

WM Training Course in Clinical Biochemistry

Outline the principles underlying HPLC. Discuss with examples the factors that are important in optimising an assay utilising HPLC.

Introduction

A simple start with a definition of what the term HPLC means. Position HPLC within the other chromatography techniques – Paper, TLC, GC, HPLC. Capillary GC has the highest number of theoretical plates but not as easy to apply as HPLC.

A schematic of a system would be good, then use this as basis to discuss the different features include: Mobile phase composition, pump characteristics, column – including discussion of different types of column and packing, Detector – include the different types and their uses.

Sample Preparation: Discuss the different ways of preparing sample and use routine applications to demonstrate depth of knowledge

For example: No preparation of minimal - urine caffeine
 Liquid-liquid extraction – vitamin A&E
 Liquid-Solid – using cartridges with methods such as catecholamines
 Protein precipitation – drugs in serum

Autosampler: Walk away is very important for routine analysis

Pumps: Includes discussion of low pulsing and ways to achieve. Also high pressure due to the backpressure of the column.

Injection valve; The key features of what is required. Typical volumes are from 1 – 25 uL these days with 50 uL being a lot.

Columns: Here length and internal diameter are worth a mention. The length and diameter of the column is intrinsically linked with the particle size of the packing material. Smaller particle size means higher back pressure – so you would use a shorter column.

Particle size: 5 micron normal, 3 micron gives greater separation per volume but at the expense of back pressure.

Length: 15cm with 4.6mm id normal for 5 micron

[This is examples of sort of facts I would expect in the essay]

Packing material: Usually based on silica – normal phase is silica and reverse phase is modified silica – for example with C18 attached to make more hydrophobic. Can have other sorts of packing for example polymer packing – these can have advantages such as working at pH lower than 3.5 which is the limit.

Column temperature: Discussion of the reason to have a constant temperature and also impact on back pressure and separating capacity of increasing temperature (back pressure reduced and also separation capacity).

Detector: Different types of detector and also examples of analytes suited to them. Here remember that there are loads of different detectors but only relatively few that have gained acceptance.

Integration and Interfacing: This is a key consideration especially to applying HPLC in routine clinical analysis in our sorts of environments.

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