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.

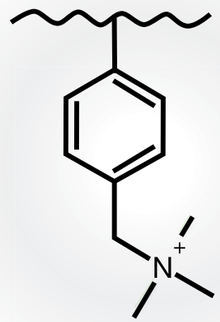
PRP-X100 columns easily separate difficult anions such as cyanide, borate and silicate at high pH (11.5). The polymeric packing is stable from pH 1 to 13, so a single column can be used for the analysis of both common and difficult anions. The PRP-X100 is compatible with many different mobile phases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

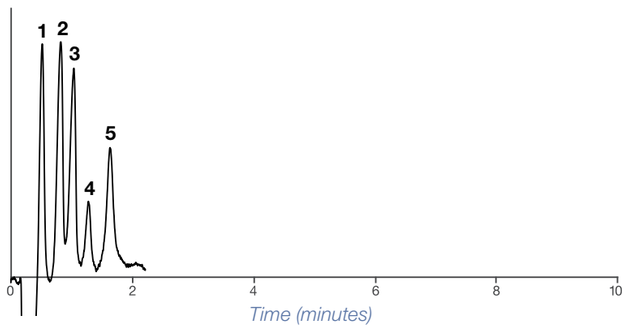
- **Pharmaceutical—** Lactate, acetate, chloride and phosphate in intravenous solution
- **Medical Research—** Monitoring analytes in bodily fluids of patients
- **Environmental—** Common anions in ground and river water, EPA 300.0 Part A
- **Food and Beverage—** Arsenic in food and water, phosphate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

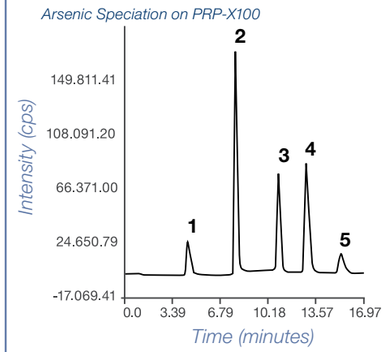
Fast Separation of Common Anions on PRP-X100



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

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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.

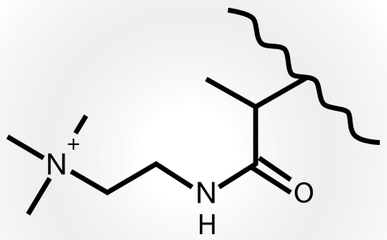
A separation of four protein standards at 0.2 mg in less than three minutes is possible with a short analytical 50 x 4.6 mm HPLC column. Recovery of sample is excellent with PRP-X500 and the support's limited permeability prevents proteins from entering the pores and unfolding, which causes peak ghosting.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

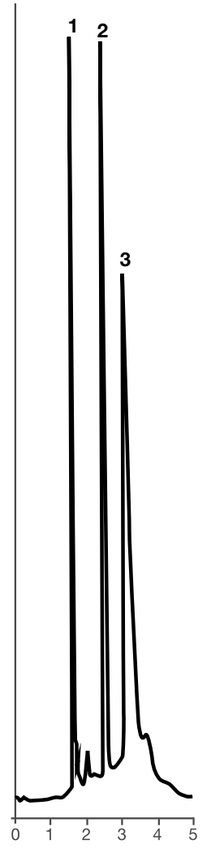
Examples of analytes that can be separated on PRP-X500 columns:

- 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.

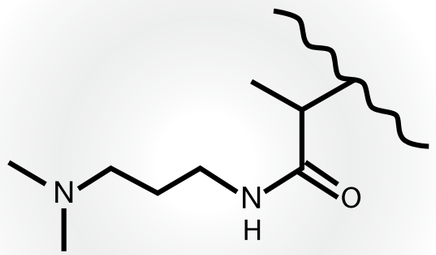
Change the mobile phase composition to alter the retention of DNA oligomers. Rapid gradient changes typically lower column efficiency; however, biomolecules run in this fashion on the PRP-X500 show very favorable separation efficiency with much shorter run times.

PRP-X600 stationary phase structure and applications

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

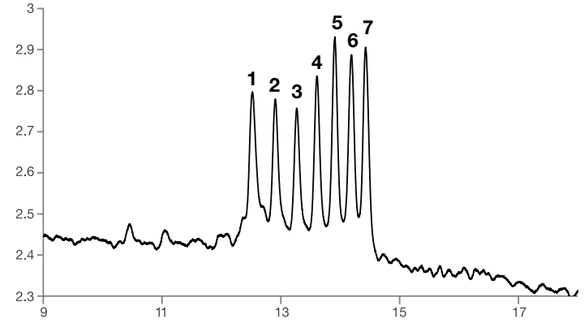
Example of analytes that can be separated on the PRP-X600:

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



ANION EXCHANGE HPLC COLUMNS

PRP-X600 application chromatograms

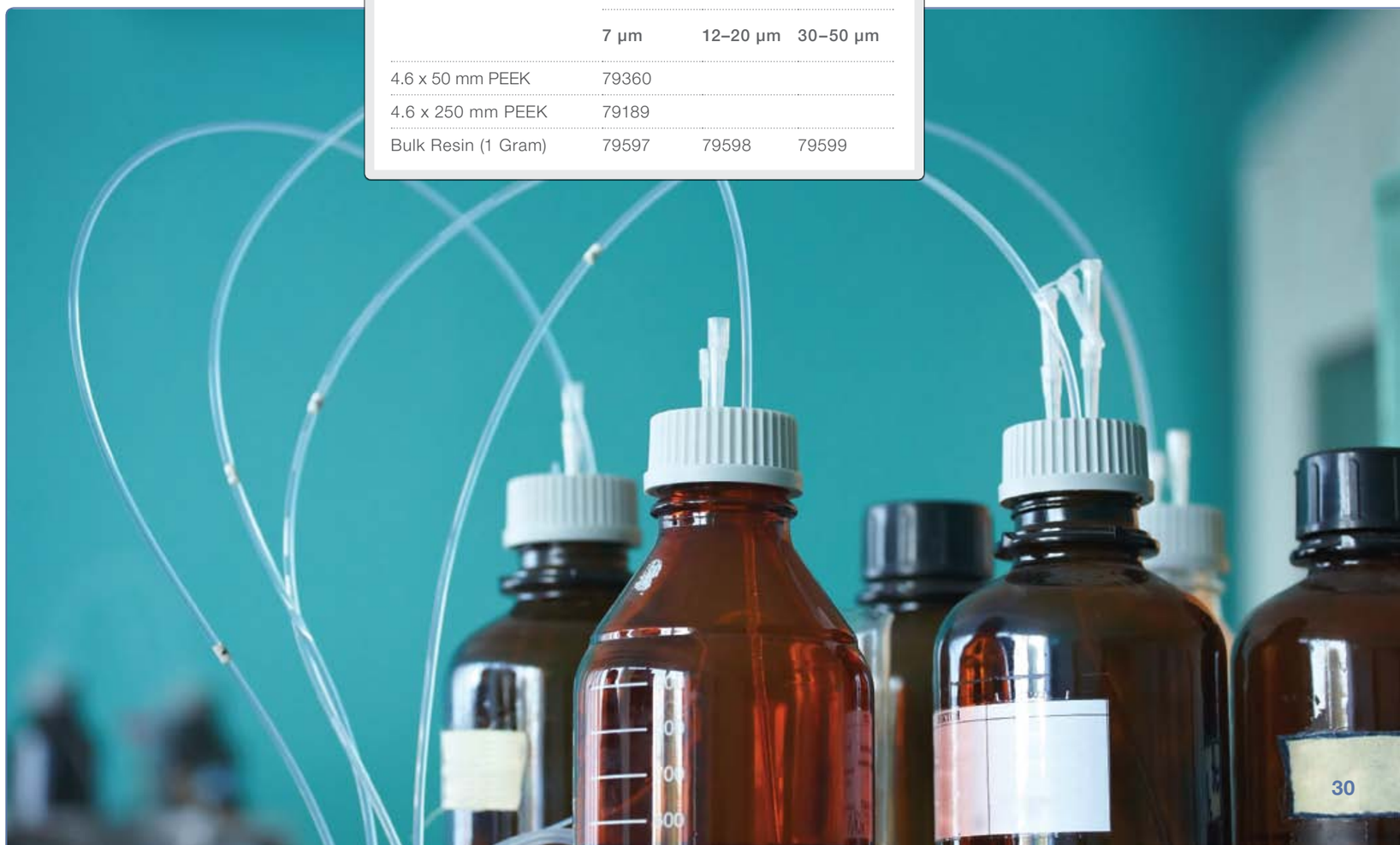


Column: PRP-X600, 7 µm, 4.6 x 50 mm
Part number: 79360
Mobile phase A: 85/15 100 mM TRIS, pH 8.0/acetonitrile
Mobile phase B: 85/15 100 mM TRIS, pH 8.0, 2.5 M lithium chloride/acetonitrile
Flow rate: 2.0 mL/min
Gradient: 0 to 40% B in 40 minutes
Temperature: Ambient
Injection volume: 10 µL
Sample concentration: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

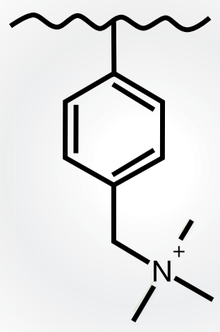
The Hamilton RCX-10 and RCX-30 carbohydrate analysis columns are designed for the isocratic or gradient separation of carbohydrates. The exchange capacity of the RCX-10/RCX-30 is greater than that of the PRP-X100, leading to characteristics better suited for the separation of carbohydrates. Simple samples 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



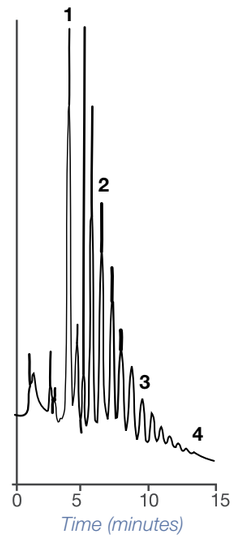
Applications:
Carbohydrates, polysaccharides, sugar oligomers up to DP8 mono and disaccharides

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

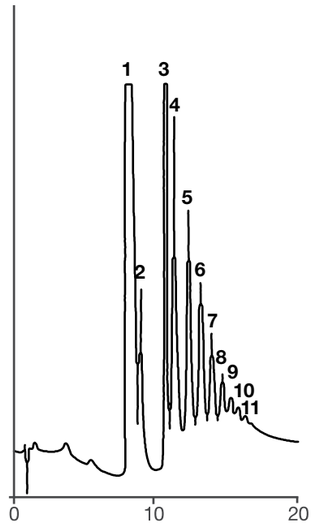
Jerusalem Artichoke Tubers on RCX-10



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.