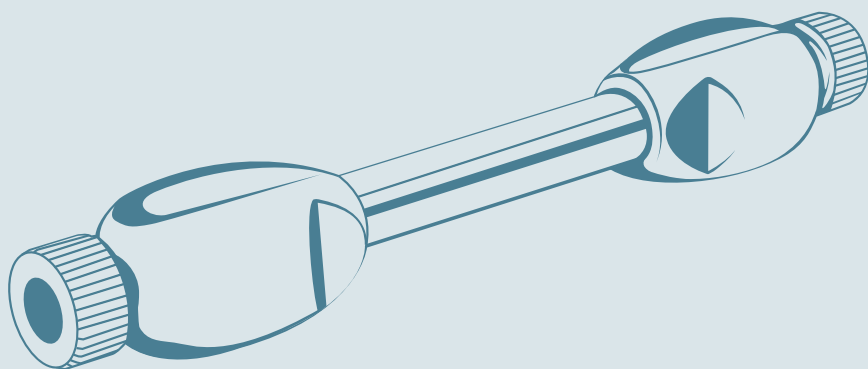


# Gemini<sup>®</sup>-NX pH-LC<sup>™</sup>

The New Standard  
for pH Method Development



## Introducing Gemini<sup>®</sup>-NX

With over 80 %\* of today's pharmaceutical compounds possessing one or more ionizable functional groups, it is essential to understand the impact ionized compounds may have on chromatographic results. Ionized compounds will display altered solubility and hydrophobicity characteristics when compared to their neutral state, often resulting in poor chromatographic results in the most common reversed phase HPLC conditions.

Gemini-NX represents the latest innovative solution for the separation of compounds using reversed phase HPLC. The stationary phase of Gemini-NX uses a second-generation organic grafting process called TWIN-NX™ Technology, resulting in a high efficiency organo-silica particle absolutely unique in the industry.

Gemini-NX overcomes pH limitations previously encountered with silica and hybrid particles, creating new paths to control ionization states of solutes by altering mobile phase pH. The result is improved performance for analytical and preparative-scale separations.

Thank you for choosing Gemini-NX for your separations!



\* Statistic from [http://en.wikipedia.org/wiki/Partition\\_coefficient](http://en.wikipedia.org/wiki/Partition_coefficient)

# **Gemini<sup>®</sup>-NX**

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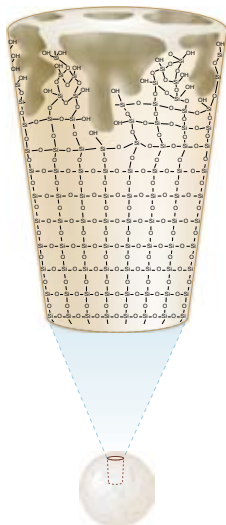
# The New Gemini®-NX

## The Evolution of TWIN-NX™ Technology

Having pH stability in chromatography media means having more options and control. In the past, a few options were available to chromatographers but with certain deficiencies.

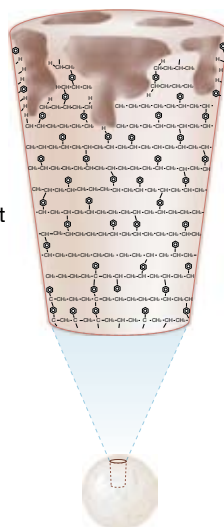
### Silica Particle

Silica particles possess excellent mechanical strength and efficiencies, but are limited in pH stability, (2-7).



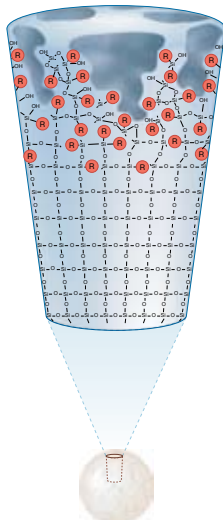
### Polymer Particle

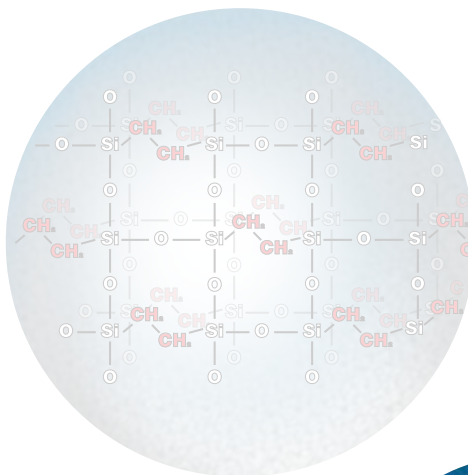
Polymer particles possess excellent pH stability (1-14) but are less mechanically strong and less efficient as compared to silica particles.



### Gemini® with TWIN™ Technology

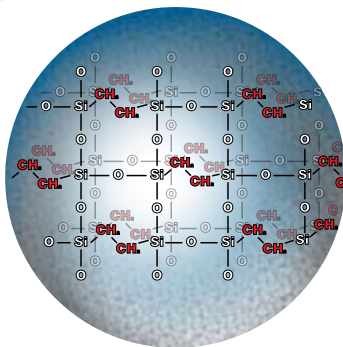
Introduced in 2004, Gemini was the first media to use TWIN™ (Two-In-One) Technology, which provided high efficiencies and mechanical strength with the extended pH stability from 1-12. This was done by grafting organo-silica layers onto a pure silica core.





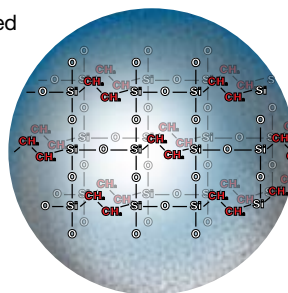
## NEW! Gemini®-NX

Gemini-NX is a new standard in high efficiency, pH method development for HPLC. This new technology creates longer lasting columns, more rugged methods, and positively impacts laboratory productivity. Users can expect an average of five times the durability of Gemini-NX over Gemini, depending on the mobile phase conditions.



## Second-Generation TWIN-NX™ Technology

Gemini-NX uses an improved patent pending organo-silica grafting process which incorporates highly stabilizing ethane cross-linking. These organic groups are evenly incorporated into the grafted layers on the silica surface while maintaining a pure silica core. This not only provides resistance to high pH attack, but also maintains the high efficiency and mechanical strength of a silica particle.

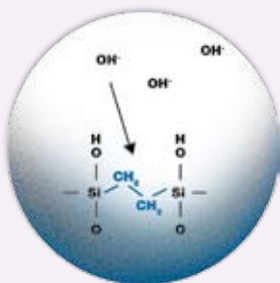


The uniform TWIN-NX grafting technology presents an ordered surface silane distribution. This provides homogeneously active bonding sites ideal for low pH resistant, multi-point ligand attachments.

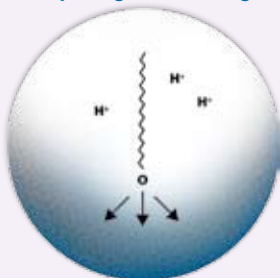
### How It Works

#### Gemini-NX

Ethane Cross-Linking Resists High pH Attack

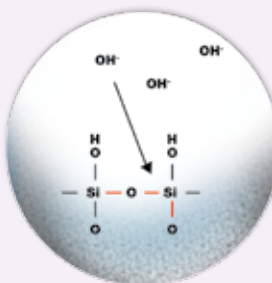


Multi-Point Ligand Attachment Resists Low pH Ligand Cleavage

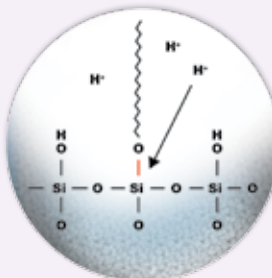


#### Standard Silica

Silica Dissolution



Ligand Cleavage



# The New Gemini®-NX

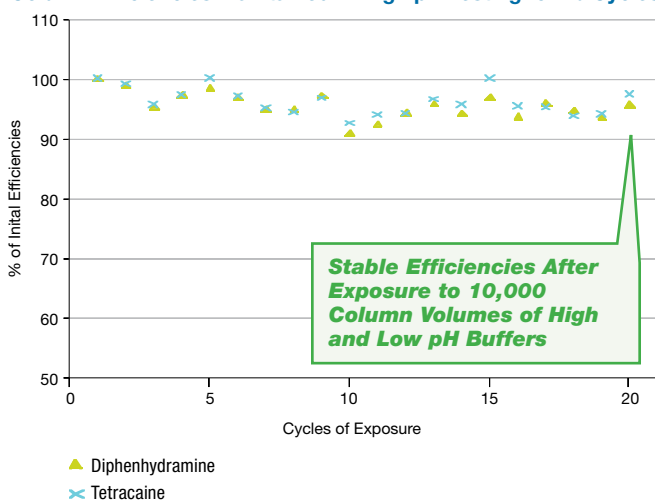
## Open-Access Performance

### An Ideal Column for Open-Access Systems

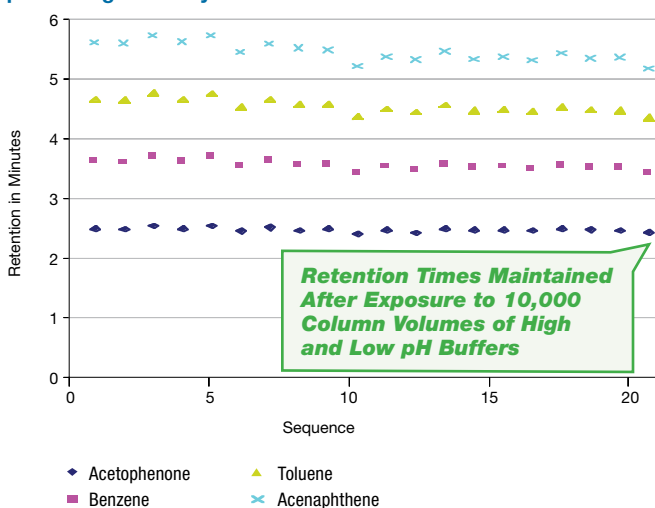
Laboratories with open-access LC systems present unique challenges for column lifetimes. Columns in these environments are subjected to changing pH, buffers, and temperatures, placing a lot of stress on both column and system. Gemini-NX was tested in conditions simulating this harsh, open-access environment.

### Gemini-NX Tested for Extreme Durability in Changing Mobile Phase pH

#### Column Efficiencies Maintained in High pH Testing for 20 Cycles



#### Retention Times of Four Probes Maintained in Neutral pH Testing for 20 Cycles



#### Column Used:

**Column:** Gemini-NX 5 µm C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4454-E0

#### Step 1

##### 24x High pH Flush Procedures

**Mobile Phase:** A: 10 mM Ammonium Bicarbonate pH 10.5  
 B: Acetonitrile  
**Gradient:** 5 % to 95 % B in 6 mins. Hold at 95 % B for 2 mins  
**Re-equilibrate:** 5 % B for 2 mins  
**Flow Rate:** 1.5 mL/min

#### Step 2

##### High pH Testing

**Isocratic:** 10 mM Ammonium Bicarbonate pH 10.5 / Acetonitrile (50:50)  
**Flow Rate:** 1.5 mL/min  
**Detection:** UV @ 230 nm  
**Samples:** 1. Tetracaine  
 2. Diphenhydramine

#### Step 3

##### 1x Neutral Flush Procedure

**Mobile Phase:** A: Water  
 B: Acetonitrile  
**Gradient:** 5 % B for 2 min  
 5 % to 100 % B in 3 min. Hold at 100 % B for 5 min  
**Flow Rate:** 1.5 mL/min

#### Step 4

##### Neutral pH Testing

**Isocratic:** Water / Acetonitrile (35:65)  
**Flow Rate:** 1.0 mL/min  
**Detection:** UV @ 254 nm  
**Samples:** 1. Acetophenone  
 2. Benzene  
 3. Toluene  
 4. Acenaphthene

#### Step 5

##### 24x Low pH Flush Procedure

**Mobile Phase:** A: 0.5 % Formic Acid in Water  
 B: 0.5 % Formic Acid in Acetonitrile  
**Gradient:** 5 % to 95 % B in 6 mins. Hold at 95 % B for 2 mins  
**Re-equilibrate:** 5 % B for 2 mins  
**Flow Rate:** 1.5 mL/min

#### Step 6

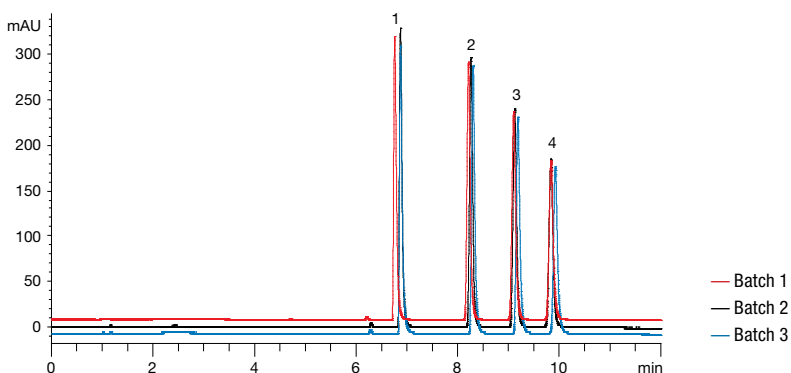
##### Neutral pH Flush Repeats

 Repeats for 20 Cycles

# Batch Reproducibility and Column Lifetimes

## Batch Reproducibility

Gemini-NX follows a highly reproducible manufacturing process that ensures column to column and batch to batch consistency. The entire process is stringently controlled and subjected to demanding QC and QA testing. The results of these efforts create an ideal column for validated and government-regulated assays.

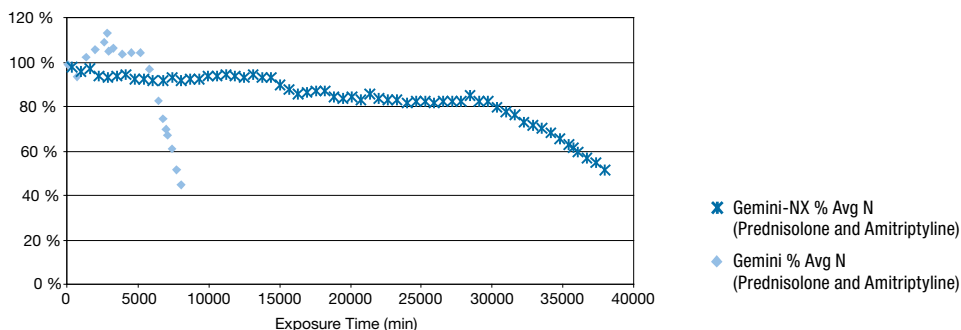


### Conditions:

<b>Column:</b> Gemini-NX 5 $\mu$ m C18	<b>Temperature:</b> Ambient
<b>Dimensions:</b> 150 x 4.6 mm	<b>Detection:</b> UV @ 254 nm
<b>Part No.:</b> 00F-4454-E0	<b>Sample:</b> 1. Nortriptyline
<b>Mobile Phase:</b> A: 20 mM Potassium phosphate pH 7.0	2. Imipramine
B: Acetonitrile	3. Amitriptyline
<b>Gradient:</b> 5 % to 80 % B in 10 min	4. Clomipramine
<b>Flow Rate:</b> 1.5 mL/min	

## Extended Lifetime

Gemini-NX is significantly more rugged under harsh mobile phase environments than Gemini. Although Gemini still provides good stability, Gemini-NX is the recommended choice when working with extreme mobile phase pH.



### % Initial N vs. Exposure Time (min) in High pH

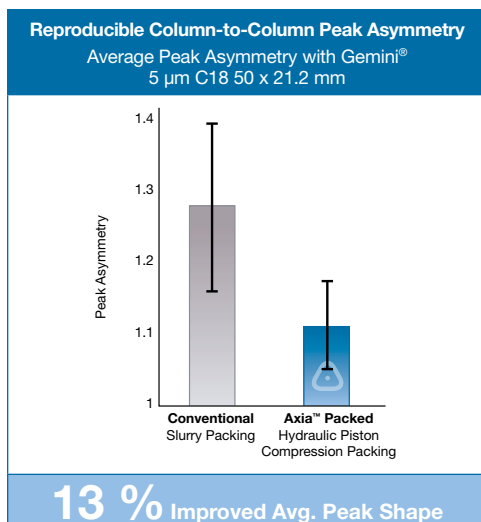
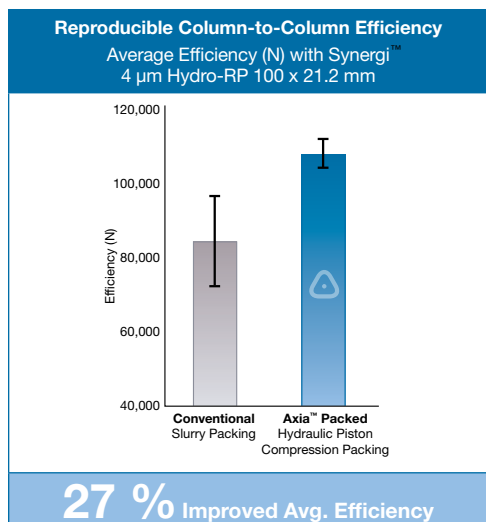
<b>Column:</b> Gemini-NX 5 $\mu$ m C18 Gemini 5 $\mu$ m C18	<b>Isocratic I:</b> 95 % B. Sample: Amitriptyline
<b>Dimensions:</b> 150 x 4.6 mm	<b>Isocratic II:</b> 55 % B. Sample: Prednisolone
<b>Part No.:</b> 00F-4454-E0 00F-4435-E0	<b>Flow Rate:</b> 1.0 mL/min
<b>Mobile Phase:</b> A: 10 mM Ammonium Bicarbonate pH 10.5	<b>Temperature:</b> 50 °C
B: 90:10 Acetonitrile/buffer	<b>Detection:</b> UV @ 254 nm
<b>Gradient:</b> 0 % to 100 % B in 10 min. Hold at 100 % B for 7 min. Re-equilibrate at 0 % B for 3 min	<b>Each Cycle:</b> 2 isocratic flushes/tests +
<b>Samples:</b> 1. Thiourea	<b>Consists:</b> 6 gradient flushes/tests
2. Prednisolone	
3. Amitriptyline	

## The New Gemini®-NX

# Unlock Preparative Chromatography Solutions

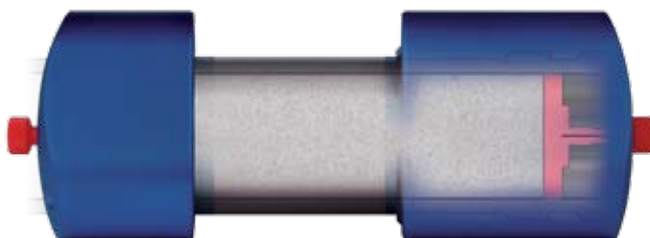
### Axia Performance

When your methods finally scale to preparative separations, you'll want a preparative column you can depend on. Although there are a variety of choices out there, only Axia-packed technology will provide guaranteed longer column lifetimes.\*



### About Axia-Packed Technology

Axia™ is an advanced column packing and hardware design that eliminates bed collapse as a source of failure in preparative columns. Using a patent pending, Hydraulic Piston Compression technology, ideal bed density is custom calculated and automated for each support, chemistry and column size. The result is an improved, more repeatable packing process, giving you significant improvements in column lifetime with efficiencies and peak symmetries on par with analytical separations.



2006 R&D 100  
Award Recipient

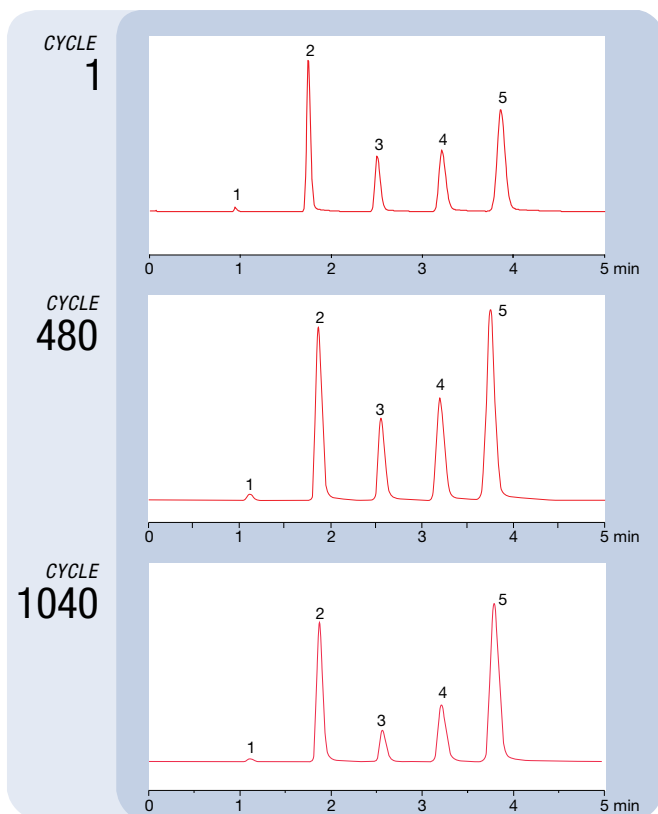
\* See Phenomenex 2008 Product Guide for full terms of guarantee.

# Gemini-NX and Axia™-Packed Technology: A Winning Combination

## Benefits of Preparative Scale-up in Basic Conditions

Gemini-NX is an ideal solution for methods that scale to preparative purification.

- Increased Loading Capacity for Basic Compounds**  
 In reversed phase LC, basic drugs are better retained in their neutral state. Gemini-NX can easily tolerate the high pH ranges necessary to do this.
- Extended Column Lifetimes**  
 Gemini-NX is so rugged, columns will perform consistently and last longer, which means more productivity.



### % Initial N vs. Exposure Time (min) in High pH

**Column:** Gemini-NX 5  $\mu$ m C18 AXIA-Packed  
**Dimension:** 100 x 21.2 mm  
**Part No.:** 00D-4454-P0-AX  
**Mobile Phase:** A: 0.2 % Ammonium Hydroxide  
 B: 0.2 % Ammonium Hydroxide in Acetonitrile  
**Gradient:** A/B (5/95) in 7 min. 3 min equilibration at 5 % B  
**Flow Rate:** 30 mL/min  
**Temperature:** 40 °C

These columns were tested daily in the following isocratic mode:

**Mobile Phase:** Acetonitrile/Water 65/35 (v/v)

**Flow Rate:** 20 mL/min

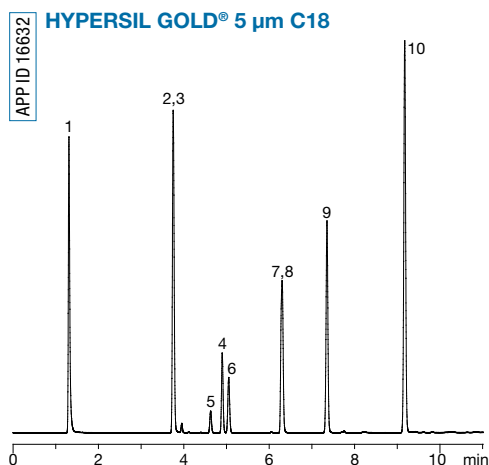
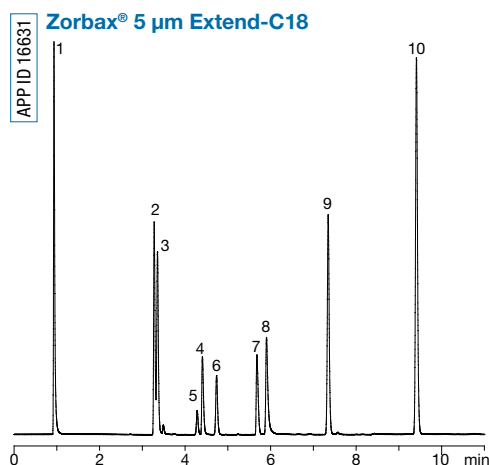
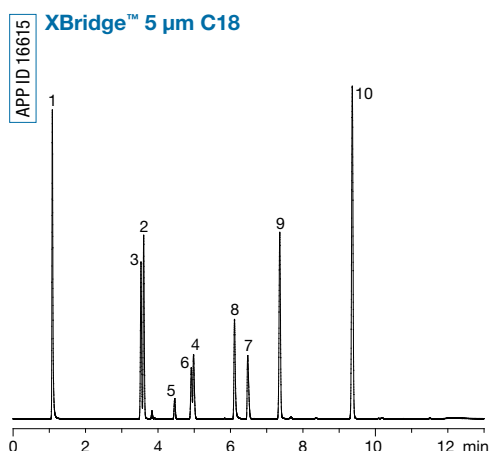
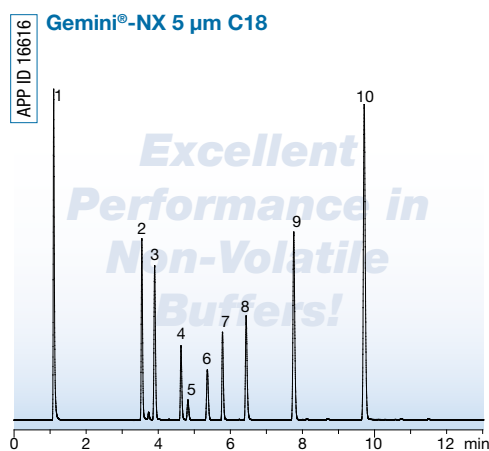
**Samples:**

1. Uracil
2. Acetophenone
3. Benzene
4. Toluene
5. Naphthalene

# Versatile Performance Applications

## Performance in Non-Volatile Buffers

Gemini-NX was engineered to be the column of choice for pharmaceutical scientists who work with difficult sample mixtures and harsh mobile phase conditions. The consistent performance and rugged phase will provide simplified method development and long column lifetimes.



### Mixtures of Acids, Neutrals, and Bases in Potassium Phosphate pH 2.5

**Columns:** Gemini-NX 5 µm C18  
Zorbax 5 µm Extend-C18  
XBridge 5 µm C18  
Hypersil Gold 5 µm C18

**Dimensions:** 150 x 4.6 mm

**Part No.:** 00F-4454-E0

**Mobile Phase:** A: 20 mM Potassium Phosphate pH 2.5  
B: Acetonitrile

**Gradient:** A/B (95:5) to (20:80) in 8 min,  
Hold for 2 min

**Flow Rate:** 1.5 mL/min

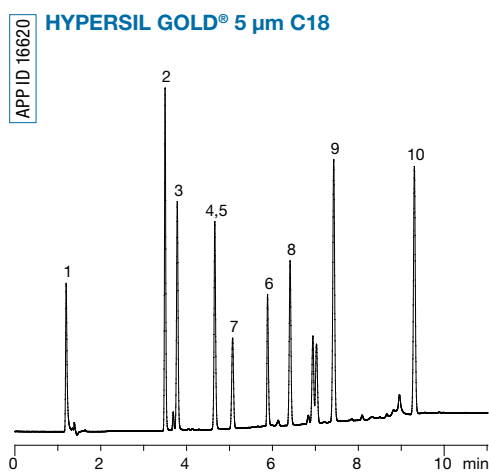
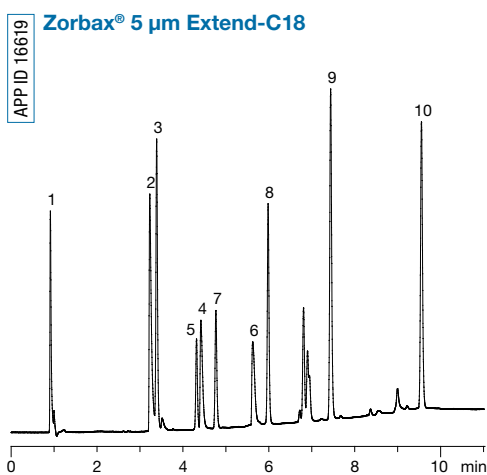
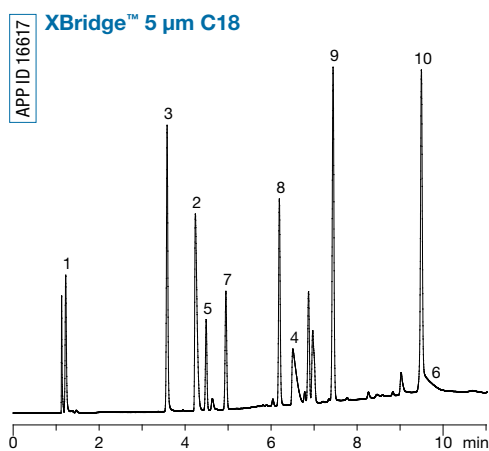
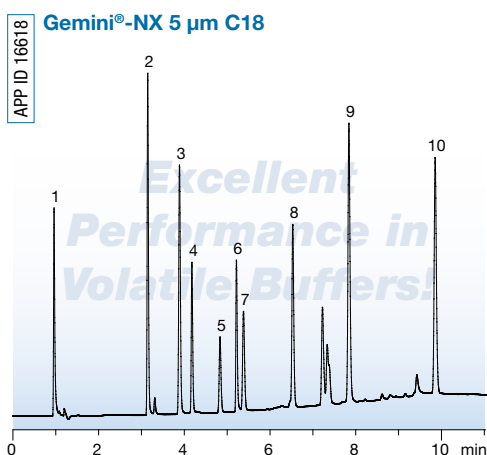
**Temperature:** Ambient

**Detection:** UV @ 254 nm

**Sample:** 1. Pyridine  
2. Quinidine  
3. Sulfathiazole  
4. Triprolidine  
5. Benzyl alcohol  
6. Phenol  
7. Nortriptyline  
8. 3-Methyl-4-nitrobenzoic acid  
9. Methylsalicylaldehyde  
10. Hexanophenone

## Performance in Volatile Buffers

With the widespread adoption of LC-MS and LC-MS/MS techniques, column performance in volatile buffers is critical.



### Mixtures of Acids, Neutrals, and Bases in Formic Acid pH 2.7

**Columns:** Gemini-NX 5 µm C18  
Zorbax 5 µm Extend-C18  
XBridge 5 µm C18  
Hypersil Gold 5 µm C18

**Dimensions:** 150 x 4.6 mm

**Part No.:** 00F-4454-E0

**Mobile Phase:** A: 0.1 % Formic Acid in Water  
B: 0.1 % Formic Acid in Acetonitrile

**Gradient:** A/B (95:5) to (20:80) in 8 min,  
Hold for 2 min

**Flow Rate:** 1.5 mL/min

**Temperature:** Ambient

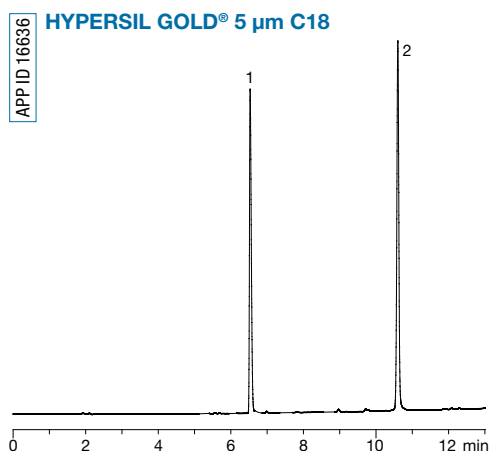
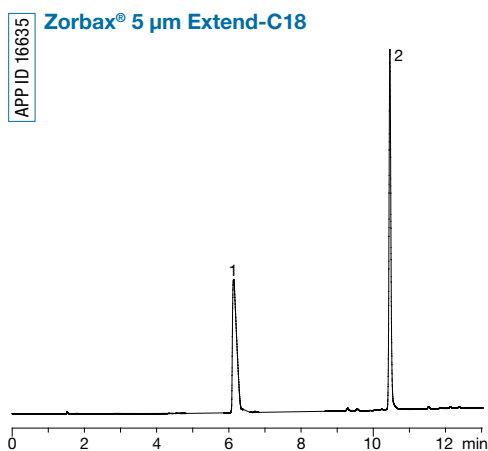
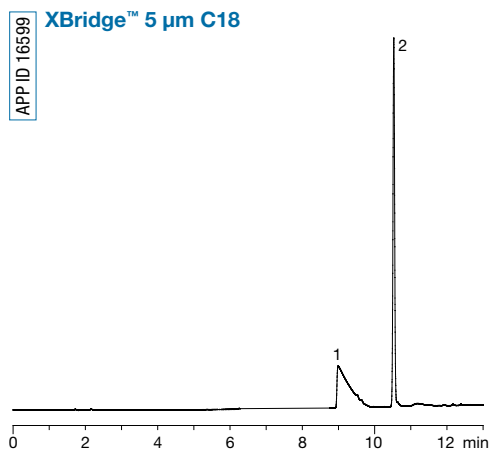
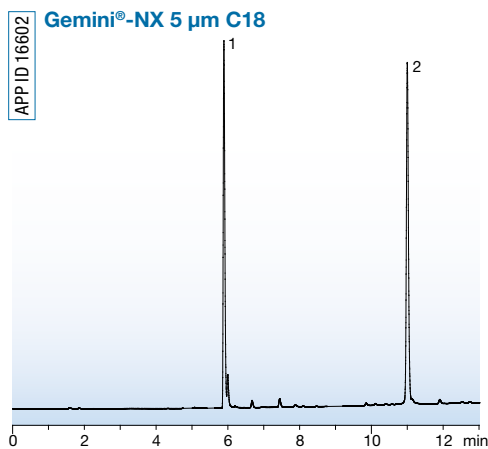
**Detection:** UV @ 254 nm

**Sample:**

1. Pyridine
2. Quinidine
3. Sulfathiazole
4. Triprolidine
5. Benzyl alcohol
6. Nortriptyline
7. Phenol
8. 3-Methyl-4-nitrobenzoic acid
9. Methylsalicylaldehyde
10. Hexanophenone

# Versatile Performance Applications (cont.)

## Hydrophobic Basic Drugs at Low pH



### Hydrophobic Bases (Diltiazem, Promethazine) in Formic Acid

**Columns:** Gemini-NX 5 µm C18  
Zorbax 5 µm Extend-C18  
XBridge 5 µm C18  
Hypersil Gold 5 µm C18

**Dimensions:** 150 x 4.6 mm

**Part No.:** 00F-4454-E0

**Mobile Phase:** A: 0.1 % Formic Acid in Water  
B: 0.1 % Formic Acid in Acetonitrile

**Gradient:** A/B (95:5) to (5:95) in 10 min

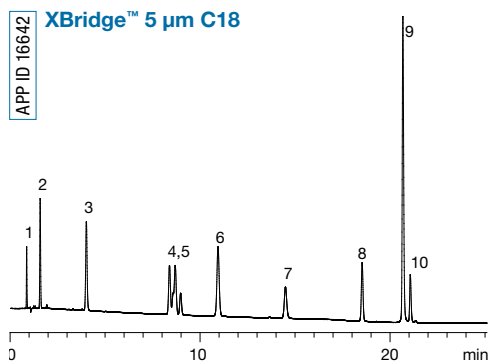
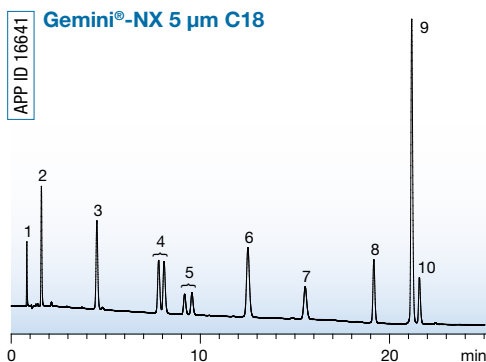
**Flow Rate:** 1.0 mL/min

**Temperature:** Ambient

**Detection:** UV @ 254 nm

**Sample:** 1. Diltiazem  
2. Promethazine

## Polar Bases at High pH



### Polar Bases (Beta Blockers) at High pH

**Columns:** Gemini-NX 5 µm C18  
XBridge 5 µm C18

**Dimensions:** 150 x 4.6 mm

**Part No.:** 00F-4454-E0

**Mobile Phase:** A: 10 mM Ammonium Bicarbonate pH 10.5  
B: Acetonitrile

**Gradient:** A/B (85:15) to (70:30) in 15 min to (50:50) in 5 min, Hold for 5 min

**Flow Rate:** 1.5 mL/min

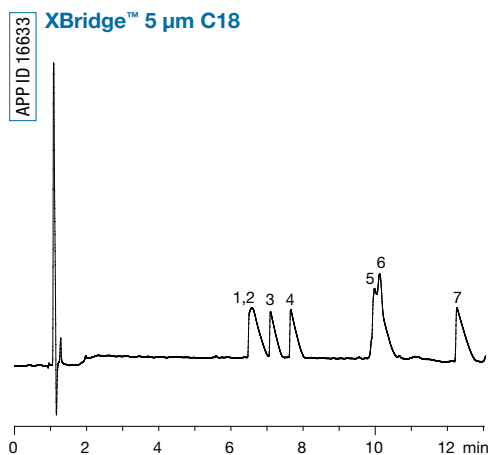
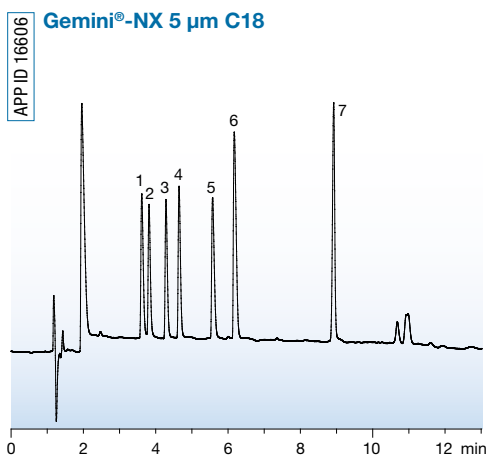
**Temperature:** Ambient

**Detection:** UV @ 230 nm

**Sample:**

1. Bisoprolol
2. Sotalol
3. Atenolol
4. Labetolol (Diastereoisomeric Pair)
5. Nadolol (Diastereoisomeric Pair)
6. Pindolol
7. Metoprolol
8. Bisoprolol
9. Propranolol
10. Alprenalol

## Polar Bases at Low pH



### Polar Bases (Antihistamines) in Formic Acid

**Columns:** Gemini-NX 5 µm C18  
XBridge 5 µm C18

**Dimensions:** 150 x 4.6 mm

**Part No.:** 00F-4454-E0

**Mobile Phase:** A: 0.1 % Formic Acid in Water  
B: 0.1 % Formic Acid in Acetonitrile

**Gradient:** A/B (90:10) to (50:50) in 10 min

**Flow Rate:** 1.5 mL/min

**Temperature:** Ambient

**Detection:** UV @ 210 nm

**Sample:**

1. Pyrilamine
2. Tripeleminamine
3. Chlorpheniramine
4. Brompheniramine
5. Chloropyramine
6. Diphenhydramine
7. Loratadine

## ***pH-LC Method Development***

# Recognizing the Importance of Solute Ionization States

In reversed phase chromatography, the goal is to achieve resolution of all analytes, with hydrophobic interaction as the primary mechanism of selectivity between the stationary phase and the solutes of interest. The ionization state of a solute has three significant effects that may alter the degree of interaction it has with the stationary phase.

### **A Solute in its Ionized State is More Polar than in its Neutral State.**

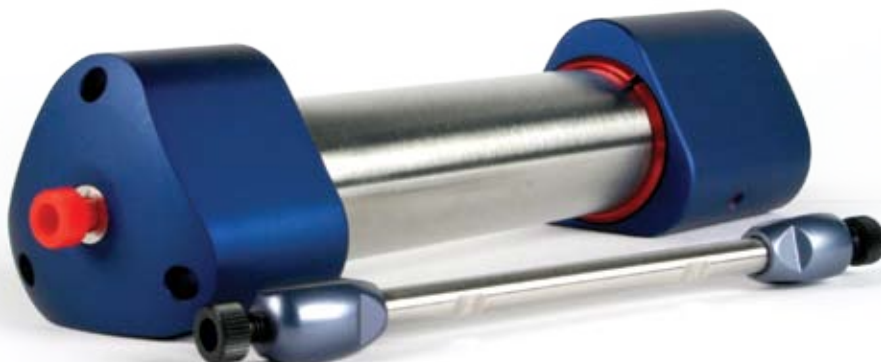
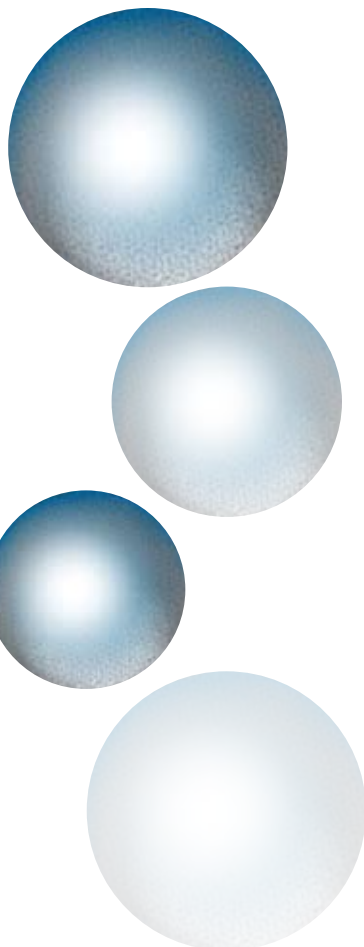
When a molecule is ionized, it is more likely to participate in hydrogen bonding. In a reversed phase, aqueous-rich mobile phase, the solute will spend less time participating in hydrophobic interactions with the stationary phase and more time hydrogen bonding in the aqueous portion relative to the neutral species. The result is less retention.

### **A Solute Should be in a Single Form (Either Ionized or Neutral) in Order to Have Good Peak Shape.**

When there are significant concentrations of both ionized and neutral forms, the ionized form will have less hydrophobic interaction with the stationary phase while the neutral form will have more hydrophobic interaction. The result is broad or asymmetrical peaks.

### **A Solute in its Ionized State may Participate in Unwanted Ionic Interactions**

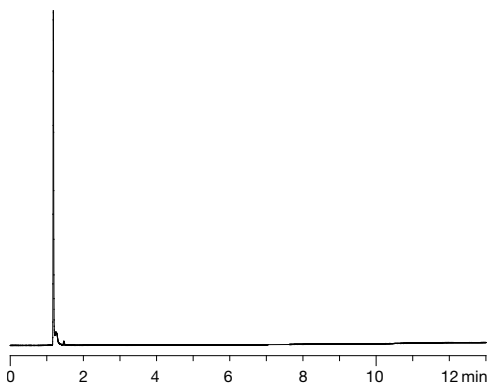
Exposed silanols groups or metal contaminants present in the silica may participate in ionic interactions with ionized solutes, which may lead to poor peak shape or irreproducibility.



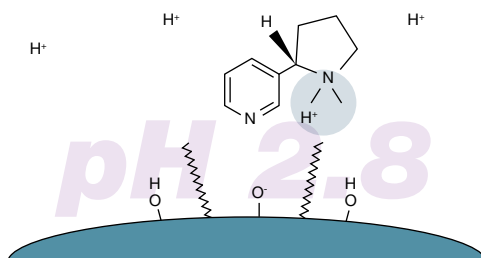
## Let's Look at Nicotine as an Example of an Ionizable Compound.

In its ionized state, nicotine exhibits distinct chromatographic behaviors:

- **Less retention** – nicotine spends less time interacting with the C18 stationary phase
- **Sharp peak** – single ionized form, but elutes in the void volume



### Charged Analyte



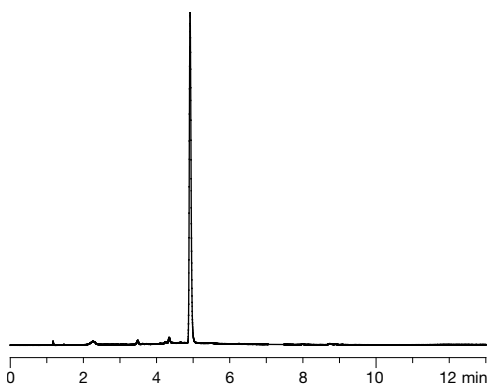
#### APP ID 16626 Nicotine in 0.1 % Formic Acid

**Column:** Gemini-NX 5  $\mu$ m C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4454-E0  
**Mobile Phase:** A: 0.1 % Formic Acid in Water  
 B: 0.1 % Formic Acid in Acetonitrile  
**Gradient:** A/B (95:5) to (5:95) in 10 min,  
 Hold for 2 min

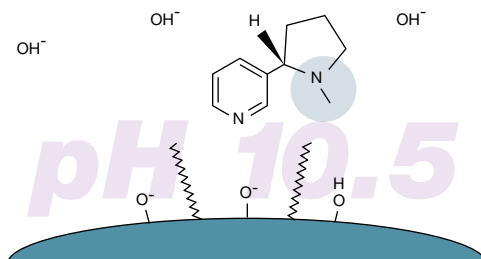
**Flow Rate:** 1.5 mL/min  
**Temperature:** Ambient  
**Detection:** UV @ 254 nm  
**Sample:** Nicotine;  $pK_a$  8.5

In its neutral state, nicotine has distinct chromatographic behaviors:

- **Increased retention** - nicotine spends more time interacting with the stationary phase
- **Sharp peak** – single neutral form, well resolved



### Uncharged Analyte



#### APP ID 16627 Nicotine in Ammonium Bicarbonate pH 10.5

**Column:** Gemini-NX 5  $\mu$ m C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4454-E0  
**Mobile Phase:** A: 10 mM Ammonium Bicarbonate pH 10.5  
 B: Acetonitrile  
**Gradient:** A/B (95:5) to (5:95) in 10 min,  
 Hold for 2 min

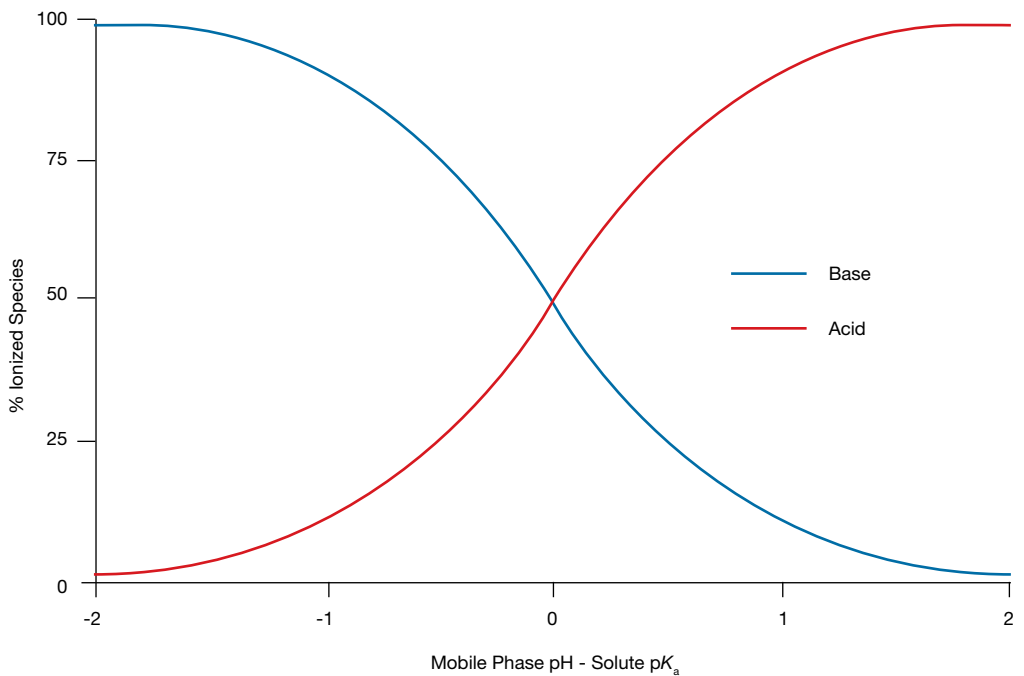
**Flow Rate:** 1.5 mL/min  
**Temperature:** Ambient  
**Detection:** UV @ 254 nm  
**Sample:** Nicotine ;  $pK_a$  8.5

## pH-LC Method Development

# Relationship Between Mobile Phase pH and Solute Ionization States

It is possible to predict the ionization state of the solute relative to the mobile phase pH by knowing the solute  $pK_a$ . In other words, you can control the ionization state simply by adjusting mobile phase pH. An important relationship between a solute  $pK_a$  and the mobile phase pH can be shown by this figure.

### Relationship Between Mobile Phase pH and Solute Ionization States



We use the terms “weak acid” and “weak base” to describe common pharmaceutical solutes and the effect of their ionizable functional groups. For non-ionizable compounds, the retention characteristics remain relatively unchanged throughout the pH range, although the use of different buffers may influence their peak shape.

### Weak Acids

The pH range in which there is partial dissociation of the acid is within 2 units above or below the  $pK_a$  value. Therefore, if we want to neutralize an acid, the mobile phase pH must be at least two units below the  $pK_a$ .

$pH = pK_a - 2$	the acid is 1 % dissociated (mostly neutral)
$pH = pK_a$	the acid is 50 % dissociated (equal amounts of ionized and neutral)
$pH = pK_a + 2$	the acid is 99 % dissociated (mostly ionized)

### Weak Bases

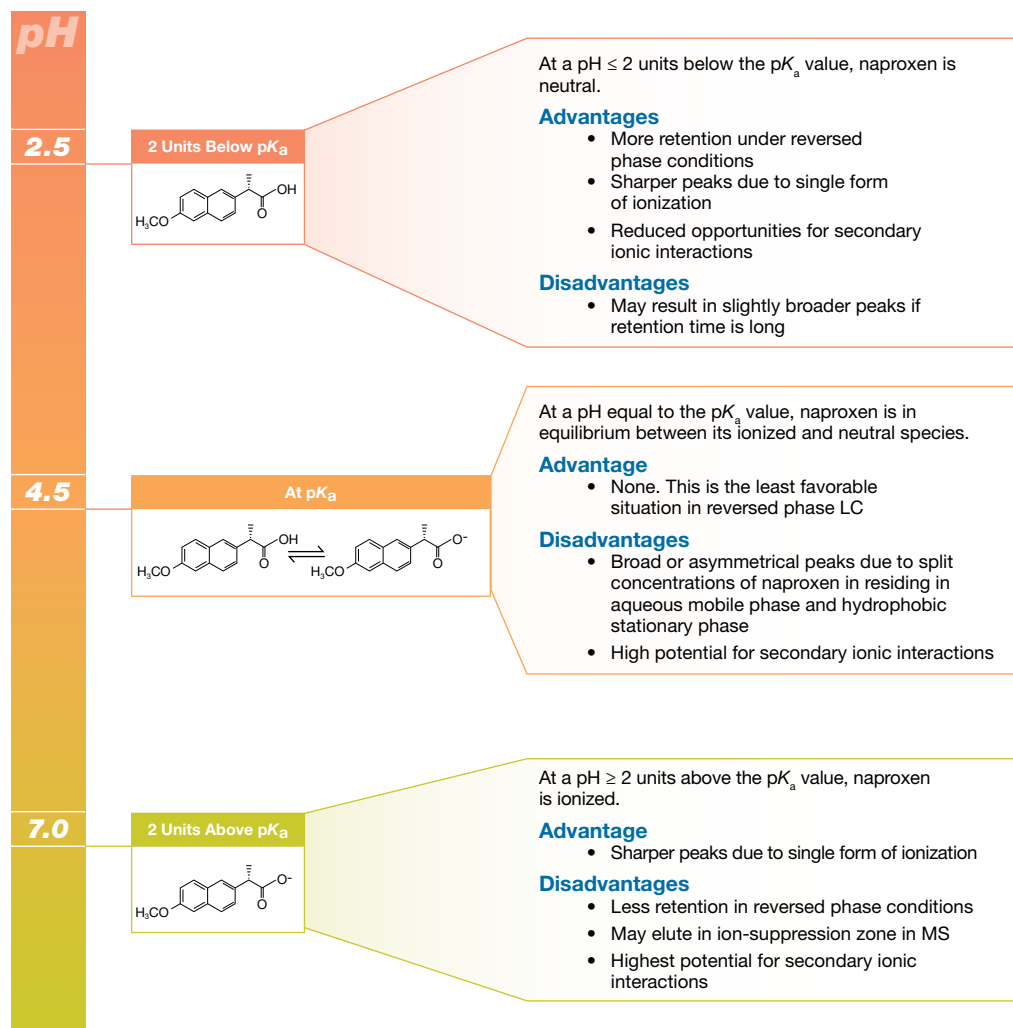
For a basic compound, the mobile phase pH must be at least two units above its  $pK_a$  in order to have it mostly neutral.

$pH = pK_a - 2$	the base is 99 % dissociated (mostly ionized)
$pH = pK_a$	the base is 50 % dissociated (equal amounts of ionized and neutral)
$pH = pK_a + 2$	the base is 1 % dissociated (mostly neutral)

## pH-LC Method Development

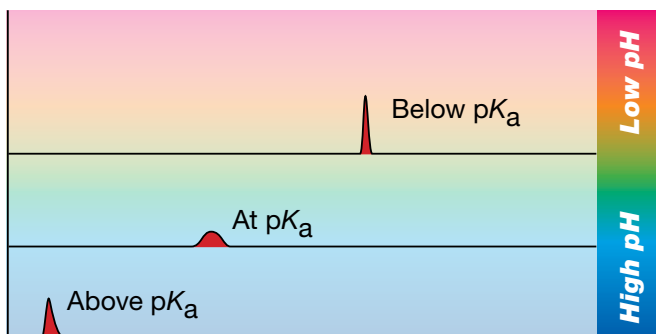
# Applying Mobile Phase pH to Control Selectivity

## Acids **Naproxen $pK_a$ 4.5**



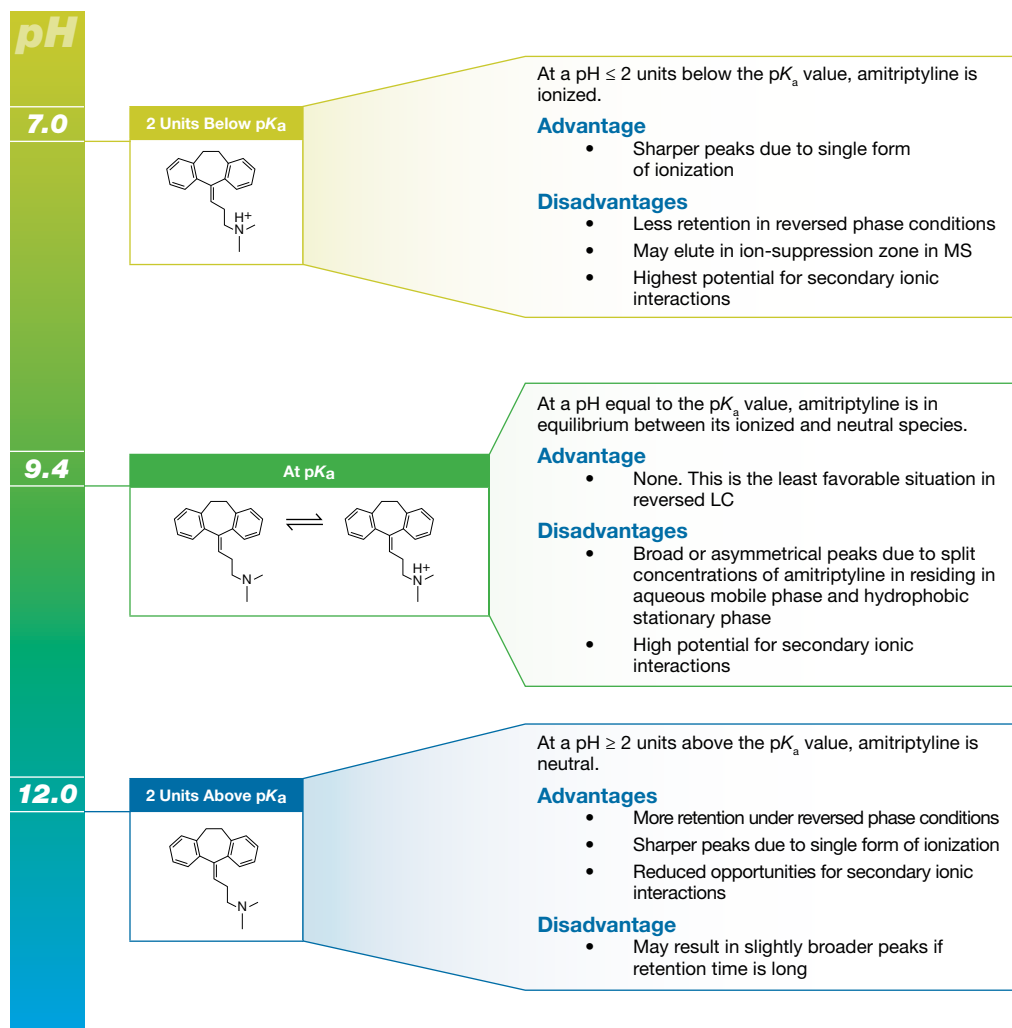
### Expected Peak Shape and Retention

#### Effects of Mobile phase pH on Acids



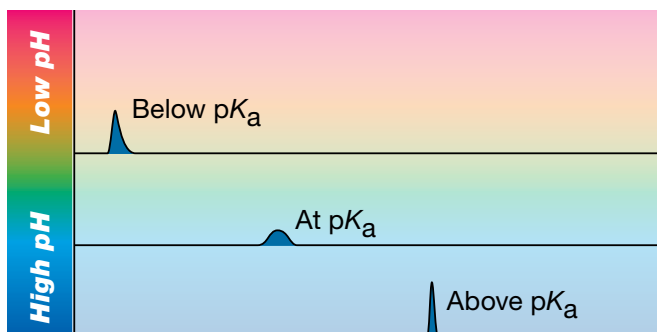
# Bases

## Amitriptyline $pK_a$ 9.4



## Expected Peak Shape and Retention

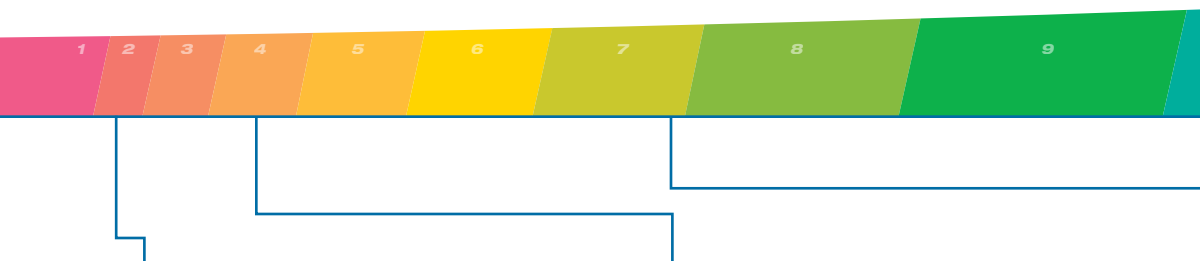
### Effects of Mobile phase pH on Bases



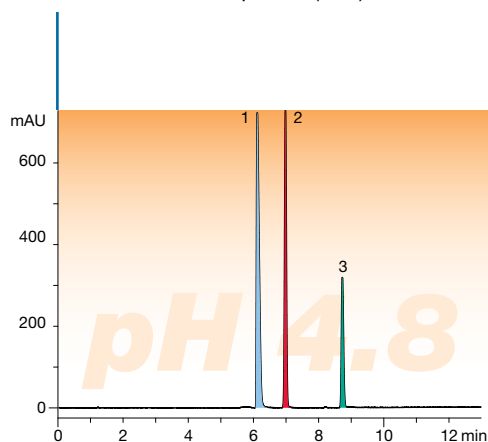
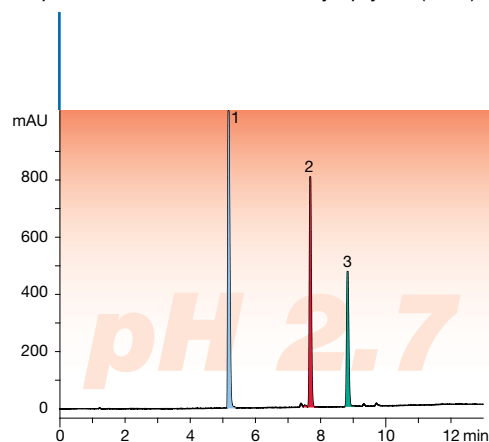
## pH-LC Method Development

# Applying Mobile Phase pH to Control Selectivity (cont.)

### Acid, Neutral, and Base in varying pH.



As pH increases, retention of amitriptyline (base) increases while retention of naproxen (acid) decreases.



APP ID 16593

**Column:** Gemini-NX 5  $\mu$ m C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4454-E0  
**Mobile Phase:** A: 0.1 % Formic Acid in Water  
B: 0.1 % Formic Acid in Acetonitrile  
**Gradient:** A/B (95:5) to (5:95) in 10 min, Hold for 2 min  
**Flow Rate:** 1.5 mL/min  
**Temperature:** Ambient  
**Detection:** UV @ 254 nm  
**Sample:** 1. Amitriptyline  
2. Naproxen  
3. Toluene

APP ID 16598

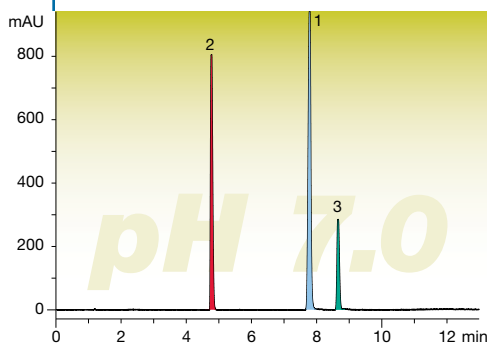
**Column:** Gemini-NX 5  $\mu$ m C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4454-E0  
**Mobile Phase:** A: 10 mM Ammonium Acetate pH 4.8  
B: Acetonitrile  
**Gradient:** A/B (95:5) to (5:95) in 10 min, Hold for 2 min  
**Flow Rate:** 1.5 mL/min  
**Temperature:** Ambient  
**Detection:** UV @ 254 nm  
**Sample:** 1. Amitriptyline  
2. Naproxen  
3. Toluene

10

11

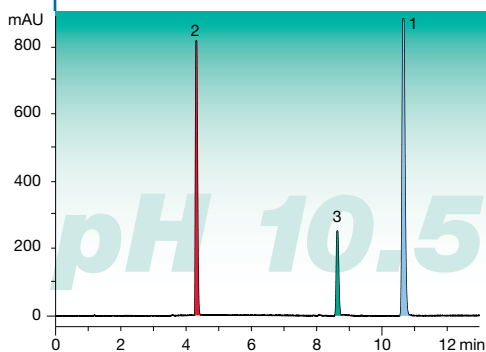
12

Using pH to control selectivity, optimized compound-dependent conditions can be developed.



APP ID 16600

**Column:** Gemini-NX 5  $\mu$ m C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4454-E0  
**Mobile Phase:** A: 20 mM Potassium Phosphate pH 7.0  
 B: Acetonitrile  
**Gradient:** A/B (95:5) to (5:95) in 10 min, Hold for 2 min  
**Flow Rate:** 1.5 mL/min  
**Temperature:** Ambient  
**Detection:** UV @ 254 nm  
**Sample:** 1. Amitriptyline  
 2. Naproxen  
 3. Toluene



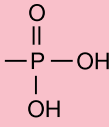
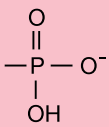
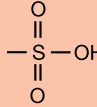
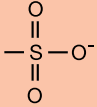
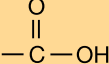
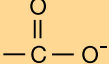
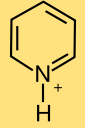

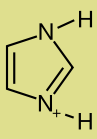
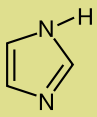
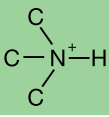
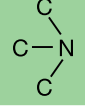
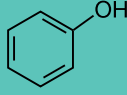
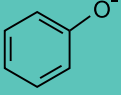
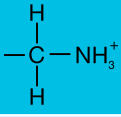
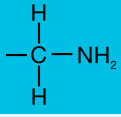
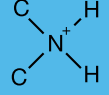
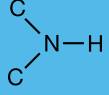
APP ID 16601

**Column:** Gemini-NX 5  $\mu$ m C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4454-E0  
**Mobile Phase:** A: 10 mM Ammonium Bicarbonate pH 10.5  
 B: Acetonitrile  
**Gradient:** A/B (95:5) to (5:95) in 10 min, Hold for 2 min  
**Flow Rate:** 1.5 mL/min  
**Temperature:** Ambient  
**Detection:** UV @ 254 nm  
**Sample:** 1. Amitriptyline  
 2. Naproxen  
 3. Toluene

## Getting Started

# Analyzing Ionizable Functional Groups

The functionalities that analytes contain will affect retention on a reversed phase column. Whether a functionality is protonated or deprotonated will usually affect the chromatographic behavior of a compound; altering the pH of a separation will often change an elution profile. A general observation is that an acidic functionality will tend to have greater retention and efficiency at a pH below its  $pK_a$  value and less retention at a pH above its  $pK_a$  value. Basic compounds follow the opposite trend: basic functionalities are often less retained at pH's below their  $pK_a$  values and demonstrate greater retention and better peak shape at pH's above their  $pK_a$  values.

Protonated Functional Group	Deprotonated Functional Group	Approx. $pK_a$	<b>Acids</b>
		1.5	
		2.0	
		4.7	
		5.3	
		7.0	
		9.8	
		10.0	
		10.6	
		10.7	

These values are approximate at best. Other nearby functionalities can DRAMATICALLY affect  $pK_a$  of functional groups.

Source: Introduction to Organic Chemistry - Streitwieser and Heathcock, MacMillan Publishing, 1981.

# Choosing the Proper Buffer

Next to organic solvent, buffer selection is the most important variable in HPLC method development. There are several criteria in selecting the appropriate buffer. The first is choosing a buffer that has a  $pK_a$  near the desired working pH. Other criteria like ionic strength, ion-pairing properties, as well as mass spectrometer compatibility should be considered before selecting any mobile phase modifier.

Buffer	$pK_a$	Buffer Range (pH)	MS Compatible
Trifluoroacetic Acid	< 2	< 2.5	•†
Phosphoric Acid ( $pK_1$ )	2.1	1.1 - 3.1	
Formic Acid	3.8	2.8 - 4.8	•
Acetic Acid	4.8	3.8 - 5.8	•
Carbonate ( $pK_1$ )	6.4	5.4 - 7.4	•
Phosphate ( $pK_2$ )	7.2	6.2 - 8.2	
Triethanolamine	7.8	6.8 - 8.8	•
Diethanolamine	8.9	7.9 - 9.9	•
Ammonia	9.2	8.2 - 10.2	•
Ethanolamine	9.5	8.5 - 10.5	•
Carbonate ( $pK_2$ )	10.3	9.3 - 11.3	•
Diethylamine	10.5	9.5 - 11.5	•
Triethylamine	11.0	10.0 - 12.0	•
Phosphate ( $pK_3$ )	12.3	11.3 - 13.3	

Sources: Practical HPLC Method Development; L.R. Snyder, JJ Kirkland, and JL Glajch. Wiley Interscience, 1997, and Introduction to Protein and Peptide HPLC; TP Bradshaw, Phenomenex, 1998.

† TFA can be used at low concentrations for LC/MS applications but can affect MS sensitivity.



# Supplying Your Laboratory

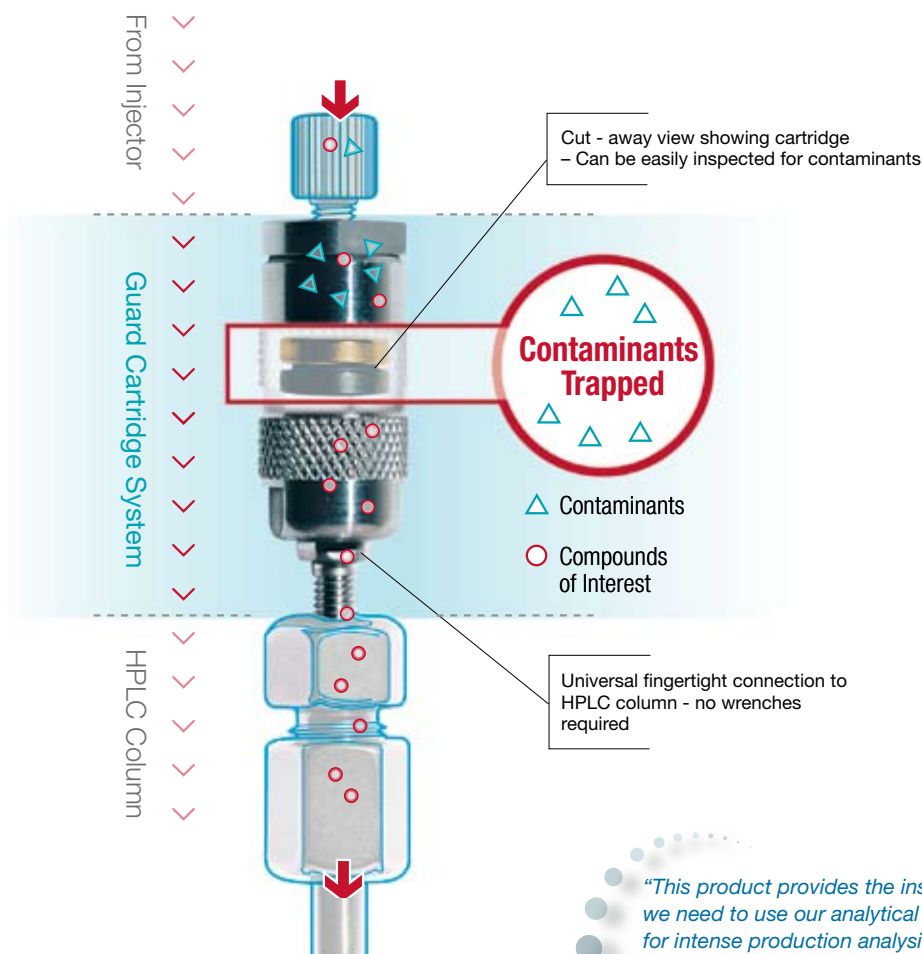
## SecurityGuard™ Universal Guard System

The SecurityGuard analytical cartridge holder directly fingertightens into any manufacturer's column with a female/inverted end-fitting. Contaminants are retained by an inexpensive disposable cartridge instead of damaging your valuable HPLC column investment.

- Extends your HPLC column lifetime
- Won't alter your chromatography
- Easy to use



### How does SecurityGuard Work?



*"This product provides the insurance we need to use our analytical columns for intense production analysis. The SecurityGuard is easy to use and cartridge replacement is simple."*  
— T. Sollima

#### Gemini-NX SecurityGuard Ordering Information

##### SecurityGuard™ Cartridges

	4 x 2.0 mm*	4 x 3.0 mm*	15 x 21.2 mm**	15 x 30.0 mm**
Phase				
C18	AJ0-8367	AJ0-8368	AJ0-8370	AJ0-8371
for ID:	2.0-3.0 mm	3.0-8.0 mm	18-30 mm	30-49 mm

\* SecurityGuard Analytical Cartridges Require Holder, Part No.: **KJ0-4282**

\*\* PREP SecurityGuard Cartridges Require Holder, Part No.: **AJ0-8223 or AJ0-8277**

## Phenex™ Sample Preparation

### Phenex Syringe Filters

#### Syringe Filter Applications and Recommended Membrances

Application / Sample	Recommended Filter	First Alternative	Second Alternative
HPLC and GC Sample Prep	RC	PTFE	PES
Aggressive or Pure Organic Solvents	PTFE	RC	NY
Protein Analysis / Biological Samples	PES	RC	GF/CA
High Particulate Loads	RC	NY	PTFE
Environmental Methods	RC	PTFE	NY
Food and Beverage	RC	PTFE	NY
Clinical / Toxicology	RC	PES	NY
Dissolution Testing	RC	PTFE	NY
Tissue Cultures, Media, Buffers	GF/CA	PES	RC



Membrane Type/Size	4 mm Diameter for < 2 mL sample volumes			15 mm Diameter for 2 - 10 mL sample volumes			25 mm Diameter for 10 - 100 mL sample volumes			
	Part No.	Unit	Price	Part No.	Unit	Price	Part No.	Unit	Price	
<b>0.45 µm</b>										
<b>Phenex-RC</b> <sup>3</sup> (Regenerated Cellulose)	AF0-3103-12	100/Pk		AF0-2103-12	100/Pk		AF0-8103-12 <sup>5</sup>	100/Pk		
	AF0-3103-52	500/Pk		AF0-2103-52	500/Pk		AF0-8103-52 <sup>5</sup>	500/Pk		
<b>Phenex-PES</b> <sup>3</sup> (Polyethersulfone)	—	—		AF2-5108-12 <sup>1</sup>	100/Pk		AF0-8108-12 <sup>5</sup>	100/Pk		
	—	—		—	—		AF0-8108-52 <sup>5</sup>	500/Pk		
<b>Phenex-PTFE</b> <sup>6</sup> (Polytetrafluoroethylene)	AF0-3102-12	100/Pk		AF0-2102-12	100/Pk		AF0-1102-12	100/Pk		
	AF0-3102-52	500/Pk		AF0-2102-52	500/Pk		AF0-1102-52	500/Pk		
<b>Phenex-NY</b> (Nylon)	AF3-3107-12	100/Pk		AF2-5107-12 <sup>1</sup>	100/Pk		AF0-1107-12	100/Pk		
	AF3-3107-52	500/Pk		AF2-5107-52 <sup>1</sup>	500/Pk		AF0-1107-52	500/Pk		
<b>Phenex-GF/CA</b> <sup>2,3,4</sup> (Glass Fiber/Cellulose Acetate)	An integrated syringe filter unit containing an inert borosilicate glass fiber prefilter and a CA membrane. Excellent for filtration of tissue culture media, general biological sample filtration and clarification.							AF0-8B09-12	100/Pk	
								AF0-8B09-52	500/Pk	
<b>0.20 µm</b>										
<b>Phenex-RC</b> <sup>3</sup> (Regenerated Cellulose)	AF0-3203-12	100/Pk		AF0-2203-12	100/Pk		AF0-8203-12 <sup>5</sup>	100/Pk		
	AF0-3203-52	500/Pk		AF0-2203-52	500/Pk		AF0-8203-52 <sup>5</sup>	500/Pk		
<b>Phenex-PES</b> <sup>3</sup> (Polyethersulfone)	—	—		—	—		AF0-8208-12 <sup>5</sup>	100/Pk		
	—	—		—	—		AF0-8208-52 <sup>5</sup>	500/Pk		
<b>Phenex-PTFE</b> <sup>6</sup> (Polytetrafluoroethylene)	AF0-3202-12	100/Pk		AF0-2202-12	100/Pk		AF0-1202-12	100/Pk		
	AF0-3202-52	500/Pk		AF0-2202-52	500/Pk		AF0-1202-52	500/Pk		
<b>Phenex-NY</b> (Nylon)	AF3-3207-12	100/Pk		AF2-5207-12 <sup>1</sup>	100/Pk		AF0-1207-12	100/Pk		
	AF3-3207-52	500/Pk		AF2-5207-52 <sup>1</sup>	500/Pk		AF0-1207-52	500/Pk		
<b>Phenex-GF/CA</b> <sup>2,3,4</sup> (Glass Fiber/Cellulose Acetate)	An integrated syringe filter unit containing an inert borosilicate glass fiber prefilter and a CA membrane. Excellent for filtration of tissue culture media, general biological sample filtration and clarification.							AF0-8A09-12	100/Pk	
								AF0-8A09-52	500/Pk	
<b>1.20 µm</b>										
<b>Phenex-GF</b> <sup>2</sup> (Glass Fiber)	Prefiltration of heavily contaminated or highly viscous samples. When used in-line preceding a membrane filter, clogging of the membrane filter is prevented and sample clean up is optimized.							AF0-8505-12	100/Pk	
								AF0-8505-52	500/Pk	

Above syringe filters are non-sterile. Housing is made of medical-grade polypropylene (PP).

- 17 mm diameter.
- Glass fiber filters are 26 mm diameter and made of borosilicate. They will remove 90 % of all particles >1.2 µm.
- Housing material is methacrylate butadiene styrene (MBS) polymerisate. Also known as Cryolite.
- Cellulose acetate is surfactant-free.
- 26 mm diameter.
- Hydrophobic membrane. Can be made hydrophilic by pre-wetting with IPA.
- Additional dimensions and membrane types are available. Please contact your local Phenomenex technical consultant or distributor for availability or assistance.
- Larger quantity purchases at significant savings are available.

# Supplying Your Laboratory

## Gemini<sup>®</sup>-NX Ordering Information

3 µm, Minibore Columns (mm)				SecurityGuard™ Cartridges
	30 x 2.0	50 x 2.0	150 x 2.0	4 x 2.0 mm*
Phase				
C18	00A-4453-B0	00B-4453-B0	00F-4453-B0	AJ0-8367 for ID: 2.0-3.0 mm

3 µm Analytical Columns (mm)			SecurityGuard™ Cartridges
	50 x 4.6	150 x 4.6	4 x 3.0 mm*
Phase			
C18	00B-4453-E0	00F-4453-E0	AJ0-8368 for ID: 3.0-8.0 mm

5 µm, Minibore Columns (mm)			SecurityGuard™ Cartridges
	50 x 2.0	150 x 2.0	4 x 2.0 mm*
Phase			
C18	00B-4454-B0	00F-4454-B0	AJ0-8367 for ID: 2.0-3.0 mm

5 µm Analytical Columns (mm)					SecurityGuard™ Cartridges
	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0 mm*
Phase					
C18	00B-4454-E0	00D-4454-E0	00F-4454-E0	00G-4454-E0	AJ0-8367 for ID: 3.0-8.0 mm

5 µm Axia™ Packed Preparative Columns (mm)								SecurityGuard™ Cartridges	
	50 x 21.2	100 x 21.2	150 x 21.2	250 x 21.2	50 x 30	75 x 30	100 x 30	15 x 21.2 mm™	15 x 30.0 mm™
Phase									
C18	00B-4454-P0-AX	00D-4454-P0-AX	00F-4454-P0-AX	00G-4454-P0-AX	00B-4454-U0-AX	00C-4454-U0-AX	00D-4454-U0-AX	AJ0-8370	AJ0-8371 for ID: 18-30 mm 30-49 mm

10 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard™ Cartridges	
	50 x 21.2	100 x 21.2	150 x 21.2	250 x 21.2	15 x 21.2 mm™	15 x 30.0 mm™
Phase						
C18	00B-4455-P0-AX	00D-4455-P0-AX	00F-4455-P0-AX	00G-4455-P0-AX	AJ0-8370	AJ0-8371 for ID: 18-30 mm 30-49 mm



If Gemini-NX does not provide at least an equivalent separation as compared to a competing column of similar particle size, similar phase and dimensions, send in your comparative data within 45 days and keep the Gemini-NX column for FREE.

### Material Characteristics:

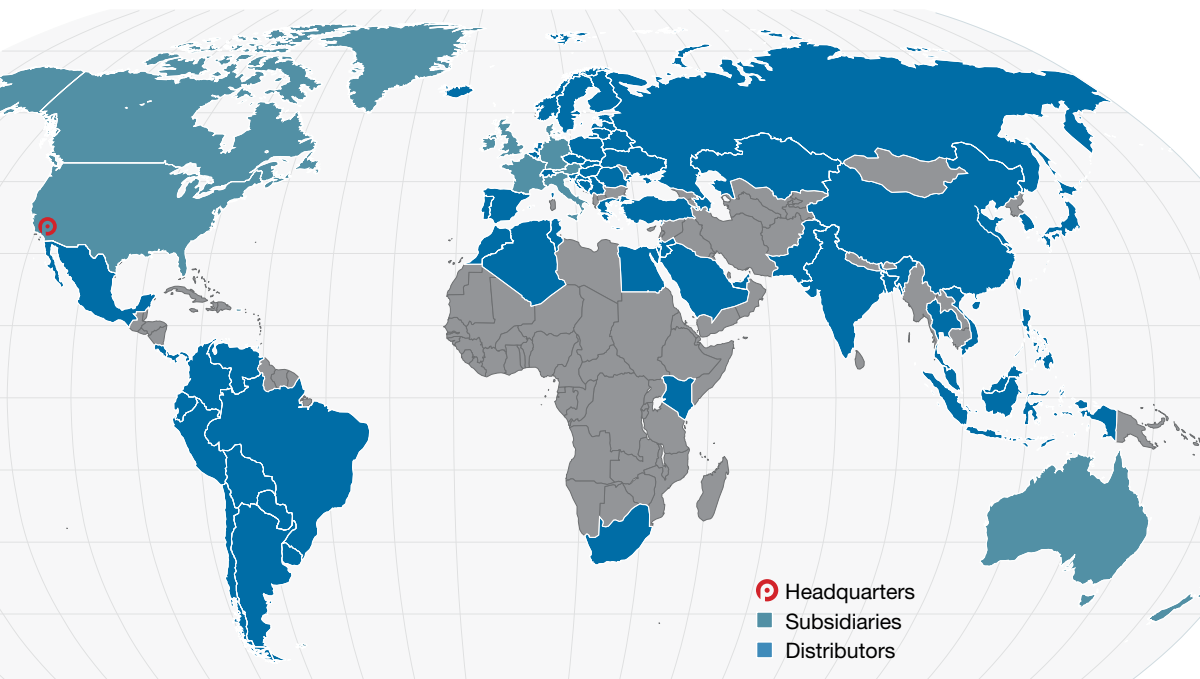
Packing Material	Gemini-NX
Particle Shape/Size (µm)	Spherical 3, 5, 10
Surface Area (m <sup>2</sup> /g)	375
Carbon Load	14 %
End Capping	TMS
Operating pH Range	1-12

\* SecurityGuard Analytical Cartridges Require Holder, Part No.: **KJ0-4282**

\*\* PREP SecurityGuard Cartridges Require Holder, Part No.: **AJ0-8223 or AJ0-8277**

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