



LAB102

High Performance Liquid Chromatography (HPLC): Operation, Calibration, Troubleshooting and Practice Maintenance

Course Introduction:

HPLC is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds which are not easily volatilized, thermally unstable and have high molecular weights.

The liquid phase is pumped at a constant rate to the column packed with the stationary phase. Before entering the column the analysis sample is injected into the carrier stream. On reaching the column the sample components are selectively retained on the basis of physic-chemical interactions between the analytic molecules and the stationary phase. The mobile phase moving at a steady rate elutes the components based on the operating conditions. Detection techniques are employed for detection and quantification of the eluted components.

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture.

The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

Course Objectives:

Upon successful completion of this course, the delegates will be able to:

- ✓ The differences between High Performance Liquid Chromatography and Gas Chromatography
- ✓ The components of the high performance liquid chromatograph (HPLC)
- ✓ The separation process
- ✓ The chromatogram
- ✓ The most common modes of HPLC

Who Should Attend?

This course is intended for Laboratory personnel and technical staff such as chemists, Analysts, chemical engineers, and anybody interested in chemical analysis, research and development, environmental studies, quality control, refineries, petrochemical plants, water and wastewater plants, hospitals and medical centers.

Course Outline:

Day 1:

Introduction

- Historical Perspective
- Overview of Liquid Chromatography

Practical and Theoretical Principles

- Resolution
- Capacity Factor
- Efficiency

Performing an Analysis

- Solvent Preparation, Isocratic and Gradient-Elution
- Sample Preparation
- Columns
- Pump Maintenance

Day 2:

High Performance Liquid Chromatography Overview

- Introduction to HPLC
- HPLC Instrumentation Overview
- Detector Details
- Laboratory: HPLC Hardware

Separation Fundamentals and Mobile Phase

- Practical HPLC Theory
- Important HPLC Parameters
- The Mobile Phase
- Laboratory: Mobile Phase Strength, Flow Rate and Temperature

Day 3:

Gradient Elution

- Purpose of Gradient Elution
- Scouting Runs and Gradient Profiles

HPLC Instrumentation

- Columns and fittings
- Solvent Delivery System
- Sample Introductions

Detectors and Qualitative and Quantitative Analysis

Day 4:

Modes of Liquid Chromatography

- Packing Materials
- Normal Phase
- Adsorption and Bonded phase
- Reverse Phase
- Principles and Rules of Retention
- Choosing a Reversed Phase Column
- Secondary Ion Effects
- Paired Ion Chromatography
- Ion Exchange Chromatography
- Size Exclusion Liquid Chromatograph
- Chiral Chromatography
- Affinity Chromatography
- Preparative Chromatography

Day 5:

Separation Modes and Columns

- Reversed Phase
- Normal Phase
- Ion Exchange
- Size Exclusions
- Laboratory: pH Effects in Reversed Phase
- Laboratory: Column Dimensions

Quantitative Analysis and Troubleshooting

- Qualitative and Quantitative Analysis
- LC/MS
- Hardware Troubleshooting
- Separation Troubleshooting
- Laboratory: Internal vs. External Standard
- Hardware Troubleshooting

Gradients and Method Development

- Gradients
- Fundamentals of Method Development
- Laboratory: Develop a Method for a Compete Unknown Mixture

Course Conclusion

Using this course overview, the instructor(s) will brief participants about the course topics that were covered during the course.

Course Requirement:

"Hand's on practical sessions, equipment and software will be applied during the course if required and as per the client's request".

Course Certificate:

International Center for Training & Development (ICTD) will award an internationally recognized certificate(s) for each delegate on completion of training.

Course Methodology:

A variety of methodologies will be used during the course that includes:

- (30%) Based on Case Studies
- (30%) Techniques
- (30%) Role Play
- (10%) Concepts
- Pre-test and Post-test
- Variety of Learning Methods
- Lectures
- Case Studies and Self Questionnaires
- Group Work
- Discussion
- Presentation

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Course Fees:

To be advised as per the course location. This rate includes participant's manual, hand-outs, buffet lunch, coffee/tea on arrival, morning & afternoon of each day.

Course Timings:

Daily Course Timings:

08:00 - 08:20	Morning Coffee/Tea
08:20 - 10:00	First Session
10:00 - 10:20	Coffee/Tea/Snacks
10:20 - 12:20	Second Session
12:20 - 13:30	Prayer & Lunch
13:30 - 15:00	Last Session