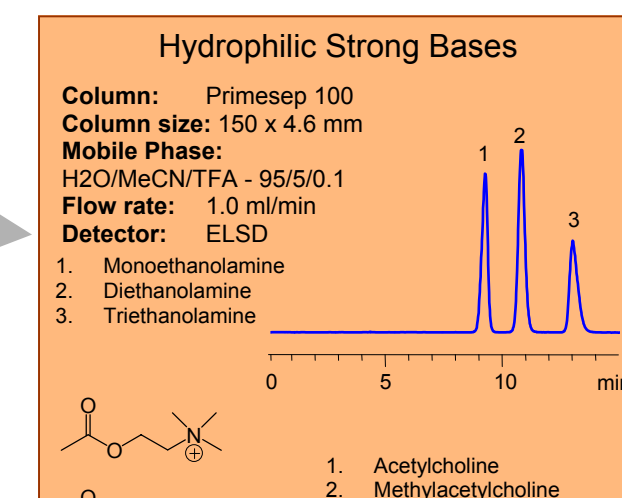
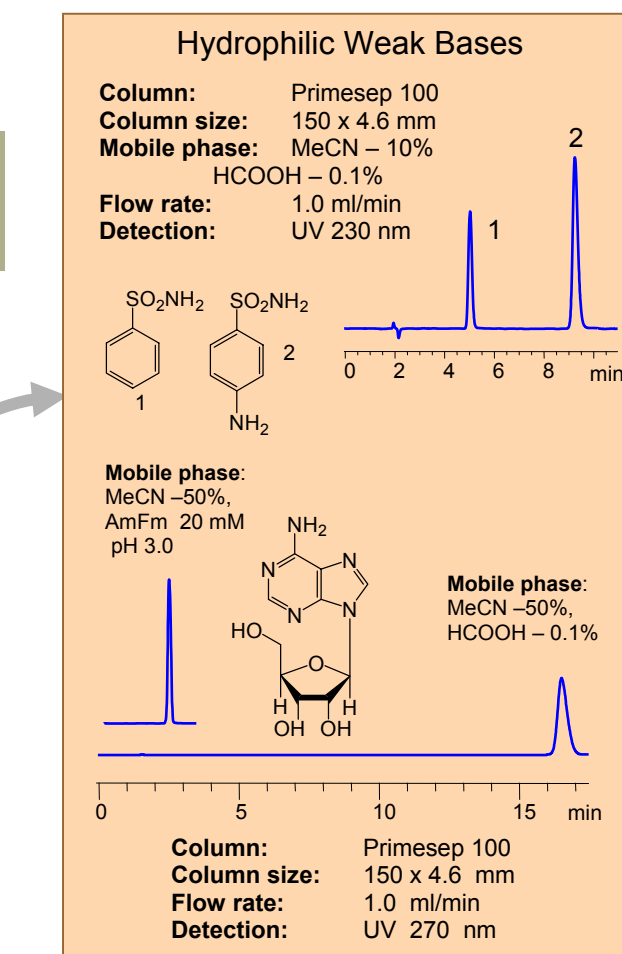
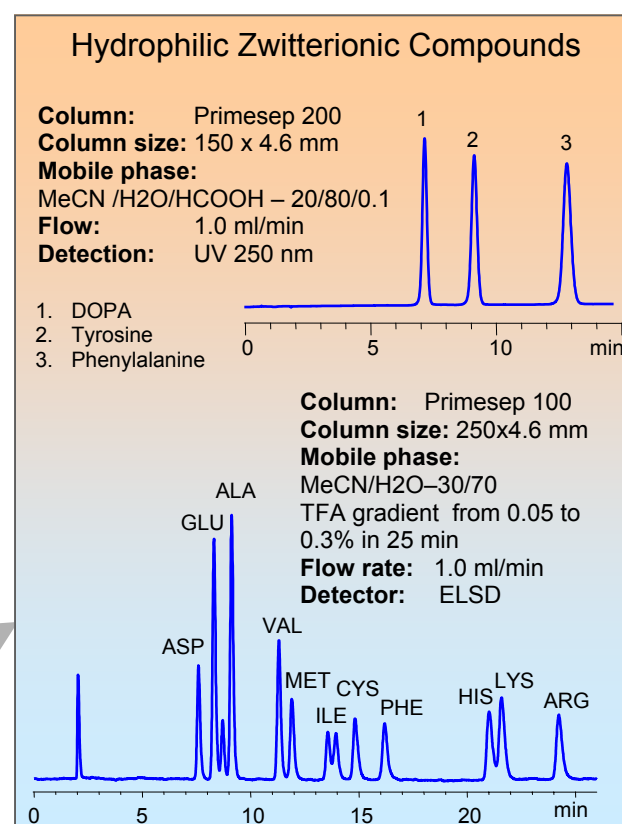
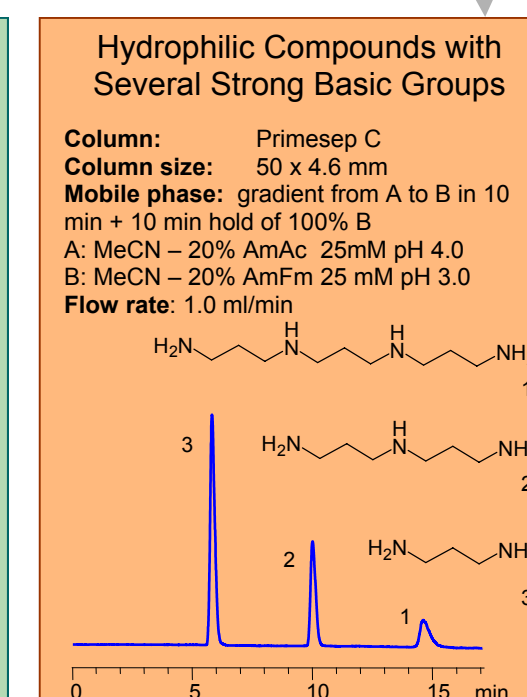
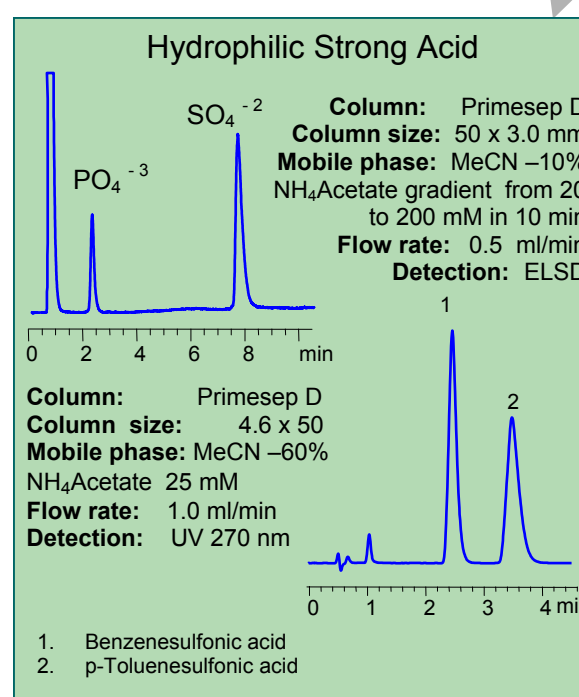
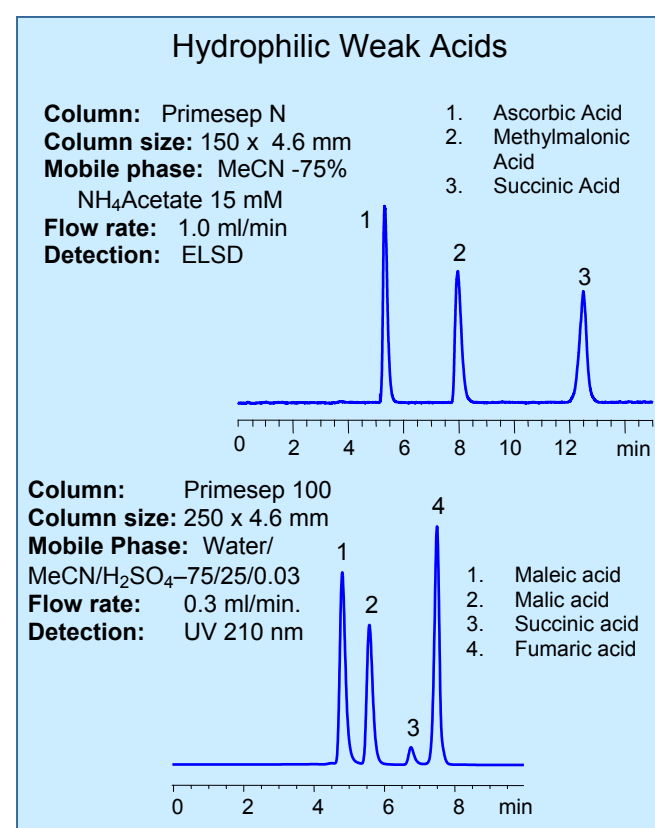
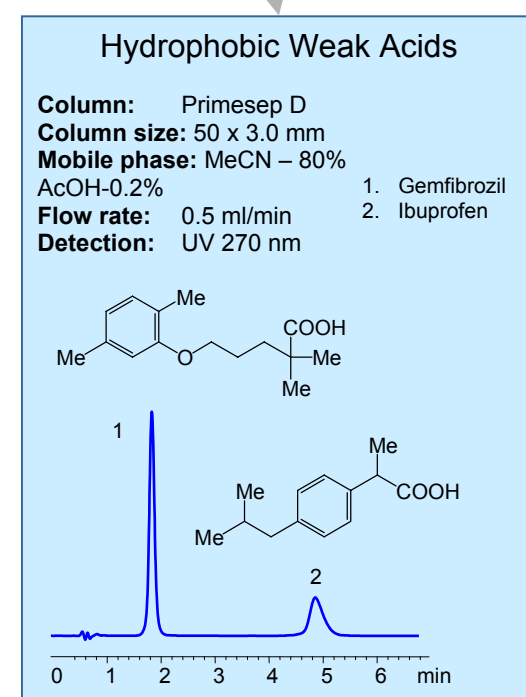
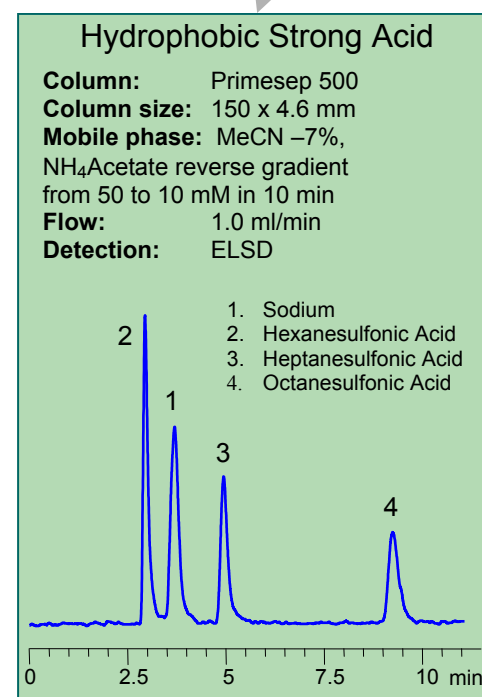
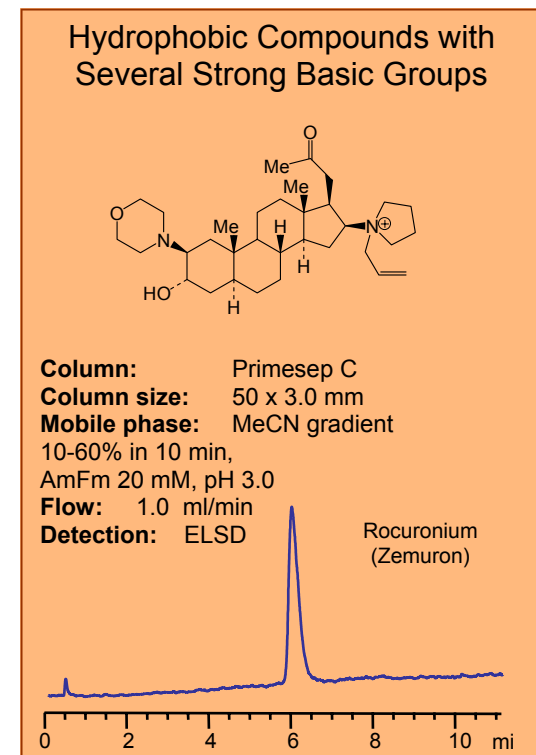
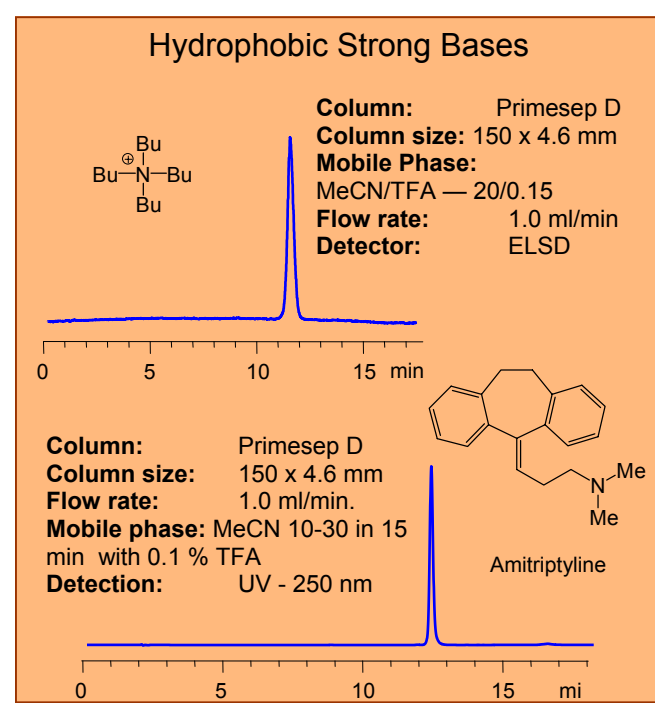
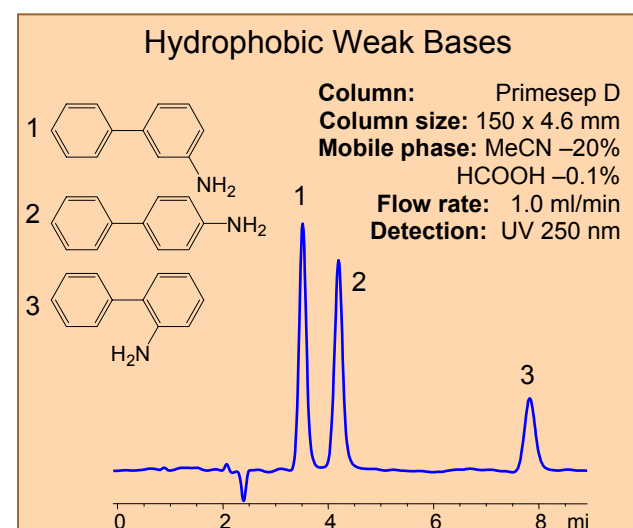
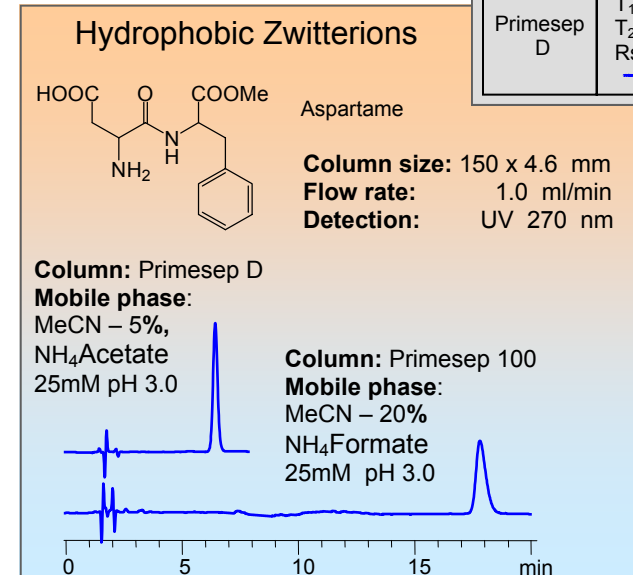
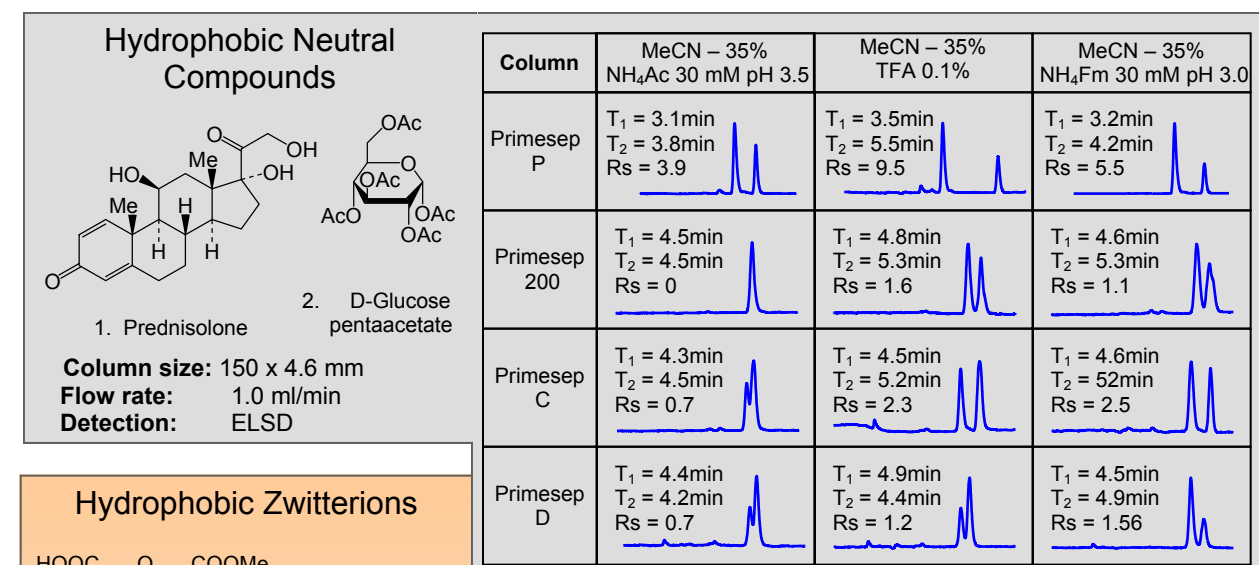


A Guide for HPLC Separation of Small Molecules



SIELC

Mixed mode chromatography is a powerful technique for the separation of various compounds. These separations are based on a combination of reverse phase, ion-exchange, ion-exclusion or pi-pi interactions. Polar and hydrophobic, acidic and basic compounds, zwitterions and neutral molecules can be separated with high selectivity and efficiency during the same run. This guide will help in developing methods for the separation of small molecules with different properties. The flexibility of mixed mode chromatography allows you to find conditions for different detection techniques (UV, MS, ELSD, IR), and provides easy scale-up and high throughput capabilities. This mixed mode technology works well with difficult sample matrices and a variety of sample diluents.



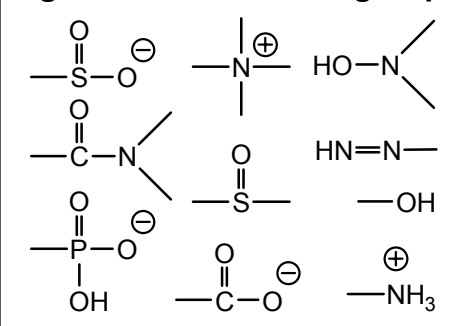
Hydrophobic ← Your compound is...? → Hydrophilic

Before you start... become familiar with the terms used in this guide:

Hydrophilic compounds - compounds with a better solubility in water than in organic solvents, or completely non-soluble in organic solvents (et. acetonitrile, ethanol). Examples of such compounds are amino acids, sugars, peptides, nucleotides, protonated amines and any other molecules with one or several polar groups (Fig. 1). The presence of some functional groups does not make a molecule significantly polar in terms of LC hydrophobic interaction (Fig. 2).

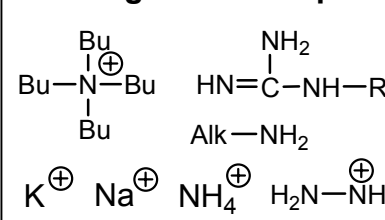
Ionizable compounds - molecules which have at least one charged functional group in a solution within a pH range from 2 to 7. Under these conditions, basic compounds are **positively** charged and acidic compounds are **negatively** charged.

Fig. 1 Polar functional groups

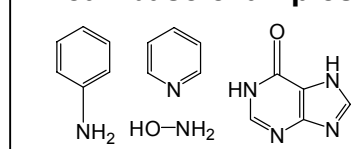


Zwitterionic compounds - compounds that bear an equal number of positively charged and negatively charged functional groups in solutions at a pH within the range of 2 to 7.

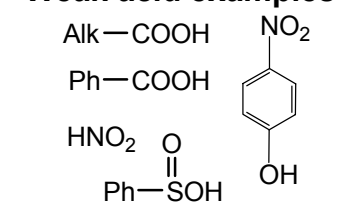
Strong base examples



Weak base examples



Weak acid examples



Zwitterion examples

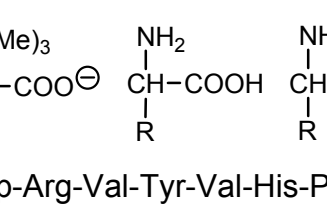
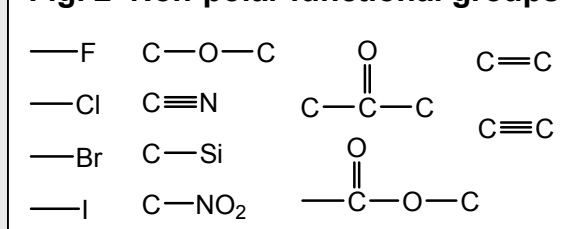


Fig. 2 Non-polar functional groups



Strong acid examples

