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HPLC Columns and Accessories

Solutions for liquid chromatography



HAMILTON®

Innovation and excellence in chromatography

Hamilton Company has been developing and manufacturing pressure-stable polymeric high performance liquid chromatography (HPLC) columns for nearly 35 years. We are an established name in science whose products are found in most of the world's top chromatography labs. From columns to syringes to septa and more, Hamilton Company offers a full line of off-the-shelf and custom chromatography products for HPLC, gas chromatography (GC) and thin layer chromatography (TLC).

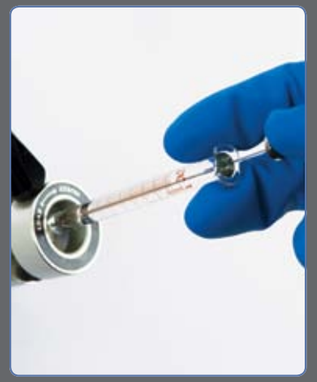
You can trust your results to Hamilton—The Measure of Excellence®





Custom columns built to your exact need

See more on page 48



HPLC syringes for every application

See more on page 53



PRP-C18 columns: Excellent for extreme pH conditions

See more on page 14

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A LEADER IN HPLC

Hamilton: A Leader in HPLC

Founded on the invention of the Microliter™ syringe, Hamilton Company has been designing and innovating the industry's best syringes since 1953—not commercial, mass produced medical syringes, but syringes designed for precision sample measurement in chromatography instrumentation. Hamilton syringes have been trusted by chromatographers for nearly 60 years and for good reason—they are The Measure of Excellence.

Need a custom method? The Hamilton HPLC team is happy to help design your unique application. Give us a call to learn more.

The commitment to chromatography expanded into HPLC columns about 34 years ago. Hamilton was one of the first companies to understand the unique qualities of polymer-based columns and how the technology could advance the field of HPLC. Following significant research and advancements by its team of engineers and scientists, Hamilton's first line of pressure-stable polymeric HPLC columns was created, making it a pioneer in the development and manufacturing of polystyrene-divinylbenzene (PS-DVB) polymers for HPLC applications.

Types of HPLC columns

Hamilton offers 17 different polymer-based HPLC columns for reversed-phase, anion exchange, cation exchange and ion exclusion separations, and two silica-based C8 and C18 columns for traditional reversed-phase separations.

The supports in a polymer-based column combine the inertness and pH stability of polymeric resins with the pressure stability and durability of silica-based materials. With Hamilton Polymeric Reversed-Phase (PRP™) HPLC columns and resins, the sample dictates the necessary separation conditions, not the limitations of the column.

Superior polymeric HPLC columns, resins and applications are a Hamilton specialty.

Nineteen different column packing materials are available for almost any challenging separation, including:

- Reversed-phase
- Anion exchange
- Cation exchange
- Ion exclusion

Specialty resins are available for a variety of difficult separations, including:

- Pharmaceuticals
- Herbicides
- Carbohydrates
- Proteins
- Alcohols

Bulk resins, column hardware and guard columns

Hamilton offers a variety of HPLC accessories to complement its column line.

- Hamilton HPLC column resins are available in bulk from 1 gram to kilogram quantities
- Column hardware in an array of specifications
  - Inside diameters: From 1.0–100 mm
  - Lengths: 30–305 mm
  - Hardware materials: Stainless steel and PEEK (polymer plastic)
- Guard columns are available to match the functionality of the analytical to preparative column sizes
  - Available dimensions: 3.0 x 8.0 mm, 2.0 x 20 mm, 4.6 x 20 mm
  - Available hardware materials: Stainless steel and PEEK

From autosampler syringes to manual injection and more, Hamilton has the chromatography syringe you need. View the full portfolio of HPLC, GC and TLC syringes at www.hamiltoncompany.com/syringes.



Hamilton: Understanding Polymer Supports

What are polymer-based columns, and how are they different than silica?

Silica has its limitations. It requires functionalization for reversed-phase separations and is prone to chemical and pH degradation. Polymer resin manufacturing employs a unique process to produce, but it is this special science that makes them a truly viable and attractive alternative to silica-based materials. Polymer-based columns work comparably to silica-based columns, and, in many applications, perform even better. They can last for years, making them an easy and long-term cost savings alternative.

Basic polymer structure

Polymer columns come in steel or PEEK hardware, just like silica-based columns, but the particles inside are a rigid polymer matrix rather than silica. Styrene (vinyl benzene) readily forms a polymer because the vinyl groups link together to form a chain. Cross-linking within the styrene groups occurs with the addition of divinyl benzene (DVB), which has a second vinyl group (i.e., meta or para to the first one). Cross-linking forms a much stronger and more rigid polymer. For HPLC applications, sufficient DVB is used to give a high density of cross-links thereby creating a more robust polymer support available for HPLC. This reaction is precisely controlled, allowing the formation of small spherical particles with a very narrow particle size distribution (within ± 1 µm).

Advantages of polymers

Hamilton HPLC columns combine the best characteristics of silica-based and polymeric columns to arrive at a column that is highly inert and long-lasting. These characteristics are especially true for difficult analyses that require high pH (8–13), labile or reactive samples (e.g., irreversible adsorption), high aqueous purifications (80–100% water) and separations with ion pairing reagents.

POLYMER SUPPORTS

Alkaline pH stability

Mobile phase pH is a powerful tool in methods development, particularly for separation of neutral forms of amines or other organic bases under alkaline conditions. Although some recent C18 columns boast stability in alkaline pH, all silica-based supports experience measurable degradation at pH > 6, where column life is still considerably shorter than if used under more favorable conditions.

Polymeric columns, on the other hand, have genuine pH and chemical stability. The stationary phase stands up to prolonged exposure to concentrations as high as 1 M NaOH and H_2SO_4, with no measurable decrease in performance. Because the support does not strip, bleed, or dissolve at any pH, it can be expected to perform reliably and reproducibly throughout the extended life of the column, regardless of mobile phase conditions.

Durable, long life polymers

Some polymers are prone to swelling in high organic solvents, rendering higher back pressures, but this is not the case with Hamilton's materials since a high degree of cross-linking prevents this from happening. Hamilton's PS-DVB supports are cross-linked to prevent shrinking or swelling, making them pressure stable up to 5,000 psi. Since the support is entirely polymeric with no silica to deteriorate, typical polymer column lifetime is approximately one year as compared to 3–4 months for an equivalent silica-based C8 or C18 column under routine or extreme method conditions.

Long column life

The highly inert polymeric support resists chemical attack from organic solvents and aqueous buffers (0–100% aqueous or organic; pH 1–13), effectively lengthening column life. If column performance deteriorates (e.g., peak broadening and a loss of symmetry), a regeneration protocol will usually return the chromatography to its original state. Even caustics can be used in extreme column fouling situations to reverse the adverse effects. This durability is especially important since it gives the chromatographer the ability to clean and regenerate a more expensive preparative column without the need to replace it.

Wide application utility

The pH stability of Hamilton's polymer HPLC columns allows samples to be analyzed at basic pH (8–13). Some samples show a dramatic increase in their absorbance characteristics at basic pH. Altering the pH of the separation may not only increase a sample's detectability, it can also radically alter a compound's retention and the elution order of a sample. Changes in elution order can be used to determine sample purity and identity.



Cleaning and more

Polymer columns can be cleaned with 1.0 M sodium hydroxide or 100% organic solvent to remove strongly retained material and can be operated at much higher temperatures as compared to silica-based materials. They provide excellent sample recoveries due to the lack of acidic silanol groups associated with silica-based materials. Swelling in organic solvents such as THF or chloroform is negligible because of cross-linking.

PS-DVB resins are similar in retention characteristics to silica C18 but do have a slightly different selectivity in some cases.

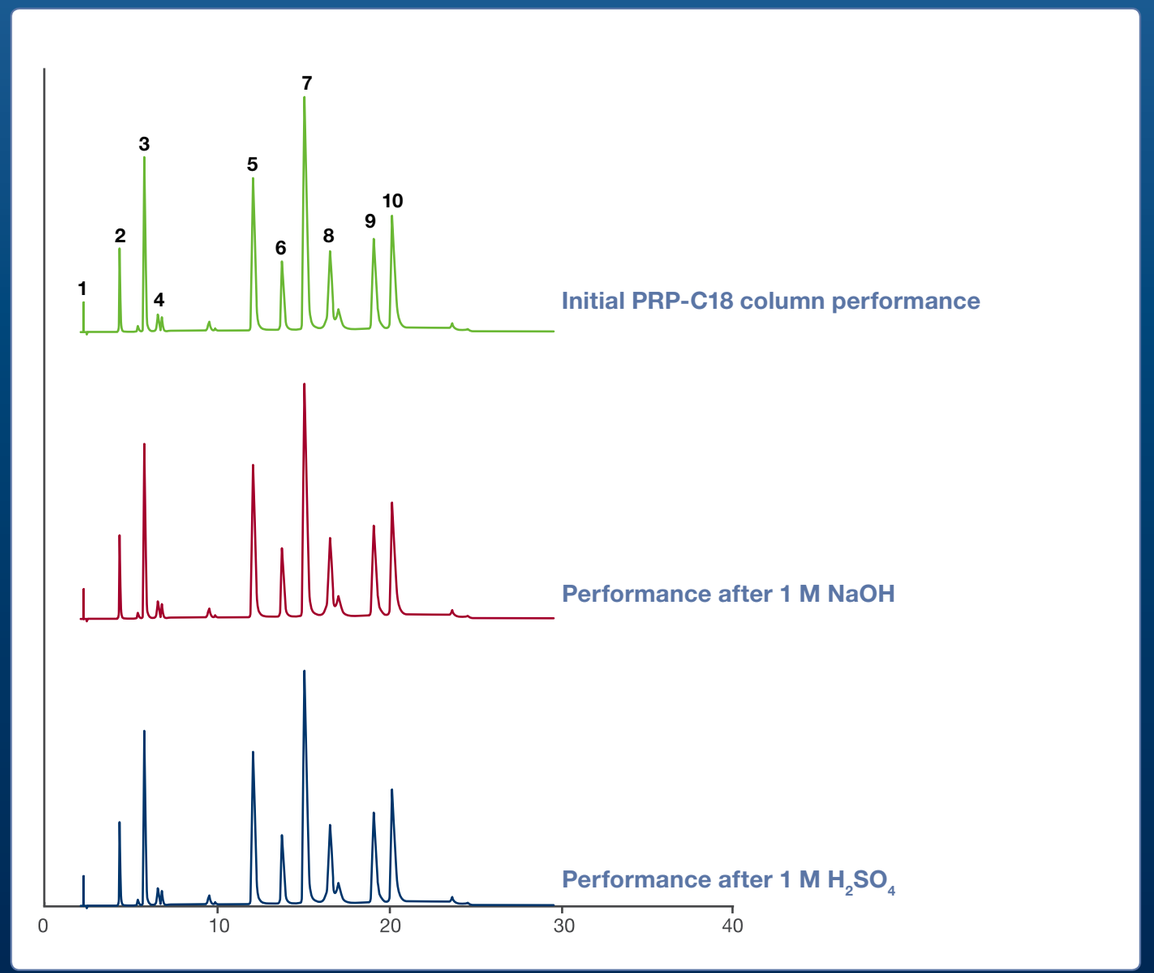


PRP-C18 Performance:
Before and after harsh conditions

To demonstrate the rugged, pH-stability of Hamilton polymeric HPLC columns, a 10-component test mixture was run on a PRP-C18 column to obtain an initial separation. The column was then subjected to 200 column volumes of 1 M sodium hydroxide and then tested once again with the initial conditions to demonstrate that there was no measurable deterioration. After the sodium hydroxide flush, the column was then subjected to 200 column volumes of 1 M sulfuric acid and then tested once again with the initial conditions to demonstrate that there was still no measurable deterioration.

These tests demonstrate that polymer-based columns, unlike silica-based columns, can be freed of otherwise irreversibly bound contaminants under conditions that normally shorten column life.

Hamilton is your partner. From determining the correct column for your application to post-purchase support and troubleshooting, the HPLC team is standing by and ready to help.



HPLC Columns by USP Listing

This guide identifies all Hamilton HPLC columns according to their United States Pharmacopeial Convention (USP) listing.

L17 Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 7 to 11 µm in diameter

- PRP-X200 ..... Page 35
- PRP-X300 ..... Page 45
- HC-75 H^+ ..... Page 41

L19 Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the calcium form, about 9 µm in diameter

- HC-75 Ca^{2+} ..... Page 41
- HC-40 Ca^{2+} ..... Page 41

L21 A rigid, spherical styrene-divinylbenzene copolymer, 3 to 10 µm in diameter

- PRP-C18 ..... Page 14
- PRP-1 ..... Page 17
- PRP-3 ..... Page 19
- PRP-h5 ..... Page 21

L22 A cation exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size

- PRP-X200 ..... Page 35
- PRP-X300 ..... Page 45

L23 An anion exchange resin made of porous polymethacrylate or polyacrylate gel with quaternary ammonium groups, about 10 µm in size

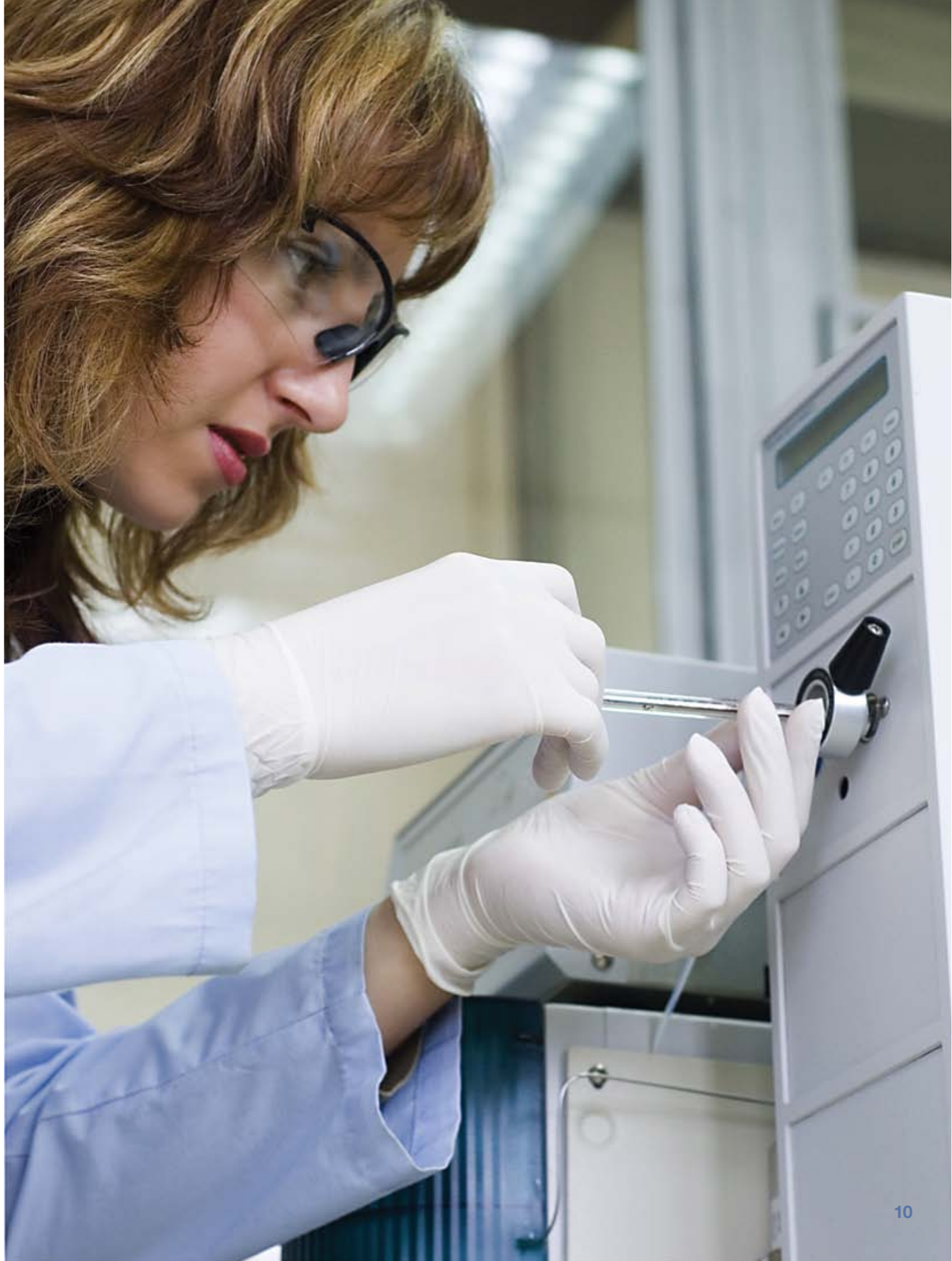
- PRP-X500 ..... Page 27

L34 Strong cation exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the lead form, about 9 µm in diameter

- HC-75 Pb^{2+} ..... Page 41

L47 High capacity anion exchange microporous substrate, fully functionalized with trimethylamine groups, 8 µm in diameter

- PRP-X100 ..... Page 24
- PRP-X110 ..... Page 24
- RCX™-10 ..... Page 31
- RCX-30 ..... Page 31

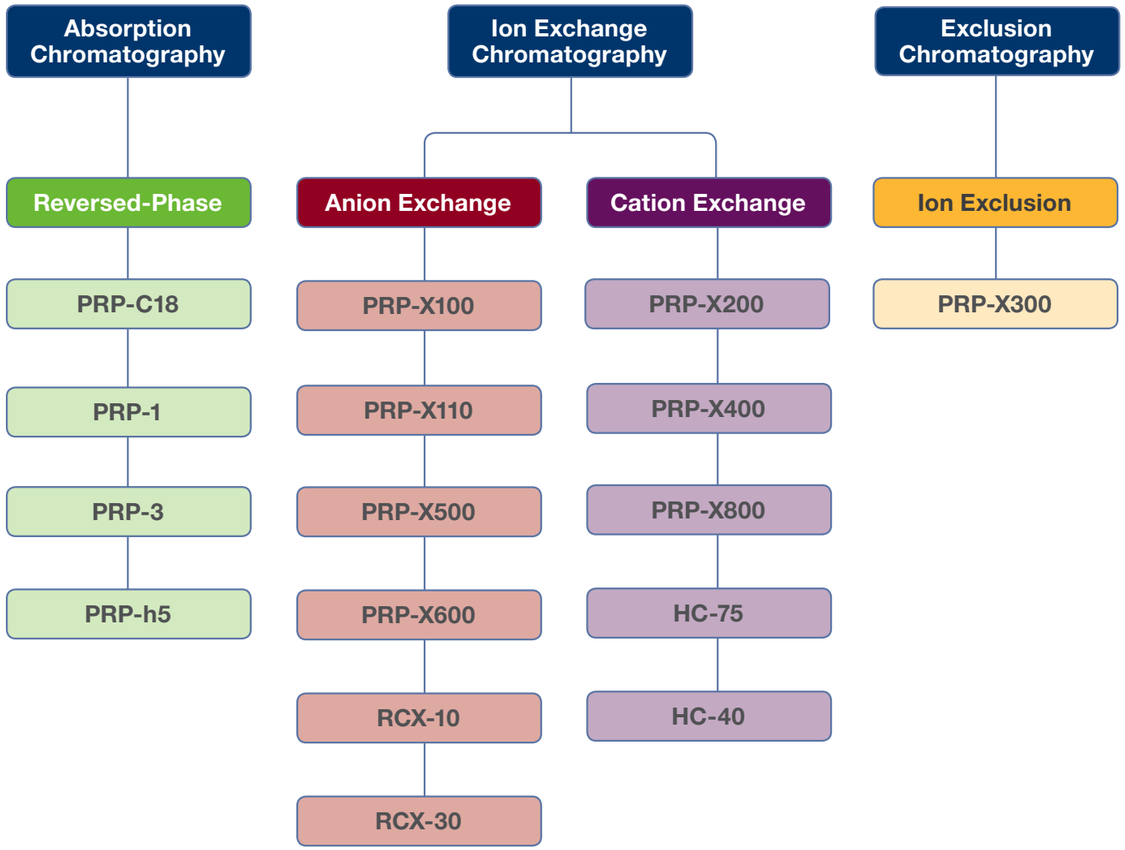


At-a-Glance Product Selection Charts

From molecular weight to separation type to product families, these simple, at-a-glance guides help determine the right Hamilton HPLC column for any need.

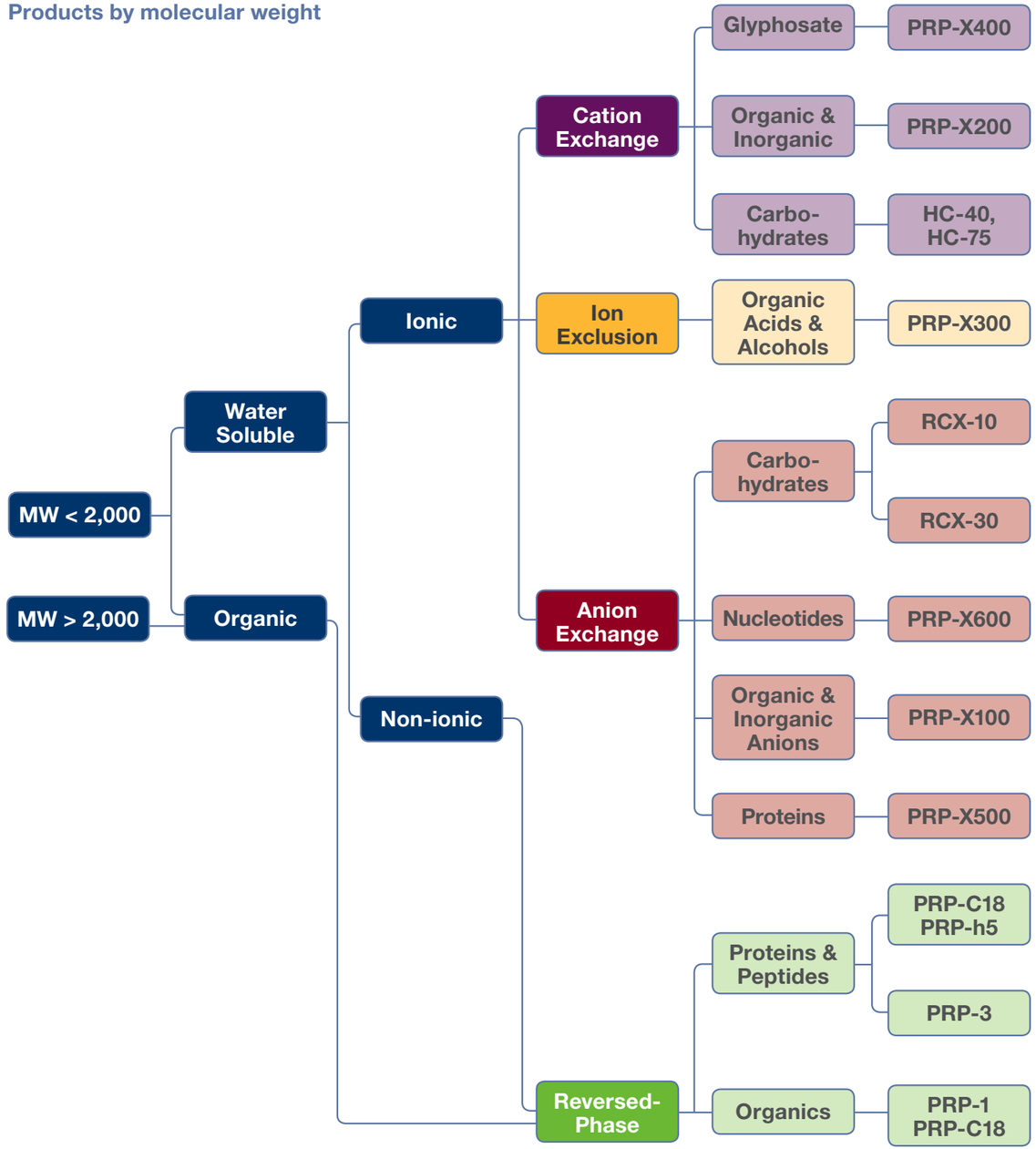
For easy reference, each style of chromatography has a corresponding color that is also present in its product section within the catalog.

Products by separation mechanism



PRODUCT SELECTION CHARTS

Products by molecular weight



Reversed-Phase HPLC Columns

Hamilton reversed-phase HPLC columns combine the best characteristics of silica-based and polymeric columns to arrive at a product that is highly inert and long-lasting. Hamilton offers four polymeric packing materials for reversed-phase separations.

Type	Recommended Application(s)
PRP-C18	Organic compounds: small molecules (< 2,000 mw), pharmaceuticals, steroids, organic halides, vitamins, amino acid analysis, herbicides
PRP-1	Organic compounds: small molecules (< 2,000 mw), pharmaceuticals, steroids, nucleic acids, vitamins, herbicides
PRP-3	Organic compounds: large molecules (> 2,000 mw), peptides, proteins, protein digests, protected and de-protected oligonucleotides, nucleic acids
PRP-h5	Organic compounds: macromolecules (> 2,000 mw), pharmaceuticals, protein digests, tryptic digests

PS-DVB resins are similar in retention characteristics to silica C18s in that retention tends to increase with lipophilicity. However, subtle differences in the chemical interaction between the analyte and stationary phase can result in differential selectivity. In many cases, analytes that co-elute on a silica C18 can be resolved on a polymeric-based support. The PRP-1 consists of a 55% cross-linked PS-DVB bead containing 100 Å pores. The properties of this base material intrinsically lend itself to reversed-phase separations with no further surface modifications. The PRP-C18 uses the PRP-1 as the base support material with the addition of octadecyl to impart characteristics more closely related to a silica-based C18, giving slightly different selectivity than the PRP-1. To make the PRP-3, the PRP-1 is modified so that the base material contains 300 Å pores, which allows for the separation of larger molecules. The PRP-h5 utilizes the PRP-3 as its base with a pentafluorinated modification, making it more hydrophobic in nature.

In the early stages of reversed-phase chromatography, prototype columns were typically a silica bead functionalized with a C18 chain. These columns were revolutionary for their time but are being replaced by modern polymeric supports.

- PS-DVB supports are as retentive as silica C8 and C18 but offer alternate selectivity
- Stable over the full pH range (1–13)
- Compatible with virtually any aqueous, organic mobile phase
- Can be operated at temperatures well over 85°C
- Improved sample recovery compared to silica-based supports

REVERSED-PHASE HPLC COLUMNS

PRP-C18 Columns

High efficiency separations at any pH

Pore size: 100 Å
Material: C18-functionalized PS-DVB

Mobile phase pH is a powerful tool in methods development, particularly for separation of neutral forms of amines or other organic bases under alkaline conditions. Although some recent C18 columns boast stability in alkaline pH, all silica-based supports experience measurable degradation at pH > 6, where column life is still considerably shorter than if used under more favorable conditions.

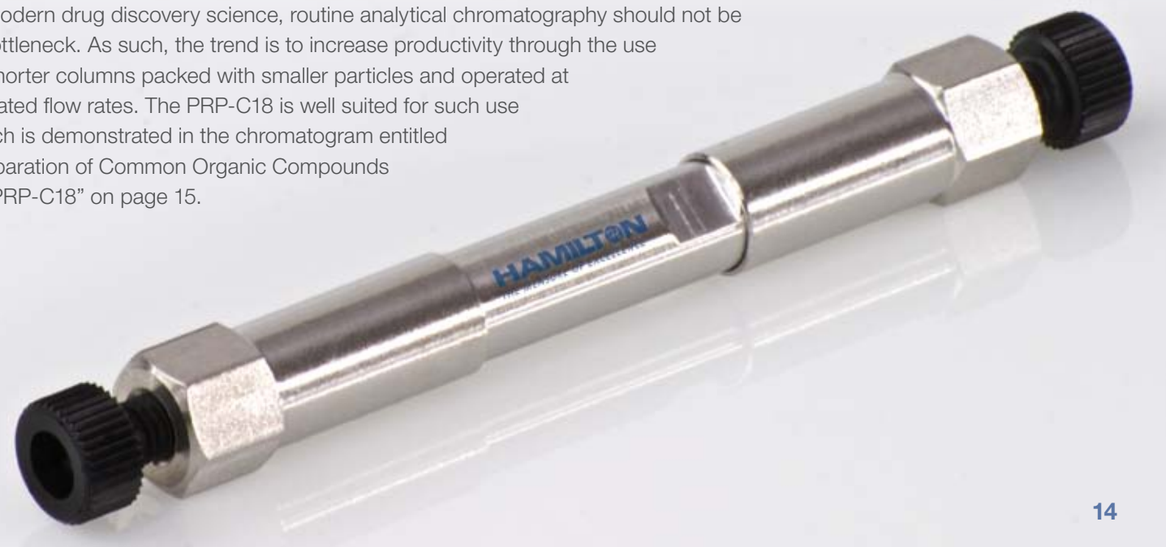
The PRP-C18, on the other hand, has genuine pH and chemical stability. The stationary phase stands up to prolonged exposure to concentrations as high as 1 M NaOH and H_2SO_4, with no measurable decrease in performance. Because the support does not strip, bleed, or dissolve at any pH, it therefore can be expected to perform reliably and reproducibly throughout the extended life of the column, regardless of mobile phase conditions.

High pH applications

More than 70% of all pharmaceutical drug compounds are cationic solutes that carry a formal positive charge below pH 7. Separation of these and other organic bases has historically been problematic. Ionization has a dominating effect in reversed-phase chromatography that tends to dictate retention. Consequently, the elution window for a sample of ionized amines is narrow. The task is further complicated by secondary interactions that occur between positively charged solutes and residual silanols on the column stationary phase. These secondary mechanisms of retention are the principle source for anomalous chromatographic activity, such as poor peak shape, shifts in retention times and loss of efficiency that progressively worsen over the life of the column, as shown in the “Rapid Separation of Basic Drug Compounds on PRP-C18” chromatogram on page 15.

Rapid elution

In modern drug discovery science, routine analytical chromatography should not be a bottleneck. As such, the trend is to increase productivity through the use of shorter columns packed with smaller particles and operated at elevated flow rates. The PRP-C18 is well suited for such use which is demonstrated in the chromatogram entitled “Separation of Common Organic Compounds on PRP-C18” on page 15.

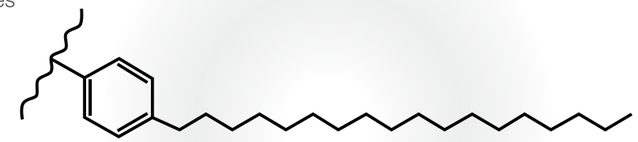


REVERSED-PHASE HPLC COLUMNS

PRP-C18 stationary phase structure and applications

Applications:

Organic compounds: small molecules (< 2,000 mw), pharmaceuticals, steroids, halides, vitamins, amino acid analysis, herbicides

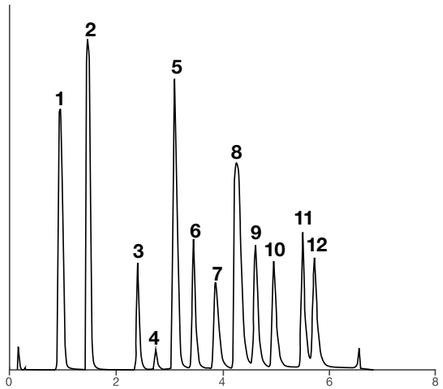


Examples of analytes that can be separated on PRP-C18 columns:

- Peptides
- DNA, RNA oligonucliotides, nucleotides
- Vitamins
- Steroids
- Herbicides
- Pharmaceutical compounds

PRP-C18 application chromatograms

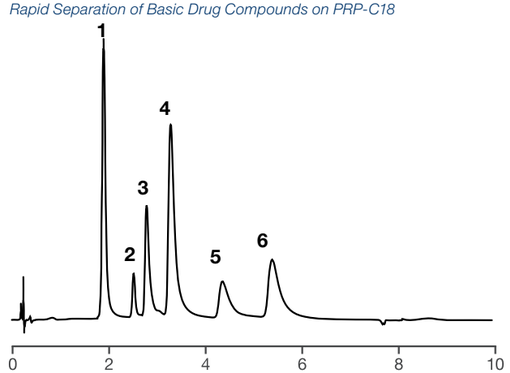
Separation of Common Organic Compounds on PRP-C18



Column: PRP-C18, 4.1 x 50 mm, 5 µm
Part number: 79675
Mobile phase A: Water + 0.2% H_3PO_4
Mobile phase B: Acetonitrile + 0.2% H_3PO_4
Flow rate: 2.5 mL/min
Gradient: 2 to 99% B in 5 minutes
Temperature: Ambient
Injection Volume: 2 µL
Detection: UV at 255 nm

Compounds:
1. Benzamide
2. Nitromethane
3. Nethyl 4-hydroxybenzoate
4. N-ethyl 4-hydroxybenzoate
5. N-propyl 4-hydroxybenzoate
6. N-butyl 4-hydroxybenzoate
7. Benzene
8. Toluene
9. Ethylbenzene
10. Propylbenzene
11. Pentylbenzene
12. Hexylbenzene

Rapid Separation of Basic Drug Compounds on PRP-C18



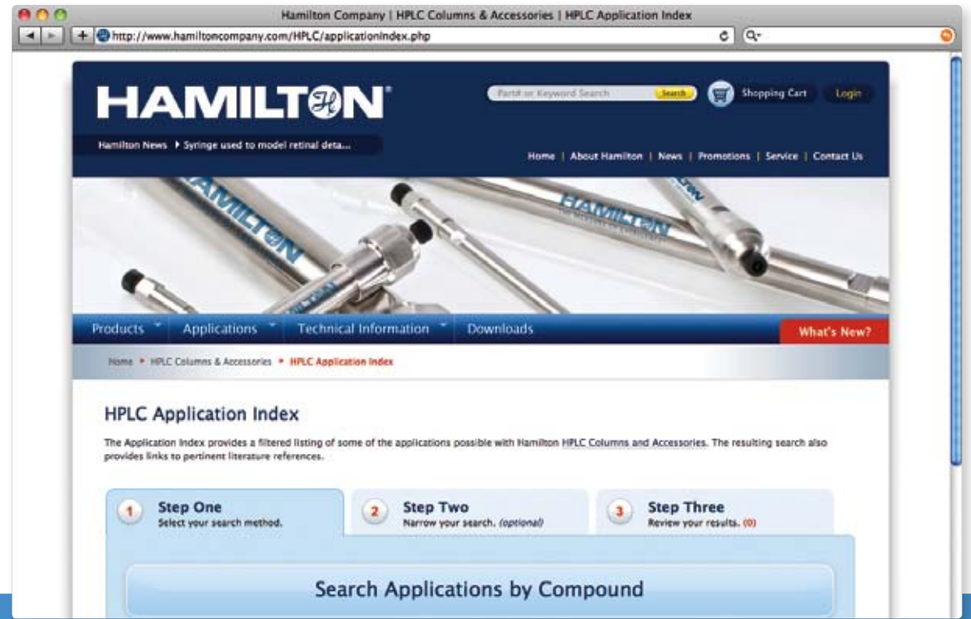
Column: PRP-C18, 4.1 x 50 mm, 5 µm
Instrumentation: Agilent 1100 quaternary pump with UV detector
Standards:
1. Nicotine
2. Metoprolol
3. Quinine
4. Doxylamin
5. Dexmethorphan
6. Amitriptyline

Mobile phase A: 30 mM Diethylamine
Mobile phase B: Mobile phase A + 95% ACN, 5% H2O
Gradient: 10 to 100% B in 5 minutes
Flow rate: 2 mL/min
Temperature: Ambient
Injection volume: 10 µL
Detection: UV at 265 nm

PRP-C18 Column Ordering Information

Dimensions	Particle Size		
	5 µm	10 µm	12–20 µm
2.1 x 50 mm	79672		
2.1 x 50 mm PEEK	79679		
2.1 x 150 mm	79673		
2.1 x 150 mm PEEK	79680		
2.1 x 250 mm	79674		
2.1 x 250 mm PEEK	79681		
4.6 x 50 mm	79675		
4.6 x 50 mm PEEK	79682		
4.6 x 150 mm	79676		
4.6 x 150 mm PEEK	79683		
4.6 x 250 mm	79677		
4.6 x 250 mm PEEK	79684		
21.2 x 250 mm			79678
Bulk Resin (1 gram)	79791	79792	79793

Learn more about PRP-C18 columns at www.hamiltoncompany.com/PRPC18.



View a keyword searchable index of applications possible with Hamilton HPLC columns at www.hamiltoncompany.com/hplcapplicationindex.

PRP-1 Columns

Superior sample recovery

Pore Size: 100 Å
Material: PS-DVB

Sample recovery is vital to sample purification. Problems arise when labile samples become irreversibly bound to the silanol groups present on C8 and C18 HPLC columns. Since Hamilton polymers are made entirely of poly styrene-divinylbenzene, there are no silanol groups to cause sample loss. Recovery and quantitation of labile and reactive samples is enhanced. The purification of protected oligonucleotides demonstrates the enhanced recovery of polymer supports. While approximately 50–80% of an oligonucleotide is recovered on a C18 column, the equivalent PRP-1 column recovers 95% or greater of the same sample.

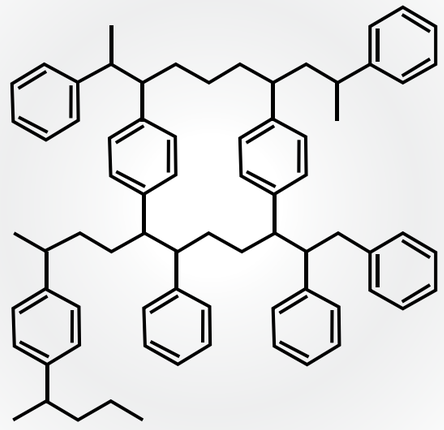
Unlike silica-based C8 or C18 columns, PRP-1 has no stationary phase coating. The integral reversed-phase characteristics of the PRP-1 column eliminate the need for special coating techniques. Since there is no stationary phase to hydrolyze, the column maintains its performance characteristics longer than many C8 or C18 columns.

PRP-1 stationary phase structure and applications

Applications:
Organic compounds: small molecule (< 2,000 mw), pharmaceuticals, steroids, nucleic acids, vitamins, herbicides

Examples of analytes that can be separated on PRP-1 columns:

- Polycyclic aromatic hydrocarbons (PAH)
- Ionizable organic compounds
- Steroids
- Peptide fragments



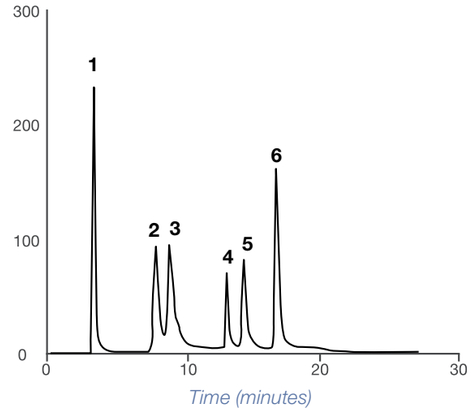
Guard columns are an easy way to prolong an analytical column's life. Refer to page 49 for more information on how guard columns protect your investment.



REVERSED-PHASE HPLC COLUMNS

PRP-1 application chromatograms

Separation of Adenine and Guanine Nucleotides on PRP-1



Column: PRP-1, 5 µm, 2.1 x 150 mm
Part number: 79366
Mobile phase A: 100 mM Monopotassium phosphate (adjust pH to 7 with potassium hydroxide), 1 mM tetrabutylammmonium phosphate, 2.5% methanol
Mobile phase B: Mobile Phase A + 20% methanol
Flow rate: 300 µL/min
Gradient:

Time (min)	%B
0–3	1
10	15
15	55
16–19	95

Injection volume: 5 µL
Sample concentration: 0.02 mM
Temperature: 50°C
Detection: UV at 254 nm

Compounds:
1. Guanosine monophosphate
2. Guanosine diphosphate
3. Adenosine monophosphate
4. Guanosine triphosphate
5. Adenosine diphosphate
6. Adenosine triphosphate

PRP-1 Column Ordering Information

Hardware Dimensions	Particle Size					
	5 µm	7 µm	10 µm	12–20 µm	30–50 µm	50–75 µm
1.0 x 50 mm		79755				
1.0 x 150 mm	79753					
2.1 x 100 mm	79790					
2.1 x 150 mm	79366					
4.1 x 50 mm	79443					
4.1 x 100 mm	79479					
4.1 x 150 mm	79444	79529	79425			
4.1 x 250 mm	79820	79422	79427			
4.6 x 100 mm PEEK	79558					
4.6 x 150 mm PEEK	79423		79351			
4.6 x 250 mm PEEK	79571	79380	79381			
7.0 x 100 mm			79495			
7.0 x 305 mm	79795		79426			
10.0 x 50 mm		79367				
10 x 100 mm	79355		79499			
10 x 250 mm		79531	79496			
21.2 x 75 mm	79154					
21.2 x 250 mm		79352	79478	79428		
30 x 250 mm				79229		
50 x 250 mm			79567	79493		
101.6 x 250 mm				79525		
101.6 x 250 mm Repack				79800		
Bulk Resin (1 Gram)	79578	79579	79580	79581	79582	79583

PRP-3 Columns

Reversed-phase column optimized for separation of macromolecules such as DNA, RNA oligos, proteins, peptides and proteomics

Pore size: 300 Å
Material: PS-DVB

Hamilton's PRP-3 is a polymeric reversed-phase HPLC column designed for the purification and isolation of proteins and peptides with very good recovery (> 90%). It is based off of the PRP-1 but utilizes a 300 Å pore size rather than the PRP-1's 100 Å pore size. The highly inert polymeric packing poly(styrene-divinylbenzene) enhances protein recovery because there are no silanol groups on the support to cause irreversible protein adsorption.

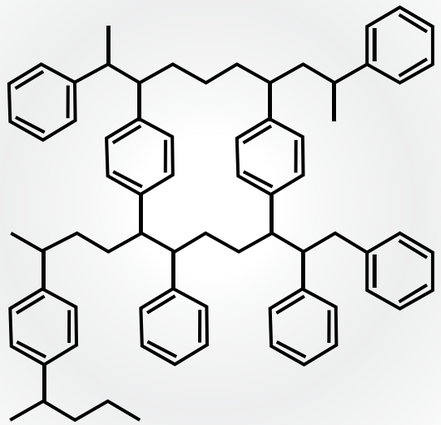
PRP-3 is a PS-DVB support that is pressure stable up to 5,000 psi and cross-linked to prevent shrinking or swelling when the mobile phase is changed. Chemically, proteins present solubility problems unlike many small molecules. Most proteins are hydrophobic on the inside, with highly charged exteriors. This often presents dissolution problems, particularly when pH is near the isoelectric point of the protein. The rugged chemical nature of the PRP-3 allows the protein chemist a much broader selection of agents for dissolution, including concentrated acids, aggressive chaotropes, as well as detergents. In proteomics, the PRP-3 has excellent potential for single-column 2D HPLC. The orthogonal selectivity between low and high pH separations is often comparable to that achieved with two different column formats (SCX, RP), but with the added bonus of MS-compatible mobile phase.

PRP-3 stationary phase structure and applications

Applications:
Organic compounds: large molecules (> 2,000 mw), peptides, proteins, protein digests, protected and de-protected oligonucleotides, nucleic acids

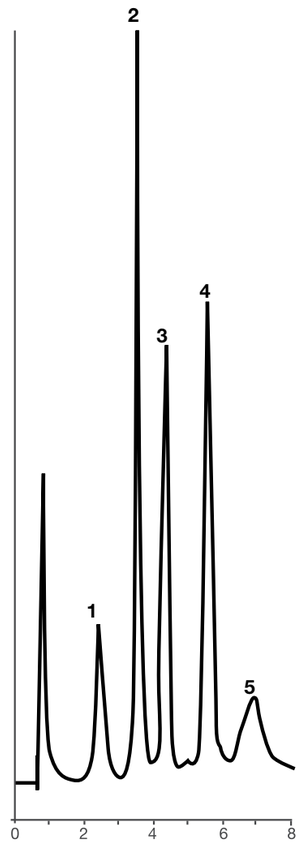
Examples of analytes that can be separated on PRP-3 columns:

- Globular proteins
- Albumins
- Antibody fragments
- Tryptic digests
- DNA
- RNA oligomers
- Synthetic high mw polymers



PRP-3 application chromatograms

Five Proteins on PRP-3



Time (minutes)

Column: PRP-3, 4.1 x 150 mm, 10 µm
Part number: 79466
Standards:
1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
4. Myoglobin
5. Ovalbumin
Mobile phase A: 0.01% TFA in water pH 2.0
Mobile phase B: 0.1% TFA in acetonitrile
Gradient: 25 to 50% B in 5 min. Hold 3 min.
Flow rate: 2 mL/min
Temperature: Ambient
Injection volume: 100 µL
Detection: UV at 215 nm

PRP-3 Column Ordering Information

Hardware Dimensions	Particle Size	
	10 µm	12–20 µm
2.1 x 150 mm	79392	
4.1 x 150 mm	79466	
4.6 x 150 mm PEEK	79382	
4.6 x 250 mm PEEK	79574	
7.0 x 305 mm	79468	
10 x 250 mm	79526	
21.2 x 250 mm	79147	
21.2 x 100 mm		79186
21.2 x 250 mm		79469
Bulk Resin (1 gram)	79701	79702

A selection of column hardware sizes is available from analytical to semi-prep and preparative. Sample scale up is easy because the PRP-3 packing is consistent from analytical to preparative columns. This saves time and eliminates the need to redevelop separations on semi-prep or preparative columns. The short analytical (50 mm) column is well-suited for the gradient elution of high molecular weight proteins and the longer (150 mm) column is best for smaller proteins.

Custom HPLC columns are available! From dimensions to particle size to packing materials, Hamilton can build you exactly what you need. *See page 48 for more information.*



PRP-h5 Columns

Pentafluoro reversed-phase column for unique selectivity

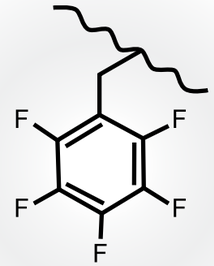
Pore size: 300 Å
Material: Pentafluorinated PS-DVB

The PRP-h5 utilizes the PRP-3 as its base with a pentafluorinated modification, making it more hydrophobic in nature. The PRP-h5, with its functionality derived from a pentafluorinated polymer bead, delivers a selectivity difference from standard silica C18 stationary phases. This gives chromatographers the desired retention characteristics necessary for certain sample types. This difference is especially pronounced for halogenated solutes.

Unlike silica-based C8 or C18 columns, PRP-h5 has no stationary phase coating. Since there is no stationary phase to hydrolyze, the column maintains its performance characteristics longer than many silica-based C8 or C18 columns.

PRP-h5 stationary phase structure and applications

Applications:
Organic compounds: macromolecules (> 2,000 mw), pharmaceuticals, protein digests, tryptic digests, proteomics

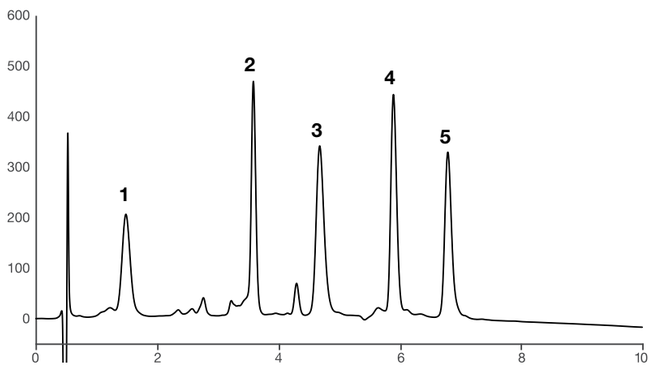


Examples of analytes that can be separated on PRP-h5 columns:

- Oligonucleotides
- Angiotensin
- apo-Transferrin
- Apomyoglobin (equine)
- Carbonic anhydrase
- Cytochrome C
- Myoglobin
- Ribonuclease A

PRP-h5 application chromatograms

Five Proteins at 80°C on PRP-h5



Column: PRP-h5, 4.6 x 50 mm, 5 µm
Part number: 79261
Standards:
1. Ribonuclease A
2. Cytochrome C
3. apo-Transferrin
4. Myoglobin
5. Carbonic Anhydrase
Mobile phase A: DI Water, 0.1% TFA
Mobile phase B: Acetonitrile, 0.1% TFA
Gradient: 22 to 55% B in 10 min.
Flow rate: 1 mL/min
Temperature: 80°C
Injection volume: 15 µL
Detection: UV at 210 nm

PRP-h5 Column Ordering Information

Hardware Dimensions	Particle Size 5 µm	12–20 µm
2.1 x 100 mm	79270	
2.1 x 150 mm	79271	
4.6 x 50 mm	79261	
4.6 x 100 mm	79262	
4.6 x 150 mm	79272	
4.6 x 250 mm	79273	
10 x 100 mm	79263	
10 x 150 mm	79274	
Bulk Resin (1 gram)	79269	79280

Hamilton is your partner. From determining the correct column for your application to post-purchase support and troubleshooting, the HPLC team is standing by and ready to help.



Anion Exchange HPLC Columns

Hamilton offers six polymeric packing materials for anion exchange separations.

Type	Recommended Application(s)
PRP-X100 **PRP-X110**	Organic and inorganic anions, organic acids, organic and inorganic arsenic species
PRP-X500	Nucleic acids: single stranded/double stranded RNA and DNA Peptides and Proteins
PRP-X600	Adjustable exchange capacity by pH Nucleic acids: single stranded/double stranded RNA and DNA Peptides and Proteins
RCX-10	Carbohydrates, polysaccharides, sugar oligomers up to DP8
RCX-30	Mono and disaccharides

In anion exchange chromatography, the stationary bed has an ionically positive (+) charged surface while the sample ions are of negative (-) charge. This technique is used almost exclusively with ionic or ionizable samples. The stronger the negative charge on the sample, the stronger it will be attracted to the positive charge on the stationary phase, and thus the longer it will take to elute. Elution in ion chromatography is effected by mobile phase pH and ionic-strength, and, to a lesser extent, operation temperature. The ability to use the full pH range and elevated temperatures are distinct advantages compared to silica-based supports.



ANION EXCHANGE HPLC COLUMNS

PRP-X100 and PRP-X110 Columns

Reliable separations of organic and inorganic anions

Pore Size: 100 Å
Material: PS-DVB/Trimethyl ammonium exchanger
PRP-X100 Exchange Capacity: 0.19 meq/gm
PRP-X110 Exchange Capacity: 0.11 meq/gm

Hamilton PRP-X100 and PRP-X110 are highly stable, inert materials. The PRP-X100 can be used with virtually any HPLC or ion chromatograph, including dedicated IC units. Technological advancements in modern polymer chemistry now deliver a more rugged column with exceptionally higher separation efficiencies than earlier predecessors. PRP-X100 and PRP-X110 columns are well suited for use in systems employing suppressed/non-suppressed conductivity, electrochemical, UV, and ICP-MS detection. Chromatographers currently using wet chemical or colorimetric methods will find ion chromatography greatly reduces sample pretreatment and improves the accuracy and precision of results.

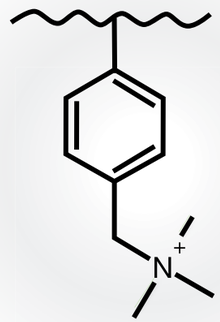
PRP-X100 columns easily separate difficult anions such as cyanide, borate and silicate at high pH (11.5). The polymeric packing is stable from pH 1 to 13, so a single column can be used for the analysis of both common and difficult anions. The PRP-X100 is compatible with many different mobile phases for suppressed and non-suppressed conductivity and direct and indirect UV detection.

For high sensitivity, Hamilton PRP-X110 ion chromatography columns are used to separate ions at concentrations from less than 20 ppb to 20 ppm. The PRP-X110 has similar selectivity to the PRP-X100 but provides lower limits of detection as a result of its lower exchange capacity. The ion exchange capacity of a stationary phase plays a significant role in determining the concentrations of competing ions used in the mobile phase for elution. Lower capacity stationary phases generally require the use of weaker mobile phase eluent to affect elution due to the lower exchange capacity, thus improving the signal to noise. This is especially true when using conductivity detectors which do not function well with high salt eluents.

PRP-X110 columns can be used in the suppressed conductivity mode for determination of inorganic anions as required in EPA 300.0 Part A (fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulfate). The PRP-X110 is a versatile column since it can be used with many different mobile phases such as carbonate, potassium hydroxide, benzoic acid, potassium hydrogen phthalate, etc. for suppressed and non-suppressed conductivity and direct and indirect UV detection.

Stationary phase structure, applications and industries

PRP-X100 and PRP-X110 applications:
Organic and inorganic anions, organic acids, organic and inorganic arsenic species



PRP-X100 and PRP-X110 columns are used to monitor ions for a variety of industries including:

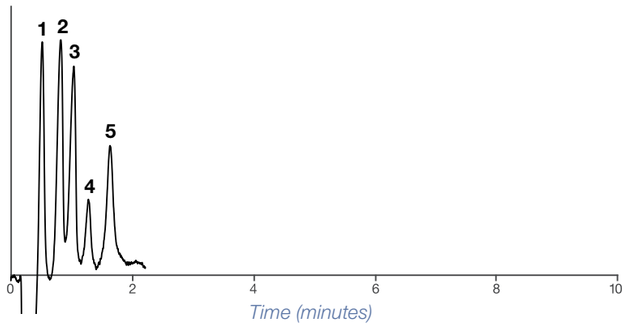
- **Pharmaceutical—** Lactate, acetate, chloride and phosphate in intravenous solution
- **Medical Research—** Monitoring analytes in bodily fluids of patients
- **Environmental—** Common anions in ground and river water, EPA 300.0 Part A
- **Food and Beverage—** Arsenic in food and water, phosphate in soft drinks and nitrates in food

Examples of analytes that can be separated on PRP-X100 and PRP-X110 anion exchange columns:

- Halides (fluoride, chloride, etc.)
- Polarizable anions (perchlorate, thiocyanate)
- Organic acids, nucleotides, carboxylic acids (pyruvate, acetate, citrate, etc.)
- Organic and inorganic arsenic species

PRP-X100 application chromatograms

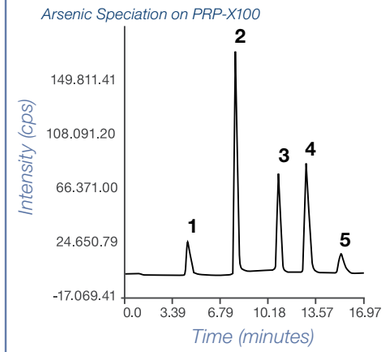
Fast Separation of Common Anions on PRP-X100



Column: PRP-X100, 5 µm, 2.1 x 50 mm
Part number: 79150
Mobile phase: 4 mM para-hydroxybenzoic acid, pH 8.5 / 4% acetonitrile
Flow rate: 0.8 mL/min
Gradient: Isocratic
Temperature: 80°C
Injection volume: 1 µL
Sample concentration: 0.2 mg/mL
Detection: UV at 305 nm

Compounds:
1. Fluoride
2. Chloride
3. Nitrite
4. Bromide
5. Nitrate

Arsenic Speciation on PRP-X100



Column: Hamilton PRP-X100, 5 µm, 4.6 x 250 mm
Part number: 79181
Flow rate: 1.0 mL/min
Mobile phase: 2 mM $(NH_4)_2CO_3$ for 0–3 min
40 mM $(NH_4)_2CO_3$ for 3–14 min
2 mM $(NH_4)_2CO_3$ for 13–17 min
Injection volume: 50 µL, 100 µg/L of each standard
Detection: ICP-MS

Compounds:
1. Trivalent arsenic
2. Dimethyl arsenic
3. Monomethyl arsenic
4. Pentavalent arsenic
5. Monomethylthioarsonic acid

Borrowed (with permission) from P. Alava *et al.* Biomed. Chromatogr. 2012; 26: 524—533

ANION EXCHANGE HPLC COLUMNS

PRP-X100 and PRP-X110 Column Ordering Information

Hardware Dimensions	PRP-X100			PRP-X110	PRP-X110S
	Particle Size				
	5 µm	10 µm	12–20 µm	7 µm	7 µm
2.1 x 100 mm PEEK				79743	
2.1 x 150 mm PEEK	79852				
2.1 x 150 mm		79421			
2.1 x 250 mm	79190	79346			
4.1 x 50 mm	79810	79365			
4.1 x 100 mm	79538	79439			
4.1 x 150 mm	79812	79434		79732	79733
4.1 x 250 mm		79433		79734	79735
4.6 x 150 mm PEEK	79174	79354		79738	
4.6 x 250 mm PEEK	79181	79455		79741	
100 Å 21.2 x 250 mm			79353		
Bulk Resin (1 Gram)	79584	79585	79586	79827	

PRP-X100 Eluent Concentrate Ordering Information

Description	Part Number
Eluent Concentrate, PRP-X100 Anion Exchange (One 60 mL bottle)	79325
Eluent Concentrate, PRP-X100 Anion Exchange (Six 60 mL bottles)	79335

For a full list of all Hamilton HPLC products or for more detailed information, visit www.hamiltoncompany.com/HPLC.

The difference between PRP-X110 versus PRP-X110S columns

PRP-X110 columns are equilibrated with a 2 mM p-hydroxybenzoic acid pH 9.3 mobile phase and are ready for use with conductivity or indirect UV detection methods.

PRP-X110S columns are equilibrated with a 1. 7 mM sodium bicarbonate, 1.8 mM sodium carbonate, 0.1 mM sodium thiocyanate mobile phase and are ready for use with suppressed conductivity detection methods.



PRP-X500 Columns

Fast separations and good sample recovery

Pore Size: Superficially porous
Material: Methacrylamido propyl trimethyl ammonium chloride (SAX)
Exchange Capacity: 1.6 meq/gm

PRP-X500 is a superficially porous polymeric anion exchange column designed for the separation, purification and isolation of proteins, peptides and DNA/RNA. The methacrylate polymeric coating of the PRP-X500 provides a more hydrophilic surface, preventing hydrophobic interaction sample losses typically seen on other commercially available protein HPLC columns.

The non-porous nature of the packing material improves mass-transfer, shortening run times and improving resolution. Both fast separations and good sample capacity are achievable with PRP-X500 columns.

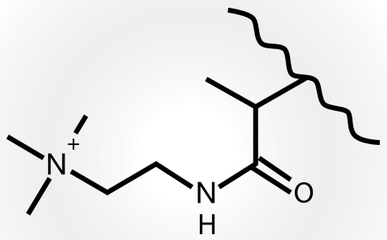
A separation of four protein standards at 0.2 mg in less than three minutes is possible with a short analytical 50 x 4.6 mm HPLC column. Recovery of sample is excellent with PRP-X500 and the support's limited permeability prevents proteins from entering the pores and unfolding, which causes peak ghosting. The superficially porous properties shorten the diffusion path of the analyte, resulting in sharp sample bands.

PRP-X500 stationary phase structure and applications

Applications:
Proteins/Peptides
Single Stranded/Double Stranded RNA/DNA

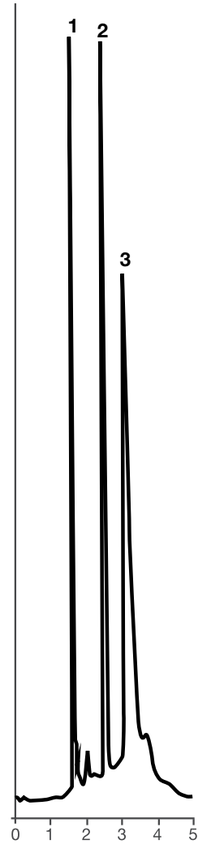
Examples of analytes that can be separated on PRP-X500 columns:

- Myoglobin
- Conalbumin
- Bovine serum albumin
- Ovalbumin



PRP-X500 application chromatograms

Myoglobin, Conalbumin and Dog Albumin on PRP-X500



Time (minutes)

Column: PRP-X500, 4.6 x 50 mm, 5 µm
Part number: 79474
Mobile phase A: 10 mM Tris pH 9.0
Mobile phase B: 70 mM Tris pH 9.0, 0.5 N Sodium Chloride
Flow rate: 2 mL/min
Gradient: 0 to 50% B in 2.5 minutes. Hold for 2.5 min.
Temperature: Ambient
Injection volume: 30 µL
Detection: UV at 254 nm

Compounds:
1. Myoglobin 7 µg
2. Conalbumin 7 µg
3. Dog albumin 77 µg

PRP-X500 Column Ordering Information

Dimensions	Particle Size		
	7 µm	12–20 µm	30–50 µm
4.6 x 150 mm, PEEK	79573		
Bulk Resin (1 gram)	79594	79595	79596

Guard columns are an easy way to prolong an analytical column's life. Refer to page 49 for more information on how guard columns protect your investment.



ANION EXCHANGE HPLC COLUMNS



PRP-X600 Columns

Separation of nucleic acids

Pore Size: Superficially porous
Material: Poly (dimethylamidopropylmethacrylamide)
Exchange Capacity: 1.6 meq/gm

PRP-X600 is a superficially porous weak-base anion exchange support that separates DNA oligomers according to negative charge. The unique porosity provides fast separation with better sample capacity than non-porous supports. The superficially porous properties shorten the diffusion path of the analyte, resulting in sharp sample bands. The PRP-X600, a hydrophilic methacrylate-based polymer, improves sample recovery due to minimized hydrophobic interactions.

Because the PRP-X600 is a weak anion exchange (WAX) resin, the exchange capacity of the resin is pH dependent. Lowering the pH will reduce the binding of proteins, reducing the run time for a complete separation.

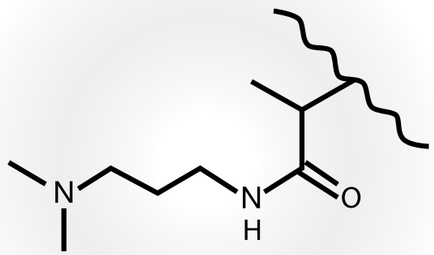
Change the mobile phase composition to alter the retention of DNA oligomers. Rapid gradient changes typically lower column efficiency; however, biomolecules run in this fashion on the PRP-X500 show very favorable separation efficiency with much shorter run times.

PRP-X600 stationary phase structure and applications

Applications:
Nucleic acids such as single stranded/double stranded RNA and DNA Peptides and proteins

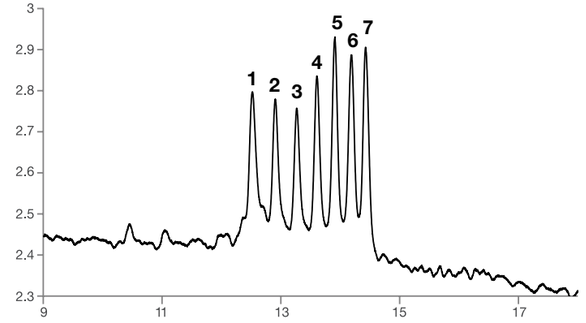
Example of analytes that can be separated on the PRP-X600:

- Synthetic RNA, DNA oligonucleotides
- Proteins and peptides
- Ovalbumin
- DNA fragments
- Oligonucleotides



ANION EXCHANGE HPLC COLUMNS

PRP-X600 application chromatograms

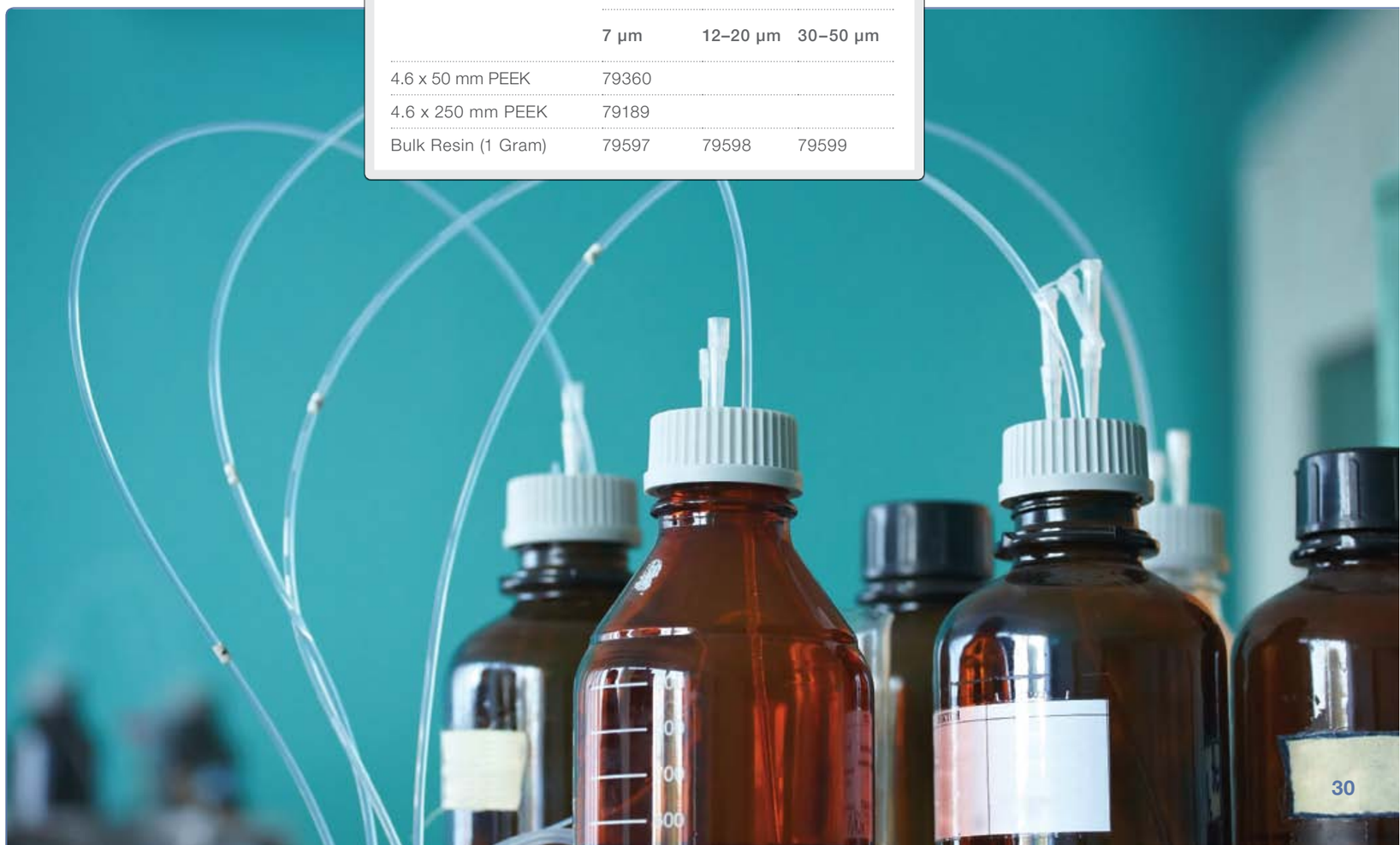


Column: PRP-X600, 7 µm, 4.6 x 50 mm
Part number: 79360
Mobile phase A: 85/15 100 mM TRIS, pH 8.0/acetonitrile
Mobile phase B: 85/15 100 mM TRIS, pH 8.0, 2.5 M lithium chloride/acetonitrile
Flow rate: 2.0 mL/min
Gradient: 0 to 40% B in 40 minutes
Temperature: Ambient
Injection volume: 10 µL
Sample concentration: 300 µg/mL
Detection: UV at 260 nm

Compounds (from oligodeoxycytidylate $(dC)_{12-18}$)
1. dC12
2. dC13
3. dC14
4. dC15
5. dC16
6. dC17
7. dC18

PRP-X600 Column Ordering Information

Dimensions	Particle Size		
	7 µm	12–20 µm	30–50 µm
4.6 x 50 mm PEEK	79360		
4.6 x 250 mm PEEK	79189		
Bulk Resin (1 Gram)	79597	79598	79599



RCX-10 and RCX-30 Columns

Designed for the isocratic or gradient separation of carbohydrates

Pore Size: 100 Å
Material: PS-DVB/Trimethyl ammonium exchanger
RCX-10 Exchange Capacity: 0.35 meq/gm
RCX-10 Exchange Capacity: 1.0 meq/gm

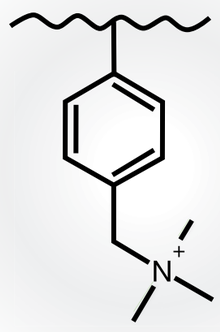
The Hamilton RCX-10 and RCX-30 carbohydrate analysis columns are designed for the isocratic or gradient separation of carbohydrates. The exchange capacity of the RCX-10/RCX-30 is greater than that of the PRP-X100, leading to characteristics better suited for the separation of carbohydrates. Simple samples with two or three carbohydrates can be quickly separated isocratically, while more complex samples require gradient elution to fully resolve all the analytes of interest. When an isocratic method is used with a conductivity, refractive index, ultraviolet, or pulsed amperometric detector (PAD), mono and disaccharides such as glucose, fructose, sucrose and lactose can be quickly determined.

To utilize the full potential of the RCX-10 column (e.g., gradient separations) a Pulsed Amperometric Detector (PAD) is recommended. The PAD allows utilization of either gradient or isocratic elution for the separation of carbohydrates in foods or food products.

A typical mobile phase is sodium hydroxide and sodium acetate. When the concentration of these mobile phases is varied, a variety of samples can be separated. Separation of carbohydrates with the RCX-10 or RCX-30 at basic pH is possible since each carbohydrate carries a different negative charge at basic pH.

RCX-30 carbohydrate analysis columns provide longer sample retention than RCX-10 columns and better resolution of complex samples like the six constituent monosaccharides of glycoconjugates. It is the increased exchange capacity of the RCX-30 that gives it these characteristics as compared to the RCX-10.

RCX-10 and RCX-30 stationary phase structure and applications



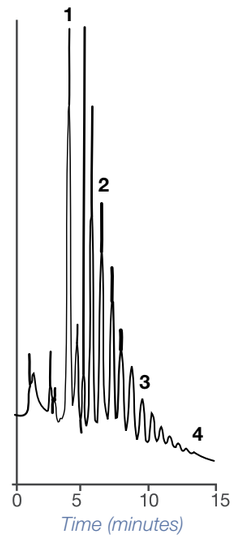
Applications:
Carbohydrates, polysaccharides, sugar oligomers up to DP8 mono and disaccharides

Example of analytes that can be separated on RCX-10 and RCX-30 columns:

- Arabinose
- Galactose
- Lactose
- Maltose
- Sucrose

RCX-10 and RCX-30 application chromatograms

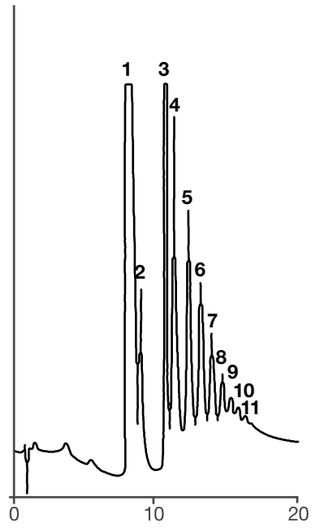
Jerusalem Artichoke Tubers on RCX-10



Column: RCX-10, 4.1 x 250 mm, 5 µm
Part number: 79440
Mobile phase A: 60 mM Sodium Hydroxide
Mobile phase B: 60 mM Sodium Hydroxide with 500 mM Sodium Acetate
Flow rate: 2 mL/min
Gradient: 0 to 100% B in 10 minutes
Temperature: Ambient
Injection volume: 20 µL
Detection: Pulsed amperometric, dual gold electrode
E1 = 50 mV T1 = 333 msec
E2 = 800 mV T2 = 166 msec
E3 = -600 mV T3 = 499 msec

Compounds:
1. DP2
2. DP5
3. DP10
4. DP15

Corn Syrup Sugars on RCX-30



Column: RCX-30, 4.6 x 150 mm, 5 µm
Part number: 79370
Mobile phase A: 60 mM Sodium Hydroxide
Mobile phase B: 500 mM Sodium Acetate in A
Flow rate: 2 mL/min
Gradient: 100% A for 4 min, then 0 to 100% B (4–15 min.)
Temperature: Ambient
Injection volume: 50 µL
Detection: Pulsed amperometric, dual gold electrode
E1 = 350 mV
T1 = 166 msec
E2 = 900 mV
T2 = 166 msec
E3 = -850 mV
T3 = 500 msec

Compounds:
1. Glucose
2. Fructose
3. Maltose
4. Maltotriose
5. Maltotetraose
6. Maltopentaose
7. Maltohexaose
8. Maltoheptaose
9. Maltooctaose
10. Maltononaose
11. Maltodecaose

RCX-10 and RCX-30 Column Ordering Information

Dimensions	RCX-10		RCX-30	
	Particle Size			
	7 µm	12–20 µm	7 µm	12–20 µm
4.1 x 250 mm	79440		79803	
4.6 x 150 mm, PEEK			79370	
4.6 x 250 mm, PEEK	79388		79877	
Bulk Resin (1 Gram)	79703	79704	79705	79706

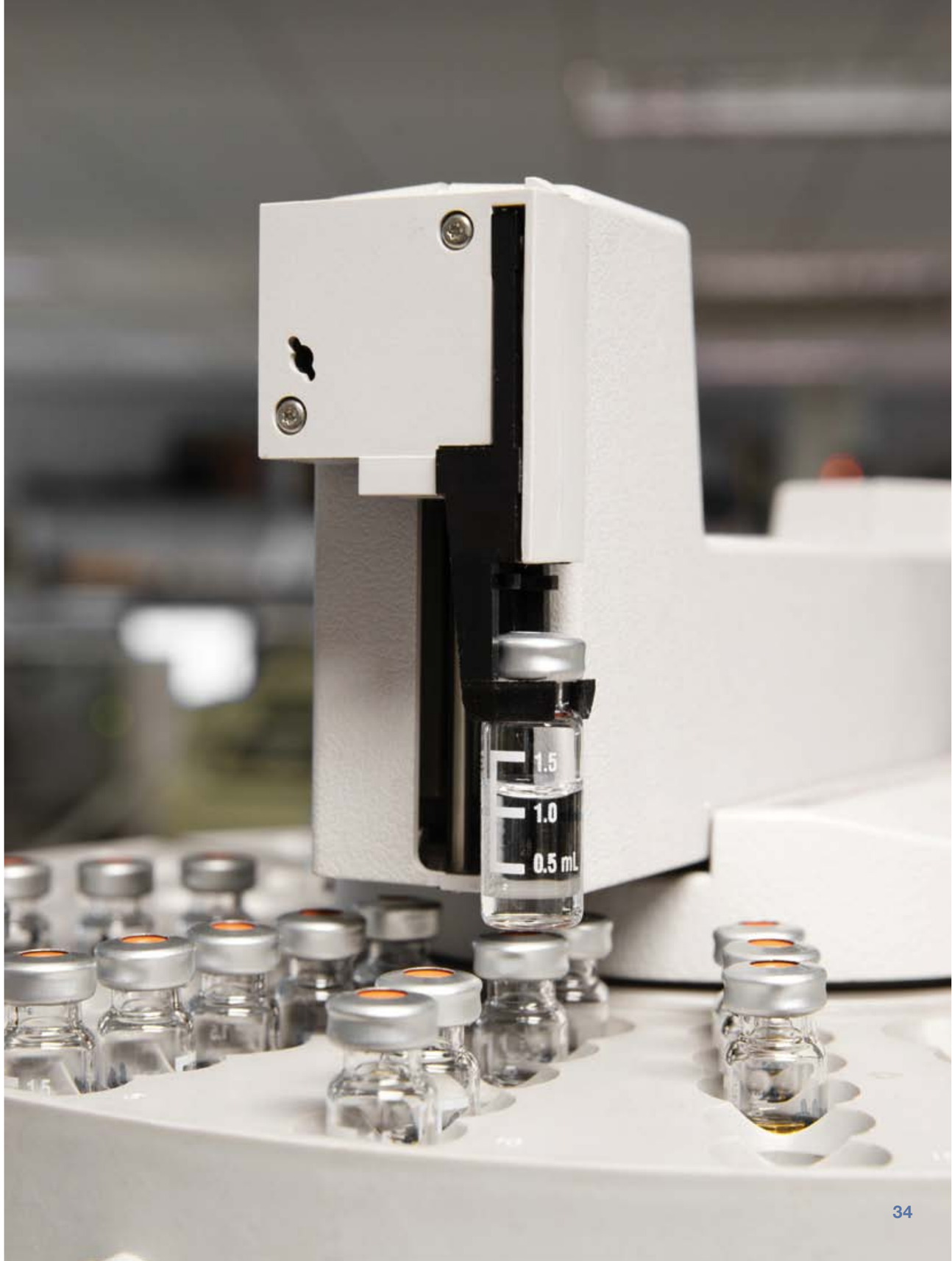
View a keyword searchable index of applications possible with Hamilton HPLC columns at www.hamiltoncompany.com/hplcapplicationindex.

Cation Exchange HPLC Columns

Hamilton offers seven polymeric packing materials for cation exchange separations.

Type	Recommended Application(s)
PRP-X200	Inorganic and organic cations using conductivity or UV detection, alkali and alkaline earth metals. Separate mono or divalent cations depending on mobile phase conditions from 20 ppb to 200 ppm.
PRP-X400	Glyphosate and its metabolite in drinking water Organic and inorganic cations using conductivity or UV detection
PRP-X800	Transition metals or mono and divalent cations in the same run
HC-40	Oligo saccharides up to DP8
HC-75 Ca^{2+}	Mono and disaccharides
HC-75 H^{+}	Organic acids, sugars and alcohols
HC-75 Pb^{2+}	Sugar alcohols and plant cell wall hydrolysates

In cation exchange chromatography, the stationary bed has an ionically negative (-) charged surface while the sample ions are of positive (+) charge. This technique is used almost exclusively with ionic or ionizable samples. The stronger the positive (+) charge on the sample, the stronger it will be attracted to the negative charge on the stationary phase, and thus the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time. Ion chromatography can employ harsh conditions requiring mobile phases that are at very high pH limits (> 11). Temperatures well above the normal operating conditions where silica materials fail can also be used.



PRP-X200 Columns

High resolution separation of alkali and alkaline earth metals

Pore Size: 100 Å
Material: PS-DVB/Sulfonic acid exchanger
Exchange Capacity: 35 µeq/gm

Hamilton PRP-X200 cation exchange HPLC columns are designed for rapid, high resolution separation of alkali and alkaline earth metals. The alkali metals and ammonium are completely resolved in less than five minutes, and the alkaline earth cations separate in under four minutes. Since the mobile phase conditions are different and unique for each of the groups of cations, interferences between these groups are eliminated.

The resolution between any two ions in the alkali metal series can be increased or decreased by changing the concentration of methanol in the mobile phase. This unique feature of the PRP-X200 mobile phase/stationary phase interaction allows the chromatographer to focus on the particular ion of interest and reduce possible interference from other ions in the sample.

The stationary phase is a sulfonated poly (styrene-divinylbenzene), so it is stable to all concentrations of organic modifiers as well as strong acids and bases. The rigid spherical polymer phase allows operation up to 5,000 psi.

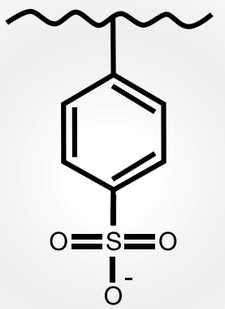
The PRP-X200 columns are designed for use in all of today's ion chromatographic equipment. Since exchange capacity is low, background signal is low. This allows low detection limits at the highest conductivity detector settings.



CATION EXCHANGE HPLC COLUMNS

PRP-X200 stationary phase structure and applications

Applications:
Inorganic and organic cations using conductivity or UV detection, alkali and alkaline earth metals. Separate mono or divalent cations depending on mobile phase conditions from 20 ppb to 200 ppm.

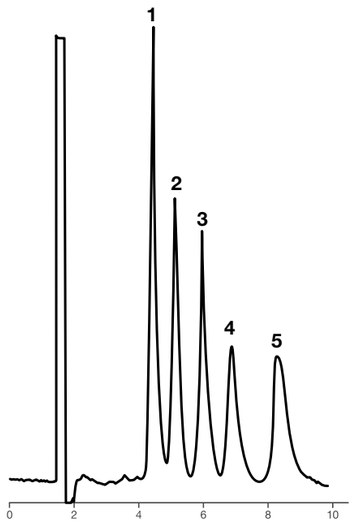


Examples of analytes that can be separated on PRP-X200 columns:

- Calcium
- Cesium
- Potassium
- Sodium

PRP-X200 application chromatograms

Monovalent Cations on PRP-X200



Column: PRP-X200, 5 µm, 4.1 mm x 150 mm
Part number: 79441
Mobile phase: (2.3:1) 4 mM Nitric acid:Methanol
Flow rate: 2 mL/min
Gradient: Isocratic
Temperature: Ambient
Injection volume: 100 µL
Detection: Conductivity

Compounds:
1. Lithium
2. Sodium
3. Ammonium
4. Potassium
5. Cesium

Need a custom method? The Hamilton HPLC team is happy to help design your unique application. Give us a call to learn more.

The 150 mm column is ideal for rapid analysis, while the 250 mm column is recommended for higher resolution or for analysis of minor components in the presence of major interferences.

PRP-X200 Column Ordering Information

Dimensions	Particle Size	
	10 µm	12–20 µm
2.1 x 150 mm	79394	
4.1 x 100 mm	79363	
4.1 x 150 mm	79441	
4.1 x 250 mm	79442	
4.6 x 150 mm PEEK	79384	
4.6 x 250 mm PEEK	79357	
Bulk Resin (1 Gram)	79587	79588

PRP-X400 Columns

Excellent separation of glyphosate and its metabolite in drinking water

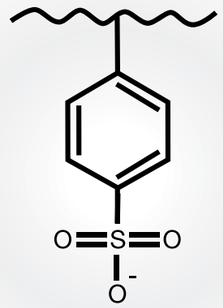
Pore Size: 100 Å
Material: PS-DVB/Sulfonic acid exchanger
Exchange Capacity: 2.5 meq/gm

The PRP-X400 column provides a fast separation for glyphosate and its metabolites. The exchange capacity of the PRP-X400 is greater than that of the PRP-X200, leading to characteristics better suited for the separation of glyphosate. It also performs well in other separations, such as inositol and sugar alcohols. The column does not have to be heated to 65°C and operates well at room temperature, so a column heater is not necessary for this method. PRP-X400 columns do not require the use of methanol in the mobile phase, and they cost much less than other glyphosate columns.

The PRP-X400 is a 7 µm poly(styrene-divinylbenzene) sulfonated cation exchange support (2.5 meq/gm) column. It separates glyphosate and aminomethylphosphonic acid according to charge in less than 10 minutes. This separation requires post-column oxidation and derivatization.

Post column reaction (oxidation) with calcium hypochlorite followed by derivatization with o-phthalaldehyde solution provides sensitive (6 ppb or lower) and selective (primary and secondary) amine detection.

PRP-X400 stationary phase structure and applications



Applications:
Glyphosate and its metabolite in drinking water. The PRP-X400 provides unique hydrophilic interaction separations. Cations, inorganic and organic using conductivity or UV detection.

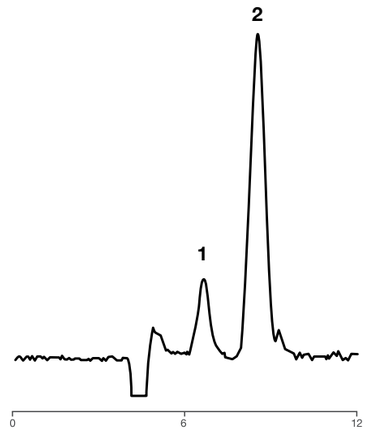
Examples of analytes that can be separated on PRP-X400 columns:

- Glyphosate
- Maltose
- Xylitol
- Mannitol

For full details on how to use the PRP-X400 for glyphosate analysis, please visit our website at www.hamiltoncompany.com/HPLC.

PRP-X400 application chromatograms

Glyphosate on PRP-X400



Column: PRP-X400, 5 µm, 4.1 mm x 250 mm
Part number: 79473
Mobile phase: 0.005 M Monobasic potassium phosphate
Flow rate: 0.5 mL/min
Gradient: Isocratic
Temperature: Ambient
Injection volume: 200 µL
Detection: Excitation wavelength—338 nm, Emission wavelength—455 nm

Compounds:
1. Glyphosate
2. Aminomethylphosphonic acid

PRP-X400 Column Ordering Information

Dimensions	Particle Size		
	7 µm	12–20 µm	30–50 µm
2.1 x 250 mm	79398		
4.1 x 150 mm	79717		
4.1 x 250 mm	79473		
4.6 x 250 mm PEEK	79387		
Bulk Resin (1 Gram)	79591	79592	79593



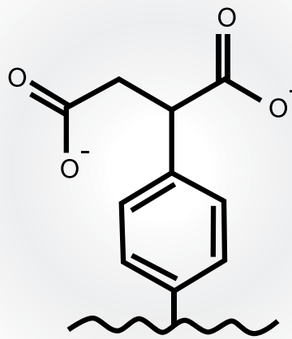
PRP-X800 Columns

Separates transition metals or mono and divalent cations in the same run

Pore Size: 100 Å
Material: PS-DVB/Itaconate exchanger (WCX)
Exchange Capacity: 1.6 meq/gm

The PRP-X800 is a polymeric cation exchange column functionalized with itaconic acid that performs the isocratic separation of mono and divalent cations such as lithium, sodium, ammonium, potassium, magnesium and calcium. The column offers excellent durability, is stable to any concentration organic solvent, and enables dynamic control of exchange capacity. Detection is via conductivity or indirect UV, depending on the mobile phase.

PRP-X800 stationary phase structure and applications



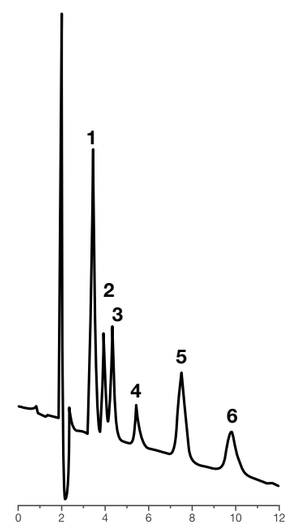
Applications:
Mono and divalent transition metals in the same run. Transition metals (e.g., manganese, zinc, cobalt and cadmium) are also resolved on the column using an ethylenediamine/tartaric acid mobile phase and conductivity detection.

Examples of analytes that can be separated on PRP-X800 columns:

- Mono and divalent metal cations (e.g., sodium, potassium, calcium)
- Transitions metals (e.g., iron, manganese, nickel, copper, zinc)

PRP-X800 application chromatograms

Mono and Divalent Cations on PRP-X800



Column: PRP-X800, 5 µm, 4.1 mm x 250 mm
Part number: 79828
Mobile phase: 2 mM Cupric Sulfate
Flow rate: 0.8 mL/min
Gradient: Isocratic
Temperature: Ambient
Injection volume: 10 µL
Sample concentration: All compounds are 5 ppm
Detection: Indirect UV at 220 nM

Compounds:
1. Lithium
2. Sodium
3. Ammonium
4. Potassium
5. Magnesium
6. Calcium

PRP-X800 Column Ordering Information

Dimensions	Particle Size: 7 µm
4.1 x 150 mm	79855
4.1 x 250 mm	79828

From autosampler syringes to manual injection and more, Hamilton has the chromatography syringe you need. View the full portfolio of HPLC, GC and TLC syringes at www.hamiltoncompany.com/syringes.

The PRP-X800 is available in PEEK hardware as a custom order. Please see page 48 for more information.



HC-40 Ca^{2+} and HC-75 (H^+, Ca^{2+}, Pb^{2+}) Columns

Separate compounds through size exclusion and ligand exchange

Pore Size: 100 Å
Material: PS-DVB/ Sulfonic acid exchanger
Exchange Capacity: 5 meq/gm

The HC-40 and HC-75 group of columns consist of four different packing types, each with a different retention characteristic and application. They are:

- HC-40—Oligo saccharides up to DP8 (e.g., corn syrup, high conversion corn syrup, beer)
- HC-75 Calcium Form—Separates mono and disaccharides (e.g., corn syrup, chewing gum sweeteners, milk product sugars, glycols and polyols, high fructose corn syrup, juices, oligosaccharides)
- HC-75 Lead Form—Sugar alcohols and plant cell wall hydrolysates
- HC-75 Hydrogen Form—Organic acids, sugars and alcohols

HC-40 and HC-75 columns separate compounds through size exclusion and ligand exchange. The 4% cross-linked HC-40 uses size exclusion as the primary mechanism of separation, while ligand exchange dominates in the more highly cross-linked HC-75. The different forms of the HC-75 (Hydrogen, Calcium and Lead) each provide a unique selectivity for separating varying types of charged analytes based on electronegativity toward the counterion. The higher carbohydrate oligomers elute first while the smaller di- and monosaccharaides elute later.

The HC-75 column provides a slightly faster 14-minute separation up to DP 5, and the HC-40 column provides a much better separation of the oligomers up to DP 8 in 16 minutes. Because carbohydrates do not contain a chromophore, UV detection cannot be used without derivatization. The recommended detection method is refractive index. The control of carbohydrate retention lies in the selection of the correct column.

The HC-40 and HC-75 columns use water as a mobile phase (gradients and salts are not required), which simplifies eluent preparation and minimizes cost. This mobile phase characteristic also lends these columns to detection techniques such as evaporative light scattering detection and mass spectrometry. A very versatile column family, the HC-75 group can be used with up to 40% acetonitrile and can be regenerated to help restore performance.

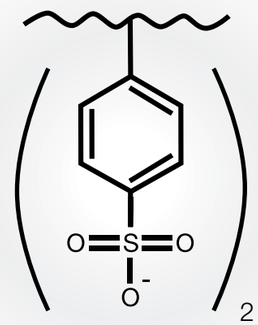
CATION EXCHANGE HPLC COLUMNS

HC-40 and HC-75 stationary phase structure and applications

Applications:
Carbohydrates, sugar oligomers up to DP8. Mono and disaccharides, organic acids, sugars, and sugar alcohols.

Examples of analytes that can be separated on HC-40 and HC-75 columns:

- Ethanol
- Maltohexose citric acid
- Glucose fructose
- Arabinose
- Sorbitol
- Acetic acid

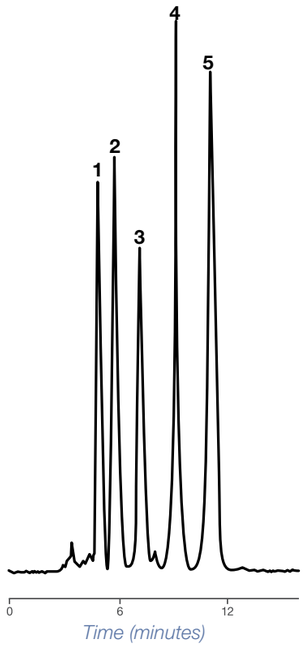


Pb^{2+}, $2H^+$ or Ca^{2+}



HC-75 application chromatograms

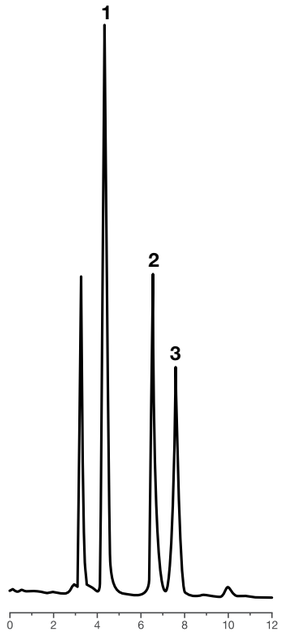
Chewing Gum Sugars on HC-75 Ca^{2+}



Column: HC-75 Calcium Form, 5 µm, 7.8 mm x 305 mm
Part number: 79436
Mobile phase: Deionized water.
Flow rate: 1.2 mL/min
Gradient: Isocratic
Temperature: 90°C
Injection volume: 2 µL
Detection: Refractive index

Compounds:
1. Sucrose
2. Glucose
3. Fructose
4. Mannitol
5. Sorbitol

Organic Acids by USP L17 on HC-75 H^+

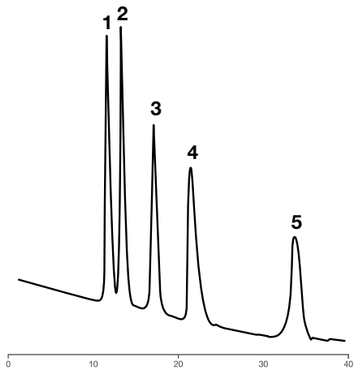


Column: HC-75 Hydrogen Form, 5 µm, 4.1 mm x 250 mm
Part number: 79476
Mobile phase: 0.01 N sulfuric
Flow rate: 0.35 mL/min
Gradient: Isocratic
Temperature: 60°C
Injection volume: 10 µL

Sample concentration: All compounds are 250 ppm
Detection: UV at 210 nm

Compounds:
1. Citric acid
2. Lactic acid
3. Acetic acid

Sugar Standards on HC-75 Pb^{2+}



Column: HC-75 Lead Form, 5 µm, 7.8 mm x 305 mm
Part number: 79438
Mobile phase: Deionized water
Flow rate: 0.6 mL/min
Gradient: Isocratic
Temperature: 80°C
Injection volume: 200 µL
Sample concentration: All compounds are 2.5 mg/mL
Detection: Refractive index

Compounds:
1. Sucrose
2. Glucose
3. Fructose
4. Inositol
5. Sorbitol

HC-75 and HC-40 Column Ordering Information

Dimensions	HC-75 Particle Size 9 µm	HC-40 9 µm
(H^+) 4.1 x 250 mm	79476	
(H^+) 7.8 x 100 mm	79547	
(H^+) 7.8 x 305 mm	79544	
(H^+) Bulk Resin (1 Gram)	79711	
(Ca^{2+}) 4.1 x 250 mm	79431	
(Ca^{2+}) 7.8 x 305 mm	79436	
(Ca^{2+}) Bulk Resin (1 Gram)	79709	79707
(Pb^{2+}) 7.8 x 100 mm	79240	
(Pb^{2+}) 7.8 x 305 mm	79438	
(Pb^{2+}) Bulk Resin (1 Gram)	79712	



Ion Exclusion HPLC Columns

Hamilton offers one polymeric packing material for ion exclusion separations.

Ion exclusion chromatography is an alternative to ion exchange chromatography in which ionized samples are excluded from the pores of the support and elute first, while the weakly ionized and nonionic compounds elute later. Mixtures of weak acids, like those in fruits and milk products, are frequently not very well separated by pure ion-exchange methods, nor in the reversed-phase mode.

PRP-X300 Columns

Ion exclusion for organic acids and alcohols

Pore Size: 100 Å
Support Material: PS-DVB/Sulfonic acid

Hamilton PRP-X300 columns offer an easy, rapid way to separate closely related alcohols and organic acids. The sulfonated poly(styrene-divinylbenzene) support separates samples via a mixed mode mechanism. Separation on the PRP-X300 is accomplished by three modes:

1. **Hydrogen Bonding**—The attraction and retention of sample compounds by the negatively charged sulfonate group.
2. **Reversed-Phase**—The interaction and retention of the sample compounds by the non-polar polymeric support.
3. **Ion Exclusion**—The process in which ionized samples are excluded from the pores of the support and elute first, while the weakly ionized and nonionic compounds elute later.

A wide variety of samples can be analyzed with PRP-X300 columns because their selectivity can be altered by changing the pH of the buffer or adding an organic modifier (e.g., methanol, acetonitrile). The support's stability to organic solvents makes it possible to analyze samples that are too highly retained on conventional ion exclusion supports.

Most separations on PRP-X300 columns are completed within five minutes. The high performance packing ensures narrow peaks under isocratic conditions. The use of isocratic conditions allows samples to be analyzed one after another without waiting for column re-equilibration. Complex samples can be run under gradient conditions if isocratic methods are insufficient. Most samples require only minimal preparation before injection, increasing sample throughput in the lab.

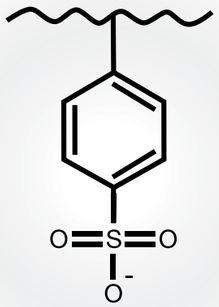
ION EXCLUSION HPLC COLUMNS

PRP-X300 stationary phase structure and applications

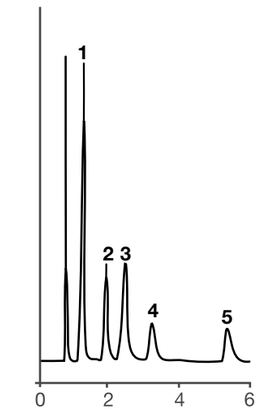
Applications:
Organic acids and alcohols

Examples of analytes that can be separated on PRP-X300 columns:

- Acetic acid
- Acrylamide
- Citric acid
- Oxalacetic acid
- Ethanol
- Propanol



PRP-X300 application chromatogram



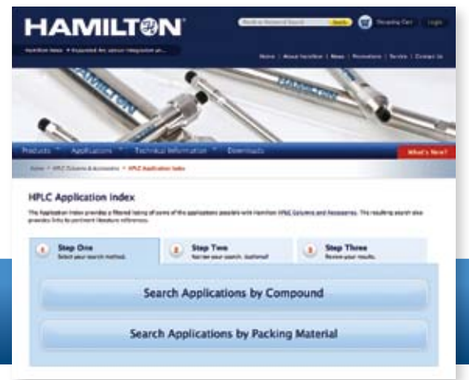
Column: PRP-X300, 3 µm, 4.1 mm x 100 mm
Part number: 79818
Mobile phase: 1 mN Sulfuric acid
Flow rate: 1 mL/min
Gradient: Isocratic
Temperature: Ambient
Injection volume: 20 µL
Detection: UV at 201 nm

Compounds:
1. Tartaric acid
2. Malic acid
3. Citric acid
4. Lactic acid
5. Acetic acid

PRP-X300 Column Ordering Information

Dimensions	Particle Size	
	7 µm	12–20 µm
4.6 x 150 mm PEEK	79475	
4.1 x 250 mm	79465	
4.1 x 150 mm	79464	
Bulk Resin (1 Gram)	79589	79590

View an index of applications possible with Hamilton HPLC columns at www.hamiltoncompany.com/hplcapplicationindex.





Custom HPLC Columns and Packing Materials

Hamilton offers an extensive line of off-the-shelf HPLC columns with different packing material chemistries, varying particle sizes and column hardware dimensions in two hardware types—stainless steel and polyether ether ketone (PEEK) plastic. However, even with all the standard options available, customers often require a custom built column where they may need one of our stationary phases packed into a column hardware combination that is not a standard product.

Custom HPLC columns offer customers the ability to have any of our stationary phases packed into most any hardware format to suit their application requirement.

Ordering custom HPLC products

Customers can specify the following information either through the online shopping cart or over the phone with a customer service representative:

- Packing material and particle size (e.g., PRP-1, 5 µm)
- Hardware dimensions and material (e.g., 4.6 x 150 mm, PEEK)

Custom HPLC Column Ordering Information

Custom Column Category	Part Number
Analytical HPLC Column, Custom Order (1.0, 2.1, 4.1, 4.6 mm ID)	79641
Semi-preparative HPLC Column, Custom Order (7.0, 7.8, 10 mm ID)	79642
Preparative HPLC Column #1, Custom Order (21.2, 30 mm ID)	79643
Preparative HPLC Column #2, Custom Order (50 mm ID)	79644
Preparative HPLC Column #3, Custom Order (100 mm ID)	79645

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HPLC Guard Columns

Hamilton guard columns protect analytical, semi-prep and preparative HPLC columns. They also remove particulate contaminants and highly adsorptive compounds from samples, prolonging column life. The designs are modular and cartridge replacement is easy and tool-free.

Cartridge holders for analytical columns are available in stainless steel and PEEK materials. Semi-prep/preparative holders are available only in stainless steel. The design of the cartridge holders is optimized to reduce dead volume and prevent extra column band broadening. Cartridge holders are reusable indefinitely.

Packing Material	Analytical Starter Kits (1 holder, 2 cartridges)		Analytical Replacement Cartridges (5/pk)		Semi-prep/ preparative Starter Kits (1 holder, 1 cartridge)	Semi-prep/ preparative Replacement Cartridges (2/pk)
	Stainless Steel	PEEK	Stainless Steel	PEEK	Stainless Steel	Stainless Steel
PRP-1	79447	79317	79445	79318	79121	79122
PRP-3	79461	79393	79454	79395	79123	79124
PRP-C18	79685	79687	79686	79688	79689	79690
PRP-h5	79267		79268		79277	79278
PRP-X100	79448	79383	79446	79385	79125	79126
PRP-X110/ X110S	79726	79727	79728	79729		
PRP-X500		79319		79320		
PRP-X600		79361		79362		
RCX-10	79462	79378	79463	79379		
RCX-30		79371		79372		
PRP-X200	79456	79368	79449	79369	79127	79128
PRP-X400		79376		79377	79131	79132
PRP-X800	79830	79831	79832	79833		
HC-75 H^+			79134		79133	79134
HC-75 CO^3					79866	79865
PRP-X300	79460	79373	79453	79374	79129	79130

Bulk Polymer Resin

Hamilton offers a range of bulk polymer resins for customers who prefer to pack their own column hardware. Chromatographic capacity and efficiency tests are conducted on both bulk and packed column resins to ensure product integrity. Samples from different lots can be purchased for method and process validation.

Please specify the quantity of resin needed in grams when ordering. Quantities from one gram to kilos are available.

Support	Support Material	Exchange Capacity	Pore Size	Particle Size						
				5 µm	7 µm	9 µm	10 µm	12–20 µm	30–50 µm	50–75 µm
PRP-C18	PS-DVB functionalized with C18	N/A	100 Å	79791						
PRP-C18	PS-DVB functionalized with C18	N/A	100 Å				79792			
PRP-C18	PS-DVB functionalized with C18	N/A	100 Å					79793		
PRP-h5	PS-DVB	N/A	300 Å					79280		
PRP-1	PS-DVB	N/A	100 Å	79578	79579		79580	79581	79582	79583
PRP-3	PS-DVB	N/A	300 Å				79701	79702		
PRP-X100	PS-DVB with Trimethylammoniun Exchanger	0.19 meq/gm	100 Å	79584			79585	79586		
PRP-X500	Poly(methacrylamidopropryl Trimethylammonium chloride)	1.6 meq/gm	Superficially porous		79594			79595	79596	
PRP-X600	Poly(dimethylamidopropyl-methacrylamide)	1.6 meq/gm	Superficially porous		79597			79598	79599	
RCX-10	PS-DVB with Trimethylammoniun Exchanger	0.35 meq/gm	100 Å		79703					
PRP-X200	PS-DVB Sulfonate Exchanger	35 µeq/gm	100 Å				79587	79588		
PRP-X400	PS-DVB Sulfonate Exchanger	2.5 meq/gm	N/A		79591			79592	79593	
HC-75 Ca^{2+}	PS-DVB Sulfonate Exchanger	5.0 meq/gm	Gel-Type			79709				
HC-75 H^+	PS-DVB Sulfonate Exchanger	5.0 meq/gm	Gel-Type			79711				
HC-75 Pb^{2+}	PS-DVB Sulfonate Exchanger	5.0 meq/gm	Gel-Type			79712				

Proper Column Care and Storage

All chromatographic columns have a finite lifetime. It is good practice to routinely monitor each column's retention characteristics and performance using appropriate analyte standards. If the column is to be stored for more than two weeks, it is necessary to use a mobile phase which will inhibit microbial growth. Solvent mobile phases containing sodium azide or high concentrations of methanol or acetonitrile are suggested.

The following precautions should be taken with Hamilton HPLC columns to achieve maximum product life:

1. Routinely monitor the column's performance.
2. Switch only between mutually miscible mobile phases.
3. Avoid the possibility of precipitation of salts in the column.
4. Use only filtered and degassed mobile phases.
5. Do not allow the column to dry out.
6. Keep the column capped with the end plugs that came with the column when not in use.
7. For prolonged storage, use a mobile phase that will inhibit bacterial and mold growth.
8. Unusually high operating pressure is an indication of a plugged inlet frit. It may be cleared by reversing flow through the column for 5–10 column volumes.
9. Using guard columns is highly recommended to remove particulate matter or impurities which may permanently bind to the polymer packing materials inside the analytical column.

Technique Tip

When executing a wash procedure, use an appropriate flow rate based on the column's inside diameter.

Custom HPLC columns are available! From dimensions to particle size to packing materials, Hamilton can build you exactly what you need. See page 48 for more information.



PROPER CARE AND STORAGE

Restoring column performance

Contamination of the stationary phase from samples or eluents can cause the column performance to diminish over time. Typically, one of the following procedures will rejuvenate the performance of a column that has deteriorated.

Column Restoration Procedures

PRP-1, PRP-C18, PRP-3, PRP-h5	PRP-X100	PRP-X200, PRP-X300	PRP-X400	RCX-10	RCX-30
Flush with 40:40:20 (ACN:IPA:H_2O)	Flush 50 mL of methanol with 1% 6 N nitric acid	Inject several times with 100 µL of 1 N nitric acid	Inject several times with 100 µL of 0.1 M potassium EDTA	Flush with 50 mL of 0.1 N sodium hydroxide	Flush with 150 mL of 0.1 N sodium hydroxide

Column Restoration Procedures

HC-75			HC-40
Calcium Form	**Hydrogen Form**	**Lead Form**	**Calcium Form**
Flush with 1% calcium chloride at 0.1 mL/min overnight	Flush with 0.1 N sulfuric acid at 0.1 mL/min overnight	Flush with 1% lead nitrate at 0.1 mL/min overnight	Flush with 1% calcium chloride at 0.1 mL/min overnight

Reverse the column so that the flow is now entering in through what was the outlet fitting, and do not connect the outlet fitting to the detector.

Technique Tip

Always make sure that the mobile phases are miscible and that precipitation will not occur. If necessary wash the column with a suitable intermediate solvent before changing over to the new phase. A minimum of five column volumes of the intermediate solvent should be used.



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