HPLC Column Selection Guide

Column Parameters

Internal Diameter
An important parameter of a HPLC column is the internal diameter (ID) as this directly influences the detection sensitivity, selectivity of separation and the quantity of analyte that can be loaded onto the column.

- Low ID columns have higher sensitivity and lower solvent consumption for analytical purposes but require low loading of sample
- Analytical scale columns (0.4 and 0.46cm) are the most common type of columns and often use a UV-Vis absorbance detector
- Narrow-bore columns with ID of ≤0.3cm are used for sensitive analytical applications and use more advanced UV-Vis, fluorescence or mass detectors
- Large ID columns (≥0.7cm) have a high loading capacity and so are used to purify usable amounts of sample material for preparative or semi-preparative applications

Particle size
The size of the particles (beads) in the stationary phase of the column influences the separation of the analyte. Smaller particles generally provide more surface area and better separations, but the pressure required for optimum separation is greater.

- Columns with 5µm particles provide high resolution of peaks and are large enough to be suitable for analytical, preparative and semi-preparative applications.
- Columns with 3µm particles are used when rapid analysis is needed and are mostly suitable for analytical applications

Porosity
An ideal stationary phase will be porous enough to provide greater surface area so that the sample material can fit into the column and be analysed. Small pores up to 120 Å provide greater surface area and lead to higher resolving ability and are typically used for smaller MW analytes. Larger pores such as 300 Å are suitable for a wider range of samples especially for larger MW analytes such as proteins and oligonucleotides.

Length
The length of a column determines the overall separation time and influences the resolution of the peaks. For the same particle size:-

- A 3 - 7.5cm length column provide faster analysis run times
- 10 – 25cm length columns provide better resolution

A compromise between the speed and resolution of analysis is normally needed to find the most efficient column i.e. the shortest column with the best resolving capability.
Applications

Small Molecule Separations (MW < 2,000)
- Pharmaceuticals
- Medical & Clinical
- Biotech & Life Sciences
- Foods, Flavours & Fragrances
- Environmental
- Biofuels & Industrial

Pharmaceuticals Applications
- Antibiotics - Mediterranea™ Sea18; Tracer Extrasil; Nucleosil
- Steroids - Mediterranea™ Sea18; Tracer Extrasil; Nucleosil
- Opiates - Mediterranea™ Sea18; Tracer Extrasil; Nucleosil
- Vaccines - Mediterranea™ Sea18
- Other Drug Agents - Mediterranea™ Sea18; Tracer Extrasil
- Nucleotides - Mediterranea™ Sea18; Tracer Extrasil ODS 1, ODS 2

Medical & Clinical Applications
- Amino Acids - Mediterranea™ Sea18; Tracer Excel ODS A, ODS B
- Water Soluble Vitamins - Mediterranea™ Sea18; Tracer Extrasil ODS 1, ODS 2; Tracer Excel ODS A, ODS B
- Fat Soluble Vitamins - Tracer Extrasil ODS 1, ODS 2

Small Molecule Biotech & Life Science Applications
- Amino Acids - Mediterranea™ Sea18; Tracer Extrasil ODS 1, ODS 2
- Nucleotides - Mediterranea™ Sea18; Tracer Extrasil
- Lipids and Oils - Tracer Extrasil, Nucleosil

Chemical, Biotech & Life Sciences Applications
- Aromatic Compounds - Mediterranea™ Sea18; Tracer Extrasil; Nucelosil
- Hormones – Tracer Extrasil; Nucelosil; Tracer Excel

Foods, Flavours & Fragrances Applications
- Carbohydrates - Transgenomic™ CARBOSep; Tracer Extrasil; Nucleosil; Tracer Excel
Organic Acids - Transgenomic™ ICSep; Mediterranea™ Sea18; Tracer Extrasil ODS 1, ODS 2; Nucleosil; Tracer Excel ODS A, ODS B
Amino Acids - Mediterranea™ Sea18; Tracer Extrasil ODS 1, ODS 2; Nucleosil; Tracer Excel ODS A, ODS B
Lipids and Oils - Tracer Extrasil; Nucleosil; Tracer Excel
Nutraceuticals - Mediterranea™ Sea18; Tracer Extrasil ODS 1, ODS 2; Nucleosil; Tracer Excel ODS A, ODS B
Water Soluble Vitamins - Mediterranea™ Sea18; Tracer Extrasil ODS 1, ODS 2; Tracer Excel ODS A, ODS B
Fat Soluble Vitamins - Tracer Extrasil ODS 1, ODS 2

Environmental Applications
Pesticides - Mediterranea™ Sea18; Tracer Extrasil ODS1, ODS2
Herbicides - Mediterranea™ Sea18; Tracer Extrasil ODS1, ODS2
PAHs - Mediterranea™ Sea18; Tracer Extrasil ODS1, ODS2

Biofuels and Industrial Applications
Mono-, di-, tri-glycerides - Tracer Extrasil ODS 1, ODS2; Tracer Excel ODS A, ODS B
FAMEs - Tracer Extrasil ODS 1, ODS 2; Tracer Excel ODS A, ODS B
Free Glycerol - Tracer Extrasil; Mediterranea™ Sea18
Sterol Glucosides - Tracer Extrasil ODS 1, ODS2; Tracer Excel ODS A, ODS B
Phospholipids - Tracer Extrasil Si
Carbohydrates - Transgenomic™ CARBOSep; Mediterranea™ Sea18; Tracer Extrasil

Large Molecule Separations (MW > 2,000)
Simple Enzymatic Digests (< 12 proteins) - Europa® Peptide, Protein; Tracer Extrasil; Hamilton PRP; Nucleosil
Complex Enzymatic Digests (≥ 12 proteins) - Europa® Peptide, Protein; Hamilton PRP
Biomolecules (0-5,000 MW) - Europa® Peptide
Biomolecules (5-10,000 MW) - Europa® Peptide, Protein; Hamilton PRP
Biomolecules (>10,000 MW) - Europa® Protein; Hamilton PRP
Antibodies - Europa® Peptide; Tracer Extrasil; Nucleosil
Oligonucleotides - Europa® Peptide; Hamilton PRP; Tracer Extrasil; Tracer Excel; Nucleosil
Oligosaccharides - Transgenomic™ CARBOSep; Tracer Extrasil; Tracer Excel; Nucleosil
Hormones - Europa® Peptide, Protein

Extreme pH Applications
Basic Compounds - Mediterranea™ Sea18; Hamilton PRP; Hyperpack Basic
Acidic Compounds - Mediterranea™ Sea18; Hamilton PRP