ANALYTICAL TRAINING SOLUTIONS

Premier Training for Analytical Chemists

Fundamentals of HPLC

Covers the essentials that every scientist needs in order to make effective use of liquid chromatography instrumentation

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WHO SHOULD TAKE THIS COURSE?

Are you new to HPLC, or do you supervise new users of HPLC? This on-line course taught by world-class experts is an opportunity to give new users a firm grounding in the fundamentals of HPLC economically and with minimum disruption to work schedules. "Fundamentals of HPLC" is intended for beginning chromatographers. No hands-on LC experience is required, but attendees should have a solid grasp of basic chemistry.

WHAT DOES IT COVER?

Fundamentals of HPLC covers the essentials that every scientist needs in order to make effective use of liquid chromatography instrumentation:

- The basics of the chromatographic process
- Terms and definitions of chromatography
- How HPLC systems work
- Practical tips on preventive maintenance
- Principles of separation chemistry
- Why "reversed phase" is the standard separation technique
- The meaning of "gradients" and why they are used
- How to get the best quantitation
- and much more.

WHAT WILL I GET FROM THIS COURSE?

You will acquire a good understanding of the fundamentals of HPLC and get a better knowledge of the key concepts involved. The course is aimed at managers, supervisors, auditors, reviewers, etc. who use information generated by HPLC but do not necessarily do chromatography themselves.

INSTRUCTORS

The course was designed by John Dolan, Tom Jupille, and Lloyd Snyder. This class is taught by Tom Jupille. Tom has been a practising chromatographer for more than 30 years, during which he has written more than 30 papers on chromatography and related subjects. He worked primarily in gas chromatography in the late '60s, switching to thin-layer chromatography in the early '70s and then to HPLC and ion chromatography in late '70s. His career has focused on instrument and column development and user support, providing a broad foundation of practical experience to call on as an instructor. He is probably best known as the moderator of the popular Chromatography Forum on-line chromatography discussion group.

COURSE DETAIL

Below are listed each section and sub-section of the class. A brief summary of each sub-section is given first. After the subsection in parentheses is the approximate running time of that module.

Section 1. Overview

What is HPLC?

High Performance Liquid Chromatography, "High Pressure" Liquid Chromatography, even "High Priced" Liquid Chromatography; all of these interpretations of the acronym "HPLC" have some validity. In this introduction, we'll take a look at some of the reasons HPLC has become the most widely used analytical technique in the world.

A brief history of HPLC

From the origins of chromatography at the beginning of the 20the century to the conceptual breakthroughs at mid-century and the revolution in technology of the 1970s, we'll trace the evolution of today's HPLC technology -- any look at the possible future.

From Apparatus to Instrument

In the final part of our overview, we'll look at the underlying reasons why HPLC technology evolved the way it did.

Section 2. Instrumentation

Pumps & Degassing

Starting at beginning of the flow patch, we'll look at the different types of solvent delivery systems used in HPLC, and pay particular attention to effective removal of dissolved gas from the solvent.

Injectors & Autosamplers

Because the interior of an HPLC system is well above ambient pressure, some provision must be made for sample introduction. We'll look at the typical design of injector valves and compare the three different types of autosamplers that are used to load those valves.

Connecting Tubing, Fittings, & Column Hardware

Continuing through the system, we'll take a look at how the characteristics of connecting tubing (inertness, pressure resistance, internal volume) fit the requirements in different parts of the HPLC system.

Detectors

Separating compounds by HPLC is only part of the story -- they must also be detected. We'll compare the most common detector technologies used: UV-VIS absorbance, fluorescence, refractive index, electrochemical, evaporative, and mass spectrometry.

Section 3. Measurements & Parameters

Retention

In many respects, retention (k')is the most important characterization parameter in HPLC. We'll look why it's defined the way it is and on the reason it has an optimum range of values.

Selectivity & Efficiency

While retention is important, it's only part of the story. We'll look at measures for selectivity (alpha) and efficiency (N) and how they affect chromatographic results.

Resolution & Asymmetry

Ultimately, the quality of a separation is measured by resolution. We'll look at how it's defined and measured, and why the measurement can be misleading. We'll end by looking at measurements that characterize non-ideal peak shape.

Section 4. All about chemistry

Reversed-phase

Reversed-phase is unquestionably the most widely used mode of HPLC. We'll look at the definition, how it got that awkward name, and the parameters that control the way it works.

pH & Ion-pair

By implication, reversed-phase chromatography deals with neutral molecules. We'll look at the impact of pH on ionizable analytes in reversed-phase, and explore the use of a ion-pair chromatography(in effect, a variant of reversed-phase) for ionized compounds.

Normal-phase & HILIC
Today, the original "liquid chromatography" would be classed as "normal-phase" chromatography. We'll look at the mechanism of normal-phase and the types of samples to which it is applicable, as well as covering it's variant, HILIC (Hydrophilic Interaction Chromatography).

Ion exchange & Size Exclusion
The last two modes of chromatography we will cover are ion-exchange and size exclusion, including both GFC (Gel Filtration Chromatography) and GPC (Gel Permeation Chromatography).

What Can Go Wrong?
While troubleshooting is outside the purview of this course (see our more extensive HPLC Basics, Equipment, & Troubleshooting course) there are some common problem areas that are of concern to auditors, managers, and supervisors, particularly in the area of ambiguous documentation.

Gradients
Up to here, we have focused entirely on isocratic separations (a big word meaning "the solvent doesn't change during the run"). In many cases, solvent gradients (changing composition) are used. We'll discuss the situations where gradients are applicable and explore similarities and differences between gradient and isocratic HPLC.

Section 5. Quantitation

Integration
In most cases, the area under a peak is proportional to the mass of that compound injected. We'll cover the algorithms used to measure area and look at some of the pitfalls that can occur.

Calibration
The response factor (the proportionality between area and amount injected) must be determined by calibration. We'll compare the pros and cons of external standard calibration and internal standard calibration.

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