

Application of Mixed-Mode HPLC Columns to Samples Containing Ionic and Hydrophobic Analytes

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ABSTRACT

While reversed-phase chromatography with a silica-based C18 column can successfully separate the analytes of interest in many samples, polar and charged analytes can be challenging to retain and, if retained, can exhibit non-ideal peak shapes that compromise their quantification and/or resolution from other analytes. Polar-embedded phases and high-pH stable phases can partially address these problems. Mixed-mode phases better address these problems. These phases are constructed to contain both reversed-phase and ion-exchange functionality. The mixed-mode columns used in this study were specifically constructed to have a long alkyl chain to impart reversed-phase retention and either an anion-exchange or a cation-exchange group at the terminus of that chain to impart ion-exchange functionality. Careful choice of eluent ionic strength, pH, and organic solvent content allows the retention and separation of ionic, polar, and hydrophobic analytes in a single injection. Here, the authors use a mixed-mode column with cation-exchange and reversed-phase properties, and a mixed-mode column with anion-exchange and reversed-phase properties to solve challenging application problems. The anion-exchange mixed mode column was used to separate and quantify the anti-corrosion components of engine coolants, a mixture of acids and neutral organic compounds, and additives to carbonated beverages, including sweeteners and preservatives. The cation-exchange mixed mode column was used to determine melamine in milk-products as both a primary and confirmatory method. Also, this column was used to monitor folic acid synthesis. This presentation will discuss the separation principles of these columns and how the above applications were developed.

EQUIPMENT

Dionex UltiMate® 3000 Standard or Rapid Separation System with the appropriate DAD-3000 diode array detector and WPS-3000 autosampler.

All the equipment was controlled and the resulting data processed using a Chromeleon® 7.0 Chromatography Data System software.

COLUMNS

Dionex Acclaim® Mixed-Mode WAX-1 and Acclaim Mixed-Mode WCX-1 in various resin particle size and column hardware formats (see each application).

Mixed-Mode Columns

There are several methods for preparing a column with both ion-exchange and reversed-phase properties (Figure 1). The Acclaim Mixed-Mode columns are prepared with the ion-exchange group at the terminus of the alkyl chain (Figure 1, Type IV). This allows distinct separation of the ion-exchange and reversed-phase properties and good control of the ion-exchange capacity. Figures 2 and 3 show diagrams of the basic construction of the Acclaim Mixed-Mode WAX-1 and WCX-1 stationary phases.

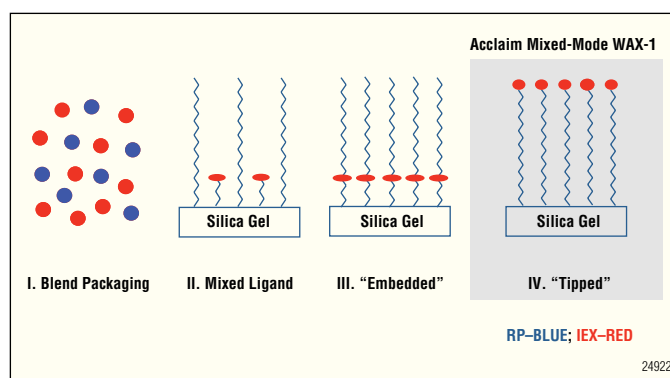


Figure 1. Silica-based mixed-mode phases.

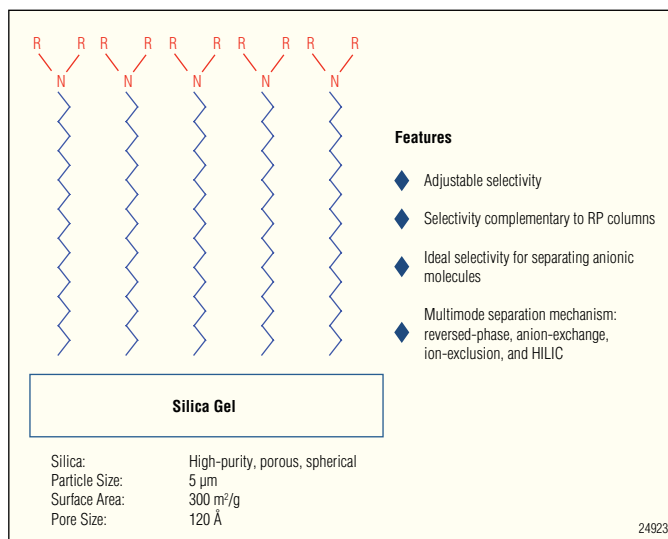


Figure 2. Acclaim Mixed-Mode WAX-1 column.

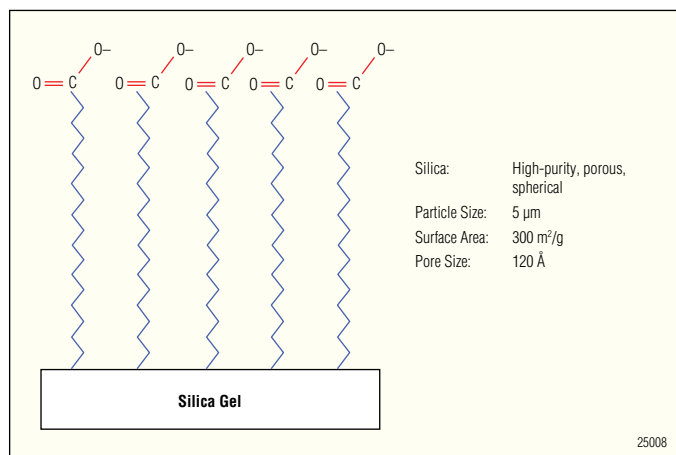


Figure 3. Column chemistry of Acclaim Mixed-Mode WAX-1 column.

Analyte retention times on these mixed-mode phases can be altered by adjusting three mobile phase properties.

1. Organic solvent content: increasing it will decrease retention of analytes bound by hydrophobic interactions.
2. pH: for some ionic analytes this can change ionization and therefore binding to the ion-exchange group (either increase or decrease depending on the pH and the analyte.)
3. Ionic strength: increasing ionic strength will reduce retention of analytes bound by ion-exchange.

For this reason, these mixed-mode columns have adjustable selectivity.

Application 1: Determination of Additives in Carbonated Beverages

Carbonated beverages such as colas and other soft drinks contain preservatives, sweeteners, and other additives (e.g., caffeine). One beverage can contain ionic, polar, and neutral compounds that must be quantified but are difficult to determine in a single traditional (C18) reversed-phase separation. Figure 4 shows an isocratic separation of seven compounds often found in carbonated beverages using the Acclaim Mixed-Mode WAX-1 column. These include three low calorie sweeteners (aspartame, saccharin, acesulfame), three preservatives (benzoate, citrate, sorbate), and caffeine. Figure 5 shows five of these seven compounds in a diet cola sample. This method was accurate for a variety of carbonated beverages.¹

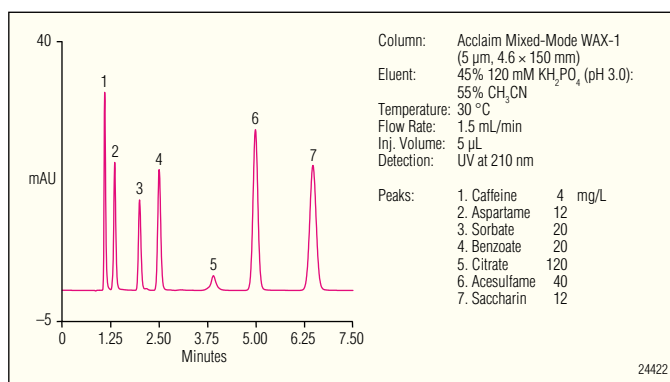


Figure 4. Chromatogram of a mixed standard.

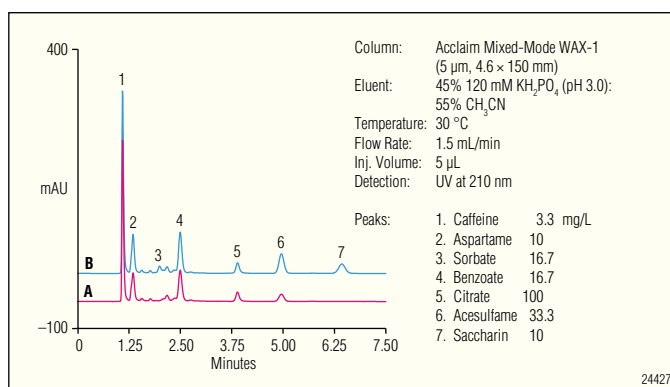


Figure 5. Overlay of chromatograms of carbonated beverage diet cola #2, diluted three-fold (chromatogram A) and the same sample spiked (chromatogram B).

Application 2: Determination of Additives in Engine Coolants

Aqueous solutions of ethylene glycol are commonly used as engine coolants. With engine use, the ethylene glycol degrades to glycolic acid which can corrode the engine. For this reason, corrosion inhibitors are added to engine coolants. As no single corrosion inhibitor is 100% effective, most engine coolants contain more than one. These inhibitors generally are from three compound classes; azoles, inorganic salts, and organic acids, and therefore represent both organic and anionic compounds. This makes a mixed-mode WAX column ideal for a simultaneous determination of glycolate and the inhibitors in an engine coolant. Figure 6 shows glycolate and four inhibitors separated using a C18 column (chromatogram A), two polar-embedded columns (B,C), and the Acclaim Mixed-Mode WAX-1. While the C18 column can separate all five compounds, the retention of glycolate is poor. The mixed-mode column is better suited for this analysis. With inclusion of benzoate in the mixture, the adjustable selectivity of this column was investigated by varying the pH, ionic strength, and organic content of the mobile phase (Figure 7). Figure 8 shows analysis of six engine coolant samples (a seventh sample, sample #4 is shown in reference 2 with sample #6) have little or no inhibitors. As these coolants were not used, no glycolate was detected.

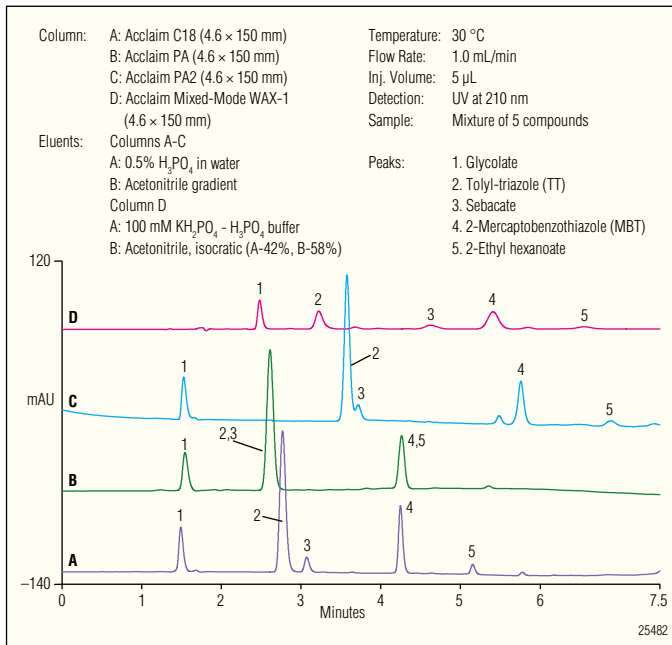


Figure 6. Chromatograms of the corrosion inhibitors and glycolate using different Acclaim columns.

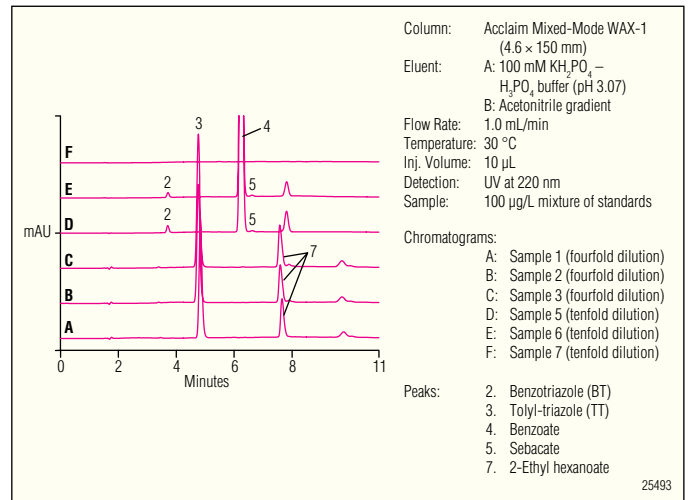


Figure 8. Chromatograms of samples 1-3 and 5-7.

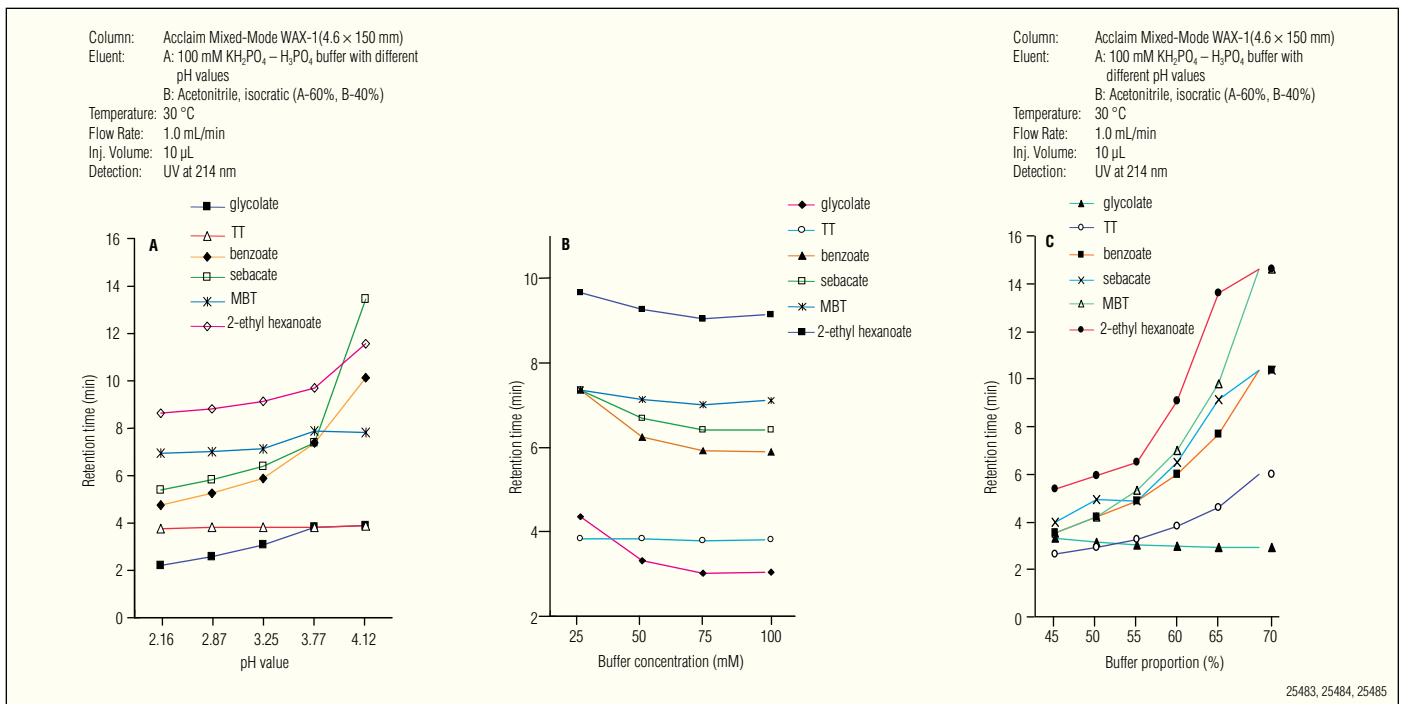


Figure 7. Effect of phosphate buffer pH (A), phosphate buffer concentration (B), and proportion of phosphate buffer in the mobile phase (C) on analyte retention.

Application 3: Determination of Melamine in Milk

In 2008, milk in China was deliberately contaminated with melamine to increase its apparent protein content. The Chinese government issued a HPLC method using a C18 column with an ion-pairing reagent to determine melamine in dried (powdered) milk,³ but the method was unable to determine melamine in liquid milk due to interferences. Melamine is a perfect candidate for a mixed-mode separation because it can be cationic. The adjustable selectivity of the Acclaim Mixed-Mode WCX-1 column allows the analyst to move melamine and other peaks so that melamine can be quantified without interference. Figure 9 shows the analysis of three powdered milk samples and two liquid milk samples. Melamine was found in all samples except for powdered milk sample #1. Melamine quantification in these samples was reproducible and accurate.⁴ The mobile phase makes this method compatible with MS detection.

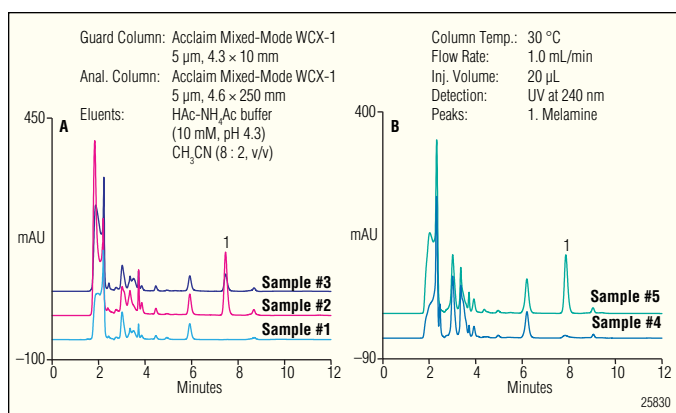


Figure 9. Chromatograms of (A) milk powder samples #1–#3, and (B) liquid milk samples #4 and #5.

Application 4: Determination of Folic Acid and Related Compounds

Folic acid is an important B vitamin, especially for the developing fetus, and therefore is a popular dietary supplement. Figure 10 shows one synthetic route for folic acid. The precursors are polar and not well retained under standard reversed-phase conditions. As these compounds can be cationic, they are therefore good candidates for separation using the Acclaim Mixed-Mode WCX-1 column. Figure 11 shows that the two precursors are well retained and separated from folic acid on the WCX-1 column. This figure also demonstrates the adjustable selectivity of the mixed-mode column which allows an unknown peak on the tail of the folic acid peak to be moved to a location that does not interfere with folic acid quantification. Additional separations can be found in reference 5.

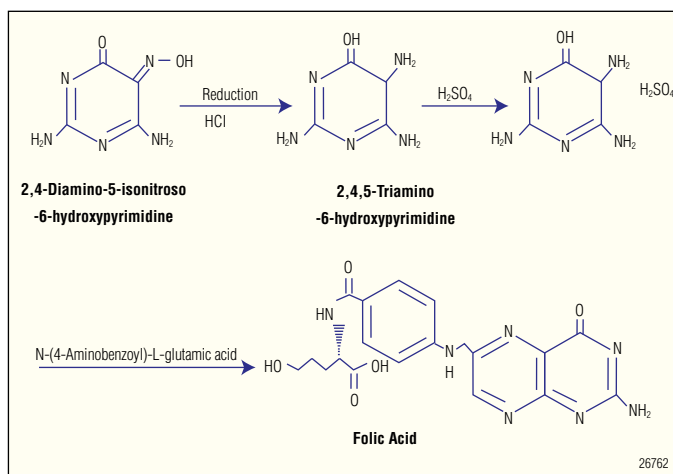


Figure 10. A synthetic process for folic acid.

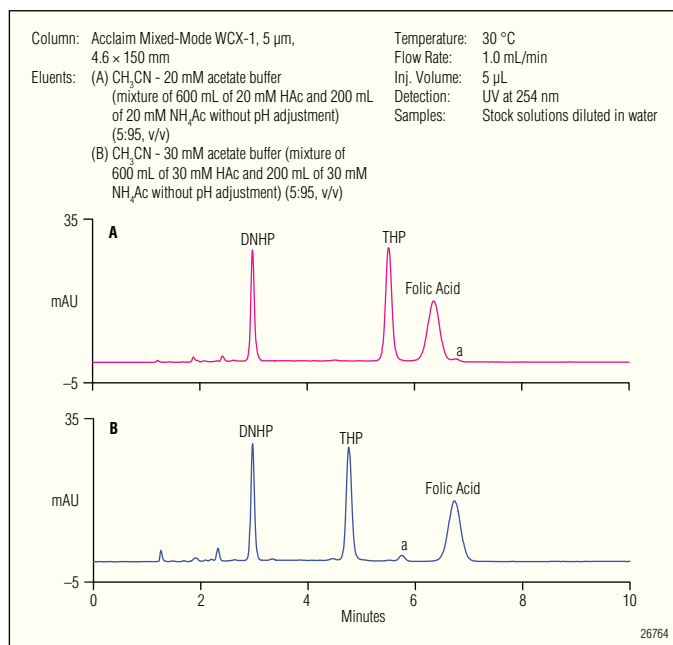


Figure 11. Chromatograms of a stock solution mixture diluted with water using (A) 20 mM NH₄Ac-HAc (~pH 4.3) and (B) 30 mM NH₄Ac-HAc (~pH 4.0) buffer in the mobile phase.

CONCLUSIONS

- Acclaim mixed-mode columns allow the simultaneous analysis of neutral, polar, and charged analytes.
- Acclaim mixed-mode columns have adjustable selectivity that allows separations to be easily altered to separate the analyte(s) of interest from interferences.

REFERENCES

1. Dionex Application Note 93, LPN 1886, 5/07.
2. Dionex Application Note 204, LPN 2074-01, 3/10.
3. Dionex Application Note 224, LPN 2184, 3/07.
4. Dionex Application Note 221, LPN 2181, 3/09.
5. Dionex Application Update 171, LPN 2383, 1/10.

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