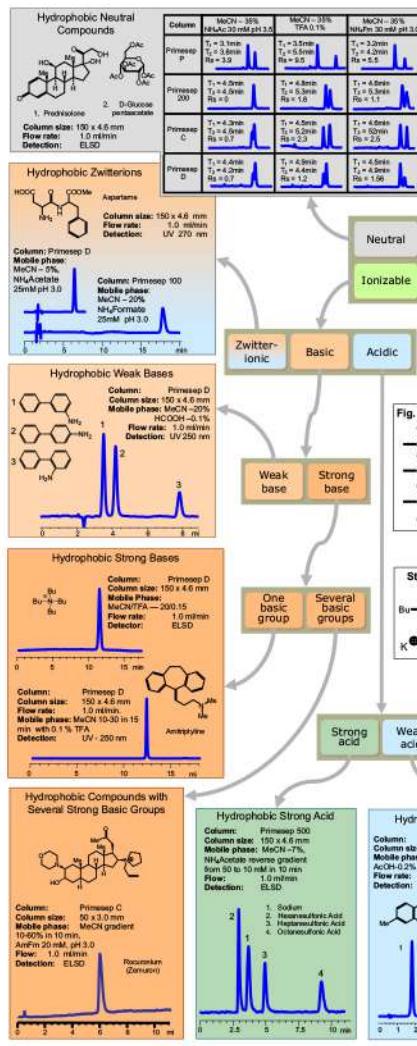


New Alternative to Ion Chromatography

Primesep ® Separation of Ions

- Primesep mixed mode columns are a powerful separation tool for analysis of polar compounds and complex mixtures with compounds of different polarity
- Combination of Primesep columns with ELSD offers simple alternative to ion-chromatography
- Anions and cations can be analyzed at the same time with the same mobile phase
- Ion-chromatography with concentration of organic modifier from 0 to 100% can be performed with possibilities to adjust resolution and peaks elution order
- Inorganic and organic compounds can be analyzed at the same time using ELSD alone or in combination with UV detector



A Guide for HPLC Separation of Small Molecules



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

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 set 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.

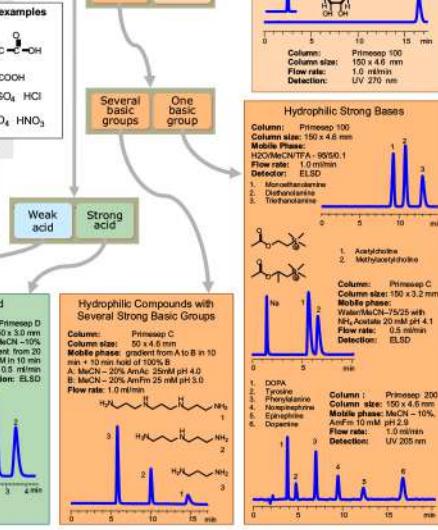
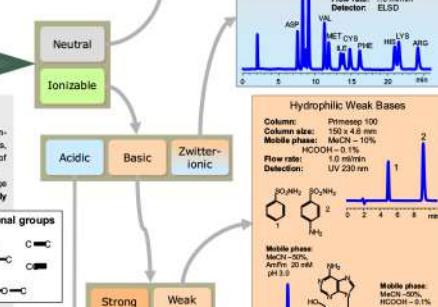
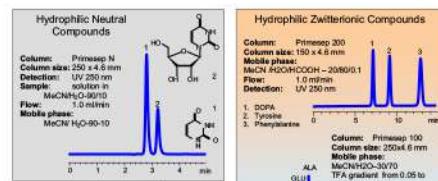


Fig. 1 Polar functional groups

Weak base compounds – compounds that go through a transition state from positively charged to neutral within a pH range from 2 to 7 (pK_a > 6).

Weak acids – compounds that go through the transition from a negatively charged state to a neutral state within a pH range from 2 to 7 (pK_a < 3). Strong acids – compounds which are significantly ionized in solutions at a pH of 1 (pK_a < 1).

Strong bases – compounds which are significantly ionized in solutions at a pH of 14 (pK_a > 14).

Fig. 2 Non-polar functional groups

Fig. 3 Strong acid examples

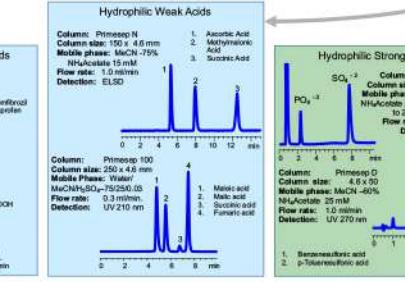
Fig. 4 Weak base examples

Fig. 5 Weak acid examples

Fig. 6 Zwitterionic examples

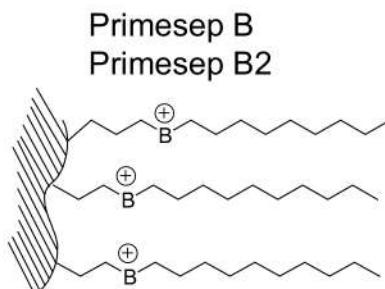
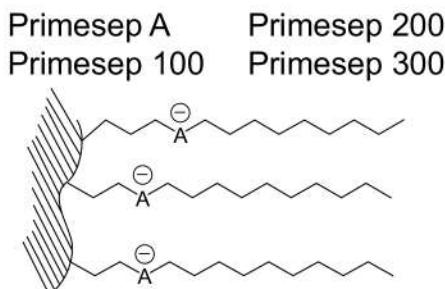
Fig. 7 Recommended buffers: Low UV methods: sulfuric acid, phosphoric acid, perchloric acid, phosphorus buffers, TFA. MS/ELSD methods: formic acid, acetic acid, ammonium formate, ammonium acetate, TFA.

Complex mixtures: each compound type can be analyzed on different Primesep columns. If you are dealing with complex mixtures that contain several different compound types, please check out additional method development resources at <http://www.HPLCMethodDevelopment.com>



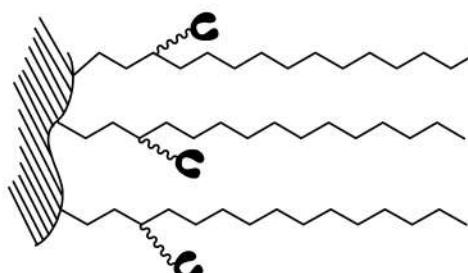
Mixed-Mode Primesep Columns

With an embedded ion-pairing group, a Primesep column requires no ion-pairing reagent in the mobile phase to retain and separate ionizable polar compounds.



- A newly developed Primesep C column ("C" stands for "complex") forms a weak complex with amino compounds and metal ions.
- The column offers a typical RP retention profile for neutral compounds.
- In addition, embedded hosting groups interact with amines and other ions, and form a unique retention pattern.
- Amines with equal hydrophobicity retain on Primesep C in the following order.
- Tertiary < secondary < primary. Alkali metals are retained in order $K^+ < Na^+ < Li^+$, which is a reverse order compared to the classical ion-exchange.

Primesep C Column



SWITCH Phase™ Technology

Columns based on SWITCH Phase™ technology change their properties depending on pH of the mobile phase. Embedded carboxylic acid is fully ionized at pH above transition point and loses charge when mobile phase pH goes below transition point. By controlling pH of the mobile phase, the polar properties of the stationary phase can be altered to tune your separation needs.

Primesep 300

Transition @ pH=3

Primesep 200

Transition @ pH=2

Primesep 100

Transition @ pH=1

Primesep A

Transition @ pH=0

Primesep 300 at pH > 3.5

Primesep 300 at pH < 2.5

