

New Tools to Enhance Selectivity in Reverse Phase HPLC

By SIELC, Inc. Prospect Heights, IL, USA
www.sielc.com

Selectivity and efficiency are two components of successful separation. In liquid chromatography (LC) the efficiency of the column is limited to about 20K theoretical plates per column regardless of the type of column or type of instrumentation used. There are technical and practical limitations that do not allow to break this natural barrier. Selectivity of the column, in the opposite, is unlimited. SIELC Technology, Inc. is offering the line of stationary phases (Fig. 1) with selectivity enhanced by additional interaction of the column with the analytes. Since the reverse phase separation (RP) is a primary type of separation in LC, we focused our efforts on developing the RP columns with additional ion-exchange interaction.

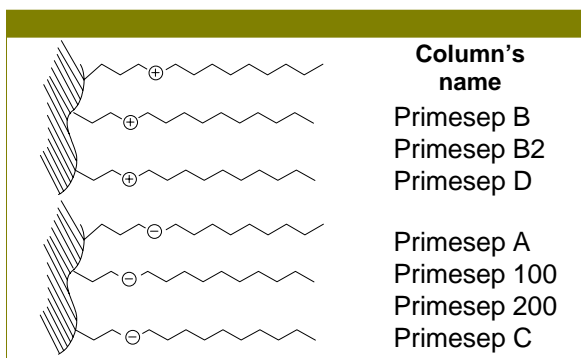


Figure 1: Simplified structure of stationary phase ligand attached to silica support

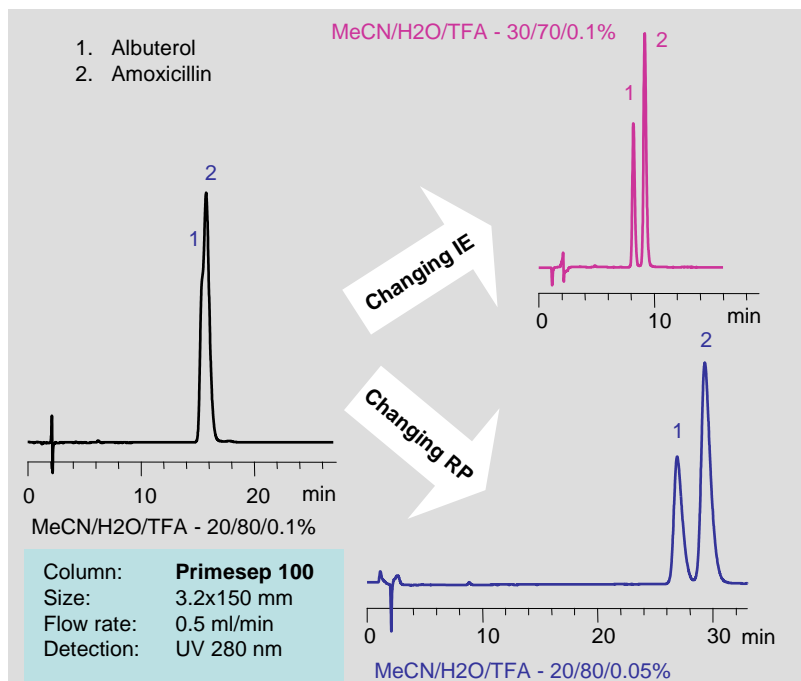


Figure 2: Non-resolved peaks can be separated by changing either ion-exchange or reverse phase component of the retention.

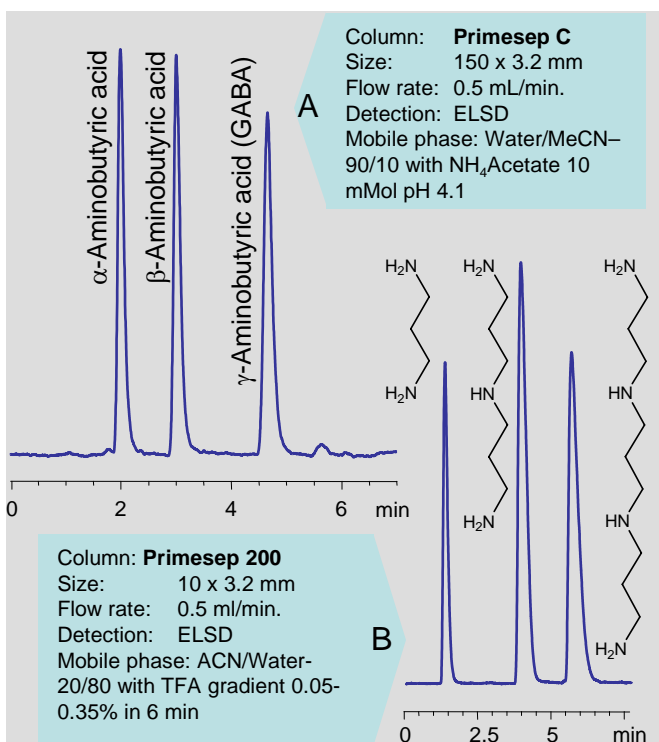


Figure 3: Using additional ion-interaction for separation of polar compounds of similar nature

Ion-exchange is the second most common separation mechanism that is commonly used towards the separation of charged analytes. Since a significant number of organic compounds have both ionic and hydrophobic nature, it is appropriate to include ion-exchange (IE) interaction in the separation equation for analytes of dual nature. The compounds like amino acids, nucleotides and nucleosides, peptides, proteins, carboxylic acids, surfactants, basic drugs, zwitterionic compounds are ionic in nature or can be converted to one by selecting appropriate pH; thus, they can be retained by either RP mechanism, IE mechanism or both.

This dual mechanism of interaction drastically increases selectivity of separation because ratio of these two interactions can be adjusted independently by two different components of the mobile phase: an organic modifier changes the RP interaction, and IE is influenced by the concentration of buffer and its pH. The increased selectivity comes from the two-dimensional freedom of selection of the composition of the mobile phase. Fig. 2 shows an example of resolution of a binary mixture of two drugs. Both molecules are hydrophobic and positively charged at the same time. Both IE and RP interactions contribute to the retention in this system. If the two compounds are co-eluted, there are more than one way to resolve the critical pair – by changing the degree of IE interaction or changing the degree of RP interaction. Baseline resolution can be achieved with either approach.

Organic compounds belonging to the same class often show similar retention on RP columns. However, if the charge is present, it's possible to achieve additional selectivity with ionic interaction only. Two examples of this type of separation are shown on Fig. 3. Even a very short column (10 mm) may provide enough selectivity if a proper mode of separation is chosen (Fig. 3B).