Pharmaceutical Analysis: What is Your Problem?

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Pharmaceutical analysis involves liquid chromatography of various compounds, from active ingredients and related impurities to starting materials and excipients. Amino acids, small amines and organic acids are essential building blocks for compounds in pharmaceutical industry. Chemists need to develop robust methods for analysis of these compounds in raw materials as well as residual content in final products. The final drug product can contain variety of components including ingredients of formulation, excipients, and impurities. Very often, these components are very different in nature, and formulation can have hydrophilic and hydrophobic, ionic and neutral compounds. That complex mixture might require several methods and columns in order to quantitate each type of molecule. For example, for analysis of hydrophilic compounds several techniques are in common use in HPLC: ion-pairing chromatography on reversed-phase columns, RP chromatography on special AQ columns, HILIC columns, and mixed-mode columns.

All these techniques have benefits and drawbacks. However, mixed-mode chromatography is the most flexible among all the techniques, and can address most of the problems (Table 1)

Comparison of different aqueous mobile phase HPLC techniques

Table 1

Factors important for pharmaceutical method development, validation, and usage	Mixed-Mode reversed-phase	Reverse phase	Reverse phase AQ	Reverse phase with ion pairing reagent	HILIC
Compatible with low UV (<210 nm)	yes	yes	yes	yes	no
Compatible with MS/ELSD	yes	yes	yes	no	yes
Compatible with alcohols in the mobile phase	some	yes	yes	yes	no
Ability to retain polar neutral compounds	some	no	some	no	yes
Ability to retain hydrophobic neutral compounds	yes	yes	yes	yes	no
Ability to retain polar charged compounds	yes	no	some	yes	yes
Ability to retain hydrophobic charged compounds	yes	yes	yes	yes	no
Low consumption of MeCN (acetonitrile)	yes	yes	yes	yes	no
Good peak shape for basic compounds	yes	no	no	yes	no
Easy cleaning of columns and system after use	yes	yes	yes	no	yes
Gradient method is possible	yes	yes	yes	no	yes
Strong effect of pH of mobile phase on retention	yes	no	no	yes	no
Good sample solubility in mobile phase	yes	some	some	yes	some
Isocratic method for compounds of different polarity	yes	no	no	yes	no
Several parameters for selectivity adjustment	yes	no	no	yes	no
Many column suppliers	no	yes	yes	yes	yes
Complex interaction mechanism	yes	no	no	yes	no
Good loadability for neutral molecules	yes	yes	yes	yes	no
Good loadability for charged molecules	yes	no	no	yes	no
Method development is complex	yes	no	no	yes	no

1. Ability to retain hydrophilic ionizable compounds along with hydrophobic compounds in one run.

Compounds in mixed-mode chromatography are retained by reversed-phase and ion-exchange mechanisms. This allows to retain compounds that are different in nature (Fig. 1). Retention time is controlled by corresponding component of the mobile phase: for hydrophobic compounds, it is the amount of organic; for ionic compounds, the amount of buffer or buffer pH. Different mechanism guarantee retention and separation of compounds that are different in nature. Mobile phase pH can be used to manipulate polarity of compounds in reversed-phase chromatography, but in many cases you cannot make polar compounds of different nature non-polar by using certain pH (Fig. 2)

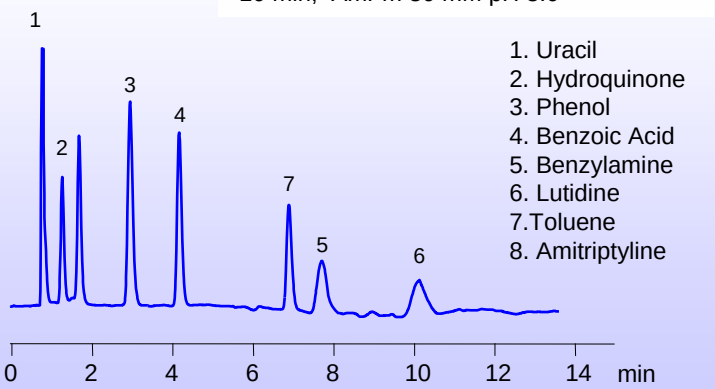

Fig. 1 Retention of basic, neutral, and acidic molecules in mixed-mode chromatography

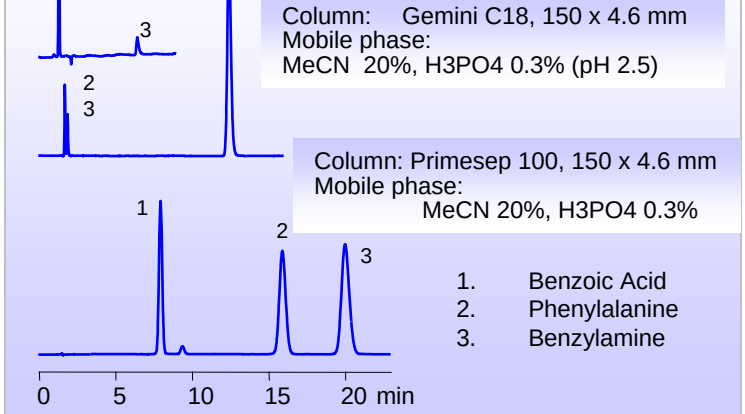

Fig. 2 Retention of basic, neutral, and acidic molecules

Presence of two mechanisms – reversed-phase and ion-exchange, allows to analyze counter-ions in one run and have one method for quantitative analysis (Fig. 3 and 4). This is not possible in reversed-phase chromatography as polar ions are not retained by reversed-phase mechanism.

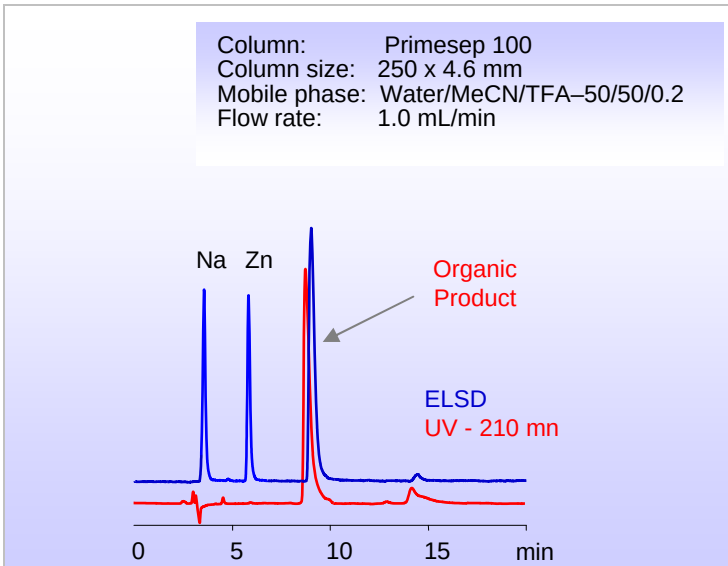

Fig. 3 Simultaneous analysis of sodium and zinc ions and drug intermediate

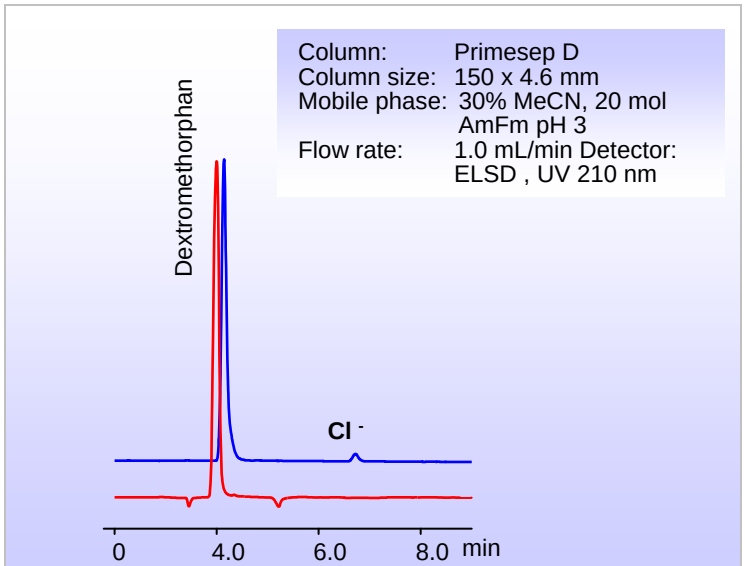

Fig. 4 Simultaneous analysis of chloride ion and dextromethorphan

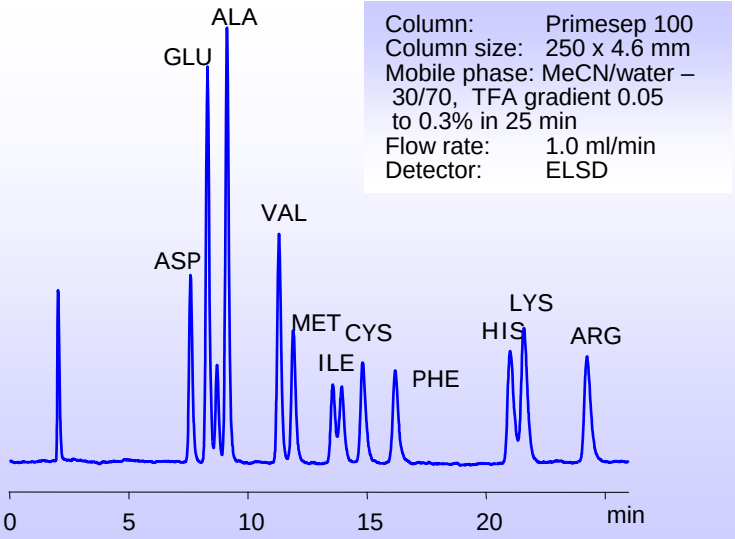

Fig. 5 Reversed-phase cation exchange mechanism of amino acid separation

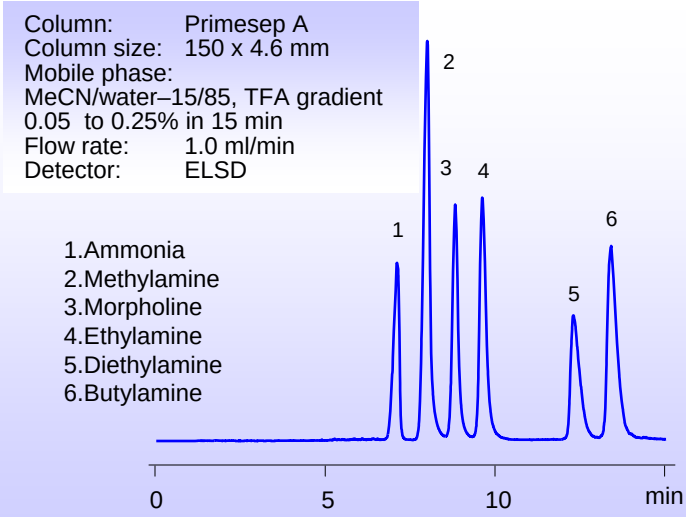

Fig. 6 Reversed-phase cation exchange mechanism of amines

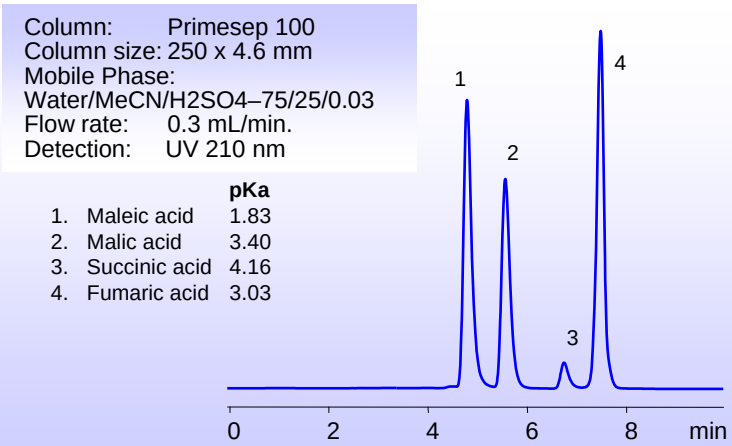

Fig. 7 Separation of carboxylic acids by anion-exclusion mechanism

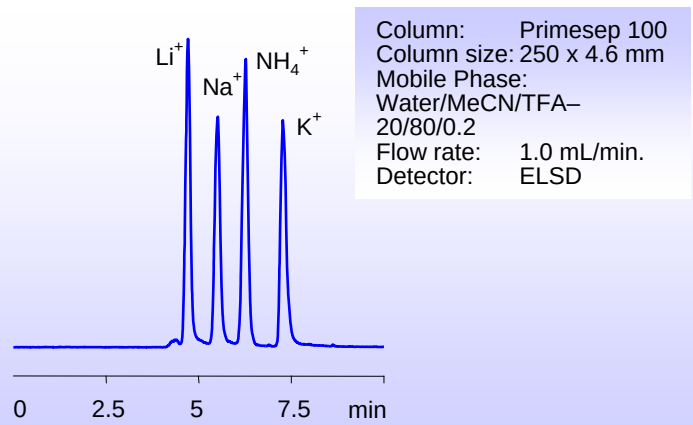

Fig. 8 Separation of inorganic ions by cation-exchange mechanism

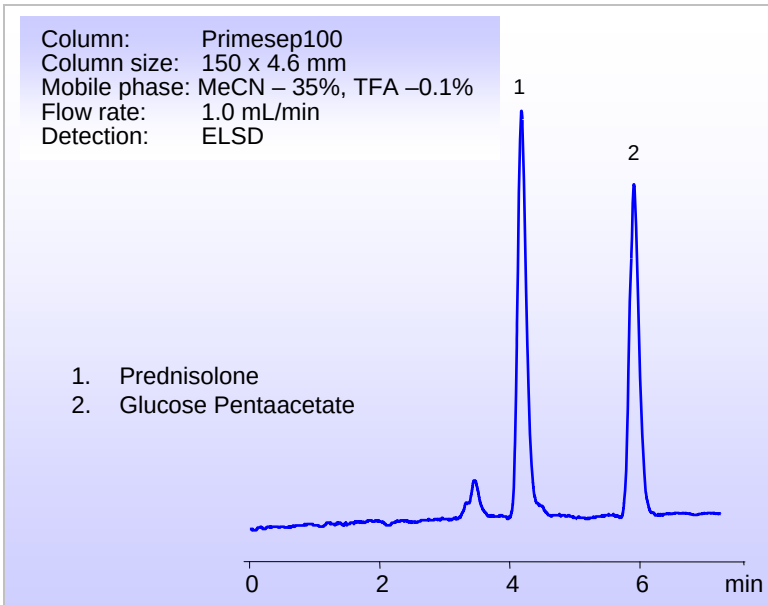

Fig. 9 Retention of neutral compounds in mixed-mode chromatography

The use of reversed-phase columns is reserved only to hydrophobic interaction. In contrast the same mixed-mode column can be used in several separation modes: reversed-phase ion-exchange (Fig. 5 and 6) ion-exclusion (Fig. 7), ion-exchange (Fig 8) and reversed-phase (fig. 9). Mixed-mode can include combination of various modes, thus mixed-mode column can replace several specialized columns including C18, ion-exchange and ion-exclusion column. This will contribute to unification of method development with the use of only handful of columns. Also two orthogonal modes: ion-exchange and ion-exclusion can be used to verify purity of compounds.

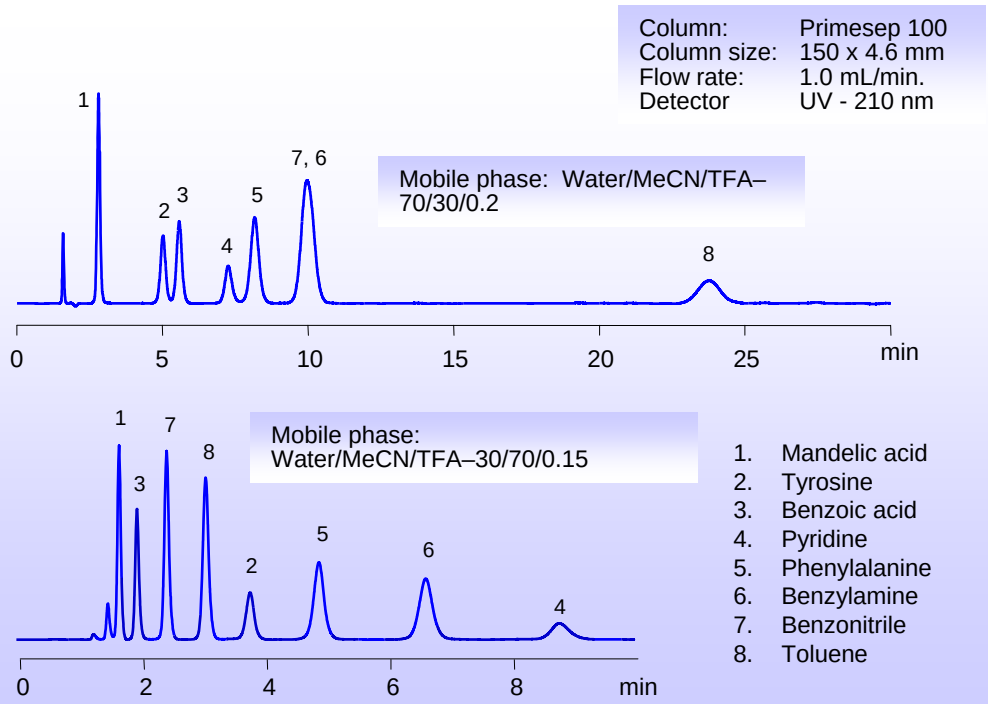

Fig. 10 Effect of mobile phase composition on separation of complex mixture

More tools to adjust selectivity of separation (amount of organic, buffer concentration, buffer pH and nature – Fig 10 and 11). Compound of different nature will respond differently to change of ACN, buffer pH and buffer concentration. Critical pairs which can be resolved by simple variation of mobile phase composition. This allows not only to elute peaks in desired order but also resolve structural isomers with great resolution (Fig. 12 and 13)

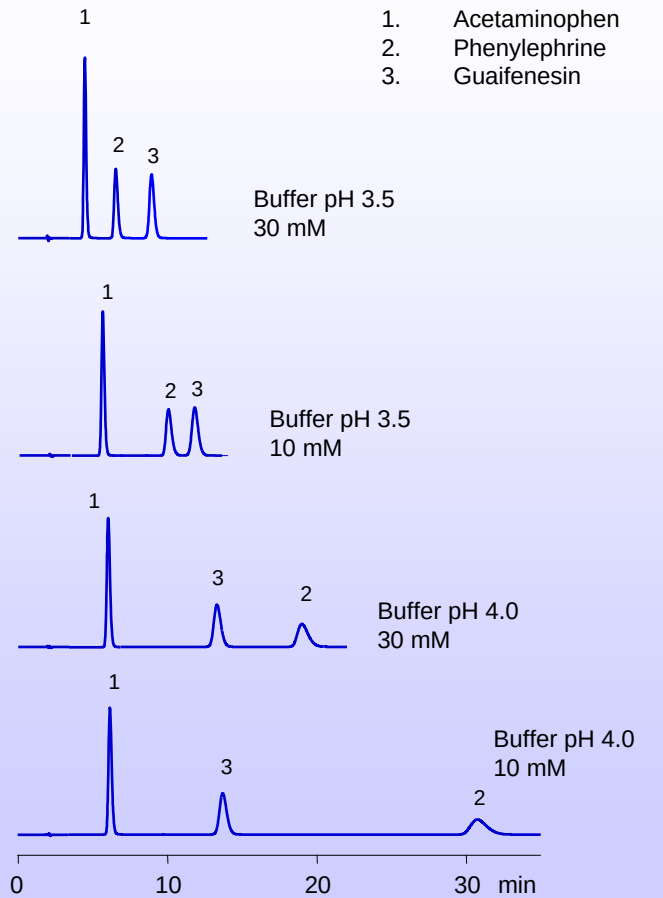

Fig. 11 Analysis of drug composition, buffer pH and concentration effect

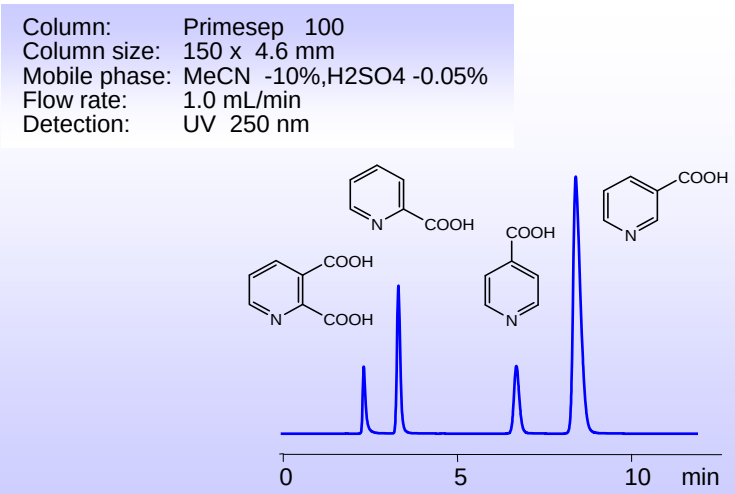

Fig. 12 Analysis of pyridinecarboxylic acids

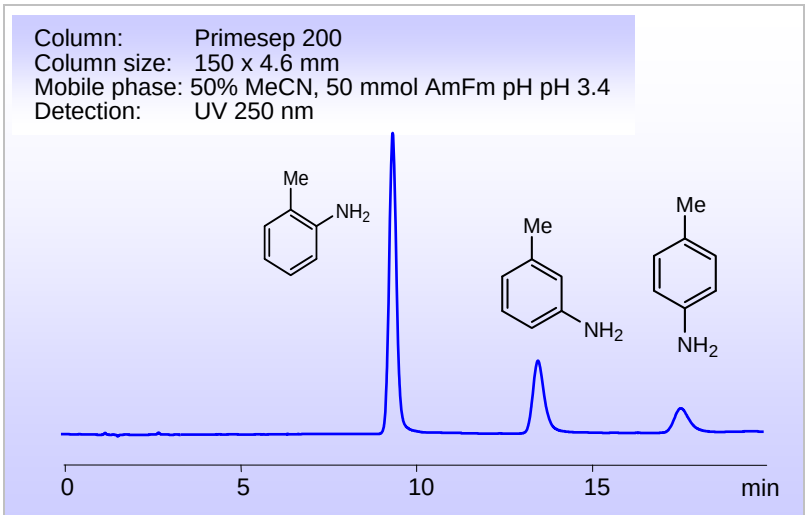

Fig. 13 Analysis of isomers of aromatic amines

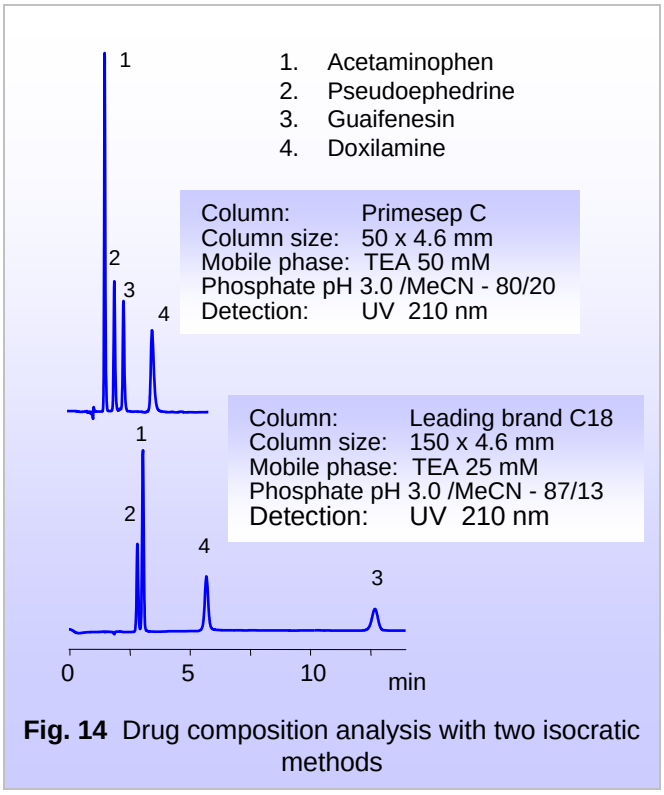

Fig. 14 Drug composition analysis with two isocratic methods

Dual mechanisms allow to replace lengthy gradient methods with simple isocratic approach drastically reduce solvent consumption and run time Fig 14)
Mixed-mode stationary phases provide multiple types of interactions with analytes. Ionizable compounds interact with the stationary phase by reverse-phase and ion-exchange mechanisms.
On these dual columns, the compounds of different polarity can be analyzed in a single isocratic mode by employing different separation mechanisms for compounds of different nature. Polar ionizable compounds can be retained to the same degree or even stronger than hydrophobic compounds do.
The amount of the ions (created by buffer salt or acid) in the mobile phase influences the retention attributed to the ion-exchange interaction to the same degree as the organic modifier affects the retention in reverse-phase separation.

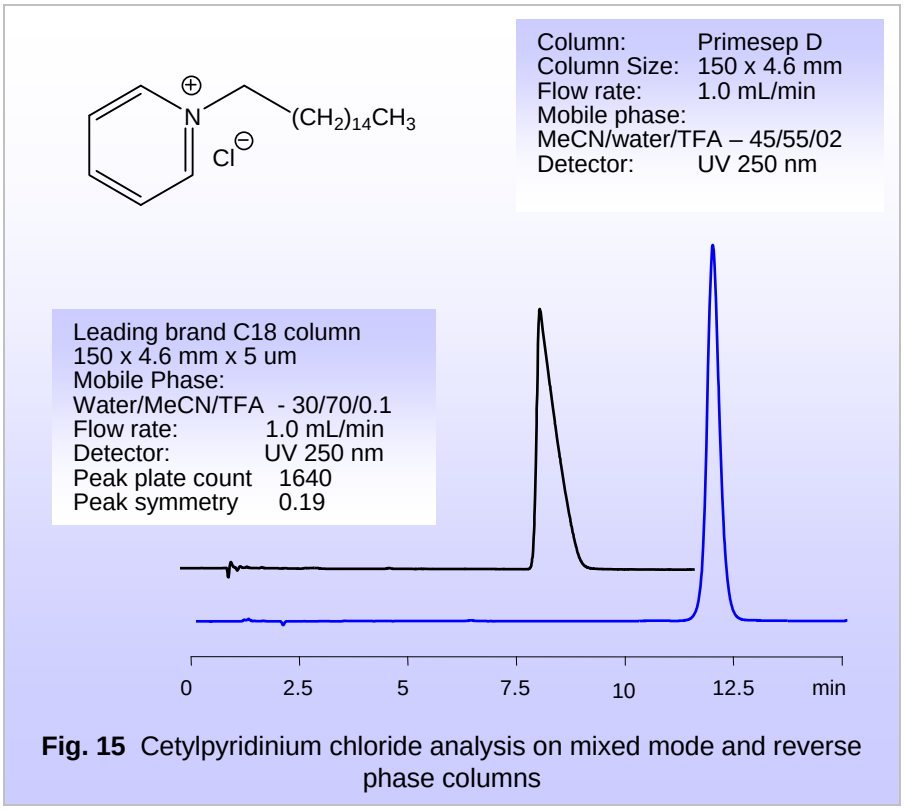

Fig. 15 Cetylpyridinium chloride analysis on mixed mode and reverse phase columns

Strong bases, like quaternary amines, do not perform well chromatographically due to the strong silanol interaction, even with the best deactivated silica-based columns. Even end-capping of the silica does not resolve this problem due to column's tendencies of losing end capping. Primesep D column with a positively charged surface completely eliminates any ion-exchange interaction of the stationary phase with positively charged analytes, and thus offers efficient separation and a symmetrical peak shape. Basic group on the surface of silica completely shields silanol groups from interaction with charged analytes. Retention is still controllable by varying the amount of organic modifier in the mobile phase that provides separation of compounds according to their hydrophobic properties. The hydrophobic interaction is reduced due to the repulsion effect of the ion-exclusion process (Fig. 15)