

Preparative Size Exclusion Chromatography for Protein Separation

SRT-10 SEC-300 Prep Column
21.2 x 400 mm (141 mL)



SRT-10 SEC-300 Prep Column

- Column size 21.2 x 400 mm (21.2 x 100mm P/N 225300-21210 + 21.2 x 300 mm P/N 225300-21230)
- Columns are connected with PEEK column coupler (Sepax part # 102002-coupler)
- Instrument GE AKTA explorer
- Pressure: 17 bar at 7 mL/min, 25 bar at 10 mL/min, well below the pressure limit for the AKTA system 100 bar or 250 bar depending on the model.



SEC prep column- BSA loading study

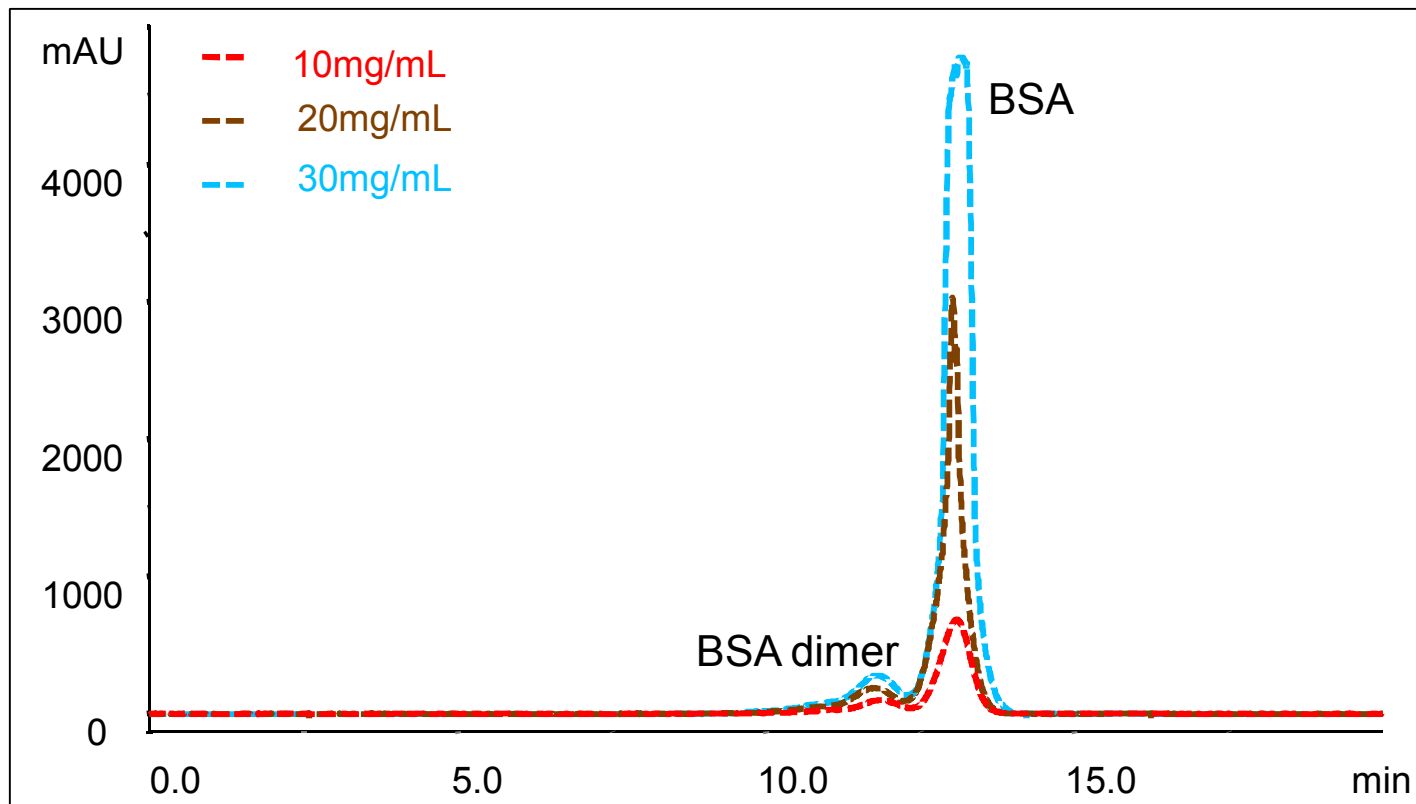
SP1019

Column: SRT-10 SEC-300 (10 μm , 300 \AA , 21.2 x 400 mm),

Mobile phase: phosphate buffer, 150mM, pH7.0

Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 $^{\circ}\text{C}$

Samples: 3 mL BSA (10, 20, or 30 mg/mL)



SEC prep column-BSA loading

Column: SRT-10 SEC-300 (10 μm , 300 \AA , 21.2 x 400 mm),

Mobile phase: phosphate buffer, 150mM, pH7.0

Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 $^{\circ}\text{C}$;

Samples: 3 mL BSA (10, 20, or 30 mg/mL)

BSA30mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter (N / m)	Asymmetry
BSA dimer	11.85	101.6082	102.906	0.8		3069	0.34
BSA	13.07	417.1044	690.324	0.55	1.06	7720	0.88
BSA60mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter (N / m)	Asymmetry
BSA dimer	11.76	195.445	190.721	0.87		2535	0.34
BSA	13.02	1163.3833	3033.566	0.26	1.31	35524	0.86
BSA90mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
BSA dimer	11.82	284.065	280.617	0.86		2640	0.33
BSA	13.19	2629.0897	4781.054	0.47	1.22	11005	0.64



SEC prep column –BSA/Uracil-7 mL/min

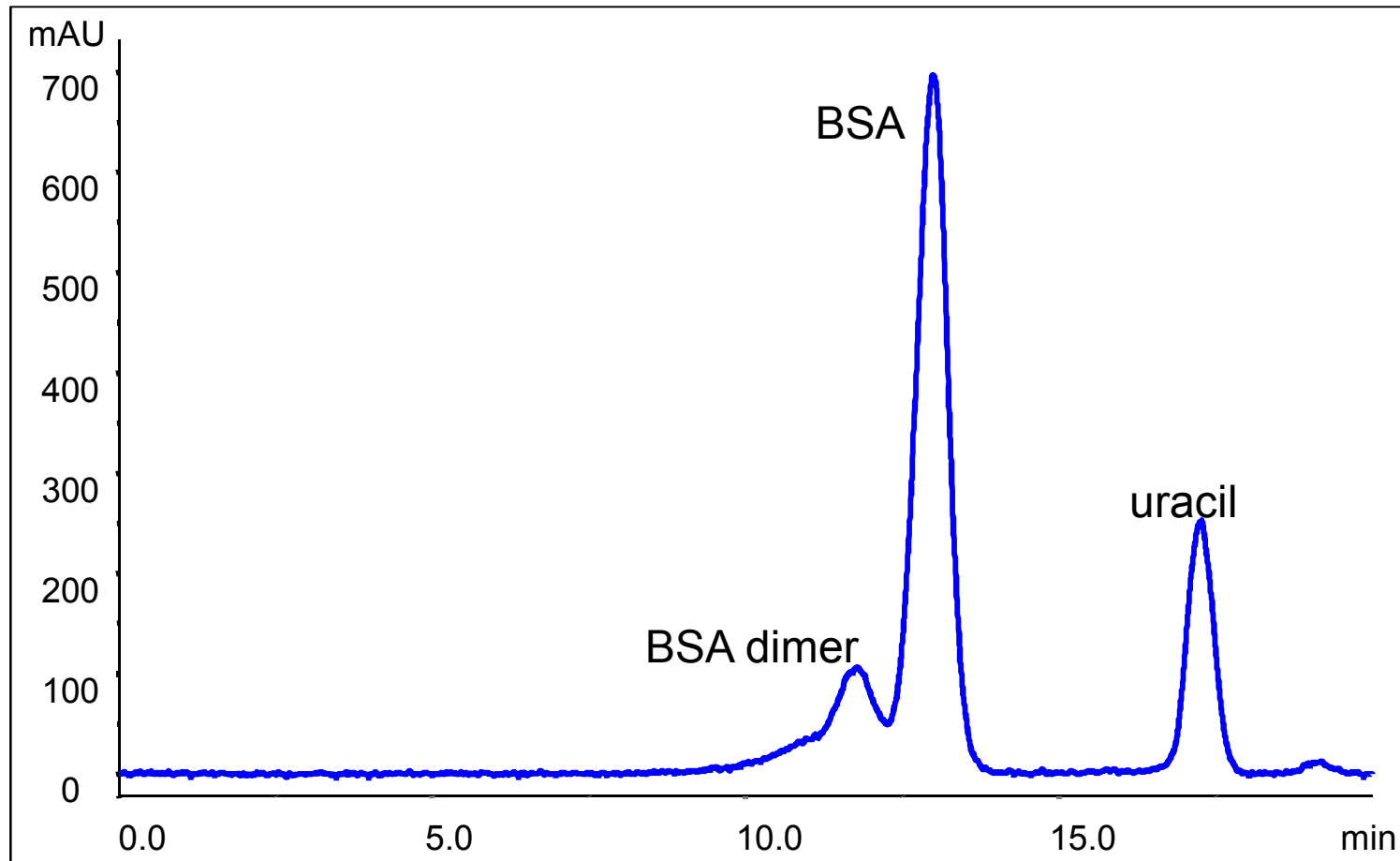
SP1019

Column: SRT-10 SEC-300 (10 μm , 300 \AA , 21.2 x 400 mm),

Mobile phase: phosphate buffer, 150mM, pH7.0

Flow rate: **7 mL/min**, 17 bar; Detector: UV 280 nm; Column temperature: 23 $^{\circ}\text{C}$

Samples: 3 mL BSA(10 mg/mL), Uracil (0.17 mg/mL)



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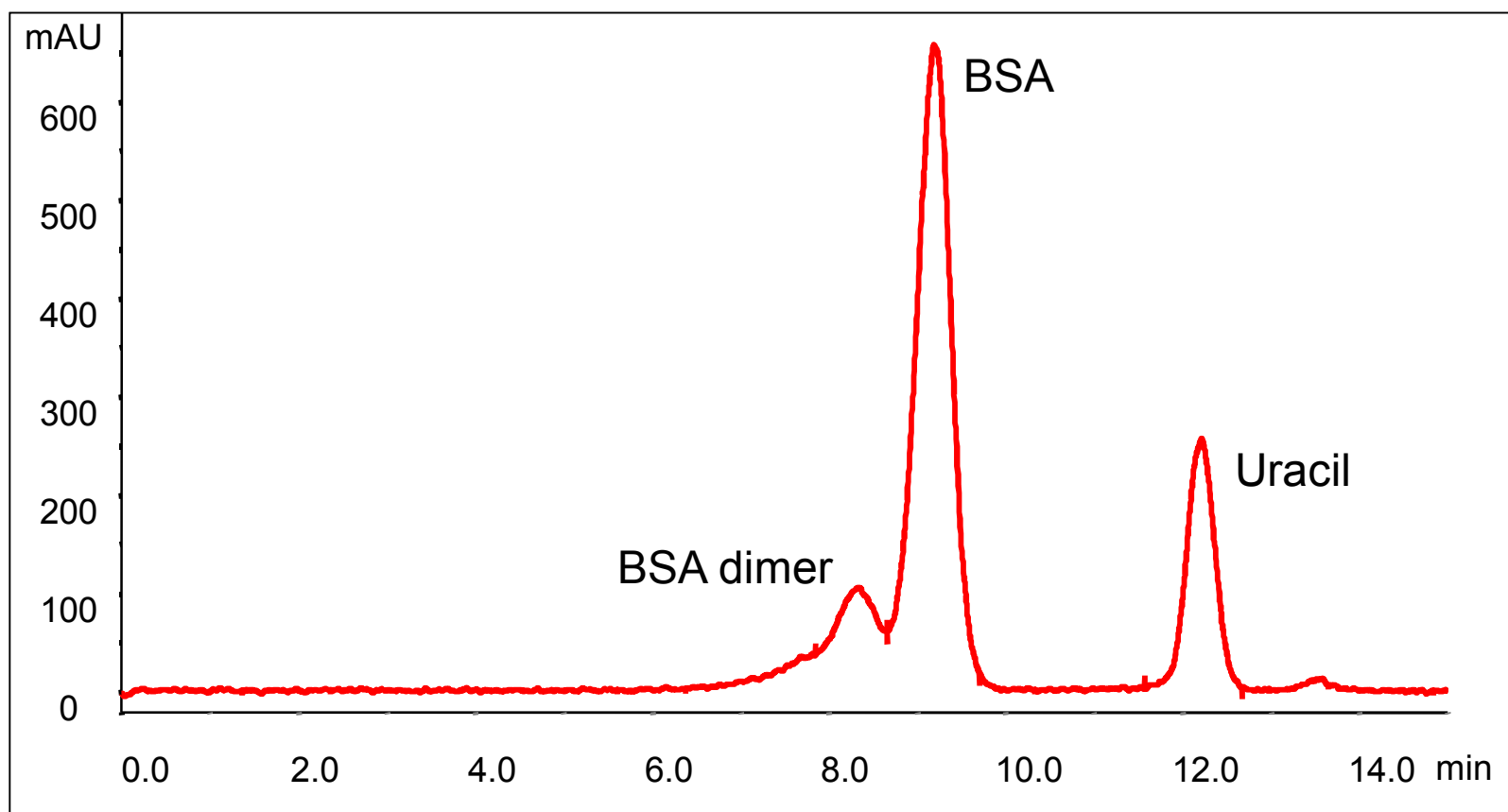
SEC prep column –BSA/Uracil-10 mL/min

Column: SRT-10 SEC-300 (10 μm , 300 \AA , 21.2 x 400 mm),

Mobile phase: phosphate buffer, 150mM, pH7.0

Flow rate: **10 mL/min**, 25 bar; Detector: UV 280 nm; Column temperature: 23 $^{\circ}\text{C}$

Samples: 3 mL BSA (10 mg/mL), Uracil (0.17 mg/mL)



BSA/uracil- overlay 7 and 10 mL/min

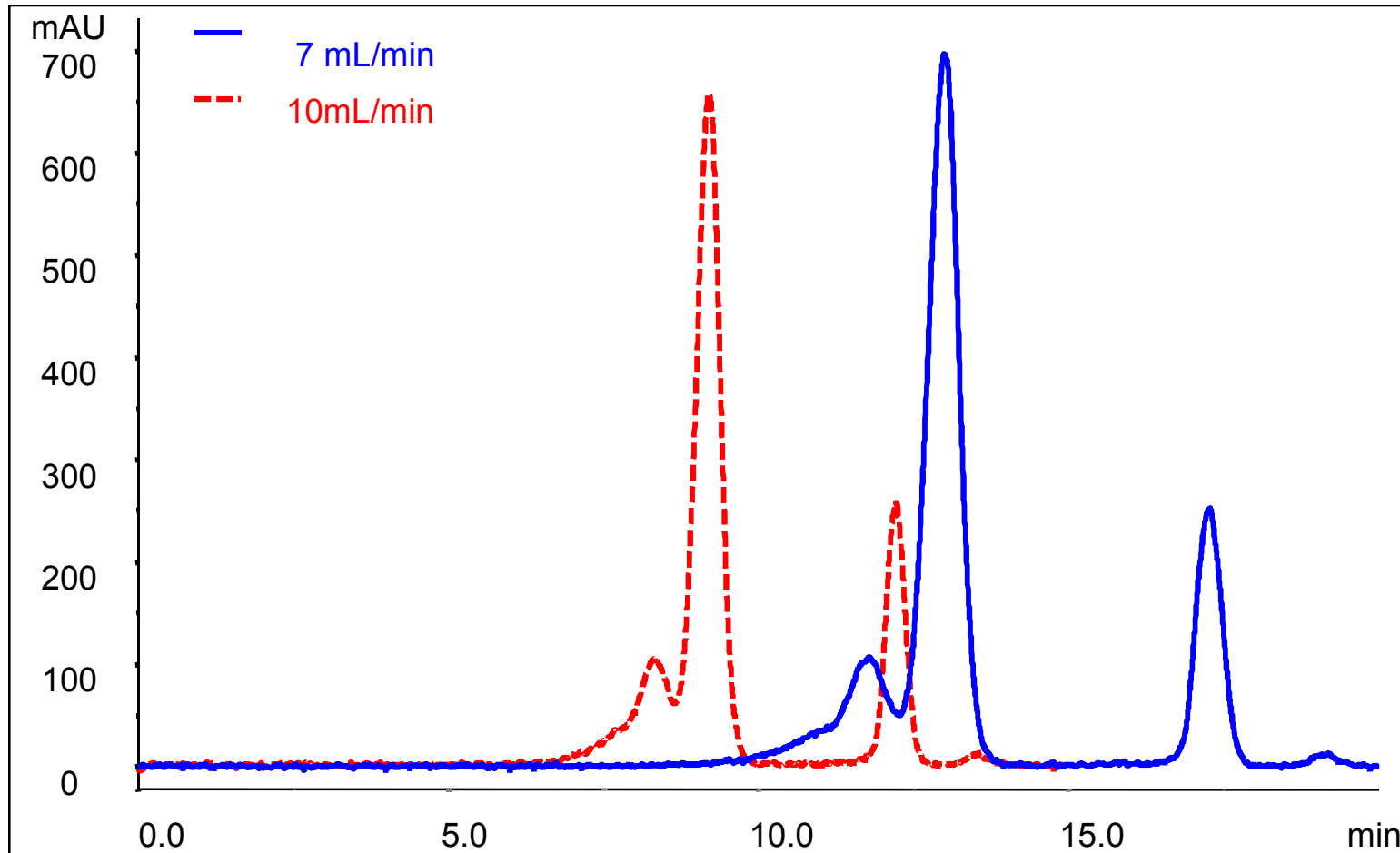
SP1019

Column: SRT-10 SEC-300 (10 μm , 300 \AA , 21.2 x 400 mm),

Mobile phase: phosphate buffer, 150mM, pH7.0

Flow rate: 7 or 10 mL/min; Detector: UV 280 nm, Column temperature: 23 $^{\circ}\text{C}$,

Samples: 3 mL BSA(10mg/mL), Uracil (0.17mg/mL)



Higher flow rate shortens the separation time while maintaining the resolution.



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SEC prep column-different flow rate

Column: SRT-10 SEC-300 (10 μ m, 300Å, 21.2 x 400 mm),

Mobile phase: phosphate buffer, 150mM, pH7.0

Flow rate: 7 or 10 mL/min; Detector: UV 280 nm, Column temperature: 23 °C

Samples: 3 mL BSA(10mg/mL), Uracil (0.17mg/mL)

flow rate 7mL/min							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
BSA dimer	11.78	114.8196	107.195	0.87		2562	0.3
BSA	12.98	431.4186	697.461	0.57	0.99	7240	0.85
Uracil	17.28	129.0461	253.549	0.48	4.83	18101	0.96
flow rate 10mL/min							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
BSA dimer	8.33	85.0802	106.557	0.66		2186	0.25
BSA	9.19	315.8112	658.445	0.44	0.91	6058	0.8
Uracil	12.21	99.1489	257.95	0.35	4.5	16609	0.98



SEC prep – QC standard mixture

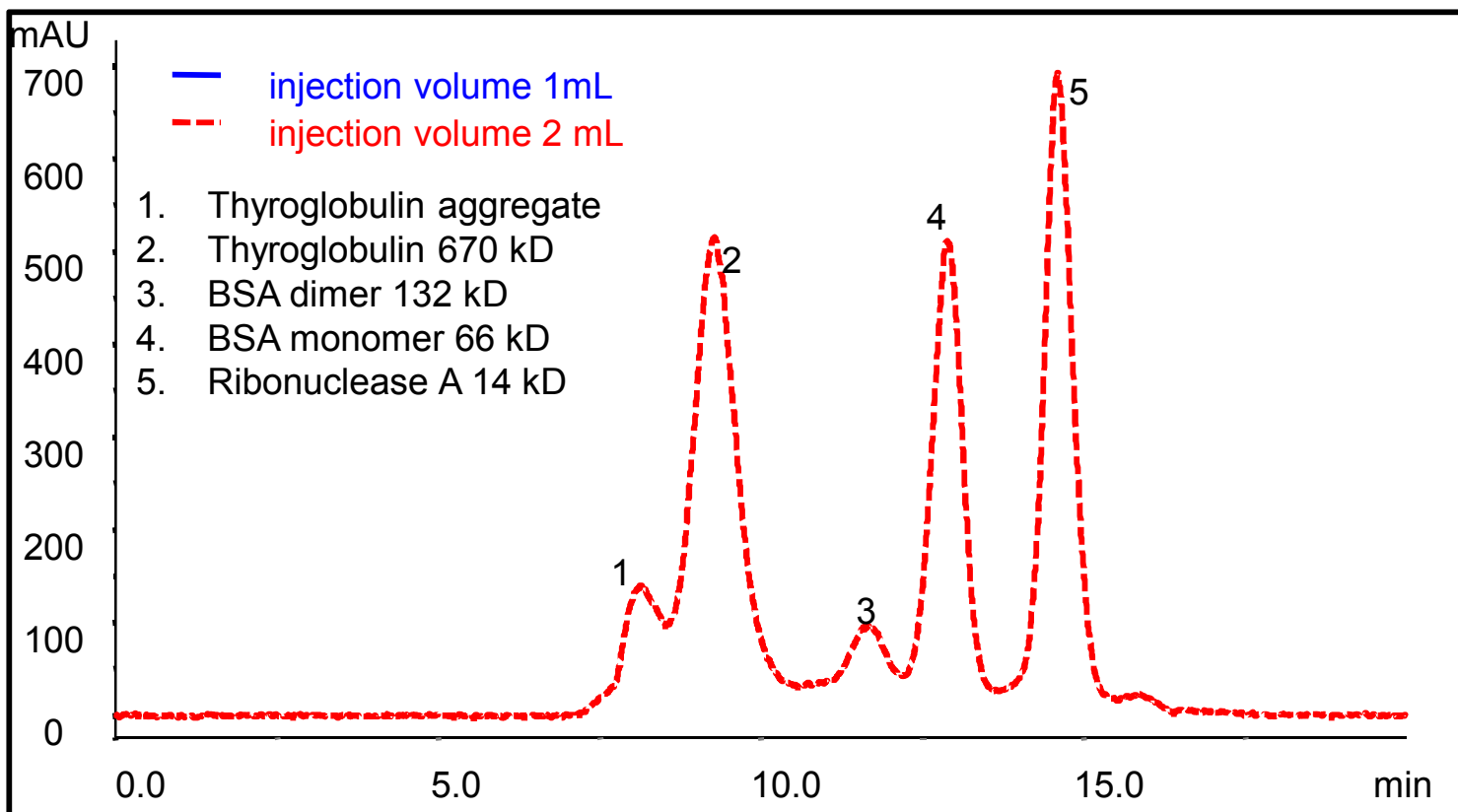
SP1019

Column: SRT-10 SEC-300 (10 μm , 300 \AA , 21.2 x 400 mm),

Mobile phase: phosphate buffer, 150mM, pH7.0

Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 $^{\circ}\text{C}$

Samples: 1 or 2 mL Thyroglobulin, BSA, Ribonuclease A (10mg/mL each)



QC standard separation parameters

SP1019

Column: SRT-10 SEC-300 (10 μ m, 300Å, 21.2 x 400 mm),

Mobile phase: phosphate buffer, 150mM, pH7.0

Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 °C

Samples: 1 or 2 mL Thyroglobulin, BSA, Ribonuclease A (10mg/mL each)

Injection volume 1mL							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
Thyroglobulin aggr	8.19	42.7311	64.826	0.72		1793	1.4
Thyroglobulin	9.33	196.6968	246.72	0.72	0.93	2352	1.46
BSA dimer	11.8	37.4138	46.508	0.76	1.98	3380	0.67
BSA	12.98	137.3646	248.467	0.51	1.1	9092	0.92
Ribonuclease A	14.71	176.2706	334.151	0.46	2.1	13873	1.13
Injection volume 2mL							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
Thyroglobulin aggr	8.13	99.9927	140.34	0.74		1689	0.65
Thyroglobulin	9.28	430.9852	516.172	0.75	0.91	2108	1.26
BSA dimer	11.66	78.8725	96.316	0.84	1.77	2690	0.77
BSA	12.89	303.9722	511.408	0.53	1.05	8078	0.89
Ribonuclease A	14.59	377.8125	693.522	0.48	1.97	12584	1.14

