New ProSwift Monolith Reversed-Phase and Ion-Exchange Columns and Their Comparative Evaluation with Other Biocolumns

Srinivasa Rao, Kelly Flook, Jim Thayer, Charanjit Saini, Maria Rey, Andy Woodruff, Yury Agroskin and Chris Pohl,
Dionex Corporation, Sunnyvale, CA, 94085

DIONEX
Passion. Power. Productivity.

ABSTRACT

Monolith columns offer significant advantages over conventional packed columns with porous substrates. These advantages include fast mass transfer, high loading capacity, improved resolution at elevated flow rates,lower backpressure, and wide pH stability. These exclusive characteristics support versatile performance in a wide range of biomolecule separations. Dionex has introduced reversed-phase anion- and cation-exchange phases of ProSwift™ monoliths (4.6 × 50 mm). Reversed-phase ProSwift columns (4.6 × 50 mm) include RP-1S, RP-2H and RP-3U versions. Each differs in pore structure and selectivity for various bioseparations. The low backpressure of the ProSwift RP columns support fast separation of analytes at high flow rates, providing increased productivity. ProSwift ion-exchange phases (4.6 × 50 mm) include weak anion- exchange (WAX-1S) and strong anion-exchange (SAX-1S) columns, and a weak cation-exchange (WCX-1S) column. The most recent additions to the monolithic column family line include the 1 × 50 mm WAX-1S and WCX-1S columns, developed especially for high resolution microanalytical biomolecule separations. The 1 mm columns offer improved sensitivity and reduced solvent consumption. Using both reversed phase and ion-exchange columns, we have developed various applications and compared with competitor columns. These results will be presented.

INTRODUCTION

ProSwift monolithic columns, available with either reversed-phase or ion-exchange chemistries, are specifically designed to provide high-resolution, high-efficiency separations of proteins, peptides, and other biomolecules, using conventional HPLC systems.

A monolith consists of aggregations of globules that resemble cauliflower in appearance.The open spaces between these aggregates are large flow-through channels, which help to minimize column backpressure. The spaces among the smaller globules are open or “through-pores” which allow the sample to interact with the surface of the media quickly. The mass transfer of the sample is primarily driven by convective flow through these open pores, instead of molecular diffusion, which is much slower. These pores are big enough to allow even large molecules to flow through freely. (Most small molecules are less than 500 nm.)

Therefore, the path lengths for mass transfer through these small globules are much shorter than the path lengths in conventional bead-based chromatographic phases. In addition, the globules are essentially non-porous (based on nitrogen adsorption (BET) measurements and scanning electron microscopy (SEM) examinations). Thus, diffusion-controlled mass transfer is minimized using these columns. By contrast, diffusion controlled mass transfer is a predominant feature of columns packed with porous beads.

Features of ProSwift Monoliths

- High speed and high resolution
- Fast mass transfer
- Low backpressures
- Wide range of operational flow rates (RP Columns)
- High throughput and improved productivity
- High loading capacity (IEX Columns)
- Excellent stability over a wide pH range
- Outstanding reproducibility
- Optimal performance

MATERIALS

Chromatographic components

RP: P680 HPG gradient pump, UVD 340 absorbance detector, UltiMate® 3000 autosampler and TCC-100 Thermostatted Column Compartment from Dionex Corporation.

IEX: ICS-3000 DP gradient pump, VWD Absorbance detector (or, UVD 340), AS autosampler, and TCC-100 Thermostatted Column Compartment from Dionex Corporation.

Chromatography was controlled by Chromeleon® Chromatography Management software (Dionex Corporation).Proteins used in standards, MES, Tris and all other analytical grade chemicals were obtained from Sigma-Aldrich Co.

ISPPP 2007 Presentation

Monolithic Columns from Dionex Corporation

ProSwift RP-1S 4.6 × 50 mm, P/N 064297

ProSwift RP-3U 4.6 × 50 mm. P/N 064298

ProSwift RP-2H 4.6 × 50 mm, P/N 064296

ProSwift SAX-1S 4.6 × 50 mm,
PEEK-lined stainless steel, P/N 064293

ProSwift WAX-1S 4.6 × 50 mm,
PEEK-lined stainless steel, P/N 064294

ProSwift WAX-1S 1 × 50 mm, PEEK, P/N 066642

ProSwift WCX-1S 4.6 × 50 mm,
PEEK-lined stainless steel, P/N 064295

ProSwift WCX-1S 1 × 50 mm, PEEK, P/N 066643

Columns from Other Manufacturers

Competitor A: RP Leading biocolumn

Competitor B: Leading strong anion-exchange column

Competitor C: Leading weak cation-exchange column

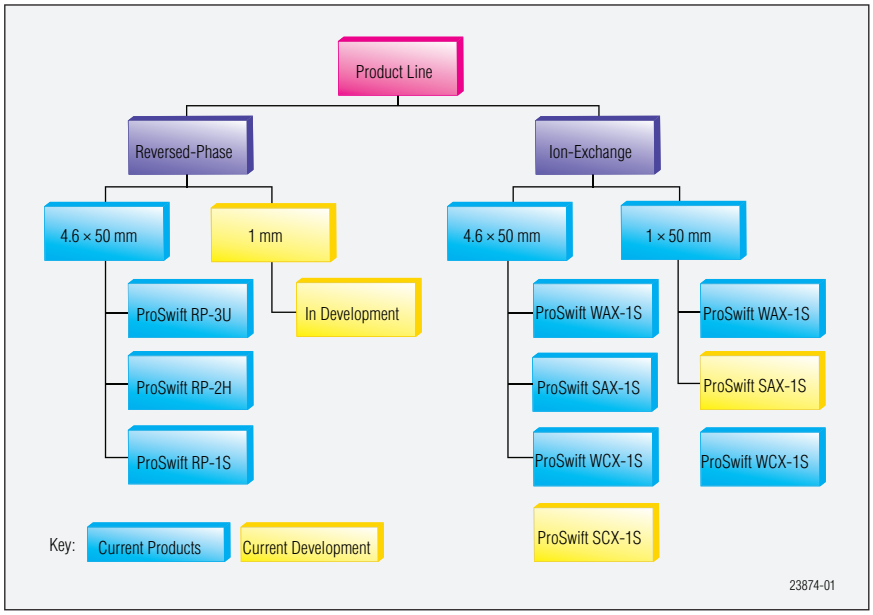


Figure 1. The ProSwift family.

REVERSED PHASE

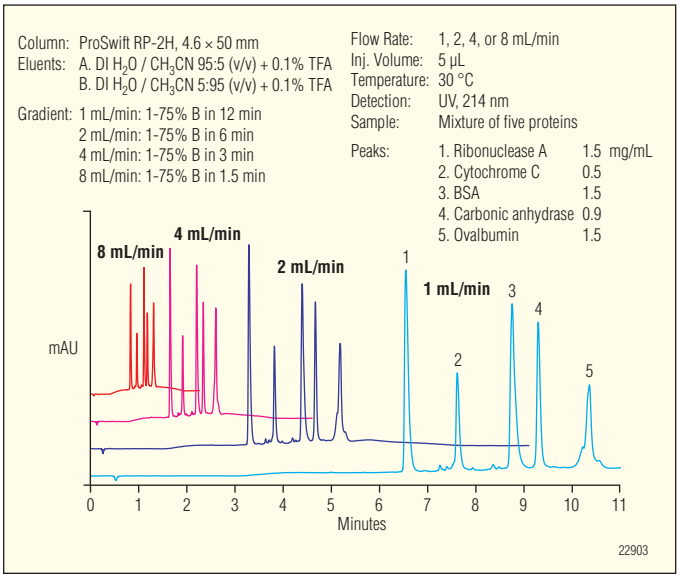


Figure 2. Separation of protein standard mixture on RP-2H with increasing flow rates.

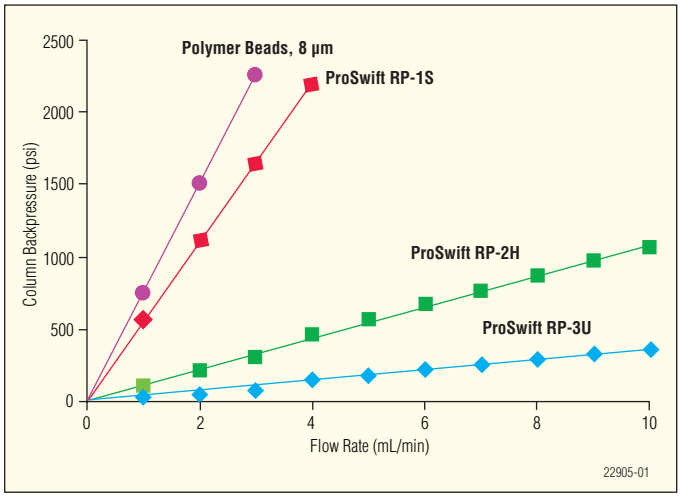


Figure 3. Column backpressure vs. flow rate.

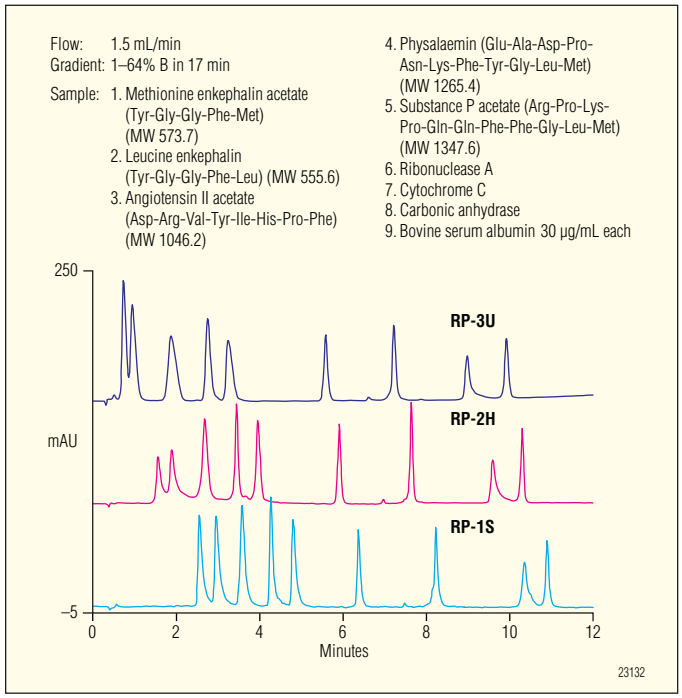


Figure 4. Peptide and protein separation on RP columns.

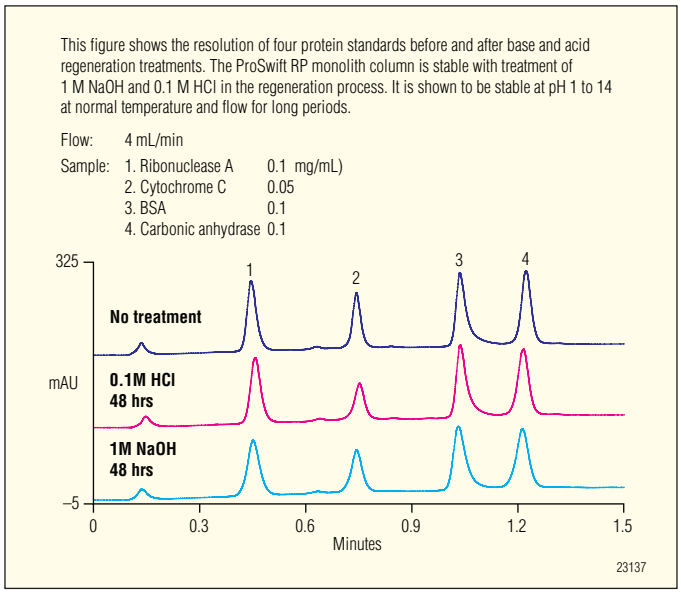


Figure 5. pH stability of the ProSwift RP-3U.

REVERSED PHASE— COMPETITOR COMPARISON

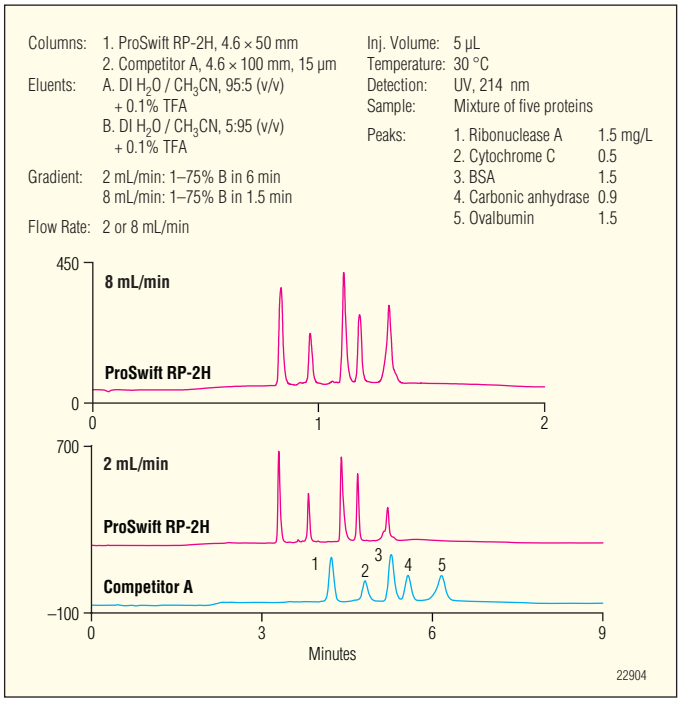


Figure 6. Comparison of RP-2H with competitor A column; separation of protein standard mixture.

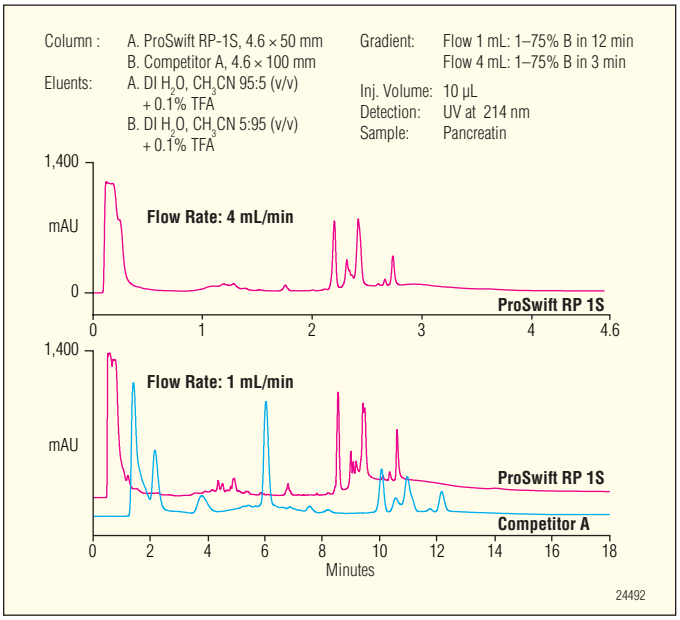


Figure 7. Comparison of ProSwift RP-1S with competitor A column; separation of pancreatin.

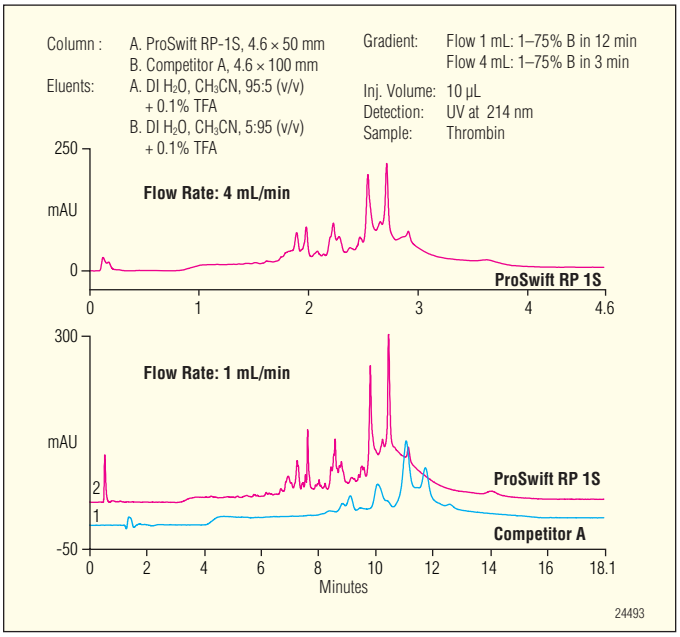


Figure 8. Comparison of ProSwift RP-1S with competitor A column; separation of thrombin.

ANION EXCHANGE

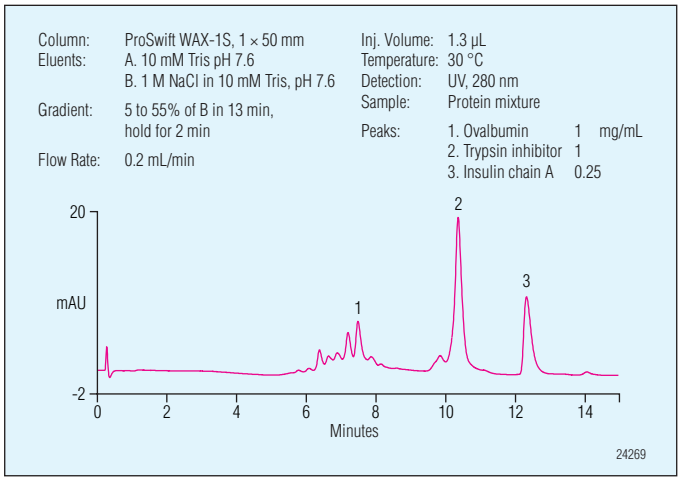


Figure 9. Separation of protein mix on WAX-1S.

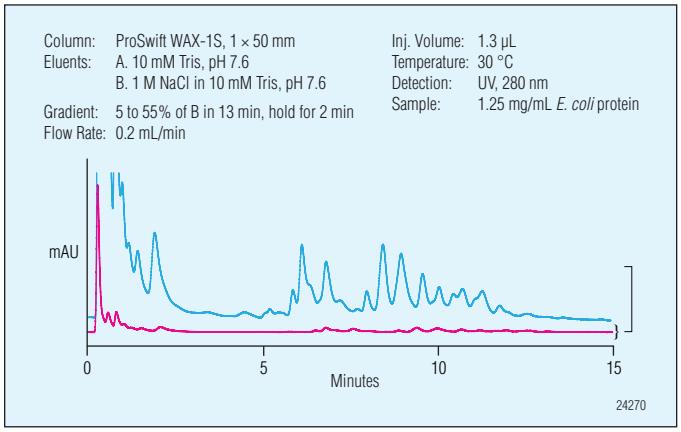


Figure 10. Separation of E. coli *proteins on the WAX-1S.*

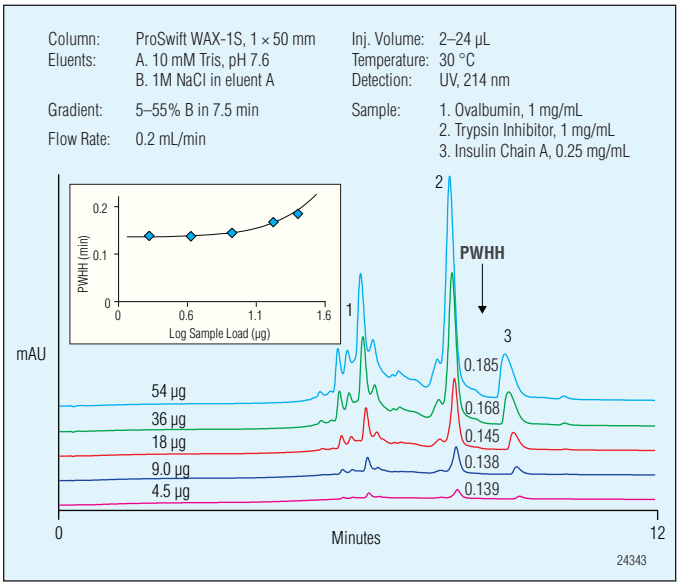


Figure 11. Protein loading on WAX-1S.

ANION EXCHANGE—COMPETITOR COMPARISON

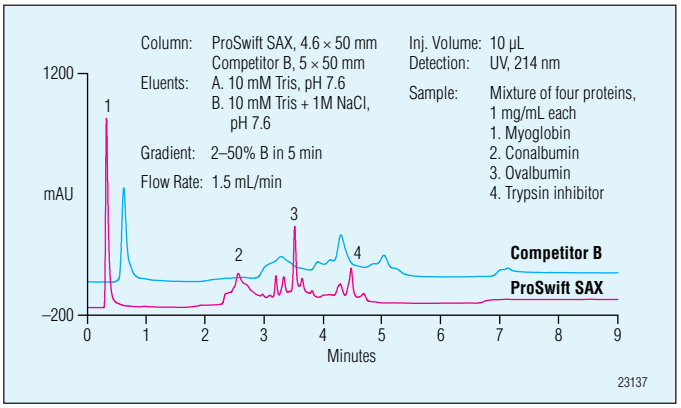


Figure 12. Comparison of ProSwift SAX and competitor B columns; separation of protein mixture.

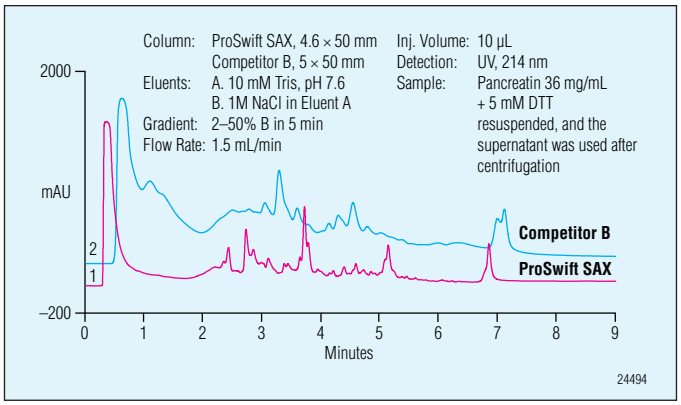


Figure 13. Comparison of ProSwift SAX and competitor B columns; separation of pancreatin.

CATION EXCHANGE

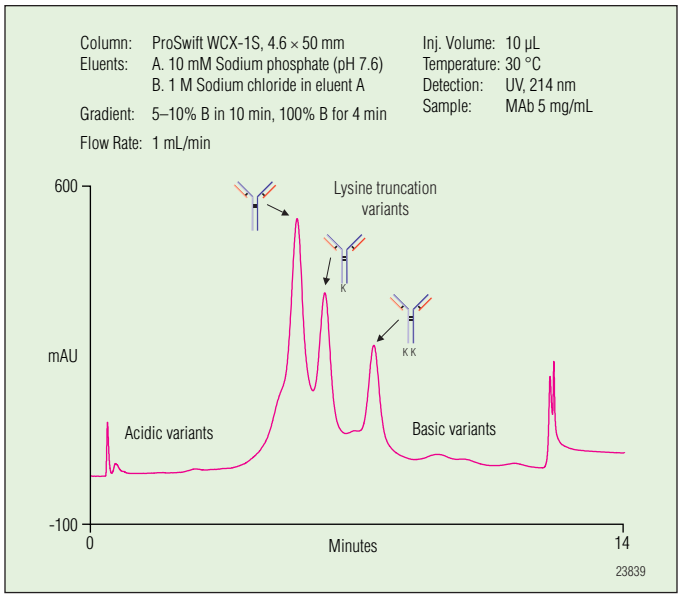


Figure 14. Separation of monoclonal antibody (MAb) on WCX-1S.

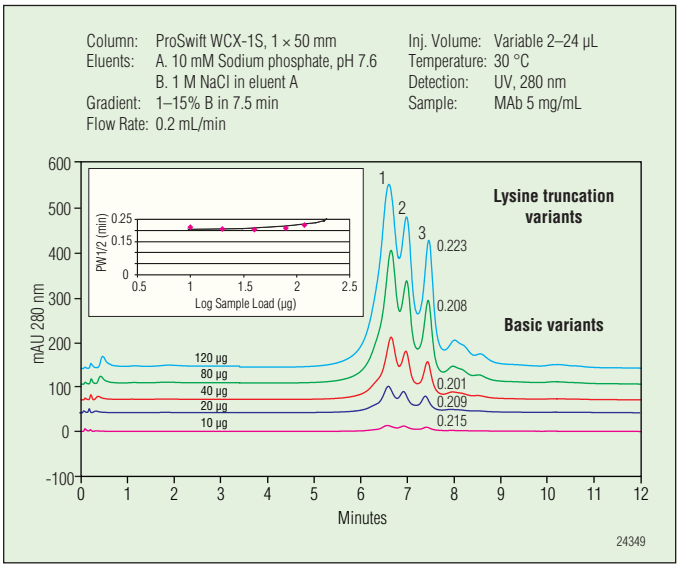


Figure 15. Loading capacity of MAb on WCX-1S.

CATION EXCHANGE—COMPETITOR COMPARISON

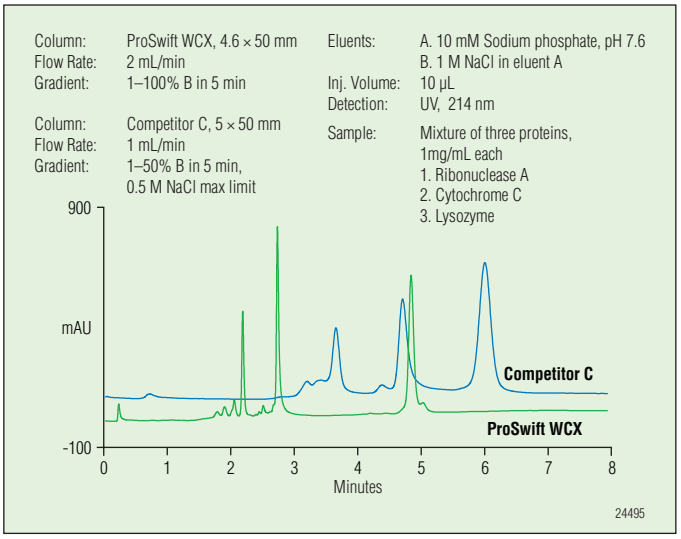


Figure 16. Comparison ProSwift WCX with competitor C column; separation of protein mixture.

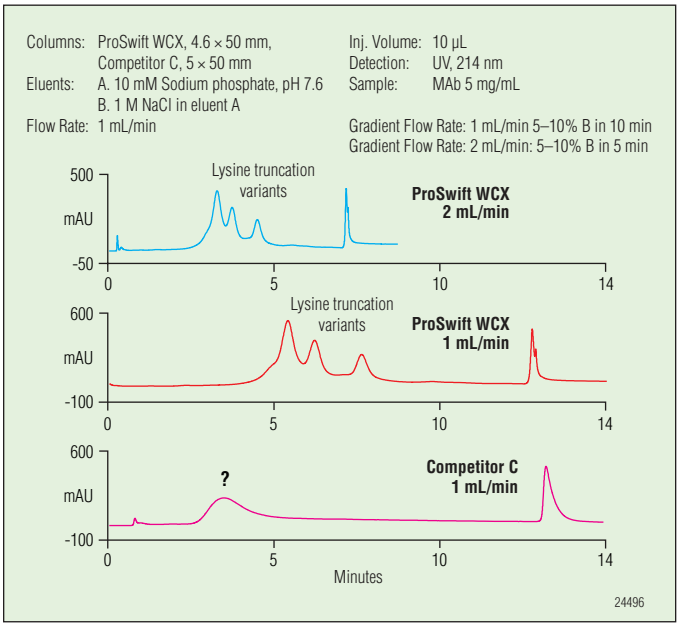
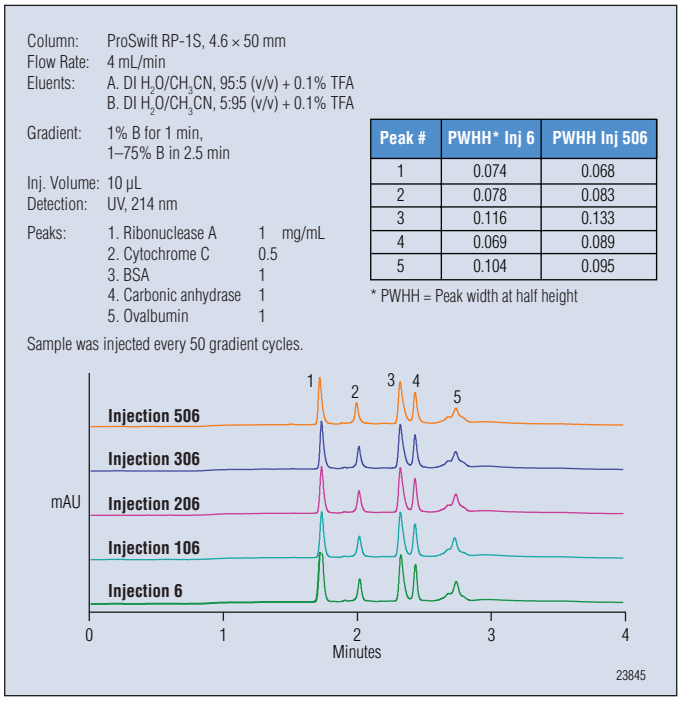


Figure 17. Comparison ProSwift WCX with competitor C column; separation of monoclonal antibody (MAb).

STABILITY AND RUGGEDNESS



Peak #	PWHH* Inj 6	PWHH Inj 506
1	0.074	0.068
2	0.078	0.083
3	0.116	0.133
4	0.069	0.089
5	0.104	0.095

Figure 18. ProSwift RP-1S robustness.

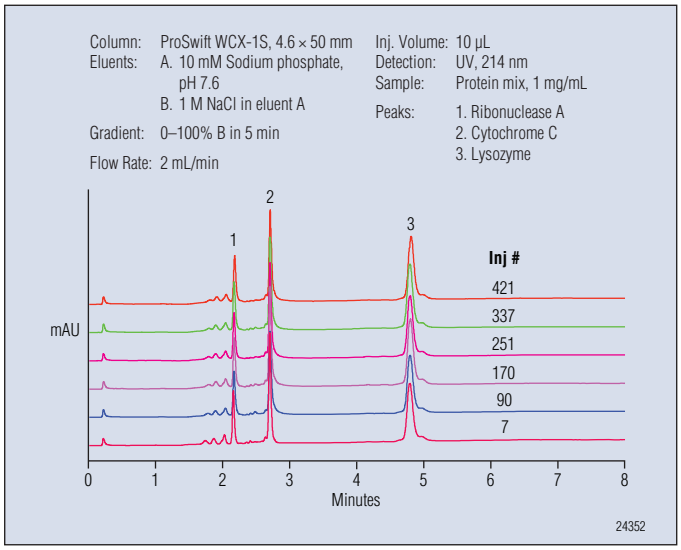


Figure 19. Stability of WCX-1S.

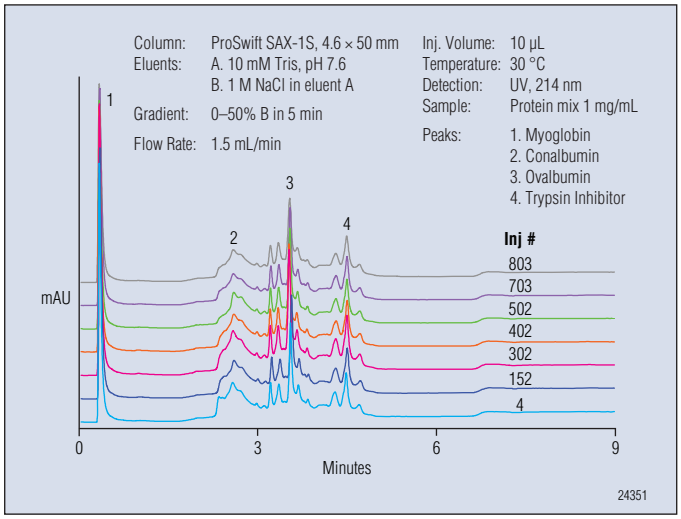


Figure 20. Stability of SAX-1S.

CONCLUSION

- ProSwift monoliths are a new family of columns developed for protein separations. They offer high speed, high resolution, and high loading capacity.
- ProSwift monolith columns are available with reversed-phase and ion-exchange chemistries.
- ProSwift RP column offerings include RP-1S, RP-2H, and RP-3U each with different pore sizes.
- ProSwift ion-exchange columns include WAX-1S, SAX-1S and WCX-1S, available in 4.6 × 50 mm (all) and 1 × 50 mm (WAX-1S and WCX 1S) dimensions. ProSwift SCX (4.6 × 50 mm) and ProSwift SAX (1 mm)columns are in development.
- ProSwift RP and IEX columns were compared with leading biocolumns for various applications. The results confirmed that ProSwift columns performed better with a higher efficiency and resolution even at a higher flow rate, which results in higher productivity (RP comparisons: Figures 6, 7, 8, and IEX comparisons: Figures 12, 13, 16, 17)
- Earlier, we introduced 1mm column dimensions for WAX and WCX chemistries. Currently, we are developing SAX as well as RP chemistries. These 1mm format columns offer improved sensitivity and reduce solvent consumption. Due to the high capacity of ProSwift IEX columns, they are ideally suited to be used in the first dimension in a multidimensional chromatography separation.
- The low backpressures inherent to ProSwift columns enable the use of high flow rates, resulting in high-throughput chromatographic separations.
- ProSwift columns offer excellent stability, reproducibility and overall performance.

Passion. Power. Productivity.

DIONEX

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America
U.S. (847) 295-7500
Canada (905) 844-9650

South America
Brazil (55) 11 3731 5140

Europe
Austria (43) 1 616 51 25 Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 885 1700 Italy (39) 02 51 62 1267 Switzerland (41) 62 205 9966
United Kingdom (44) 1276 691722

Asia Pacific
Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 28475235
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.dionex.com

ISO 9001 REGISTERED

LPN 1990-01 10/07