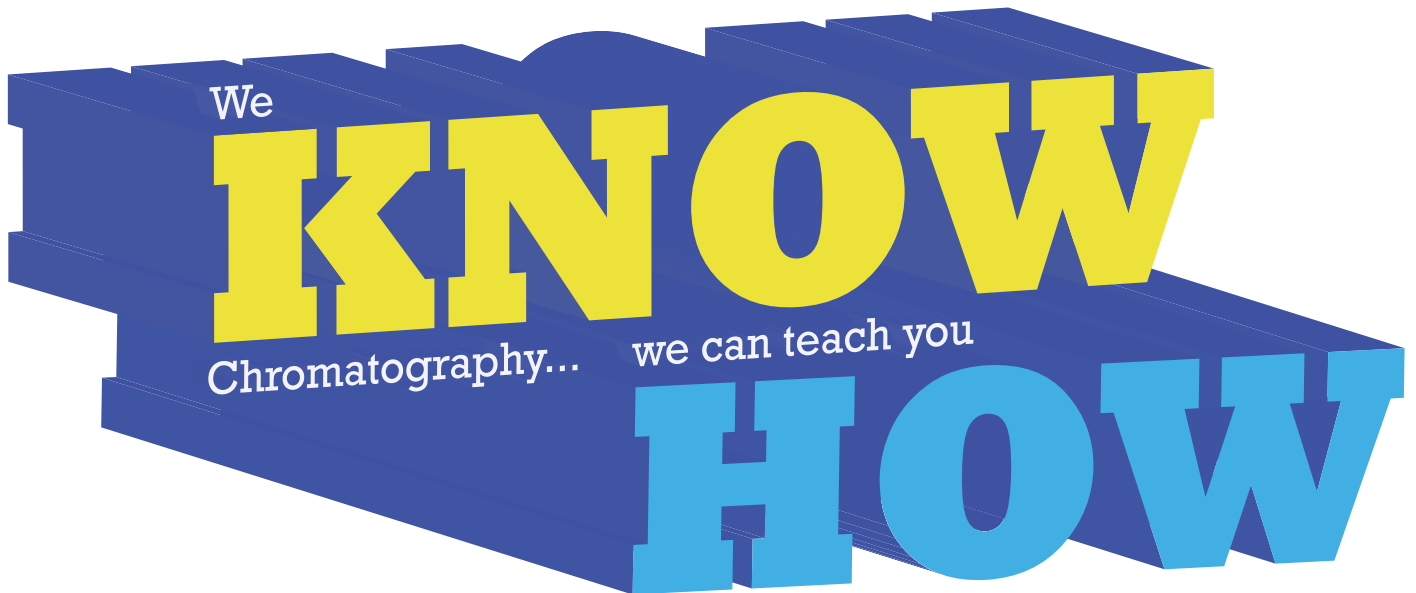




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Crawford Scientific experts

# 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
- » LC-MS Data Interpretation
  
- » GC-MS For the Chromatographer
- » GC-MS Data Interpretation
  
- » Introductory Statistics for Analytical Chemists
- » Advanced Statistics for Analytical Chemists
- » Analytical Method Validation

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.



Email our Training Team



## HPLC Fundamentals

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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

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### 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
- Principles of ionisation (Ion Suppression Chromatography)
- Ion 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

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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

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### 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

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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

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### 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

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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

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### 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

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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

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### 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

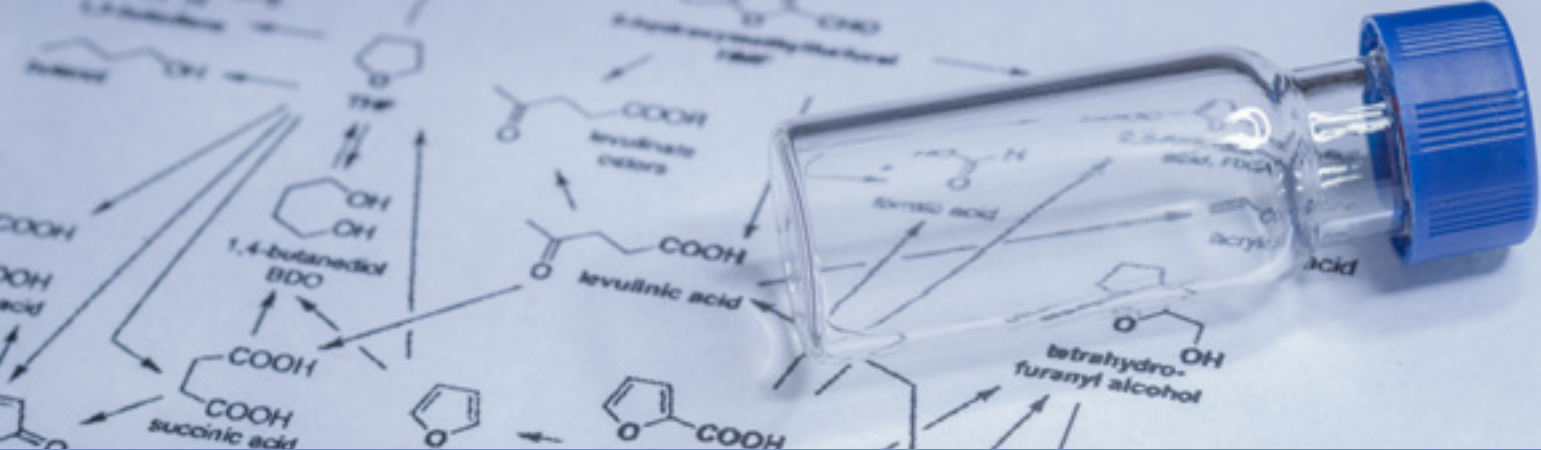
- Baseline spikes
- Noisy baselines
- Cycling baselines
- Rising / falling baselines

### 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

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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

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### Introduction – Fundamentals Review

- Commonly used terms and concepts
- Atmospheric Pressure Ionisation 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



## LC-MS Data Interpretation

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Attendees on this courses will benefit from a greater understanding of how to interpret the mass spectra produced by Electrospray Ionisation (ESI) and Atmospheric Pressure Chemical Ionisation (APCI).

This course will give the delegate increased confidence and understanding of:

- Mass spectral data analysis
- Structural elucidation
- Product ion generation by Collision Induced Dissociation (CID) characterization
- Advantages and limitations of common mass analyser types

## Course Contents

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**Interpretation of low molecular weight compound mass spectra considers the importance of:**

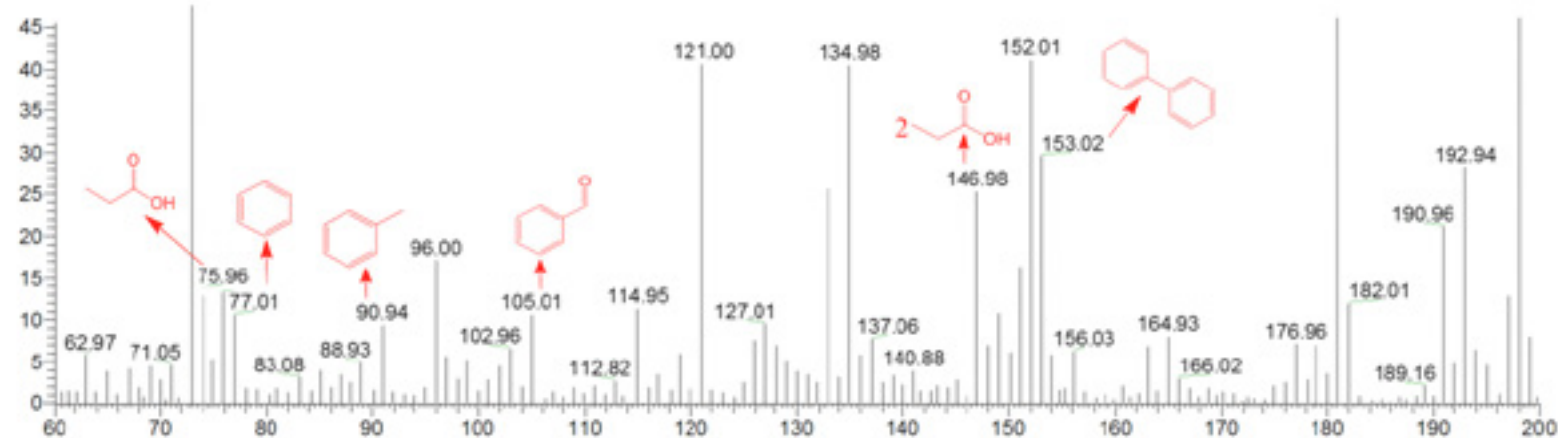
- Understanding isotope patterns and their relative signal responses in assigning elemental composition and confirming chemical structure
- Understanding how common characteristic production fragmentation series are produced through atomic electronic effects
- A mechanistic understanding of the inductive and alpha cleavage
- How CID fragmentation can be invaluable for sample identification purposes through first principles interpretation and database searching
- Inclusion of peptide sequencing by tandem mass spectrometry as required

**Interpretation of high molecular weight compound mass spectra considers the importance of:**

- When to report monoisotopic mass or average mass
- The process of deconvolution of an ESI multiply charged ion series to derive the molecular weight of a compound

**Further**

- Explanation of the formation of common adduct ions under different solvent and buffer conditions
- Solution and source compound dimerisation effects
- The process of data dependent acquisition and modern detector systems
- Comprehensive set of tutor-led tutorial questions to facilitate the learning process



## 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. Ionisation 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

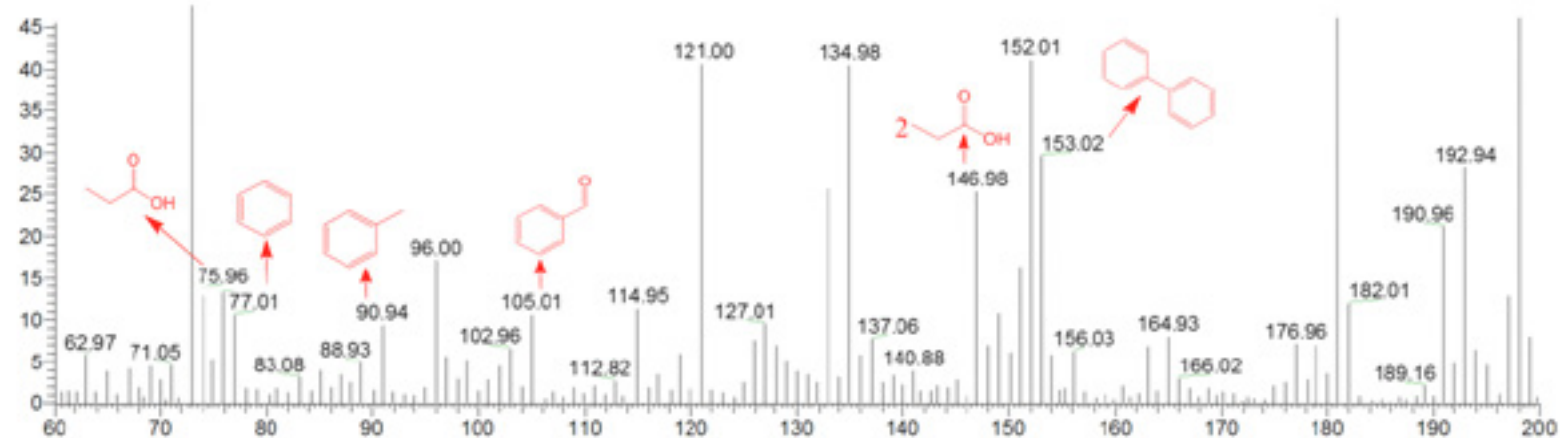
- Electron multipliers and detector electronics
- Matthieu stability diagrams
- X-ray lens and high energy dynodes
- AMU gain and offset
- Spectral resolution

### 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



## GC-MS Data Interpretation

This course provides the basic knowledge required for the interpretation of unknown spectra. Starting from the first principles, it introduces the fundamental chemistry of mass spectrometry and builds up to an examination of the most frequently encountered fragmentation patterns.

This course is not instrument-specific and is suitable for anyone involved in mass spectrometry. A good working knowledge of the technique is required and attendance on our "GC/MS for the chromatographer" course is recommended prior to attendance on this course.

## Course Contents

### Basic Chemistry

- Review of the chemistry of ions, electrons and radicals and isotopes vital to understanding of molecular fragmentation and ionisation
- The principles of isotopic normalisation

### Principles of Ionisation

- Techniques and impact on ionisation and fragmentation of Electron Impact (EI) and Chemically Induced (CI) ionisation mechanisms

### Fragmentation Mechanisms

- Homo and heterolytic fission
- Alpha cleavage
- Inductive cleavage
- McLafferty rearrangement and ortho effects
- Retro Diels-Alder reactions

### Interpretation Rules

- General appearance
- Isotopic abundances
- Isotopic normalisation
- Common ion series
- Rings and double bonds
- The nitrogen rule
- Logical (Illogical) neutral molecular losses
- Logical (Illogical) neutral fragment losses
- Ion stability

### Interpretation Practice

- At all points during the course, practice spectra are given to highlight and allow the student to learn the principles under investigation
- The course ends with practice exercises on several spectra to confirm overall understanding



## Introductory Statistics for Analytical Chemists

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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

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#### 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  $\mu$  / Comparison of  $x_1$  and  $x_2$
- 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



## Advanced Statistics for Analytical Chemists

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This course is designed for scientists involved in experimental design, decision making and process optimisation.

The course shows how to use analysis of variance, randomisation and manipulation of controllable variables to reduce variability, time and cost of design and development.

### Course Contents

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#### Quality Control Methods

- Shewhart charts for mean values
- Shewhart charts for ranges
- Warning and action lines
- Cusum charts
- Zone control charts (J-charts)
- Specification setting

#### Experimental Design and Optimisation

- Introduction
- Randomisation and blocking
- Two-way ANOVA
- Latin squares and other designs
- Simulated annealing
- Interactions
- Factorial versus one-at-a-time design
- Factorial design and optimisation
- Optimisation: basic principles and univariate methods
- Optimisation using the alternating variable search method
- The method of steepest ascent
- Simplex optimisation

#### The Multivariate Analysis

- Introduction
- Initial analysis
- Principal component analysis
- Cluster analysis
- Discriminant analysis
- K-nearest neighbour method
- Disjoint class modelling
- Regression methods
- Multiple linear regression (MLR)
- Principal components regression (PCR)
- Partial least squares (PLS) regression
- Artificial neural networks



## Analytical Method Validation

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This one day course covers all of the basics required to produce an analytical method validation protocol, implement the validation studies and report to FDA standards.

Analytical Best Practices will also be discussed and described and statistical treatments introduced.

## Course Contents

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### Validation Parameters

- Selectivity (Specificity)
- Linearity
- Accuracy (Trueness)
- Precision
- Sensitivity
- Robustness (including experimental design and statistical analysis)

### Experimental Design for Analytical Method Validation

- ANOVA, Plackett-Burman, full factorial methods etc. Statistical Methods and Tests for Validation
- When and how to use t-tests / f-tests to provide rigour to validation results
- Overview of Equipment Validation

### The following topics will be considered for each of the major validation parameters

- Definitions
- Applicability
- ICH guidelines
- Eurachem guidelines
- ISO / IUPAC guidelines
- Important Comments and Considerations
- Procedure
- Statistical Analysis (where applicable)
- Validation Protocol Statements and Reporting
- Conclusions
- Workshop / Tutorial Exercise (where applicable)



## Don't see the course for you?

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The courses highlighted in this brochure have been chosen by us as those which are the most popular with our customers.

Crawford Scientific's training team have years of chromatographic experience in all aspects of analytical chemistry, which means we can also offer training on a much wider range of topics.

For example, some other common courses are:

- Sample Preparation
- Dissolution
- ICP-MS
- Basic Laboratory Skills
- Ion Chromatography
- Quality by Design
- Forced Degradation
- Bio-chromatography

So, if you don't see a training course here that is right for you, just get in touch.

Don't see the course you want? Just get in touch.



Email our Training Team





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## 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.



**Josep Miquel Serret**  
Training and Technical Consultant

HPLC and GC, LC-MS and GC-MS method development.

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# LC GC's CHROMacademy

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