SWITCH Phase™ HPLC Technology

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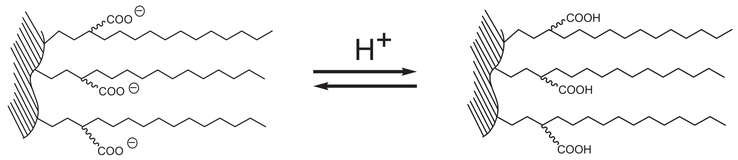
Stationary phases based on SWITCH Phases technology represent a new approach in the column chemistry. These stationary phases combine reverse phase and ion-exchange properties in a single ligand attached to the silica surface. Columns based on SWITCH Phase technology change their properties depending on the composition of the mobile phase. With the right mobile phase selection SWITCH Phase columns can behave as pure reverse phase, pure ion-exchange, normal phase, HILIC or mixed mode column. For example, by changing the pH of the mobile phase you can gradually shut down or enhance ion-exchange properties of the column and tune your separation needs. This unique property of the stationary phase becomes important when the analytes are basic compounds with very different pKa values. The presentation will discuss various ways to adjust selectivity of the column by simple modifications of mobile phase. The effect of organic component, buffer concentration, nature of buffer, pH of the mobile phase will be discusses with various examples of pharmaceutically important compounds. If selectivity or retention of particular set of compounds is not achievable with one separation mode the mobile phase can be changed and new separation mode can be used without changing the column itself. We will present application of SWITCH Phase technology to separation of various polar, non-polar, hydrophobic, and hydrophilic compounds.



Column @ pH abow transition pH

Column @ pH below transition pH

Common buffers for two modes of operation:

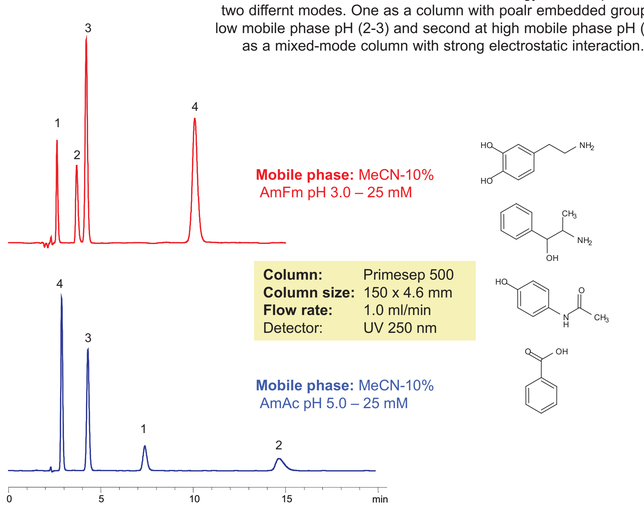
No buffer;
Ammonium acetate pH 4-5;
Sodium phosphate pH 6-7;

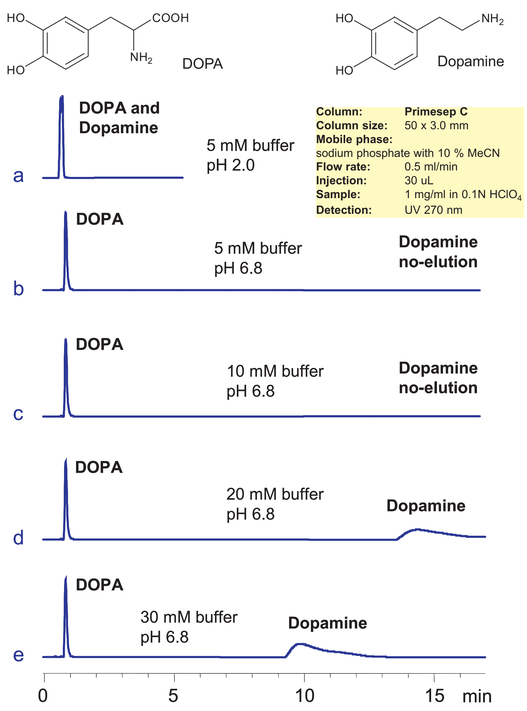
Phosphoric acid; TFA; Sulfuric acid,
Perchloric acid; Sodium phosphate pH 2-3
Ammonium formate pH 2.8-3.5

Columns based on SWITCH Phase™ technology change their ionization depending on pH of the mobile phase.
Embedded carboxylic acid is ionized at the pH above the transition point and loses charge when the pH of the mobile phase goes below the transition point.

Primesep 500	**Transition @ pH=4.5**	**pKa = 4.5**
Primesep C	**Transition @ pH=3.5**	**pKa = 3.5**
Primesep 200	Transition @ pH=2	pKa = 2
Primesep 100, P	Transition @ pH=1	pKa = 1
Primesep A	Transition @ pH=0	pKa = 0

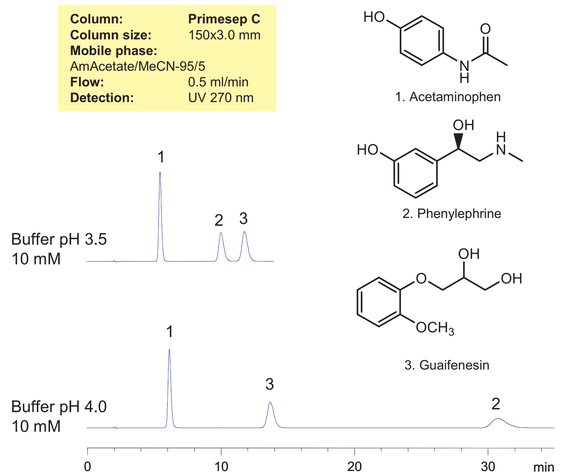
Columns based on SWITCH Phase™ technology can be operated in two differnt modes. One as a column with poalr embedded group at low mobile phase pH (2-3) and second at high mobile phase pH (5-7) as a mixed-mode column with strong electrostatic interaction.

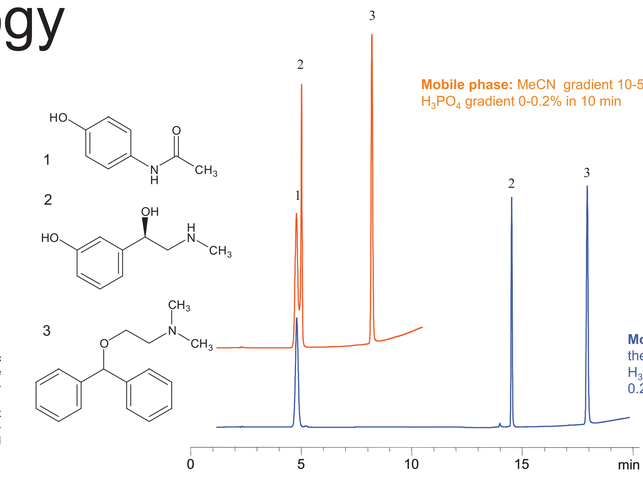


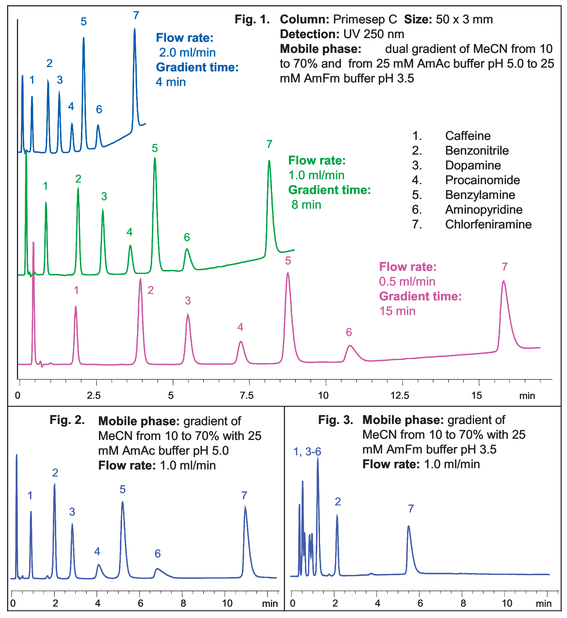


Mobile phase pH can be used to trap basic compounds on a column with SWITCH Phase properties or it can work as a fine separation tuning tool.

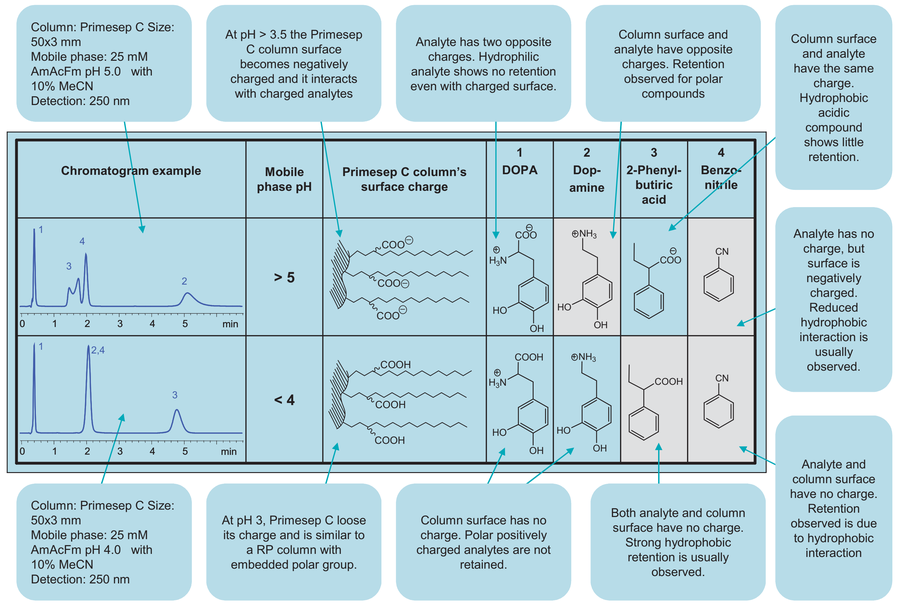
Amino acids and neutral compounds are not effectsd by mobile phase pH while basic compounds retention strongly depends on pH and concentration of buffer in the mobile phase.

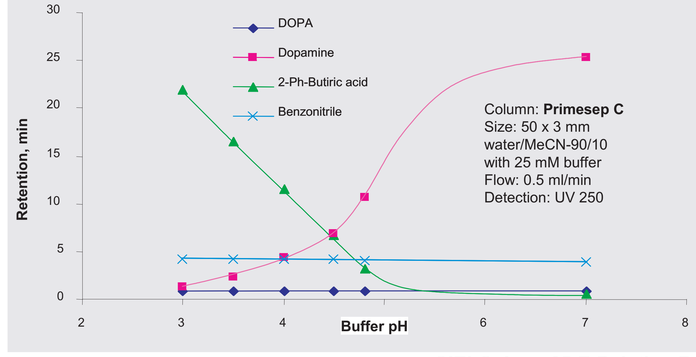






Primesep C is a special type of mixed-mode column. This column is complimentary to other dual mode columns such as Primesep 100 and Primesep 200. The important difference is the ion exchange embedded functional group, which can be switch off completely by the pH of the mobile phase. At a pH below 3 the ion-exchange properties of Primesep C column are significantly suppressed and RP is only mechanism of retention. At a pH over 4 both the IE and RP mechanism can be employed for separation. This unique property of the stationary phase becomes important when the analytes are basic compounds with very different pKa values. The pKa for basic compounds can vary significantly, as can the number of basic functional groups in the molecule. It is very difficult to separate compounds with vastly different basic properties using a single ion-exchange column. Primesep C with a pH gradient mobile phase allows one to retain the majority of basic compounds using MS friendly conditions.





Buffer in pH range from 3-5 is ammonium acetate-formate (AmAcFm), made by adjusting the pH of 100 mM ammonium acetate with formic acid, and then diluting with MeCN and water to 25 mM. The mobile phase at pH 7 is obtained by dissolving ammonium acetate in water and MeCN without pH adjustment.

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