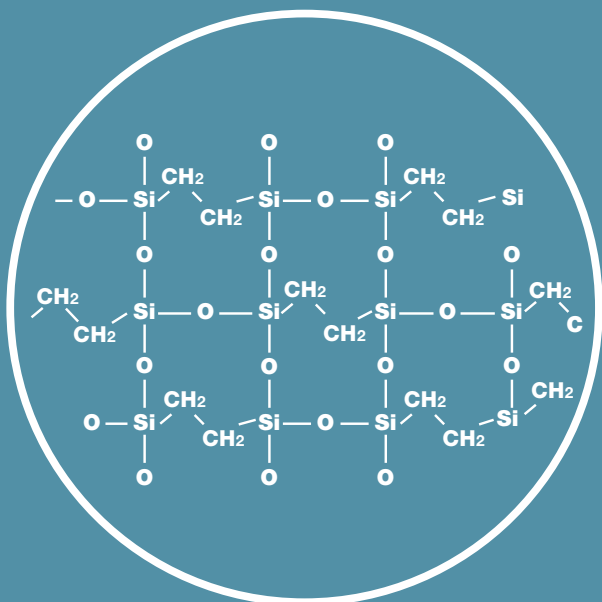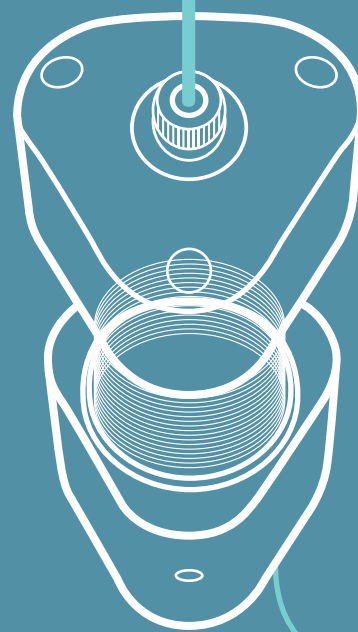# Gemini®-NX

Axia™ Packed Technology Improves Yield, Throughput, and Column Lifetime

Gemini®-NX
pH-LC™

+

AXIA™

Gemini-NX Performance *paired with* Axia Technology

Gemini®-NX Axia™ Packed Technology Improves Yield, Throughput, and Column Lifetime

While reversed phase separation using HPLC remains one of the most efficient ways of obtaining large-scale purification, several limitations can impact overall productivity:

• Column loading capacity

• Media durability

• Column lifetimes

By choosing Gemini-NX in Axia packed preparative columns, these limitations can be overcome; purification yield and throughput can significantly increase.

An Equation for Success

The success begins with the Gemini-NX media. Through a patent-pending process of grafting a network of ethane crosslinking on a pure silica core, a new type of media is created that boasts high column efficiencies, strong hydrophobic selectivity, and a durable pH stability of 1-12.

Axia technology complements the package with a self-contained axial-compression piston that locks the media bed in place and eliminates premature column failure due to bed collapse, resulting in a high efficiency, long lasting preparative column.

Together, these two technologies complete the equation for increasing productivity for preparative purifications.

Increase Sample Loading and Throughput with High pH

- Increase loading capacity
- Sharpen peak shapes
- Increase productivity

Most pharmaceutical compounds are ionizable (either acidic or basic). The ionization state of a compound greatly affects its reversed phase retention. When compounds are ionized, they exhibit less retention. When compounds are neutral, they exhibit greater retention. The ionization state of a compound can be controlled through the mobile phase pH, simply by determining the pK_a and adjusting the pH accordingly:

For bases, adjust mobile phase pH 2 units above the pK_a to neutralize the charge*
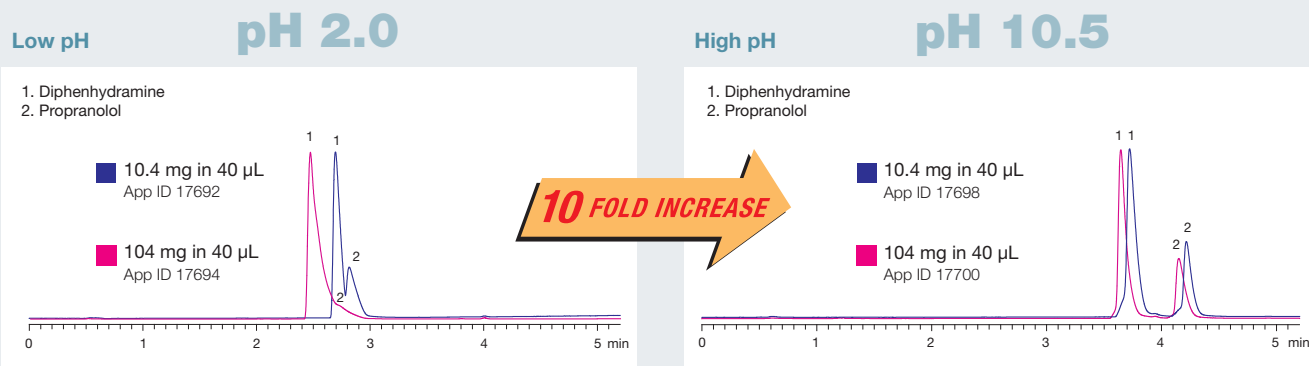
For acids, adjust the mobile phase pH 2 units below the pK_a to neutralize the charge*

Because the compound ionization state significantly impacts its reversed phase selectivity, preparative purifications can be optimized to increase yields.

When the basic compounds shown in the separation below (diphenhydramine; pK_a 8.3 and propranolol; pK_a 9.0) are run under acidic conditions, reversed phase selectivity is minimal, due to the decrease in overall hydrophobicity of the solutes.

By increasing the mobile phase pH two units above their respective pK_a values to 10.5, both bases are neutralized increasing reversed phase selectivity, resulting in greater overall resolution and increased sample loading.

Impact of pH on Selectivity*
using a Gemini®-NX Axia™ packed column

Low pH   pH 2.0

1. Diphenhydramine
2. Propranolol

10.4 mg in 40 µL
App ID 17692

104 mg in 40 µL
App ID 17694

High pH   pH 10.5

1. Diphenhydramine
2. Propranolol

10.4 mg in 40 µL
App ID 17698

104 mg in 40 µL
App ID 17700

10 FOLD INCREASE

At low pH, when the sample mass is increased to 104 mg, the resolution between the compounds is lost since the two compounds elute so close together.

Increasing pH allowed higher mass loading, reducing the number of purification cycles required. The increased resolution at the higher pH also resulted in higher purity and yield for desired basic compounds.

* For more detailed information, request technical note TN-1050, "Impact of pH on the Purity and Yield for Preparative Separations".
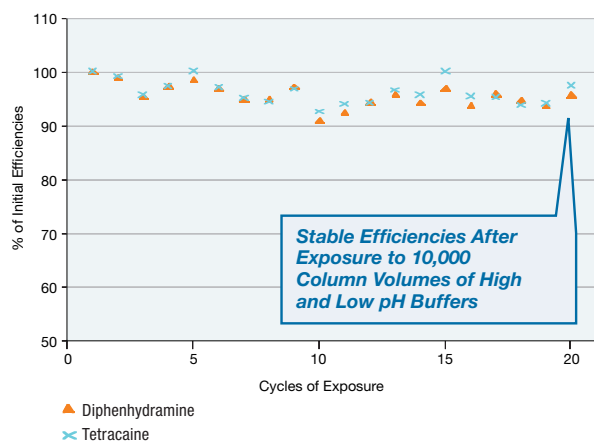
Gemini®-NX Offers Extreme Durability and Robustness in Ever-Changing Environments

In today's demanding environments, columns need to deliver more. Consistent performance and long lifetime in the presence of harsh conditions is a requirement for columns that are used routinely for method development or in open-access systems.
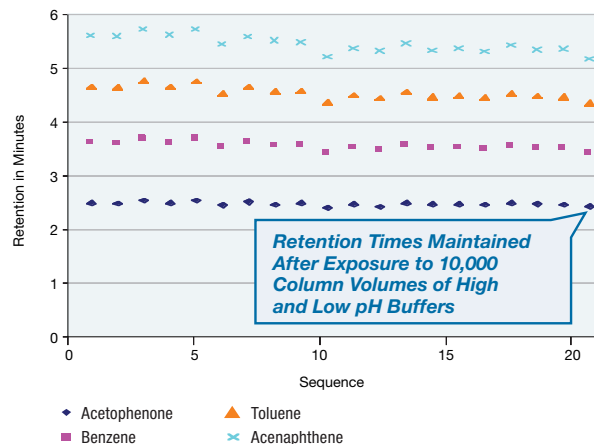
Gemini-NX columns have been subjected to the most extreme conditions (shown below) to demonstrate their place as your number one choice for reversed phase methods, analytical to preparative.

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

Column Efficiencies Maintained in High pH Testing for 20 Cycles

Stable Efficiencies After Exposure to 10,000 Column Volumes of High and Low pH Buffers

▲ Diphenhydramine
✕ Tetracaine

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

Retention Times Maintained After Exposure to 10,000 Column Volumes of High and Low pH Buffers

◆ Acetophenone   ▲ Toluene
■ Benzene   ✕ Acenaphthene

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

Column Testing Cycle

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 min
Hold at 95 % B for 2 min
Re-equilibrate: 5 % B for 2 min
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, pH 2.0
Gradient: 5 % to 95 % B in 6 min
Hold at 95 % B for 2 min
Re-equilibrate: 5 % B for 2 min
Flow Rate: 1.5 mL/min

STEP 6. Neutral pH Flush Repeats

Repeats for 20 Cycles

Over 1000 Cycles with No Loss of Performance

What happens when you combine the durable Gemini®-NX media with the advanced technology of Axia™ packing? You have a preparative column solution that can provide analytical-like efficiencies over extended periods of use. With less frequent column changes, costs per sample decrease and overall productivity increases.

## Gemini-NX Offers Robust Media for Extreme Conditions at pH 10.5 (40 °C)

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

Column:	Gemini-NX 5 µm 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 (95:5) to (5:95) in 7 min, equilibration in 3 min
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

APP ID 17994

CYCLE 1

CYCLE 480

CYCLE 1040
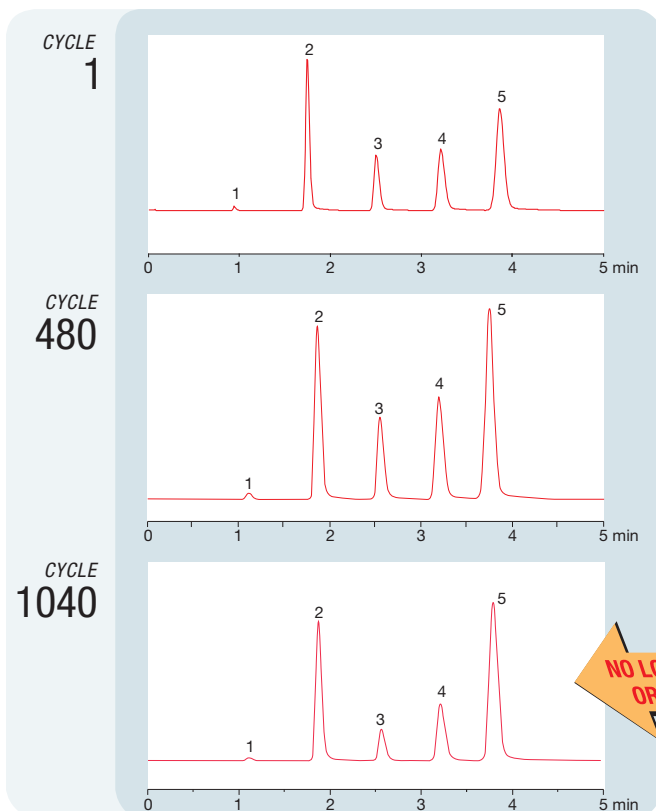
NO LOSS OF RETENTION OR RESOLUTION

Y-axis normalized for all chromatograms.

What is Axia™ Technology? Why Do I Need It?

Axia Technology Benefits:

- Eliminates media collapse for longer lifetimes
- Drastically decreases column variability
- Achieves proper packing density and uniformity

Axia technology is beautiful, both on the outside and on the inside. It's an improved, more repeatable packing process that has been recognized by the scientific community as a unique, innovative technology with numerous benefits.

The Axia packing process provides preparative chromatographers significant improvements in column lifetime, efficiencies, and peak symmetries that are on par with analytical separations. Overall column-to-column consistency is also dramatically improved.

With Axia packed columns, the ideal sorbent density is custom calculated and automated for each chemistry and column size. Computer control of the entire process ensures the proper sorbent density and uniformity for an excellent performing preparative column every time. No other company offers this packing technology.

2006 R&D 100 Award Recipient

We invite you to view the award winning packing process at: www.phenomenex.com/info/Axia

Calculating your scaling factor

Scaling a separation to meet preparative demands requires the redimensioning of chromatographic parameters such as flow rate/column ID/sample load.

When only the **column ID** is changing:

$$SF = (d_2/d_1)^2$$

When both **ID** and **length** are changing:

$$SF = (d_2/d_1)^2 * (L_2/L_1)$$

SF= Scaling factor
d_1= diameter of starting column
d_2= diameter scaling to

L_1= length of starting column
L_2= length you are scaling to

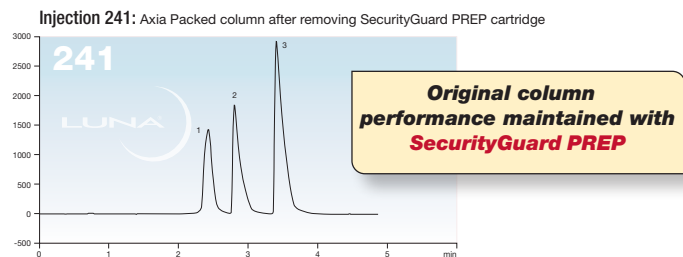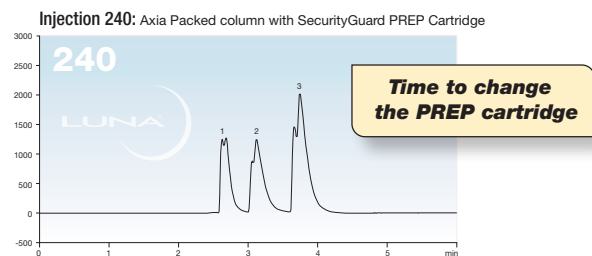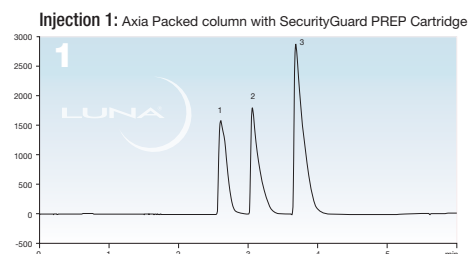| Length (mm)	Internal Diameter (mm)				Sample Load (mg)
	4.6 mm	**21.2 mm**	**30 mm**	**50 mm**	
50 mm	0.5-2	10-45	20-90	60-270	
75 mm	0.75-3	15-70	30-140	90-420	
100 mm	2-6	20-105	40-210	120-630	
150 mm	3-6	30-160	60-320	180-960	
250 mm	2.5-10	50-240	100-480	300-1400	
	1-1.5	20-40	40-80	120-270	
	Flow Rate (typical to high) (mL/min)				

Estimating Sample Load (mg) for 4.6 mm ID column to Axia Preparative Columns (21.2 - 50 mm ID)

Please email us at info@phenomenex.com for further information on large scale preparative and bulk materials.

SecurityGuard™ Extends Column Lifetime

Notice in the study below how the column would have been fouled under the aggressive sample loading at 240 injections. With Security-Guard PREP the inexpensive cartridge was ruined while the integrity of the prep column was maintained. SecurityGuard PREP offers even more injections per column!

Forced Degradation Study APP ID 17993

Injection 1: Axia Packed column with SecurityGuard PREP Cartridge

Injection 240: Axia Packed column with SecurityGuard PREP Cartridge

Time to change the PREP cartridge

Injection 241: Axia Packed column after removing SecurityGuard PREP cartridge

Original column performance maintained with SecurityGuard PREP

Conditions

Column:	Luna® 10 µm C18(2) Axia™ packed
Dimension:	50 x 21.2 mm
Part No.:	00B-4253-P0-AX
	AJ0-8370 (SecurityGuard Cartridge)
Mobile Phase:	A: 0.1 % TFA in Water
	B: 0.1 % TFA in Water/Acetonitrile (25:75)
Gradient:	Linear 93:7 (A/B) to 100 % B over 5 minutes
Injection Volume:	420 µL
Flow Rate:	60 mL/min
Temperature:	Ambient
Detection:	UV@ 270 nm
Sample:	1. Nadolol
	2. Metoprolol
	3. Propranolol

guarantee

If Axia™ packed columns do not provide LONGER LIFETIME when used with SecurityGuard PREP, as compared to a competing column of the same particle size, phase and dimensions, send in your comparative data and the column within 45 days for a FULL REFUND.

Supplying Your Laboratory
Ordering Information

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	100 x 30	150 x 30	250 x 30	15 x 21.2 **	15 x 30.0 ♦
Phase										
C18	00B-4454-P0-AX	00D-4454-P0-AX	00F-4454-P0-AX	00G-4454-P0-AX	00B-4454-U0-AX	00D-4454-U0-AX	00F-4454-U0-AX	00G-4454-U0-AX	AJ0-8370	AJ0-8371
								for ID:	18-29 mm	30-49 mm

10 µm Axia Packed Preparative Columns (mm)							SecurityGuard Cartridges	
	250 x 21.2	250 x 30	50 x 50	100 x 50	150 x 50	250 x 50	15 x 21.2 **	15 x 30.0 ♦
Phase								
C18	00G-4455-P0-AX	00G-4455-U0-AX	00B-4455-V0-AX	00D-4455-V0-AX	00F-4455-V0-AX	00G-4455-V0-AX	AJ0-8370	AJ0-8371
						for ID:	18-29 mm	30-49 mm

** 30-49 mm ID PREP SecurityGuard Cartridges Require Holder, Part No.: **AJ0-8277**

♦ PREP SecurityGuard™ Cartridges require holder, Part No.: **AJ0-8277**

Australia
t: 02-9428-6444
f: 02-9428-6445
   auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
   anfrage@phenomenex.com

Belgium
t: +31 (0)30-2418700
f: +31 (0)30-2383749
   beinfo@phenomenex.com

Canada
t: (800) 543-3681
f: (310) 328-7768
   info@phenomenex.com

Denmark
t: 4824 8048
f: 4810 6265
   dkinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
   franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
   anfrage@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
   eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
   italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
   nlinfo@phenomenex.com

Netherlands
t: 030-2418700
f: 030-2383749
   nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
   nzinfo@phenomenex.com

Puerto Rico
t: (800) 541-HPLC
f: (310) 328-7768
   info@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
   ukinfo@phenomenex.com

All other countries:
Corporate Office USA [⊕]
t: (310) 212-0555
f: (310) 328-7768
   info@phenomenex.com

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