



# Size Exclusion Separation of Protein Mixtures: Comparison of Sepax SRT<sup>®</sup> SEC Columns and Tosoh TSKgel<sup>®</sup> Columns

Protein Separation

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## Abstract

There are increasing numbers of biological large molecules such as proteins and antibodies manufactured as pharmaceutical therapeutics. It is often a challenge to separate these molecules from lysates and partially purified protein mixes, or differentiate them from variants with different post-translational modifications. The data presented here focus on the use of analytical SRT<sup>®</sup> SEC 150 and 300Å pore sizes with 5 µm particle size to separate several commercially available proteins with different molecular weights. The separation performance is compared with that of Tosoh TSK G2000SWxl and G3000SWxl respectively.



## Introduction

Size exclusion chromatography (SEC) has been shown as an effective way to separate different sizes of proteins, as well as buffer exchanges at the same time. Since SEC technique can be achieved with high resolution with few protein components or partially purified protein mixtures, often researchers use it in the final polishing step in a protein purification scheme or Quality Control departments use it in the detection of the sample uniformity. Samples of interest can be separated from aggregates or variants with different modifications occurred during the process – a crucial step in ensuring the quality of biological products.

Sepax SRT<sup>®</sup> SEC columns are based on uniform, hydrophilic, and neutral nanometer thick films chemically bonded on high purity and mechanically stabilized silica. The unique property of the resin minimizes the non-specific interactions between biological samples and the column medium. SRT<sup>®</sup> SEC provides a wide selection of pore sizes: 100, 150, 300, 500, 1,000 and 2,000Å pore sizes for different molecular weight separation limits. In this application note, the performance of SRT<sup>®</sup> SEC 150 and 300Å pore sizes with 5 µm particle size is compared with that of Tosoh TSK G2000SWxl and G3000SWxl respectively with the same column dimensions.

## Experimental

### HPLC system:

Agilent 1200 HPLC with binary pump

### SEC columns:

Sepax SRT<sup>®</sup> SEC-150 (150 Å, 5 µm), Tosoh TSK G2000SWxl (125 Å, 5 µm), 7.8x300 mm  
Sepax SRT<sup>®</sup> SEC-300 (300 Å, 5 µm), Tosoh TSK G3000SWxl (300 Å, 5 µm), 7.8x300 mm

### Chemicals and Reagents:

Thyroglobulin, BSA, Ribonuclease A, ply-DL alanine and Uracil were purchased from Sigma-Aldrich. Monobasic and dibasic sodium phosphate were purchased from EMD.

## LC Method:

For SRT<sup>®</sup> SEC-150 and Tosoh TSK G2000SWxl, the flow rate is 1ml/min, mobile phase is 150 mM sodium phosphate, pH 7.0, and the temperature is ambient at 23 degree C.

Sample injection volume is 10 µl with following components: Thyroglobulin, BSA, Ribonuclease A at 1.0 mg/ml each and Uracil at 2.5 µg/ml. For SRT<sup>®</sup> SEC-300 and TSK G3000SWxl, the column running conditions are the same as that for SRT<sup>®</sup> SEC-150, except the sample concentration is as follows: thyroglobulin, BSA, ribonuclease A (1 mg/mL); uracil (0.1 mg/mL), injection volume is 5 µl.

## Results

In comparison of SRT<sup>®</sup> SEC-150 and Tosoh TSK G2000SWxl, figure 1 shows the similar separation profiles from both columns under the same LC running conditions. SRT<sup>®</sup> SEC-150 exhibits a slightly better resolution for Ribonuclease A and Poly-DL-alanine. Table 2 summarizes the retention time for each eluted protein peak. With column dimension at 7.8x300 mm, the retention time for each protein peak is similar using both columns.

SRT<sup>®</sup> SEC-300 and TSK G3000SWxl have the same column dimension, particle size and porosity. The elution profiles are exhibiting similar chromatographic patterns, under 1 ml/min flow rate and 150 mM phosphate buffer, pH7.0. SRT<sup>®</sup> SEC-300 shows a better separation resolution with Thyroglobulin and its aggregated forms 9 (figure 2).

## Conclusion

In comparison to Tosoh G2000SWxl and G3000SWxl, Sepax SRT<sup>®</sup> SEC-150 and SRT<sup>®</sup> SEC-300 demonstrate a similar or slightly better separation with the same protein mixtures respectively. Sepax SRT<sup>®</sup> SEC columns can be a great alternative for biologics separations, which currently use Tosoh TSK columns.

**For more information on SRT<sup>®</sup> SEC products, please visit Sepax website: <http://www.sepax-tech.com/SRT.php> or contact us at 1-877-SEPAX-US**

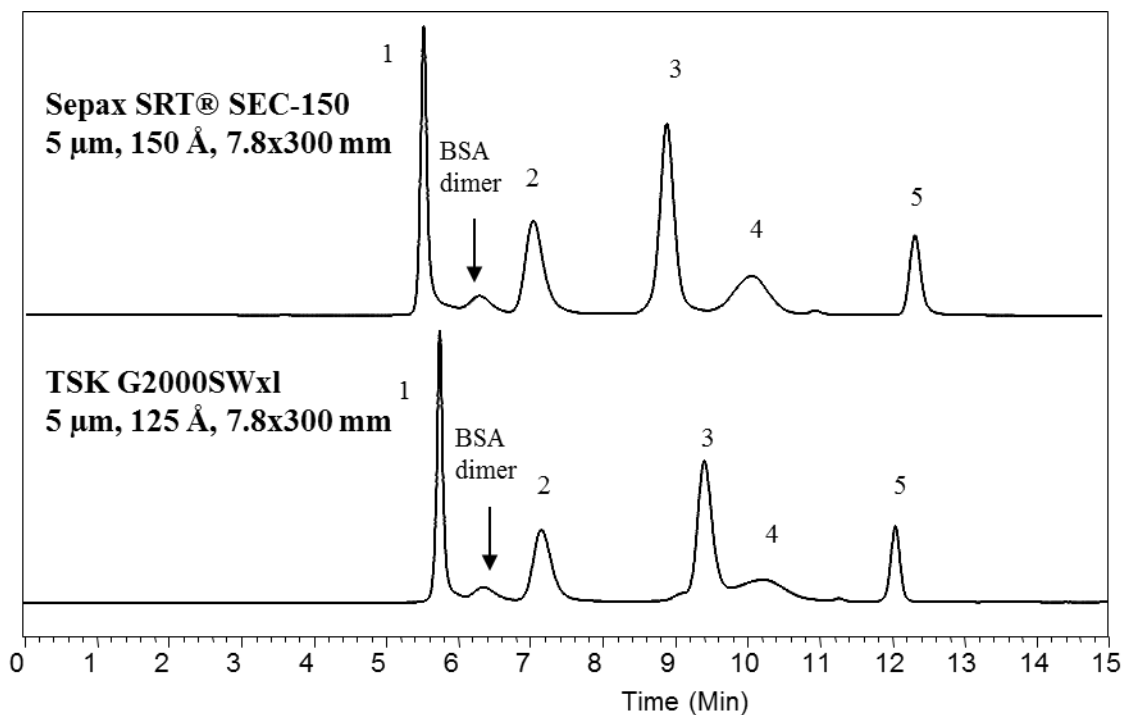


Figure 1. Comparison of chromatograms of SRT<sup>®</sup> SEC-150 and Tosoh TSK G2000SWxl, 7.8x300 mm using protein mixture 1) Thyroglobulin, 2) BSA monomer, 3) Ribonuclease A, 4) Poly-DL-alanine, 5) Uracil

Peak	Protein	MW (kD)	SRT SEC-150 RT (min)	TSKG2000SWxl RT (min)
1	Thyroglobulin	670	5.43	5.64
2	BSA monomer	66	6.93	7.02
3	Ribonuclease A	13.7	8.74	9.22
4	Poly-DL-alanine	1-5	9.9	10.02
5	Uracil	0.12	12.13	11.81

Table 1. Comparison of retention time of eluted proteins with SRT<sup>®</sup> SEC-150 and TSK G2000SWxl, 7.8x300 mm.

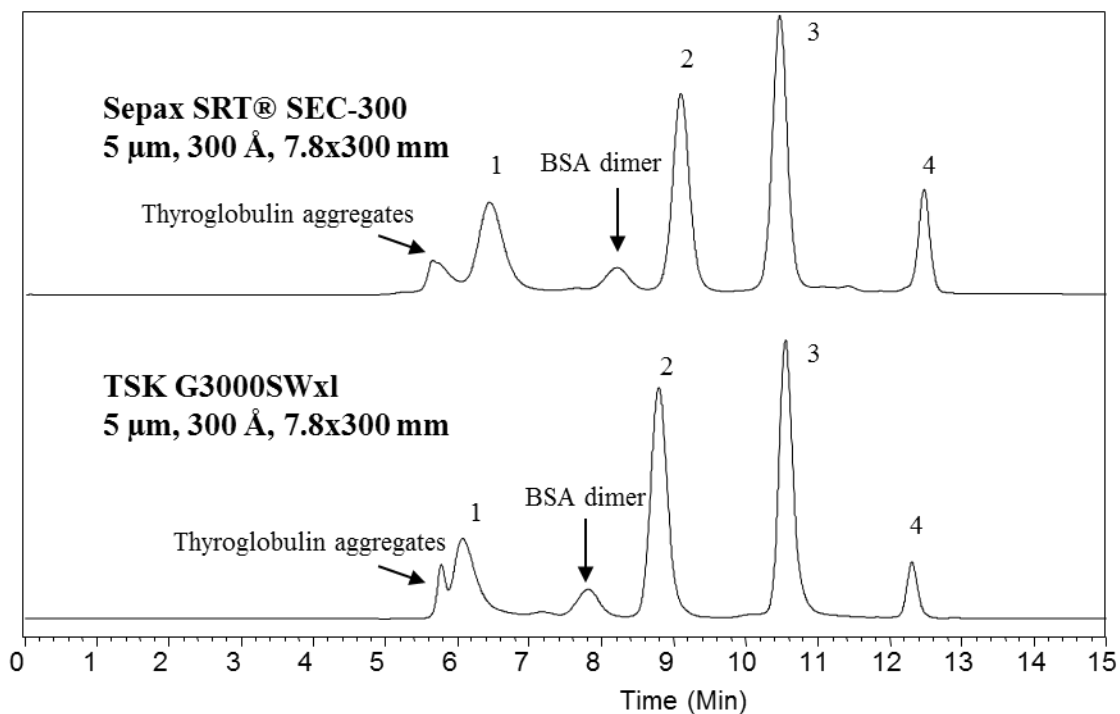


Figure 2. . Comparison of chromatograms of SRT® SEC-300 and Tosoh TSK G3000SWxl, 7.8x300 mm using protein mixture 1) Thyroglobulin, 2) BSA monomer, 3) Ribonuclease A, 4) Uracil

Peak	Protein	MW (kD)	SRT SEC-300 RT (min)	TSK G3000SWxl RT (min)
1	Thyroglobulin	670	6.43	6.08
2	BSA	66	9.08	8.79
3	Ribonuclease A	13.7	10.46	11.07
4	Uracil	0.12	12.46	12.31

Table 2. Comparison of retention time of eluted proteins with SRT® SEC-300 and TSK G3000SWxl, 7.8x300 mm



Sepax SEC to Tosoh Biosciences (TSK) cross Reference

<b>Sepax Resin chemistry</b>		<b>TSK Resin Chemistry</b>	
SRT SEC-150, 5 $\mu$ m, 150 $\text{\AA}$		G2000SW, 5 $\mu$ m, ~125 $\text{\AA}$	
SRT SEC-300 5 $\mu$ m, 300 $\text{\AA}$		G3000SW, 5 $\mu$ m, ~300 $\text{\AA}$	
SRT SEC-500, 5 $\mu$ m, 500 $\text{\AA}$		G4000SW, 8 $\mu$ m, ~450 $\text{\AA}$	
Zenix SEC-150, 3 $\mu$ m, 150 $\text{\AA}$		SuperSW2000, 4 $\mu$ m, ~150 $\text{\AA}$	
Zenix SEC-300, 3 $\mu$ m, 300 $\text{\AA}$		SuperSW3000, 4 $\mu$ m, ~300 $\text{\AA}$	
<b>Detailed part number based cross:</b>			
<b>Sepax Product</b>	<b>Sepax P/N #</b>	<b>TSK Product</b>	<b>TSK P/N #</b>
SRT SEC-150, 7.8mmx30cm, 5 $\mu$ m, 150 $\text{\AA}$	215150-7830	G2000SWxl, 7.8mmx30cm, 5 $\mu$ m	8540
SRT SEC-300, 7.8mmx30cm, 5 $\mu$ m, 300 $\text{\AA}$	215300-7830	G3000SWxl, 7.8mmx30cm, 5 $\mu$ m	8541
SRT SEC-500, 7.8mmx30cm, 5 $\mu$ m, 500 $\text{\AA}$	215500-7830	G4000SWxl, 7.8mmx30cm, 8 $\mu$ m	8542
Zenix SEC-150, 4.6mmx30cm, 3 $\mu$ m, 150 $\text{\AA}$	213150-4630	SuperSW2000, 4.6mmx30cm, 4 $\mu$ m	18674
Zenix SEC-300, 1.0mmx30cm, 3 $\mu$ m, 300 $\text{\AA}$	213300-1030	SuperSW3000, 1mmx30cm, 4 $\mu$ m	21845
Zenix SEC-300, 2.1mmx30cm, 3 $\mu$ m, 300 $\text{\AA}$	213300-2130	SuperSW3000, 2mmx30cm, 4 $\mu$ m	21485
Zenix SEC-300, 4.6mmx30cm, 3 $\mu$ m, 300 $\text{\AA}$	213300-4630	SuperSW3000, 4.6mmx30cm, 4 $\mu$ m	18675
<b>Sepax Available column ID (SS&amp;PEEK tubing): 1.0 mm; 2.1 mm; 4.6 mm; 7.8 mm; 21.2 mm; 50 mm</b>			