

# iLearn

## BASIC HPLC FOR PHARMACEUTICAL ANALYSIS

### Introduction

HPLC is probably the most important analytical technique used in pharmaceutical analysis. A skilled operator is required to perform HPLC analysis and therefore effective training is required by analysts who use the technique. This HPLC training course provides a comprehensive treatment of HPLC theory and application in a pharmaceutical analysis environment, concentrating on the theory and practical implementation of the technique. The course is made up of four modules which are described below.

### MODULE 1

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#### What is HPLC?

The learning objective for this module is to understand what is meant by: chromatography, High Performance Liquid Chromatography (HPLC); and the application of HPLC to the chemical analysis of pharmaceuticals.

The theory of chromatography is introduced and the different types are briefly described. The concept of polarity is revised due to its importance in HPLC. High Performance Liquid Chromatography (HPLC) is defined and the different types, namely partition, adsorption, size-exclusion, and ion-exchange, are discussed. The nature and structure of drug molecules are reviewed together with consideration of how their structures make them suitable for analysis by one or more types of HPLC.

### MODULE 2

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#### HPLC Stationary Phase

The learning objective for this module is to understand the wide range of available stationary phases and the differences between them, and use this information to identify a particular HPLC column using the relevant descriptive parameters.

The HPLC stationary phase is introduced in terms of the column where each parameter is introduced in turn e.g. packing, bonded phase, particle size, length, etc. The packing is

considered in some detail with a discussion of the different materials commonly used, e.g. silica, zirconia, hybrid technologies, etc. This is followed by a discussion of the different types of available bonded phases and the differences between them, including the technologies used for particular problems such as polar analytes and pH stability. The physical parameters of the column such as particle size, pore size, length and internal diameter, and their effects, are then considered.

## **MODULE 3**

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### **HPLC Mobile Phase**

The learning objective for this module is to prepare mobile phase for HPLC using different types of solvents, buffers and additives and understand the effect these have on the chromatographic separation.

The most commonly used solvents in HPLC are each introduced and their properties, including polarity and UV cutoff values, discussed. Then the solvents which are commonly used for each of the different types of HPLC, namely partition, adsorption, size-exclusion, and ion-exchange, are considered. The effect of ionisable analytes such as acids and bases in reversed phase partition HPLC is investigated and the use of buffers and other additives explored. Isocratic and gradient elution methods are introduced. Best practice methods for the preparation of mobile phase are described including methods of mixing mobile phases required in gradient analysis. Finally methods for, and the purpose of, mobile phase degassing is considered.

## **MODULE 4**

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### **HPLC Systems & Instrumentation**

The learning objective for this module is to identify and understand the purpose of each component of the instrumentation used for HPLC, and how they are connected to each other.

The purpose of each component in a typical HPLC system is explained by introducing each part of a typical instrument in terms of the role that it plays in enabling the interaction of the sample with the stationary phase and mobile phase. The function and key features of each component is discussed in turn, namely mobile phase reservoirs; in-line degassers; pumps and solvent management systems; injection systems; column compartment; column fittings; detectors; waste collection; data processors and chromatography data systems (CDS).