

New Weak Cation-Exchange/Reversed-Phase Mixed-Mode Column for Pharmaceutical Applications

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INTRODUCTION

Basic compounds are widely used in many areas such as pharmaceutical development (e.g., basic drugs), biological research (e.g., catecholamines), consumer products (e.g., cationic surfactants), etc. Although reversed-phase liquid chromatography (RPLC) is the most commonly used analytical separation technique for a broad spectrum of applications, the task becomes highly challenging when it comes to basic analytes. It is well-known that a basic molecule often elutes as a tailing peak at a mid-pH on a silica-based RP column, mainly due to the secondary interaction resulting from residual silanol groups on the silica surface. This difficulty is minimized by increasing surface coverage using high-purity silica gel. However, the resulting material is incompatible with highly aqueous mobile phases because of the de-wetting phenomenon on a hydrophobic surface. Another challenge relates to inadequate retention of hydrophilic, basic molecules on an RP column. Many pharmaceutical drugs are hydrophilic basic molecules. Very often, an RP column fails to give sufficient retention mainly due to its retention mechanism.

Although ion-pairing chromatography is frequently used to increase retention for hydrophilic charged molecules, it often requires long equilibration time, a dedicated column and a complicated mobile phase that is incompatible with a mass spectrometer. Conventional ion-exchange chromatography is used to separate highly basic compounds. However, commonly used silica-based cation exchangers exhibit inadequate hydrophobicity while the polymer resin-based ones frequently exhibit low chromatographic efficiency. Mixed-mode chromatography is a combination of ion-exchange and conventional RP chromatography. A mixed-mode stationary phase has both hydrophobic and ion-exchange properties and facilitates the independent control of retention for ionizable and neutral molecules. Therefore many application challenges involving hydrophilic ionizable compounds that are difficult for an RP column can be easily resolved on a mixed-mode column.

This paper presents a new mixed-mode silica column that combines weak cation-exchange (WCX) and reversed-phase properties. The new phase features unique selectivity not only for the separation of basic compounds with different hydrophobicity, but also for simultaneous separation of basic, acidic, and neutral pharmaceuticals. Furthermore, it exhibits orthogonal selectivity compared to RP columns. Column chemistry, chromatographic properties, and some example pharmaceutical applications will be discussed.

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ACCLAIM[®] MIXED-MODE WCX-1

Features:

- Adjustable selectivity
- Orthogonal selectivity
- Ideal for basic molecules
- Multi-mode retention mechanism
- Simultaneous separation of basic, neutral, and acidic molecules

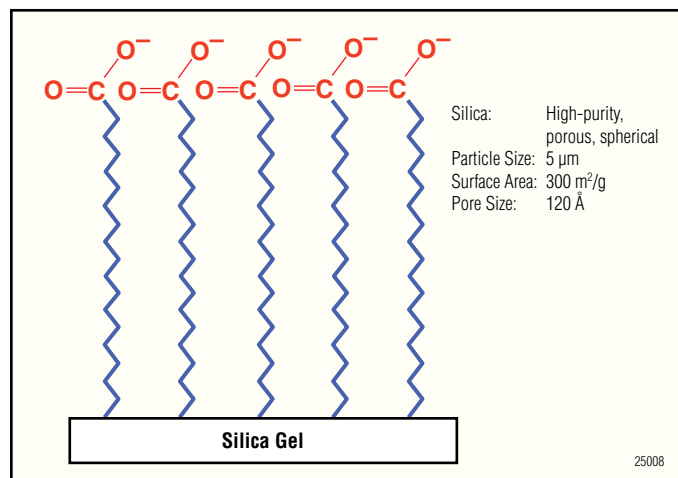


Figure 1. Column chemistry of Acclaim Mixed-Mode WCX-1.

CHROMATOGRAPHIC PROPERTIES

• Adjustable selectivity

The Acclaim Mixed-Mode WCX-1 combines both hydrophobic and WCX characteristics, facilitating adjustable selectivity through changes in mobile phase ionic strength (Figure 2), pH (Figure 3), and the organic solvent content (Figure 4), individually or concurrently.

• Orthogonal selectivity

As shown in Figures 5 and 6, the Mixed-Mode WCX-1 provides orthogonal selectivity compared to both a RP column and its weak anion-exchange (WAX) counterpart.

• Ideal for basic molecules

The Acclaim Mixed-Mode WCX-1 not only retains basic molecules (from highly hydrophilic ones, such as sodium ions, to highly hydrophobic ones, such as amitriptyline), but it also elutes them with symmetrical peak shapes and excellent efficiency (Figures 10 and 14).

• Multi-mode retention mechanism

Because of its novel column chemistry, the Acclaim Mixed-Mode WCX-1 column offers multi-mode retention mechanisms, including RP, cation-exchange, ion-exclusion, and HILIC. Figure 7 demonstrates that in highly organic mobile phase, the Mixed-Mode WCX-1 functions as a HILIC column.

• Simultaneous separation of basic, neutral, and acidic molecules

Separation of different types of molecules, including bases, neutrals, and acids in a single chromatographic run, on the same column, is highly desirable but challenging. Figure 8 demonstrates the simultaneous separation of a mixture of basic, neutral, and acidic molecules using an isocratic method, with excellent peak shape and resolution on the Acclaim Mixed-Mode WCX-1 column.

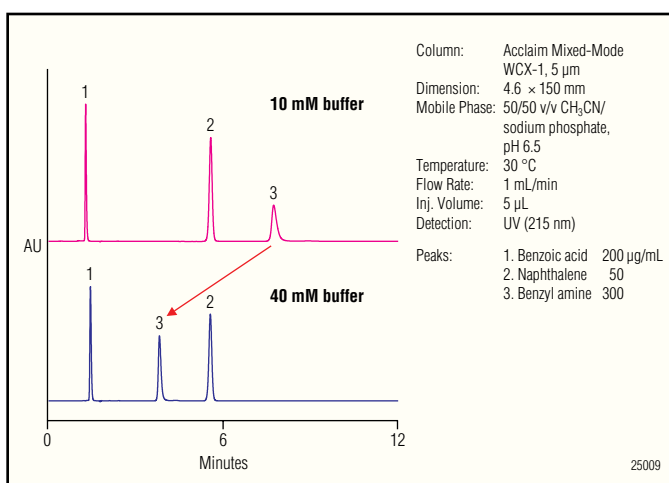


Figure 2. Adjustable selectivity—ionic strength effect. Changing ionic strength affects cation retention without affecting neutral or anionic compounds.

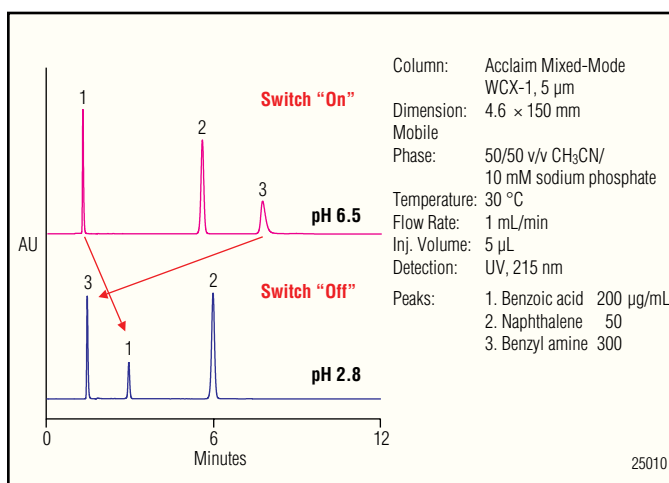


Figure 3. Adjustable selectivity—pH effect. Changing the pH causes the capacity of weak cation-exchange functionality on the stationary phase, and can be switched “on” or “off.”

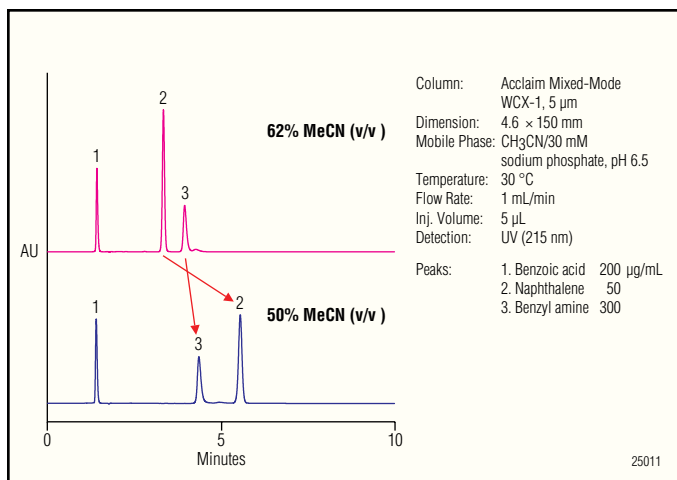


Figure 4. Adjustable selectivity—organic solvent effect. Changing the mobile phase organic content affects selectivity.

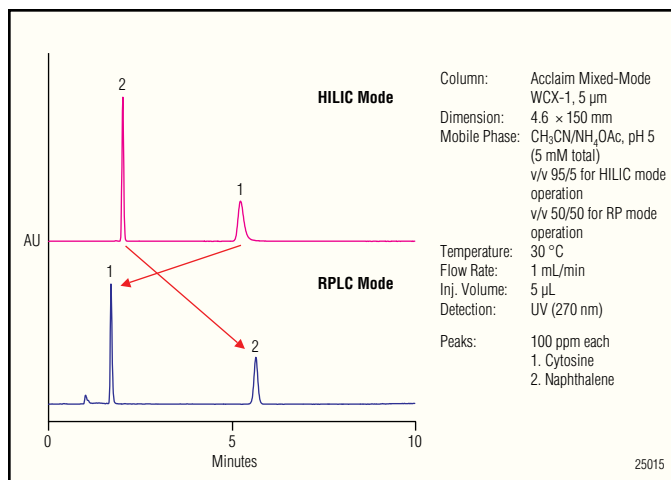


Figure 7. RPLC mode vs HILIC mode.

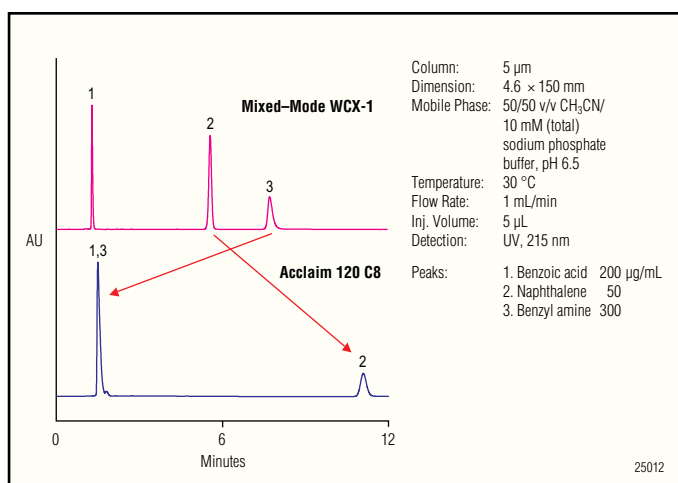


Figure 5. WCX vs RP—orthogonal selectivity.

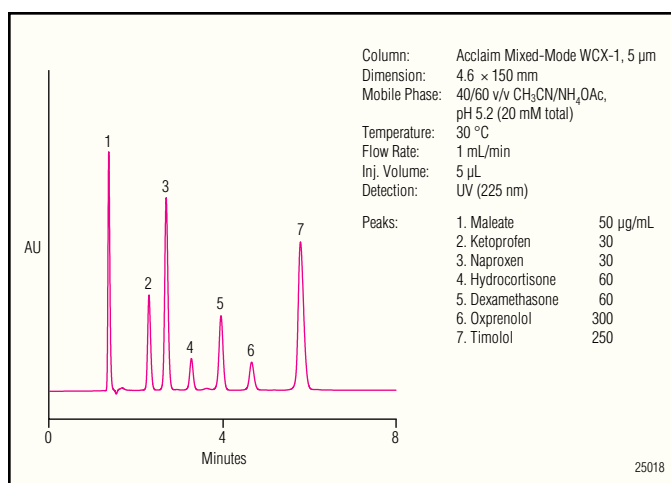


Figure 8. Simultaneous separation of acidic, neutral, and basic pharmaceuticals.

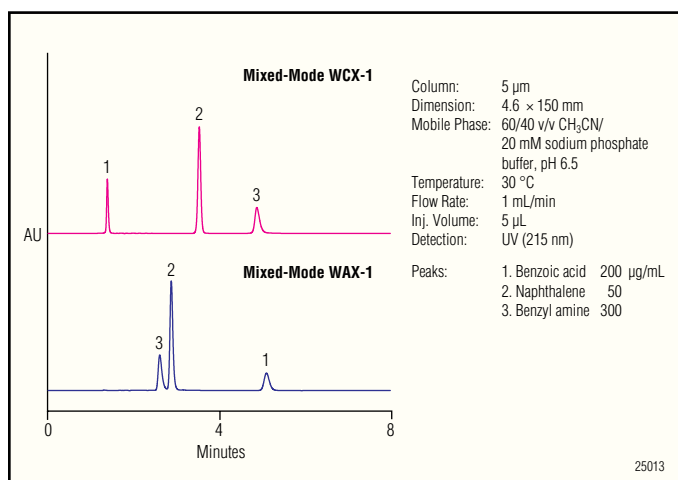


Figure 6. WCX vs WAX—orthogonal selectivity.

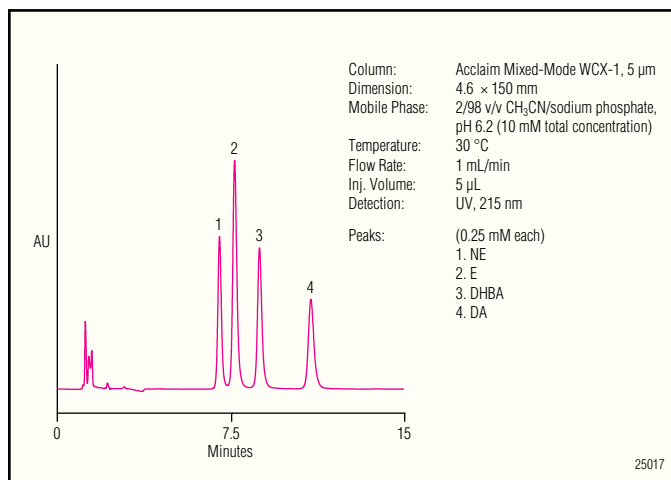


Figure 9. Separation of catecholamines.

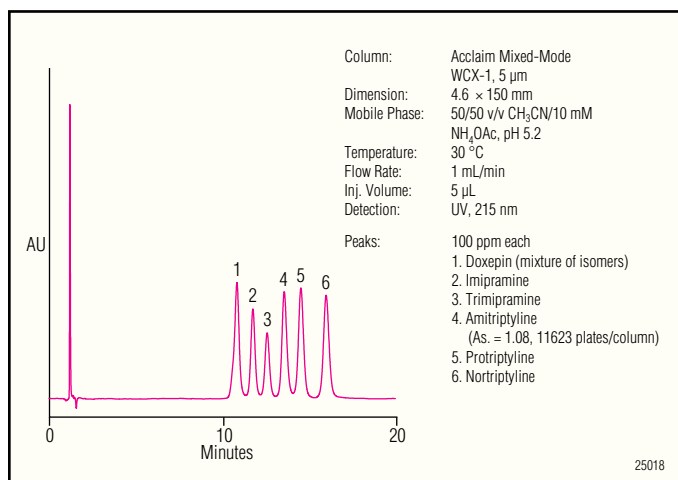


Figure 10. Separation of antidepressants.

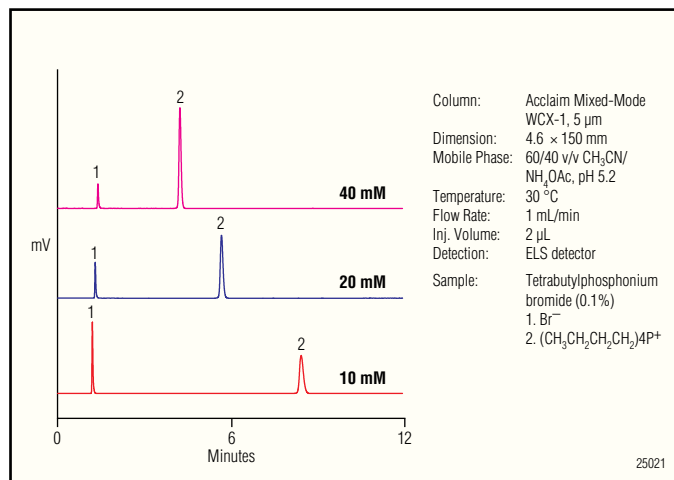


Figure 13. Analysis of phosphonium salt.

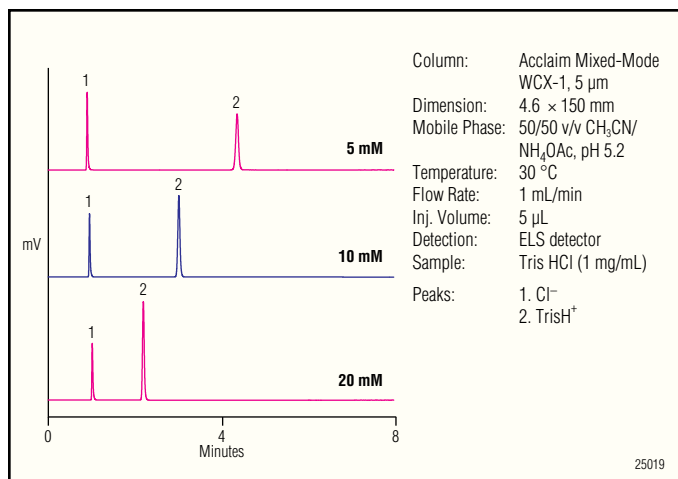


Figure 11. Analysis of tris HCl salt.

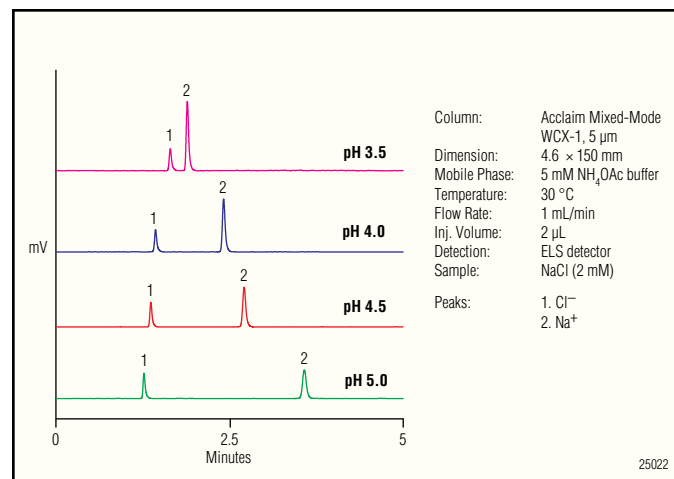


Figure 14. Analysis of NaCl.

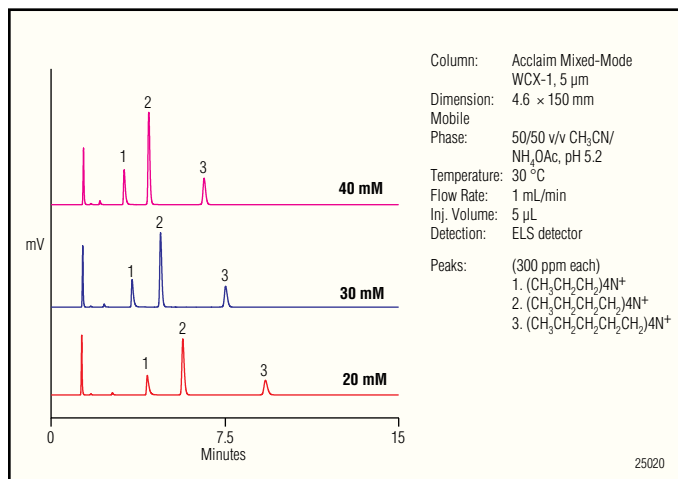


Figure 12. Analysis of quaternary amines.

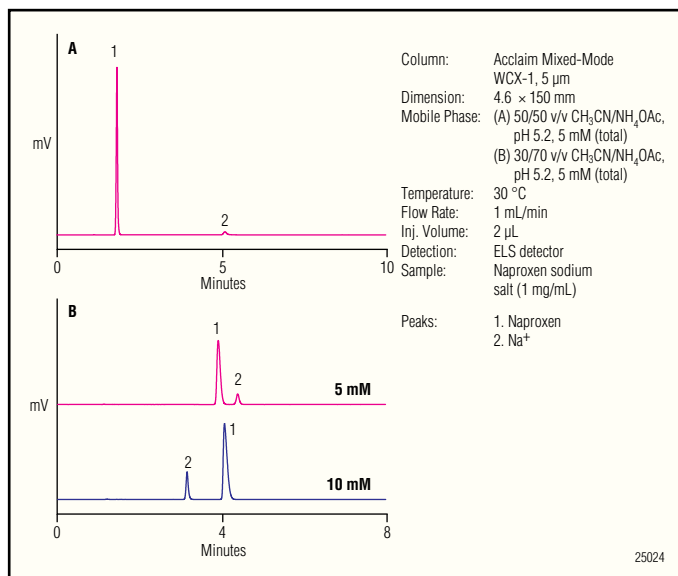


Figure 15. Analysis of Naproxen sodium salt. A) Analysis of counterion only. B) Simultaneous separations of both API and its counterion (with adjustable selectivity).

CONCLUSION

The Acclaim Mixed-Mode WCX-1 column functions as a primary column for a variety of basic analytes and as a secondary column that provides selectivity orthogonal to that of reversed-phase columns. Thus, it is an invaluable tool for HPLC method development for a broad range of applications, such as pharmaceutical, food and beverage, and chemical analyses.

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