PRODUCT MANUAL

for

ProPac® SCX-20

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PRODUCT MANUAL

for

ProPac® SCX-20

PROPAC SCX-20 ANALYTICAL COLUMN
(4 x 250 mm, P/N 074628)

PROPAC SCX-20 GUARD COLUMN
(4 x 50 mm, P/N 074643)

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Document No. 065403
Revision 01
September 2010

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SECTION 1 – INTRODUCTION

1.1. Features of the ProPac SCX-20, Strong Cation Exchange Columns

The ProPac® SCX-20 column is a strong cation exchange (SCX) column designed specifically to provide high-resolution separations of proteins. A grafted cation-exchange surface provides pH-based selectivity control and fast mass transfer for high-efficiency separation and moderate capacity.
The stationary phase is composed of non-porous, solvent compatible ethylvinylbenzene-divinylbenzene copolymer beads that are uniformly coated with a highly hydrophilic layer to reduce non-specific interactions between the surface and the biopolymer. The top surface of the beads is grafted with SCX functionalized polymer layer providing well-controlled graft density and uniform distribution of chain lengths.

1.2. ProPac SCX-20 Operating Limits and Specifications

1.2.1. Operating Conditions

Short Term Storage Solution (overnight):	Your eluent (pH 3-10)
Typical buffers:	Sodium or potassium salts of phosphate, MES ***Always maintain a minimum ionic strength of at least 20 mM, to ensure optimum resolution.***
Solvents:	50% acetonitrile if needed for cleaning. ***Avoid using H_2O alone for washing the column.***
Detergent Compatibility	Nonionic, anionic or zwitterionic detergents. ***Do not use cationic detergents.***
Temperature Range:	Ambient to 60 ºC
Pressure Limit:	3,000 psi
pH Range	2-12
Dynamic capacity (4 x 250 mm col. volume =3.14 mL)	Depending on the protein, 10 – 100 µg protein can be injected

NOTE

Assistance is available for any problem during the shipment or operation of DIONEX instrumentation, columns, and consumables through the DIONEX North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or through any of the DIONEX Offices listed in, "DIONEX Worldwide Offices" on the Dionex Reference Library CD-ROM.

1.2.2. Physical Characteristics

Substrate Pore Size	Non-porous, 10 µm
Substrate Monomers	ethylvinylbenzene-divinylbenzene
Substrate Cross-linking	55%
Mode of Interaction	Cation Exchange
Functional Group	SCX - Sulfonic Acid

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1.3. Formats of the ProPac SCX-20 Columns

Currently, the ProPac SCX-20 strong cation exchange analytical column is available in 4 x 250 mm format. Dionex recommends purchasing columns from at least 3 different lots before setting method specifications.

1.3.1. ProPac SCX-20 Strong Cation-exchange Columns (Sulfonate Functionality)

Description	Part Number
ProPac SCX-20 Analytical Column (4x250 mm)	074628
ProPac SCX-20G Guard Column (4x50 mm)	074643

1.4. ProPac SCX-20 Operating Conditions

ProPac SCX-20 strong cation exchange columns are stable between pH 2 and 12, and are compatible with both aqueous mobile phases and those containing solvents, such as acetonitrile. The ProPac SCX-20 strong cation exchange columns can be operated at any flow rate, as long as the backpressure remains below 3,000 psi. When one sets up a system to be used with this column, the special precautions listed in Section 3, "Operation", should be considered. PEEK™ (polyetheretherketone) is used as a material for the column housing. PEEK has excellent resistance to most organic solvents and inorganic solutions. It is inert and does not contribute to metal contamination during operation.

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SECTION 2 – SYSTEM REQUIREMENT

2.1. An Inert System is Strongly Recommended

The ProPac SCX-20 columns were designed to be used with a standard bore HPLC system having a gradient pump module, injection valve, and a UV detector.

A metal-free/inert system is recommended for halide-salt mobile phases which may otherwise cause corrosion of metallic components. Metal leaching from the system will lead to decreased performance from metal contamination. A metal-free pump is also recommended to avoid denaturation of the protein samples. Use of stainless steel tubing, ferrules, and bolt assemblies is not recommended.

Typical Flow Rate:	1 mL/min
Injection Volume:	5–25 µL
Autosampler:	AS
System Void Volume:	Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.
Pumps:	SP (single pump) or DP (dual pump)
Detectors:	VWD (Variable Wavelength Detector)

2.2. System Void Volume

Tubing between the injection valve and detector should be < 0.010” ID PEEK tubing. Minimize the length of all liquid lines, but especially the tubing between the column and the detector. The use of larger diameter and/or longer tubing may decrease peak efficiency and peak resolution.

2.3. Mobile Phase Limitations

The ProPac SCX-20 columns is compatible with typical mobile phases, such as MES, Tris, Phosphate or acetate buffers; and with sodium or potassium salts of phosphate up to the limit of their solubility. Use of organic solvents in the mobile phase is usually unnecessary. If you choose to use one, test the solubility limit of the mobile phase in the presence of the chosen organic solvent. Some combinations of salts and organic solvents are not miscible.

WARNING

Do not use Cationic detergents. Cationic detergents will irreversibly bind to the ProPac strong cation exchange columns and their use should be avoided.

2.4. Chemical Purity Requirements

Reliable, reproducible results require mobile phases that are free from impurities and prepared consistently.

2.4.1. Deionized water

The deionized water used to prepare your mobile phase should be Type I reagent grade water with a specific resistance of 18 megohm-cm. The water should be free from ionized impurities, organics, microorganisms and particulate matter. UV treatment in the water purification unit is recommended. Follow the manufacturer’s instructions regarding the replacement of ion exchange and adsorbent cartridges. All filters used for water purification must be free from UV-absorbing components. Contaminated water in the mobile phase causes high background signals, gradient artifacts, and even sample degradation.

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SECTION 3 – OPERATION

3.1. Mobile Phase Selection

The mobile phase for the ProPac SCX-20 strong cation exchange columns consists of a buffer component and a salt component. The buffer selection depends upon the pI of the proteins to be separated, and should provide minimal UV interference at the wavelength to be monitored. MES, Tris, phosphate containing buffers are routinely used.

Proteins are eluted using a gradient of increasing ionic strength. Optimum performance is obtained if a minimum salt concentration is maintained in buffer A at all times. Dionex recommends a minimum concentration of 20 mM NaCl or equivalent ionic strength in buffer A. Failure to maintain a minimum ionic strength in buffers will result in alteration of the stationary phase conformation resulting in an increase in the column backpressure beyond the maximum recommended value and effects protein separations. If this occurs, remove the column from the system, flush the buffer from the system and replenish with buffer B containing your high salt concentration. Replace the column and pump buffer B through the column at low flow rate (0.1-0.2 mL/min), until the backpressure falls back to normal.

Mobile Phase Constituent	Recommendations
Buffer	MES, Tris, Phosphate
Salt	Potassium or sodium salts of chloride, acetate
pH Modifier	Phosphoric acid, HCl, or NaOH
Column Cleaning / Pretreatment	10 mM Sodium hydroxide at room temperature
Solvent	Up to 50% acetonitrile
Detergent	Non-ionic, anionic, or zwitterionic detergents
Bacteriostatic agent	0.1% sodium azide

WARNING

Do not operate the ProPac SCX-20 in the absence of a minimal ionic strength (At least 20 mM). If the ionic strength is too low, the conformation of the stationary phase will be affected, causing a significant increase in backpressure. This effect can be reversed by pumping 500 mM NaCl in a buffer through the column at a low flow rate (0.1-0.2mL/min) until the backpressure is reduced

3.2. Mobile Phase Preparation

3.2.1. Adjusting the pH of the Mobile Phase

The mobile phase should contain all the electrolytes before adjusting the pH. Salts may be added after the pH adjustment. It is important to prepare buffers gravimetrically (by weight) when possible and without need to adjust the pH each time. Slight variations in the pH meter adjustment can lead to substantial differences in the reproducibility of runs. If a pH meter is used in order to make sure that the pH reading is correct, the pH meter should be calibrated at least once a day using recommended procedures. Stirring and temperature correction should be employed. *(Note that pH measurements of buffers containing Tris should not be performed with a Ross electrode, as this electrode produces erroneous results with amine containing solutions.)*

3.2.2. Filtering the Mobile Phase

To extend the lifetime of your column as well as your HPLC pump, the high salt concentration buffers must be filtered using a 0.2 µm membrane filter to remove insoluble contaminants from the eluents.

3.2.3. Degassing the Mobile Phase

Before using them, the buffers must be degassed. The degassing can be done either using the Dionex pump degas function as described in the manual, or by using a vacuum pump. Vacuum degas the solvent by placing the mobile phase reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump for 5-10 minutes while sonicating.

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3.3. Validating Column Performance

Dionex recommends that you perform an efficiency test on your ProPac SCX-20 strong cation exchange column before you use it. The purpose of column performance validation is to make sure that no damage has been done to the column during shipping. Test the column using the conditions described on the Quality Assurance Report enclosed in the column box, and also included in the appendix of this manual. Repeat the test periodically to track the column performance over time. Note that slight variations may be obtained on two different HPLC systems due to system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Please see the example Quality Assurance Report in Appendix C.

3.3.1. Procedure for Validating Column Performance

1. Connect the column to the LC system.
2. Remove the storage solution from the column with the mobile phase listed on the QA report for 20 to 40 column volumes. This is best achieved during the column equilibration.
3. After the equilibration, inject the test mix shown in the QA report and collect the data.
4. Compare your result with the QA report provided in the column box.
5. If the chromatograms look similar, you can use the column for your application work.

3.4. Equilibrating the Column

Equilibrate the column after installing it for the first time. Always re-equilibrate the column prior to use following periods of storage. Equilibrate the column with at least 10-15 column volumes of mobile phase A, or until a stable baseline is achieved.

3.5. Caring for the Column

To ensure the high performance of the ProPac SCX-20 strong cation exchange columns, the following guidelines should be followed.

1. Protect the column from contamination using a ProPac SCX-20 guard column.
2. Make sure that solvents are miscible when changing mobile phases.
3. Always degas and filter mobile phases through a 0.22-mm membrane filter.
4. When switching to a new mobile phase, the column should be equilibrated with at least 30 column volumes before injecting the sample.
5. The recommended pH range is from pH 2 to 12. However, it is preferred that the column be used between pH 3 and pH 11 to achieve longer lifetime.
6. The column can be stored in mobile phase for short-term storage (e.g. 48 h). However, it is highly recommended that the column be stored in 20 mM Na_2HPO_4/H_3PO, pH 6.5 with 0.1% sodium azide (more than 2 days).
7. The recommended operating maximum temperature is below 50 ^{o}C. In most cases, temperature control between ambient and 30 ºC gives good results.
8. The recommended maximum backpressure is 4000 psi.

WARNING

NEVER USE DI H_2O ALONE for washing the column. This will lead to significant increase in back pressure. This effect can be reversed by washing the column for long periods of time with buffered high ionic strength eluents. Please make sure to start at a low flow rate to keep the pressure under control. Gradually increase the flow rate as the column pressure drops further.

WARNING

Always maintain minimum ionic strength (20 mM Sodium phosphate, or equivalent) in the eluents.

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SECTION 4 – APPLICATIONS

4.1. Selectivity Differences between ProPac SCX-10 and ProPac SCX-20

Figure 1 shows the separation of a standard protein mixture containing cytochrome C, ribonuclease A and lysozyme on ProPac SCX-10 and ProPac SCX-20 columns and documents the selectivity differences between these two columns. In ProPac SCX-10 column, cytochrome C elutes first, followed by ribonuclease A and lysozyme; The order of elution for the new ProPac SCX-20 is altered. Ribonuclease A elutes first, followed by cytochrome C and lysozyme. Such selectivity differences are quite useful for protein separation and purification applications. Also, the peak capacity for ProPac SCX-20 is superior when compared to ProPac SCX-10 making it the column of choice for protein separation applications.

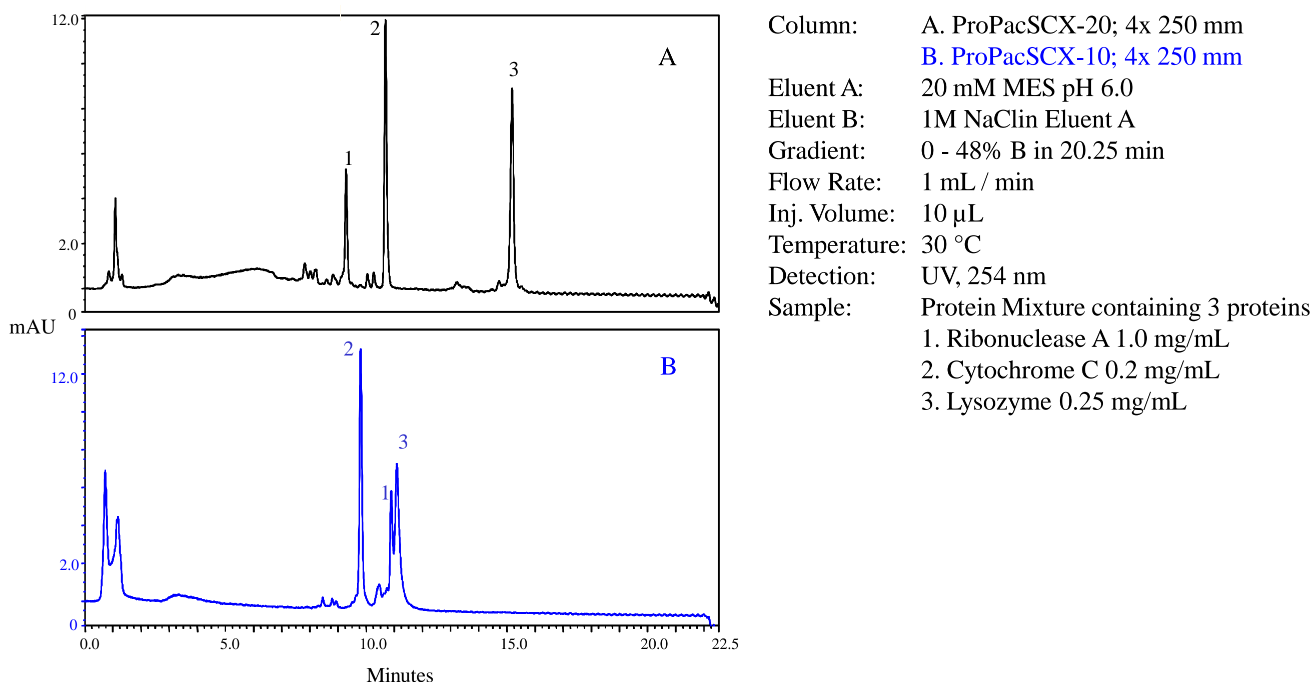

Figure 1
Selectivity differences between ProPac SCX-10 and ProPac SCX-20 columns; Separation of a Protein Mixture.

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4.2. Analysis of Hemoglobin Variants

Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of vertebrates and in the tissues of some invertebrates. Hemoglobin transports oxygen to the rest of the body and releases the oxygen for cell use. In mammals, hemoglobin makes up about 97% of the red blood cell's dry content.

Clinical laboratories frequently separate and quantify the levels of different hemoglobin variants. Separation of hemoglobin sequence variants is shown in Figure 2. Separating and identifying hemoglobins associated with serious hemopathies, including sickle cell, hemoglobin C, and Barts disease are also extremely important in the diagnosis, treatment and counseling of afflicted children.

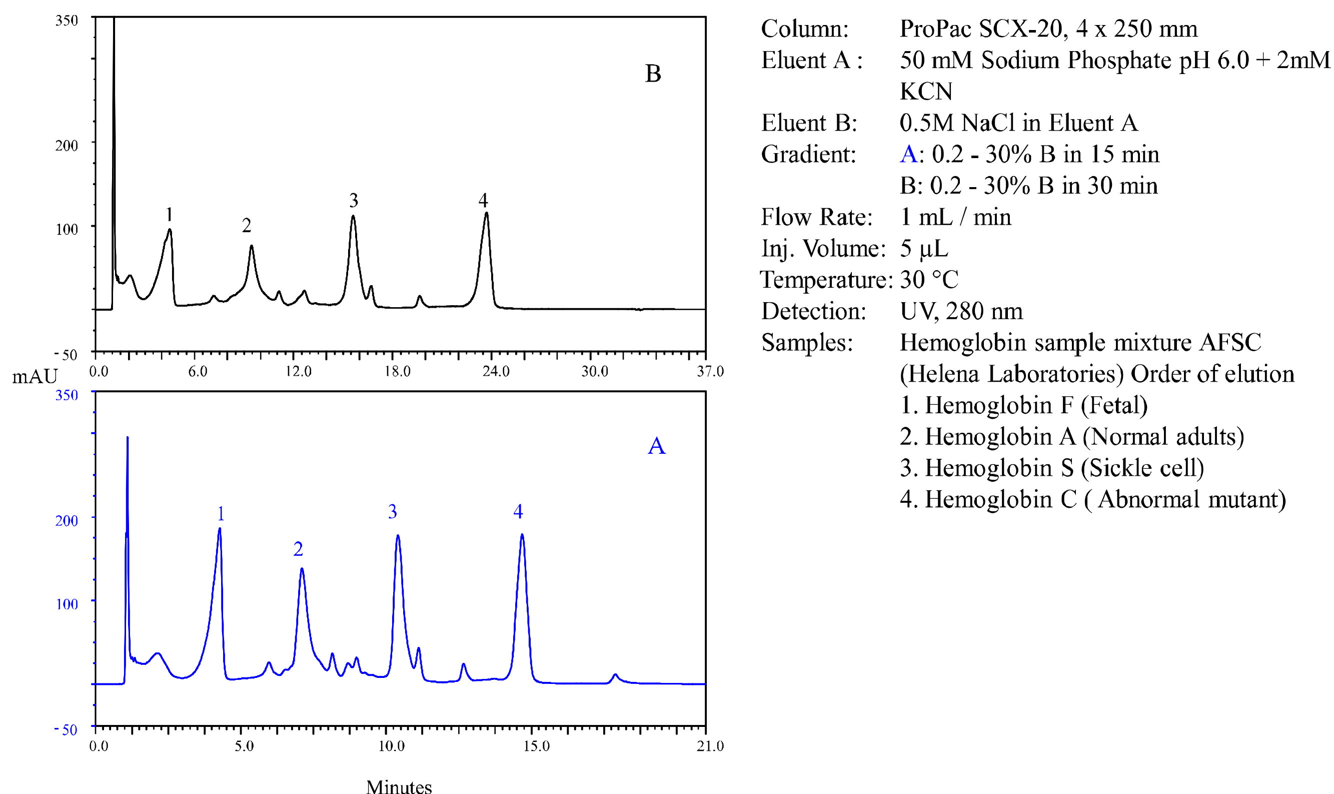

Figure 2
Separation of Hemoglobins AFSC on ProPac SCX-20 Column using different gradient conditions

Typically, isoelectric focusing gel electrophoresis (IEF) is used for the analysis of hemoglobin sequences, including Hb S, C, F and A. However, two IEF steps, using cellulose acetate electrophoresis with alkaline pH, followed by confirmation using citrate agar electrophoresis at acidic pH are necessary. The ProPac SCX-20 column successfully resolves these hemoglobin species in a single run within 20 minutes.

CAUTION

Potassium cyanide is used in the eluents. Dispose of the waste eluents containing potassium cyanide using basic conditions. Treat the waste as hazardous material.

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Figure 3 shows the separation of hemoglobin A2 using both ProPac SCX-10 and ProPac SCX-20 columns. A faster separation could be achieved on ProPac SCX-20 within 16 minutes with a different selectivity and better resolution of the main peak.

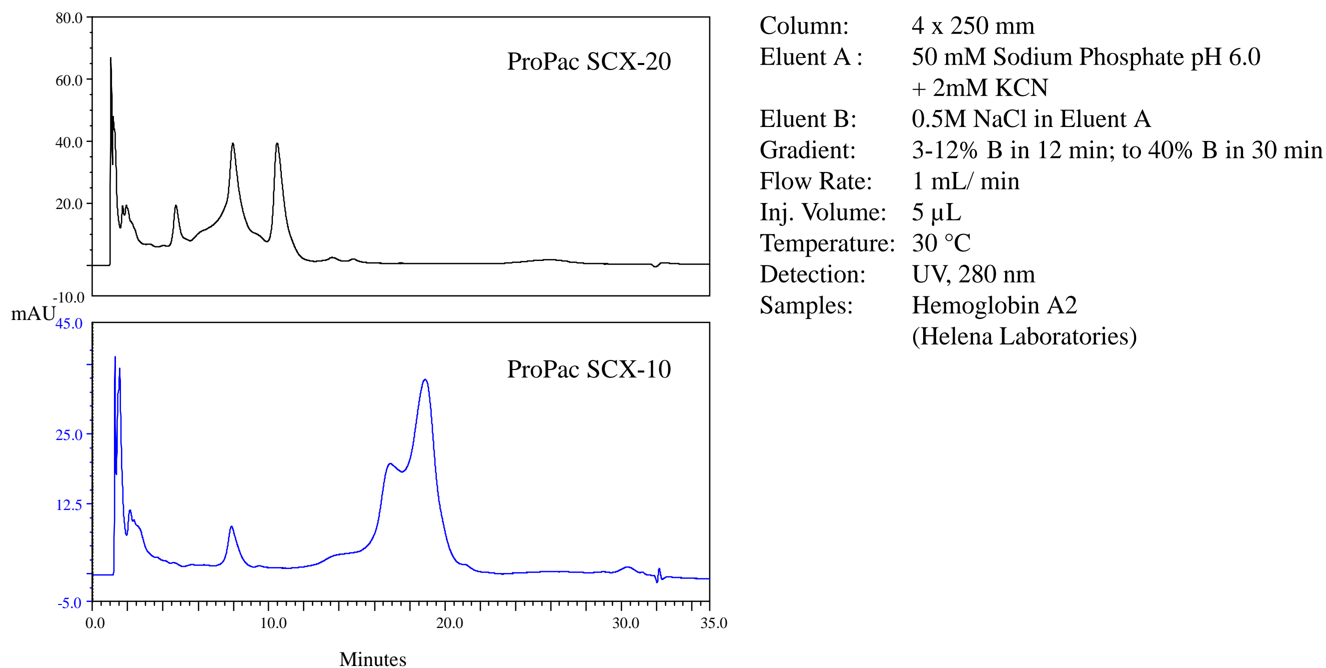

Figure 3
Separation of Hemoglobin A2 on ProPac SCX-10 and ProPac SCX-20 columns

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Separation of Human hemoglobin and Porcine hemoglobin is achieved with ProPac SCX-20 column (Figure 4). Several closely related variant separations could be achieved in less than 20 minutes.

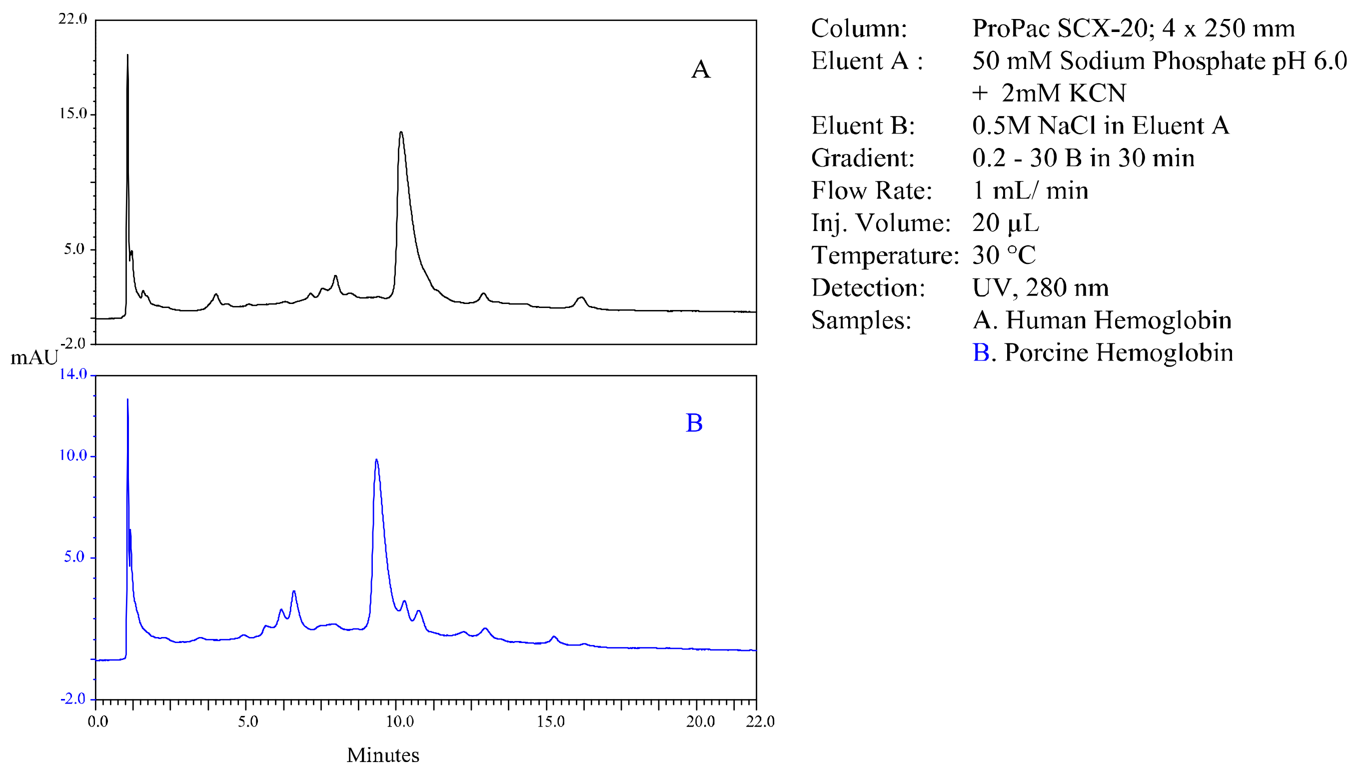

Figure 4
Separation of Human and Porcine Hemoglobins on a ProPac SCX-20 column

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4.3. Monitoring Forced Aspargine Deamidation of Glycoproteins by Cation Exchange Chromatography

Deamidation of Asn residues or the isomerization of Asp residues occurs in a variety of protein-based pharmaceuticals including human growth hormone [1], tissue plasminogen activator [2], hirudin [3], monoclonal antibodies [4], acidic fibroblast growth factor [5], and interleukin 1 [6], with varying effects on the activity or stability of the therapeutic protein. Hence, monitoring the deamidation of Asn residues in proteins is of interest to analytical and protein chemists in quality control and process departments at biotechnology and pharmaceutical companies [7].

As described by A. D. Donato et al. [8], separation of the Asn67 deamidation products of ribonuclease A required cation exchange on Mono S followed by hydrophobic interaction chromatography to resolve the two deamidation variants (Asp and isoAsp at residue 67). In contrast, using only a ProPac WCX-10 Column, deamidation variant forms having Asp or isoAsp at Asn67 were baseline-resolved from each other and from native ribonuclease A in a single chromatographic analysis (9, 10).

Figure 5 shows the separation of ribonuclease A and its deamidation products on ProPac SCX-20 at various incubation time points during the course of the forced deamidation. The baseline separation made it possible to quantify the change in amounts of each form within the mixture as a function of time. This forced deamidation of RNase A follows the method described by Di Donato et al (8). For the deamidation experiment, combine 334 μL of 15 mg/mL RNase A in DI H_2O, 100 μL of 10% ammonium bicarbonate and 566 uL of DI H_2O in a 1.5 mL microcentrifuge tube to make a 5 mg/mL RNase A solution and incubated at 37 ºC. Aliquots were withdrawn (50 μL) intermittently and kept frozen until used for analysis. When ready to analyze the samples, aliquots were thawed, diluted five-fold with the Eluent A (20 mM MES pH 5.6 + 60 mM NaCl, See figure 5) in auto-sampler vials and used for analysis.

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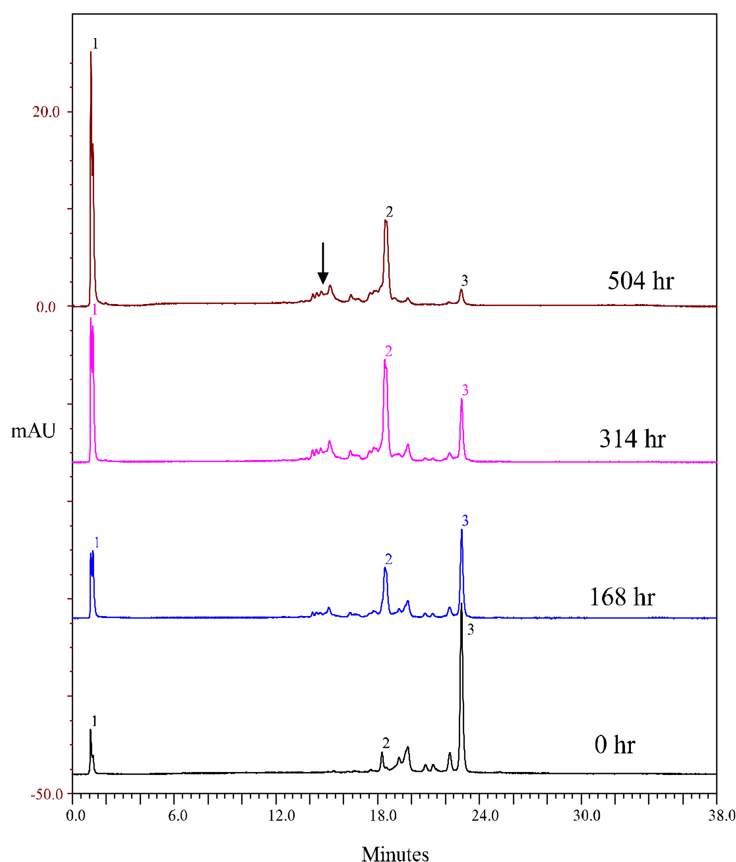

Column: ProPac SCX-20; 4 x 250 mm
Eluent A: 20 mMMES + 60 mM NaCl pH 5.6
Eluent B: 20 mMMES + 300 mM NaCl, pH 5.6
Gradient: 2 - 100% B in 30 min
Flow Rate: 1 mL/ min
Inj. Volume: 5 µL
Temperature: 30 °C
Detection: UV, 280 nm
Samples: RibonucleaseA 1 mg/mL
0 hr (Control)
168 hr
314 hr
504 hr

Peaks: Peak 1: Unbound;
Peak 2: One of the deamidation Peaks;
Peak 3: RibonucleaseMain Peak;

Arrow shows the second deamidation peak, seen as multiple peaks.

Time (hr)	Peak 1 Area (%)	Peak 2 Area (%)	Peak 3 Area (%)
0	0.64 (100%)	0.24(100%)	3.2 (100%)
168	1.37 (214)	1.36(567)	1.66 (52)
314	2.99 (467)	2.78(1158)	1.14 (36)
504	4.39 (686)	2.36(983)	0.31 (10)

Figure 5 and Table 1.
Separation of deamidation products by ProPac SCX-20 column.

Forced deamidation products are formed as a function of time when Ribonuclease A (5 mg/mL) was incubated in 1% ammonium bicarbonate buffer, pH 8.0, at 37 ºC. Peak areas at these increased incubation time points are shown in the accompanying table 1. Percent increase of the unbound peak (Peak 1), deamidation peak (Peak 2), and decrease in parent main peak (Peak 3) are shown in the parenthesis. Arrow shows the 2nd deamidation peak (separated into multiple small peaks)

Figure 5 displays the chromatographic profiles of samples taken during the 37 °C incubation of RNase A in 1% ammonium bicarbonate. One of the deamidation peaks (Peak 2) increases the peak area by more than 11 fold at 314 hr time point. While the native RNase A (Peak 3) peak area decreased by 3 fold. Peak 1 (Unbound) area increased with incubation time. RNase A stability in H_2O at 37 °C is unaffected in the control over the 314 hr incubation period (data not shown). At 0 hr time point deamidated RNase A (Peak 2) is present as a small peak. The resolution of ribonuclease A on ProPac SCX-20 is better than ProPac SCX-10 (See Figure 1). The multiple peaks seen as second deamidation variant (shown with an arrow in Figure 5) could be due to the separation deamidated variants of the protein.

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4.4. Separation of Snake Venom Proteins using ProPac SCX-20 column.

Separation of snake venoms from Naja naja and Russell's viper is shown in Figure 6. Snake venoms are composed of hydrolytic enzymes, a complex mixture of polypeptides (proteases, polypeptidases), peptidases, and nucleases. Some of these components contribute to the toxicity of the venom. These are separated and used for developing anti-venom therapies.

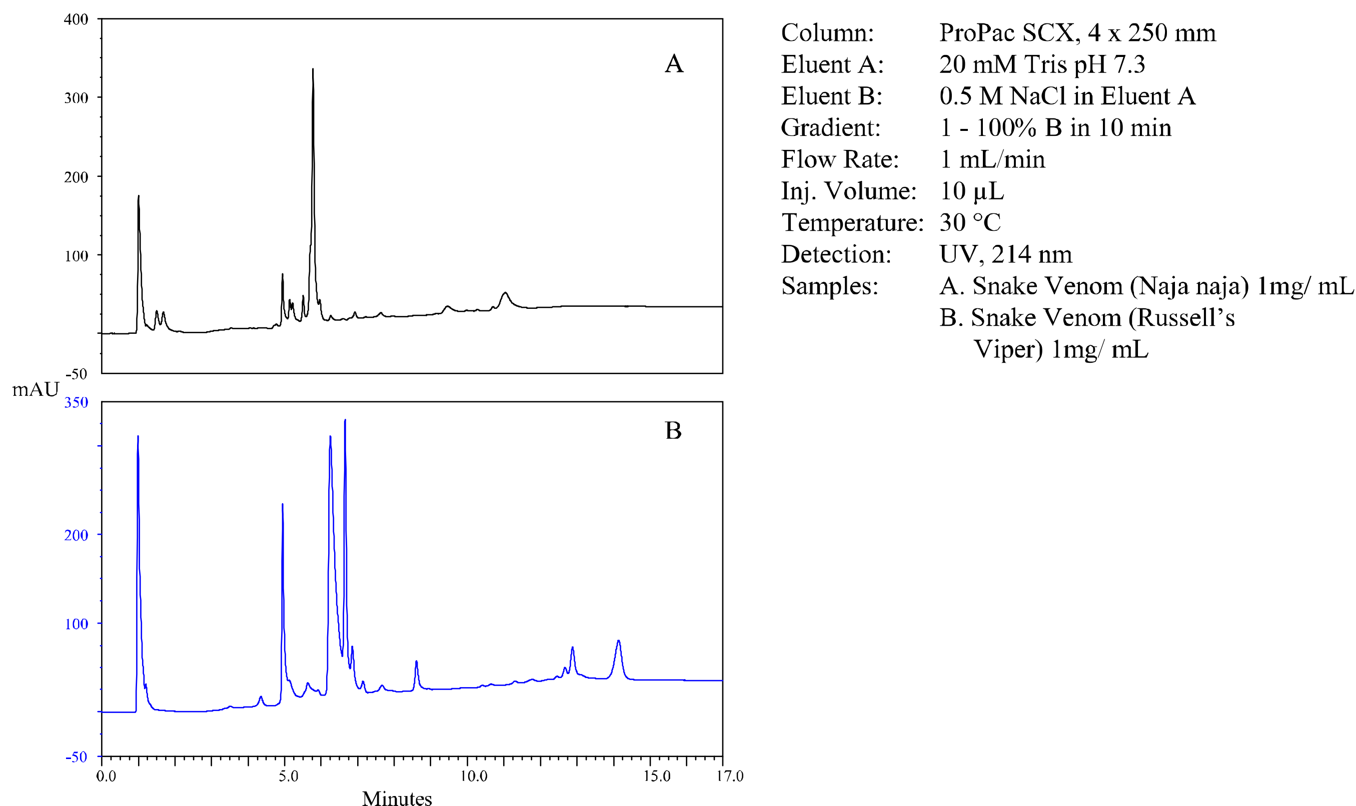

Figure 6
Separation of Snake Venom Proteins on a ProPac SCX-20 column.

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SECTION 5 – TROUBLESHOOTING GUIDE

5.1. Finding the Source of High System Backpressure

WARNING

NEVER WASH THE PROPAC WCX/SCX COLUMN WITH H_2O. Always maintain minimum ionic strength (20 mM, or equivalent) in the eluents.

1. If you observe high back pressure, wash the column with an eluent containing high salt (Buffer containing 1M NaCl) at a lower flow rate (0.1 to 0.5 mL/ min) until the pressure becomes normal.
2. A significant increase in the system backpressure may be caused by a plugged inlet frit (bed support).
3. Before replacing the inlet bed support assembly of the column, make sure that the column is the cause of the excessive backpressure.
4. Check for pinched tubing or obstructed fittings from the pump outlet, throughout the eluent flow path to the detector cell outlet. To do this, disconnect the eluent line at the pump outlet and observe the backpressure at the usual flow rate. It should not exceed 50 psi. Continue adding components (injection valve, column, and detector) one by one while monitoring the system backpressure. The 4 x 250 mm ProPac SCX-20 should add no more than 2200 psi backpressure at 1 mL/min. The 4 x 50 mm ProPac SCX-20 columns should add no more than 600 psi back pressure at 1 mL/min. No other component should add more than 100 psi to the system backpressure.
5. If the high backpressure is due to the column, first try cleaning the column. If the high backpressure persists, replace the column bed support at the inlet of the column.

WARNING

One of the sources of decreased performance could be metal leaching from the system. To avoid denaturation of the protein samples and corrosion of components with halide-salt mobile phases we strongly recommend a metal-free system, including pump, tubing, ferrules, and bolt assemblies.

6. If changes to the system plumbing have been made, check for excess lengths of tubing, tubing diameters larger than 0.010 ID in., larger than normal tubing diameter, and for leaks.
7. Check the flow rate and the gradient profile to make sure your gradient pump is working correctly.
8. The column may be fouled. Clean the column using the recommended cleaning conditions.
9. If there seems to be a permanent loss of efficiency, check to see if headspace has developed in the column. This is usually due to improper use of the column such as submitting it to high backpressure. If the resin doesn't fill the column body all the way to the top, the resin bed has collapsed, creating a headspace. The column must be replaced.
10. If the peak shape looks good, but the efficiency number is low, check and optimize the integration parameters. If necessary, correct the integration manually, so the start-, maximum-, and end of the peak are correctly identified.

5.1.1. Unidentified peaks appear as well as the expected analyte peaks.

1. The sample may be degrading. Proteins tend to degrade faster in solutions; therefore, store your protein samples in the freezer in dry form, and prepare only a small amount of solution/mixture for analysis.
2. The eluent may be contaminated. Prepare fresh, filtered eluent.
3. Run a blank gradient to determine if the column is contaminated. If ghost peaks appear, clean the column.

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5.1.2. Peak efficiency and resolution is poor.

1. Try to use different eluents (buffer, pH, concentration etc.); to make sure you are using the optimum conditions for your separation problem.
2. The column may be overloaded. Dilute the sample and/or inject smaller volumes.

5.1.3. Peak retention time varies from run-to-run.

1. The column may not be adequately equilibrated or washed.
2. Make sure that the equilibration time is adequate and remains constant after every gradient run. Re-equilibration should be part of the method.
3. Column washing is usually not necessary between every run, unless your sample is extremely "dirty." If you need to use a wash, a consistent and adequate method for washing and equilibrating should be part of the method.

5.2. No Peaks, Small Peaks, Noisy Baseline

5.2.1. Detection Problem

Make sure that you are using the correct wavelength for your sample/buffer system. Adjust the selected detector range (AU) according to your injected sample amount. Check your lamp: aged UV lamps tend to give noisier response. Replace the lamp if necessary.

5.2.2. Chromatographic Problem

Make sure that your sample can be eluted with the buffers and conditions you are using. Before trying a gradient separation, try isocratic elution with 100% B (high salt) buffer: the sample should elute at, or near to, t0 (void). If not, try a higher salt concentration or different pH.

5.2.3. Pump Problem

A noisy baseline can be caused by an improperly working pump. Be sure the pump is primed. Test the flow rate and also the pump head pressure fluctuation according to your pump manual.

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APPENDIX A. REFERENCES

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9. Dionex Corporation; ProPac WCX/SCX Manual: See applications.

10. Dionex Corporation; Application Note 125

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APPENDIX B. PROPAC SCX-20 COLUMN CARE

B.1 New Column Equilibration

The columns are shipped in 10 mM Na_2HPO_4/H3PO4 pH=6.5 buffer containing 0.1% sodium azide. Before use, wash the column with approximately 20 mL of the starting eluent (20 min at 1 mL/min).

B.2 Column Clean-Up

NOTE

When cleaning an analytical and guard column in series, move the guard column after the analytical column in the eluent flow path. Otherwise contaminants that have accumulated on the guard column will be eluted onto the analytical column.

1. For minor contamination, use a mild cleaning protocol by running consecutive gradient runs, using a high (1-2 M) salt concentration at the end of the gradient.
2. For more severe contamination, inject 100-500 µL (or more as needed) of 0.1-0.5 M NaOH consecutively.
3. If necessary, the column can be washed with strong acid and/or base such as 1.0 M HCl and/or 0.1 - 0.5 M NaOH. Usually 5 - 30 min at 1 mL/min is sufficient. Do not exceed 20x the column volume of 0.5 M NaOH (60 mL). The use of high concentrations of base and/or larger volumes of base is not recommended. The ProPac SCX-20 column can be washed at temperatures up to 60 °C. The above mentioned strong acid or base cleaning solutions should be used at room temperature (<30 °C). After the wash, rinse the column with at least 20 mL of the starting buffer solution.

NOTE

Do not store the column in strong acid or base solution.

B.3 Column Storage

Short Term Storage: For short term storage, use the low salt concentration eluent (pH = 3 - 10) as the column storage solution.

Long Term Storage: For long term storage, use 20 mM Na_2HPO_4/H3PO4 pH=6.5 eluent (or other low salt concentration eluent with pH=6.0-7.5) with 0.1% sodium azide added to avoid bacteria growth on the column.

Flush the column with at least 10 mL of the storage eluent. Cap both ends, securely, using the plugs supplied with the column.

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B.4 Replacing Column Bed Support Assemblies

NOTE

Replace the inlet bed support ONLY if the column is determined to be the cause of high system backpressure, AND cleaning of the column does not solve the problem.

1. Carefully unscrew the inlet (top) column fitting. Use two open end wrenches.
2. Remove the bed support. Tap the end fitting against a hard, flat surface to remove the bed support and seal assembly. Do not scratch the wall or threads of the end fitting. Discard the old bed support assembly.
3. Removal of the bed support may permit a small amount of resin to extrude from the column. Carefully remove this with a flat surface such as a razor blade. Make sure the end of the column is clean and free of any particulate matter. Any resin on the end of the column tube will prevent a proper seal. Insert a new bed support assembly into the end fitting and carefully thread the end fitting and bed support assembly onto the supported column.
4. Tighten the end fitting finger-tight, then an additional ¼ turn (25 in x lb.). Tighten further only if leaks are observed.

WARNING

If the end of the column tube is not clean when inserted into the end fitting, particulate matter may prevent a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or break the end fitting.

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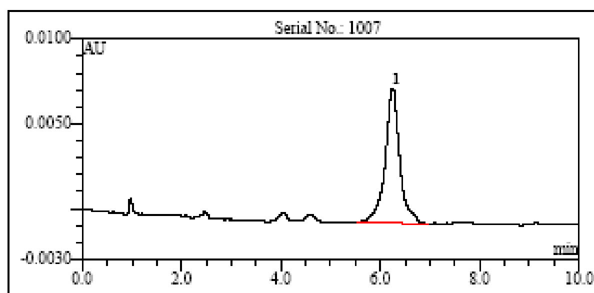
APPENDIX C. QUALITY ASSURANCE REPORTS

ProPac SCX-20
Analytical (4 x 250 mm)
Product No. 074628

Date: 03-Jun-10 13:22
Serial No. : 001007
Lot No. : 2010-09-03

Eluent:	162.8 milli Molal NaCl + 10.17 milli Molal Na2HPO4 (pH = 6.5)
Flow Rate:	1.0 mL/min
Temperature:	25 °C
Detection:	Absorbance Detection using AD25 UV at 254 nm
Injection Volume:	10 µL
Storage Solution:	10mM Na2HPO4 pH = 6.0 + 0.1% NaN3



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Concentration (mg/mL)
1	Cytochrome C	6.2	1.0	n.a.	2895	0.250

QA Results:

Analyte	Parameter	Specification	Results
Cytochrome C	Efficiency	>=2070	Passed
Cytochrome C	Asymmetry	0.9-1.8	Passed
Cytochrome C	Retention Time	6.1-8.3	Passed
	Pressure	<=2200	1563

Production Reference:

Datasource: QAR
Directory: ProPac\SCX-20
Sequence: PP_SCX20
Sample No.: 1

6.80 SR9a Build 2680 (163077) (Demo-Installation)

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074630-01 (QAR)

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Lot Validation
ProPac® SCX-20
(Using 4 x 250 mm)

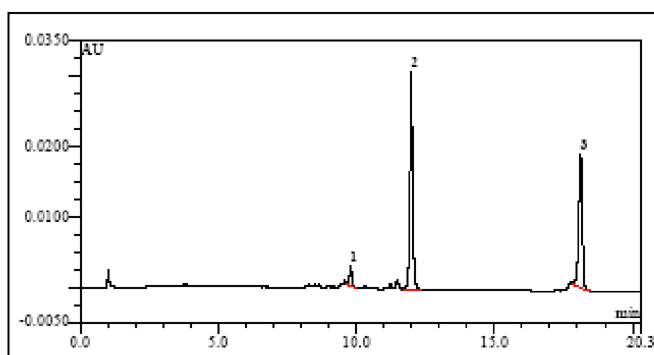
Date: 30-Jun-10 11:01
Lot No. : 2010-09-03

Eluent Flow Rate: 1.0 mL/min
Temperature: 25 °C
Detection: Absorbance Detection
UV at 254 nm
Injection Volume: 10 µL
Storage Solution: N/A

Buffers:
%A: 10 mm (milli Molal) Na2HPO4 (pH=6.0+/-0.02)
%B: 1002 mm (milli Molal)NaCl in 10 mM Na2HPO4 (pH=6.0+/-0.02)

Buffer Gradient

Time	%A	%B	Comment
-2.00	100	0	
0.00	100	0	
20.25	52	48	
20.30	0	100	
25.00	0	100	
25.10	100	0	
30.00	100	0	



No.	Peak Name	Ret.Time (min)	Peak Width (50%)	Resolution (EP)	Efficiency (EP)	Concentration (mg/mL)
1	Ribonuclease A	9.79	0.08	14.92	75801	0.5
2	Cytochrome C	11.99	0.09	32.72	96583	0.5
3	Lysozyme	18.13	0.13	n.a.	106926	0.5

QA Results:

Analyte	Parameter	Specification	Results
Ret. of (Lyso-Cyto)/(Cyto-Ribo)	Selectivity	2.69-2.91	Passed

Production Reference:
Datasource: QAR
Directory: ProPac\SCX-20
Sequence: PP_SCX20_GRADIENT
Sample No.: 1

6.80 SR9a Build 2680 (163077) (Demo-Installation)

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