

Atlantis Premier BEH C₁₈ AX Columns

CONTENTS

- I. INTRODUCTION**
- II. GETTING STARTED**
 - a. eCord Installation
 - b. Column Installation
 - c. Column Equilibration
 - d. Initial Column Efficiency Determination
- III. COLUMN USE**
 - a. Sample Preparation
 - b. pH Range
 - c. Solvents
 - d. Pressure
 - e. Temperature
 - f. Replacing the VanGuard FIT Cartridge
- IV. COLUMN CLEANING, REGENERATION, AND STORAGE**
 - a. Cleaning and Regeneration
 - b. Storage
- V. eCORD INTELLIGENT CHIP TECHNOLOGY**
 - a. Introduction
 - b. Installation
 - c. Manufacturing Information
 - d. Column Use Information
- VI. TIPS AND TRICKS**
- VII. CAUTIONARY NOTE**

I. INTRODUCTION

Thank you for choosing a Waters™ Atlantis™ Premier BEH C₁₈ AX Column. The Atlantis Premier BEH C₁₈ AX Column packing material is manufactured in a cGMP, ISO 9001 certified plant using ultra-pure reagents. Each batch is tested chromatographically and the results are held to narrow specification ranges to assure excellent, reproducible performance. Every column is individually tested, and a Performance Chromatogram and Certificate of Batch Analysis are provided in the column box or on the eCord™ Intelligent Chip.

The Atlantis Premier BEH C₁₈ AX Column is offered with or without a VanGuard™ Fully Integrated Technology [FIT] Cartridge. To address the desire to extend the operating lifetimes of analytical columns, the VanGuard FIT Cartridge is designed to prevent the non-desired introduction of sample matrix or particulates onto the column without degrading the separation. The VanGuard FIT Cartridge can be easily replaced to restore separation performance and extend the analytical column's lifetime.

The Atlantis Premier BEH C₁₈ AX Column utilizes MaxPeak™ High Performance Surfaces, which is an innovative technology designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

Atlantis™ PREMIER
Columns



II. GETTING STARTED

A Certificate of Analysis and Performance Test Chromatogram are available with each Atlantis Premier BEH C₁₈ AX Column in the column box or located on the column's eCord Intelligent Chip. The Certificate of Analysis is specific to each batch of packing material and includes the batch number, analysis of unbonded particles, analysis of bonded particles, and chromatographic results and conditions. The Performance Test Chromatogram is specific to each individual column and contains the batch number, column serial number, USP tangent efficiency, USP tailing factor, retention factor, and chromatographic conditions. These data should be stored for future reference. For those not able to access the information on the eCord Intelligent Chip, the Certificate of Analysis and Performance Test Chromatogram are available on request at www.waters.com/coa.

a. eCord installation

(May not be available for all column configurations)

The eCord Intelligent Chip button is designed for use on ACQUITY™ UPLC™ and ACQUITY Arc™ Systems and should be attached to the side of the instruments' column heater module. The eCord button is magnetized and does not require specific orientation. For more information on eCord Intelligent Chip functionality, go to section V in this care and use manual.

b. Column installation

(with or without a VanGuard FIT Cartridge)

Note: Prior to handling Atlantis Premier BEH C₁₈ AX Columns and any chemical, consult with your safety department and/or local regulations on the use of proper protective equipment.

Atlantis Premier BEH C₁₈ AX Columns are shipped in 100% acetonitrile. The flow rates given in the procedure below are for 2.1 mm I.D. columns. They should be multiplied by 4.8 for 4.6 mm I.D. columns.

1. Purge the pumping system of any buffer-containing mobile phases and connect the inlet end of the column to the injector outlet.
2. Flush column with 100% organic mobile phase (methanol or acetonitrile) by setting the pump flow rate to 0.1 mL/min and increase the flow rate to 0.5 mL/min over five minutes.

3. When the mobile phase is flowing freely from the column outlet, stop the flow and attach the column outlet to the detector. This prevents entry of air into the detection system.
4. Gradually increase the flow rate as described in Step 2.
5. Once a steady backpressure and baseline have been achieved, proceed to the next section.

c. Column equilibration

It is important to ensure mobile-phase compatibility before changing to a different mobile-phase system. Equilibrate the column with a minimum of 10-column volumes of the mobile phase to be used (refer to Table 1 for a list of column volumes). The column may be considered thermally equilibrated once a constant backpressure is achieved.

Note: These columns may require longer initial equilibration times than conventional reversed-phase columns.

**Table 1. Empty Column Volumes in mL
(multiply by 10 for flush solvent volumes)**

Column length (mm)	Internal diameter	
	2.1 mm	4.6 mm
30	0.10	–
50	0.17	0.83
75	0.26	1.25
100	0.35	1.66
150	0.52	2.49
250	–	4.15

To avoid precipitating mobile-phase buffers on your column or in your system, flush the column with five column volumes of a water/organic solvent mixture, using the same or lower solvent content as in the desired buffered mobile phase. (For example, flush the column and system with 60% methanol in water prior to introducing 60% methanol/40% buffer mobile phase.)

d. Initial column efficiency determination

1. Perform an efficiency test on the column before using it. This test may consist of:
 - a. An analyte test mixture that is commonly used in your laboratory, and/or
 - b. The analyte mixture as found on the "Performance Test Chromatogram" that accompanied your column.

Note: If (b) is performed, the isocratic efficiencies measured in your laboratory may be less than those given on the Waters "Performance Test Chromatogram." This is normal. The Waters isocratic column testing systems have been modified to achieve extremely low system volumes. This presents a more challenging test of how well the column was packed. This guarantees the highest quality packed column. These special testing systems have been modified to such an extent that they are not commercially viable and have limited method flexibility other than isocratic column testing.

2. Determine the number of theoretical plates (N) and use this value for periodic comparisons.
3. Repeat the test at predetermined intervals to track column performance over time. Slight variations may be observed due to differences in the quality of the connections, operating environment, system electronics, reagent quality, column condition, and operator technique.

III. COLUMN USE

To ensure the continued high performance of Atlantis Premier BEH C₁₈ AX Columns, follow these guidelines:

a. Sample preparation

1. Sample impurities often contribute to column contamination. One option to avoid this is to use Oasis™ Solid-Phase Extraction Cartridges/Columns or Sep-Pak™ Cartridges of the appropriate chemistry to clean up the sample before analysis. For more information, visit www.waters.com/sampleprep
2. It is preferable to prepare the sample in the operating mobile phase or a solvent that is weaker than the mobile phase for the best peak shape and sensitivity. Acetone should not be used as a sample solvent/diluent unless a Hexane Tetrahydrofuran Compatibility Kit has been installed.
3. If the sample is not dissolved in the mobile phase, ensure that the sample, solvent, and mobile phases are miscible to avoid sample and/or buffer precipitation.
4. Filter sample with a 0.2 µm membrane to remove particulates. If the sample is dissolved in a solvent that contains an organic modifier (e.g., acetonitrile, methanol, etc.) ensure that the membrane material does not dissolve in the solvent. Contact the membrane manufacturer with solvent compatibility questions. Alternatively, consider centrifugation for 20 minutes at 8000 rpm, followed by the transfer of the supernatant liquid to an appropriate vial.

b. pH range

The recommended operating pH range for Atlantis Premier BEH C₁₈ AX Columns is 2–10. A listing of commonly used buffers and additives is provided in Table 2. Note that the column lifetime varies depending upon the operating temperature, type, and concentration of buffer.

Table 2. Buffer Recommendations for Use with Atlantis Premier BEH C₁₈ AX Columns

Additive/Buffer	pKa	Buffer range	Volatility	Used for Mass Spec	Comments
TFA	0.3	—	Volatile	Yes	Ion pair additive, can suppress MS signal.
Acetic acid	4.76	—	Volatile	Yes	Maximum buffering obtained when used with ammonium acetate salt.
Formic acid	3.75	—	Volatile	Yes	Maximum buffering obtained when used with ammonium formate salt.
Acetate (NH ₄ CH ₃ CO ₂)	4.76	3.76–5.76	Volatile	Yes	Note that sodium or potassium salts are not volatile.
Formate (NH ₄ HCO ₂)	3.75	2.75–4.75	Volatile	Yes	Note that sodium or potassium salts are not volatile.
Phosphate 1	2.15	1.15–3.15	Non-volatile	No	Traditional low pH buffer, good UV transparency.
Phosphate 2	7.2	6.2–8.2	Non-volatile	No	Above pH 7, reduce temperature/concentration and use a guard column to maximize lifetime.
4-Methylmorpholine	~8.4	7.4–9.4	Volatile	Yes	Generally used at 10 mM or less.
Ammonia (NH ₄ OH)	9.2	8.2–10.2	Volatile	Yes	For MS work keep source >150 °C.
Ammonium Bicarbonate 1	6.4	5.4–7.4	Volatile	Yes	For MS work keep source >150 °C. Adjust pH with ammonium hydroxide or acetic acid. Good buffering capacity at pH 10.
Ammonium Bicarbonate 2	10.3	9.3–11.3	Volatile	Yes	<i>Note: Use ammonium bicarbonate (NH₄HCO₃), not ammonium carbonate ((NH₄)₂CO₃).</i>
Ammonium (Acetate)	9.2	8.2–10.2	Volatile	Yes	For MS, use in the 1-30 mM range.
Ammonium (Formate)	9.2	8.2–10.2	Volatile	Yes	For MS, use in the 1-30 mM range.
Borate	9.2	8.2–10.2	Non-volatile	No	Reduce temperature/concentration and use a guard column to maximize lifetime.
CAPSO	9.7	8.7–10.7	Non-volatile	No	Zwitterionic buffer, compatible with acetonitrile. Low odor.
Glycine	2.4, 9.8	8.8–10.8	Non-volatile	No	Zwitterionic buffer, can give longer lifetimes than borate buffer.

c. Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all aqueous buffers prior to use through a 0.2 µm filter. Solvents containing suspended particulate materials will generally clog the inlet frit of the column. This will result in higher operating pressure and poorer performance.

d. Pressure

2.1 mm I. D. Atlantis Premier BEH C₁₈ AX 1.7 µm and 2.5 µm columns can tolerate operating pressures up to 18000 psi (1241 bar or 124 MPa). The 4.6 mm i.d. columns containing 2.5 µm and 5 µm particles can tolerate pressures up to 10,000 psi (700 bar or 70 MPa).

Note: Working at the extremes of pressure, pH and/or temperature may result in shorter column lifetimes.

e. Temperature

Temperatures between 4 °C – 60 °C are recommended for operating Atlantis Premier BEH C₁₈ AX Columns to enhance selectivity, lower solvent viscosity, and increase mass transfer rates. When operating near the pH limits, lower operating temperatures are recommended for longer column lifetime. Working at higher temperatures (e.g., >60 °C) may result in shorter column lifetimes.

f. Replacing the VanGuard FIT Cartridge

When desired, the VanGuard FIT Cartridge can be replaced on the Atlantis Premier BEH C₁₈ AX Columns using two 3/8" wrenches. Simply apply the wrenches to the flats on the guard and column end nut and turn in a counter clockwise direction (see Figure 1). This will allow the VanGuard FIT Cartridge to be removed and appropriately discarded when following good laboratory practices.

A new VanGuard FIT Cartridge can now be used to replace the discarded one. Extra cartridges can be obtained separately as needed. Hand-tighten the new cartridge in a clockwise direction, then tighten using two 3/8" wrenches. Proper sealing should not require more than a 1/4 turn past the hand-tightened position.

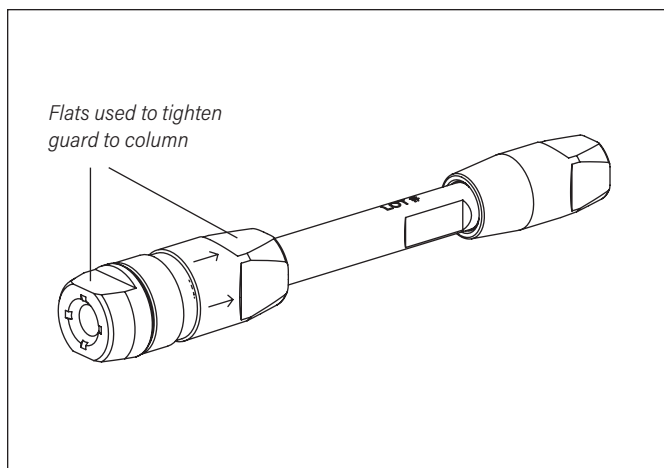


Figure 1. Recommended 3/8" wrench placement to remove the VanGuard FIT Cartridge from the Atlantis Premier BEH C₁₈ AX Column.

IV. COLUMN CLEANING, REGENERATION, AND STORAGE

a. Cleaning and regeneration

Changes in peak shape, peak splitting, shoulders on the peak, shifts in retention, change in resolution, or increasing backpressure may indicate contamination of the column. Flushing with a neat organic solvent, taking care not to precipitate buffers, is usually sufficient to remove contaminants. If the flushing procedure does not solve the problem, purge the column using the following cleaning and regeneration procedures.

Use the cleaning routine that matches the properties of what you believe is contaminating the column (see Table 3). Flush columns with 20-column volumes of solvent. Increasing column temperature increases cleaning efficiency. If the column performance is poor after regenerating and cleaning, call your local Waters office for additional support.

b. Column storage

Do not store columns in buffered eluents. If the mobile phase contained a buffer salt, flush Atlantis Premier BEH C₁₈ AX Columns with 10-column volumes of HPLC grade water (see Table 1) and then with 10-column volumes of 100% acetonitrile prior to storage. Failure to perform this intermediate step could result in precipitation of the buffer salt in the column when 100% acetonitrile is introduced. Completely seal column to avoid evaporation and drying out of the bed.

Note: If a column has been run with a mobile phase that contains formate (e.g., ammonium formate, formic acid, etc.) and is then flushed with 100% acetonitrile, slightly longer equilibration times may be necessary when the column is re-installed and run again with a formate-containing mobile phase.

Table 3. Column Cleaning Instructions

Polar contaminants	Non-polar contaminants**	Proteinaceous contaminants
1. Water	1. Isopropanol (or an appropriate isopropanol/ water mixture*)	Option 1: Inject repeated aliquots of dimethylsulfoxide (DMSO)
2. Methanol	2. Tetrahydrofuran (THF)	Option 2: Gradient of 10% to 90% B where:
3. Tetrahydrofuran (THF)	3. Dichloromethane	A = 0.1% trifluoroacetic acid (TFA) in water
4. Methanol	4. Hexane	B = 0.1% trifluoroacetic acid (TFA) in acetonitrile (CH ₃ CN)
5. Water	5. Isopropanol (followed by an appropriate isopropanol/ water mixture*)	Option 3: Flush column with 7 M guanidine hydrochloride, or 7 M urea
6. Mobile phase	6. Mobile phase	

* Use low organic solvent content to avoid precipitating buffers.

** Unless a Hexane Tetrahydrofuran Compatibility Kit (p/n: 205000464) has been installed, using solvents such as THF or hexane should only be considered when the column cannot be cleaned by using neat, reversed-phase organic solvents such as acetonitrile. When using THF and/or hexane, reduce the flow rate, use a low operating temperature, and limit the time to avoid damage to the system.

V. eCORD INTELLIGENT CHIP TECHNOLOGY (Not available for all column configurations)

a. Introduction

The eCord Intelligent Chip stores the history of a column's performance throughout its lifetime. The eCord is permanently attached to the column to ensure the column's performance history is maintained if the column is moved from one instrument to another.

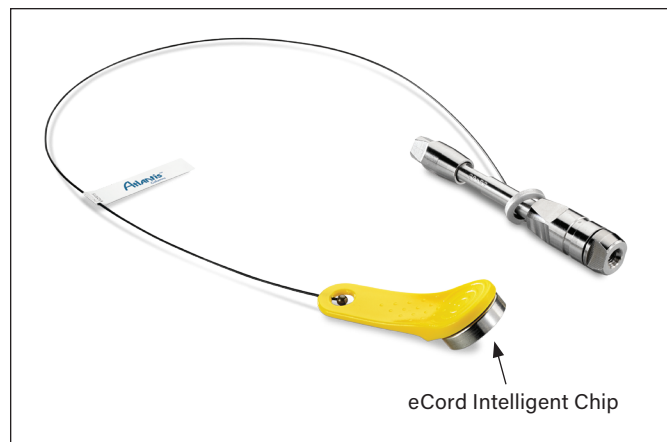


Figure 2. Waters eCord Intelligent Chip.

At the time of manufacture, tracking and quality control information is downloaded to the eCord. Storing this information on the chip eliminates the need for a paper Certificate of Analysis. Once the column is installed, the software will automatically download key parameters into a column history file stored on the chip. In this section, we explain how the eCord provides a solution for easily tracking the history of the column, reduces the frustration of paperwork trails, and gives reassurance that a well performing column is installed.

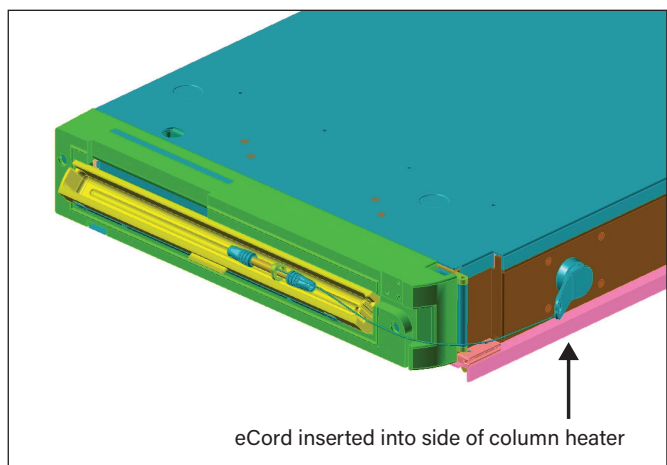


Figure 3. eCord inserted into side of column heater.

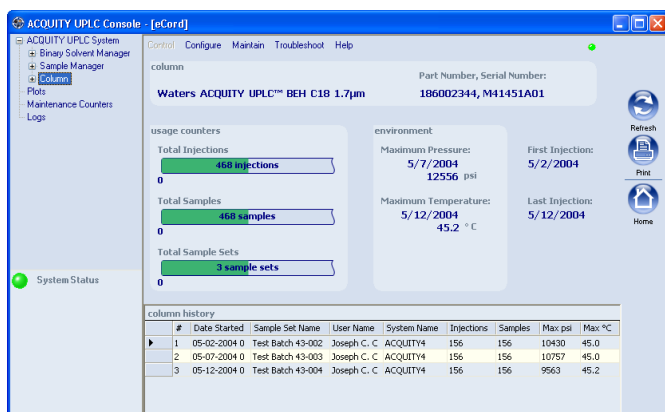
b. Installation

Install the column into the column heater and plug the eCord into the side of the column heater. Once the eCord is inserted into the column heater the identification and overall column usage information will be available in the ACQUITY™ Console, allowing access to column information.

c. Manufacturing information

The eCord Intelligent Chip retains the results for the Performance Test Chromatogram and the Certificate of Analysis. In addition, the chromatographic test conditions used to determine the results are given. They include mobile phases, analytes, and system parameters used.

d. Column use information



The eCord Intelligent Chip stores column use data. The top of the screen identifies the column including chemistry type, column dimensions, and serial number. The overall column usage information includes the total number of samples, total number of injections, total sample sets, date of first injection, date of last injection, maximum pressure, and temperature. The information also details the column history by sample set including date started, sample set name, user name, system name, number of injections in the sample set, number of samples in the sample set, maximum pressure and temperature in the sample set, and if the column met basic system suitability requirements.

VI. START UP: TIPS AND TRICKS FOR SUCCESS

Atlantis Premier BEH C₁₈ AX Columns are reversed-phase (RP) columns. They differ from other C₁₈ bonded, reversed-phase columns by utilizing an additional positive charge on the surface of the stationary phase. This surface charge allows you to utilize another interaction mechanism: anion exchange (AX) in addition to hydrophobic interaction to manipulate selectivity and retention.

The surface charge of the Atlantis Premier BEH C₁₈ AX stationary phase is influenced by the pH of the mobile phase. The anion-exchange groups of this stationary phase are ionized from pH 2–8.5. Analytes that are negatively charged will have increased retention when operating in this pH window. Conversely, if the analyte is positively charged, the retention could be reduced due to ionic repulsion. The following tips on mobile phase provide guidance when using the Atlantis Premier BEH C₁₈ AX mixed-mode columns.

METHOD DEVELOPMENT OVERVIEW

To optimize chromatographic methods, mobile phase buffer concentration, pH, and organic modifier are three key variables that can be adjusted independently or concurrently.

A. Buffer concentration

An important mobile phase variable is the concentration of the buffer, which strongly affects the ion-exchange mechanism. Figure 4 shows the separation of a polar mixture using a series of mobile phases containing pH 3 ammonium formate at concentrations ranging from 5–20 mM. Two of the analytes, adenosine 5'-monophosphate (AMP) and procainamide, show a strong dependence of retention on buffer concentration, with opposite trends. The retention factor of negatively charged AMP decreases with increasing buffer concentration, due to the formate anions displacing the AMP from the AX sites on the stationary phase. In contrast, the retention factor of positively charged procainamide increases with increasing buffer concentration. This is attributed to a weakening of the ionic repulsion of protonated procainamide as the buffer concentration increases. Figure 5 shows retention factors vs buffer concentration for the three charged analytes.

An increase in buffer concentration can create the following results:

1. Retention decreases for negatively charged molecules
2. Retention increases for positively charged molecules
3. Minimal effect for neutral molecules

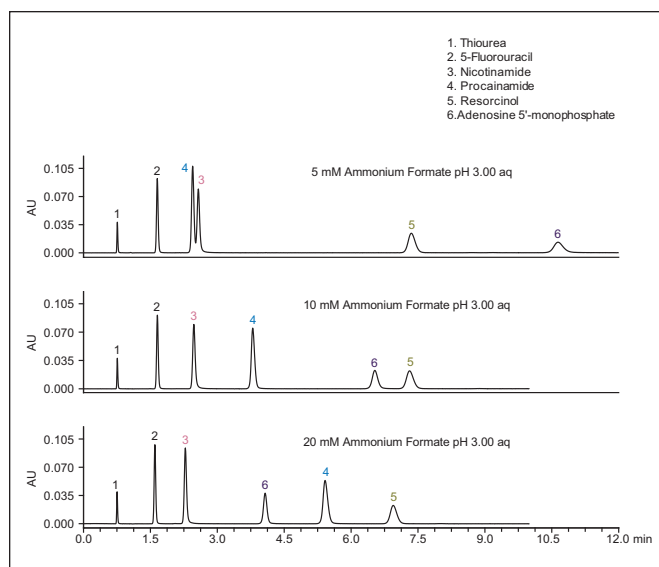


Figure 4. Separation of acidic, basic, and neutral compounds varying buffer concentration.

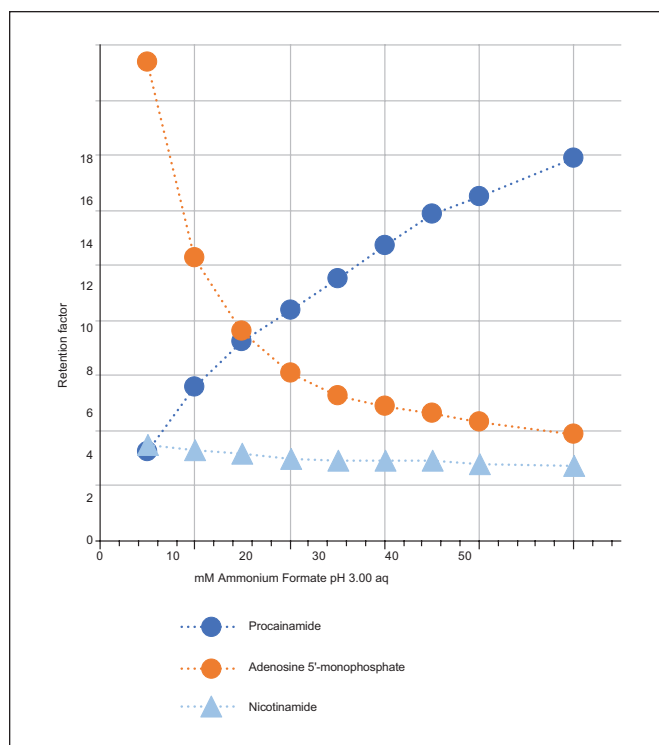


Figure 5. The impact of buffer concentration on the retention of positively and negatively charged compounds.

B. Mobile phase pH

Although pH has little effect on the retention of neutral molecules, it does affect the retention of ionizable molecules significantly. For example, with a pH decrease, molecules containing carboxyl groups may become less negatively charged, giving rise to decreased ion-exchange retention.

Knowing the ionization state of the analyte as a function of pH will help determine the optimal mobile phase pH (see Table 2 for buffer and mobile phase additive considerations). Figure 6 shows the ionization states for acids and bases with different pK_a values as a function of pH. The anion-exchange groups on the stationary phase are active from pH 2-8.5, and will provide increased retention for negatively charged analytes in this pH range due to interactions with the positively charged stationary phase.

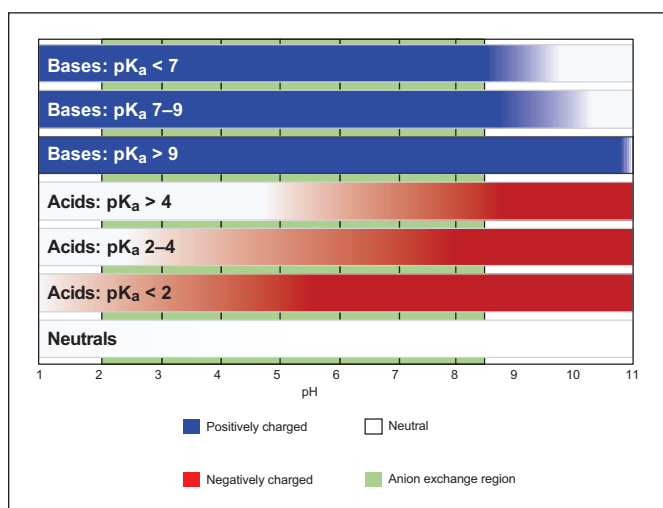


Figure 6. Ionization states of acids and bases with different pK_a values as a function of pH.

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C. Organic modifier

Hydrophobic retention is markedly affected by organic modifier concentration in the mobile phase. In general, all types of molecules (acids, bases, and neutrals) are less retained on this column with increased organic content in the mobile phase, when keeping other conditions constant (e.g., ionic strength, pH, temperature, etc).

D. Isocratic vs. gradient

For many applications that involve small numbers of analytes, it is usually easier to develop an isocratic method on an Atlantis Premier BEH C_{18} AX Column versus a gradient method. For more complicated separations, such as one that involves a mixture of molecules with different types and numbers of charged groups, as well as different hydrophobicities, a gradient method could be advantageous. Gradients of organic solvent concentration, buffer concentration and/or buffer pH are all options for optimizing separations for Atlantis Premier BEH C_{18} AX Columns.

VII. CAUTIONARY NOTE

Depending on the user's application, these products may be classified as hazardous following their use, and as such are intended to be used by professional laboratory personnel trained in the competent handling of such materials. Responsibility for the safe use and disposal of products rests entirely with the purchaser and user. The Safety Data Sheet (SDS) for this product is available at www.waters.com/sds.

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