SEPAX APPLICATION NOTE

Fc Fusion Protein Separation on Sepax Proteomix SAX Columns

Two Fc Fusion Protein Samples
MW: 60-70 KDa, pI: 5.4-5.8

Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm
Part Number: 403NP5-4625

Highlights

- Fc fusion proteins, a growing class of biological therapeutics, are challenging to characterize because of their heterogeneity. Some of the fusion proteins can be highly glycosylated with broad distributions of charge variants.
- In today's biotechnology industry, there is an increasing need to structurally characterize the charge heterogeneity of these variants by methods other than electrophoresis, especially for biosimilar products.
- In this study, two different Fc Fusion Proteins (pI below 7) were being analyzed on Sepax Proteomix Anion Exchange (SAX and WAX) columns by both salt gradient based and pH gradient based systems. The primary bio-innovator drug and one of its biosimilar products of the second Fc Fusion Protein project were being evaluated as well.
- Proteomix SAX provided high resolution charge variant separation of the two different Fc fusion proteins. Charge variant separation can be achieved with either salt or pH gradient depending on the individual sample and its structure. These columns can be used for both analytical and preparative purposes and is available in various preparative sizes for peak fractionation and further structurally characterization.

Sample	Salt Gradient	pH Gradient
Fc Fusion Protein-1 **SAX** *(Salt Gradient outperforms)*	A: 20mM Tris, pH 8.0 B: A+ 500mM NaCl, pH 8.0	A: 20mM Triethanolamine, Bis-Tris Propane, 1-Methylpiperazine, pH 9.7 B: A, pH 3.7
Fc Fusion Protein-2 - **BioInnovaor** - **Biosimilar** **SAX and WAX** *(pH Gradient on SAX outperforms)*	A: 50 mM Tris-HCl, pH 8.5; B: A+ 1M NaCl, pH 8.5 A: 50 mM Tris-HCl, pH 9.0; B: A+ 1M NaCl, pH 9.0	A: 20 mM Bis-Tris Propane + 20 mM triethanolamine + 20 mM piperazine + 20 mM N-methylpiperazine, pH 9.0 by HCl; B: A, pH 3.4 by HCl on SAX

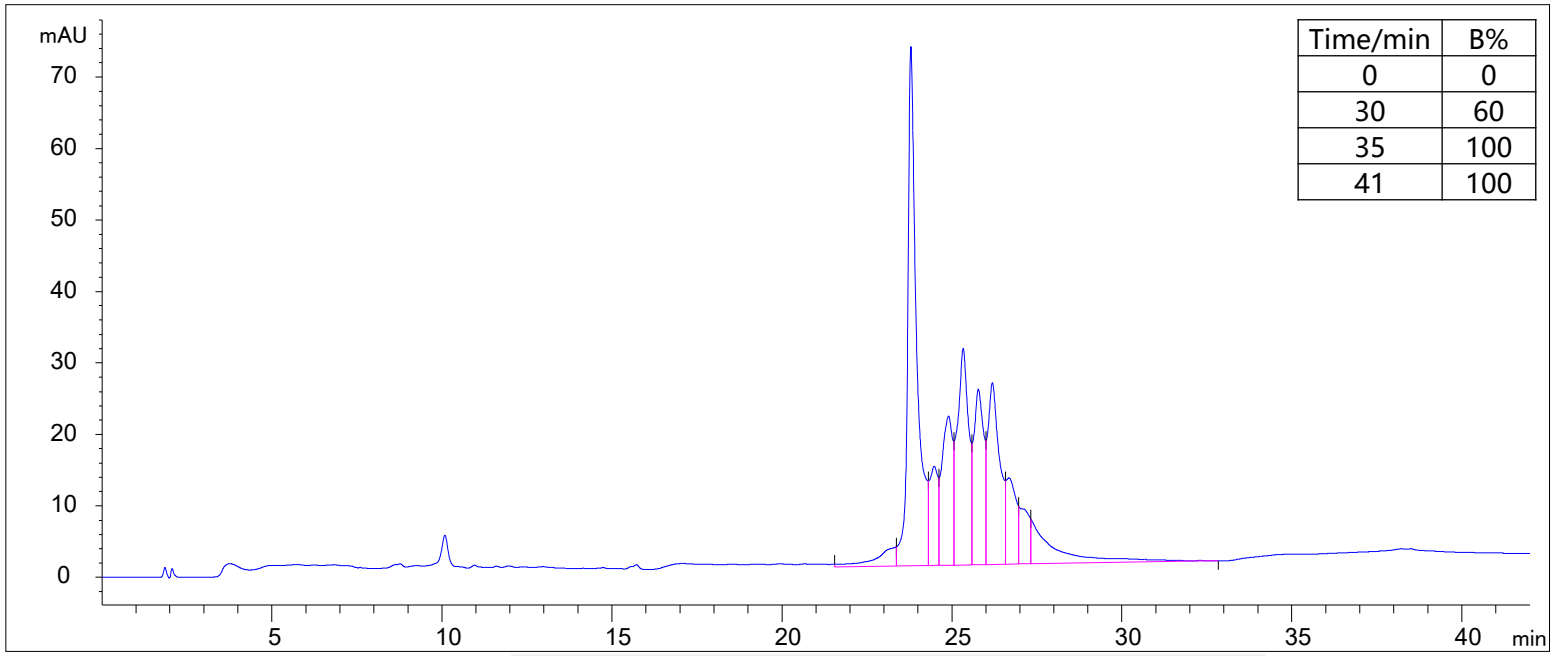
Fc Fusion Protein-1

Proteomix SAX-NP5, 5 µm, 4.6 × 250 mm

Part Number: 403NP5-4625

Fc Fusion Protein-1 on Proteomix SAX-NP5 - Salt Gradient 1

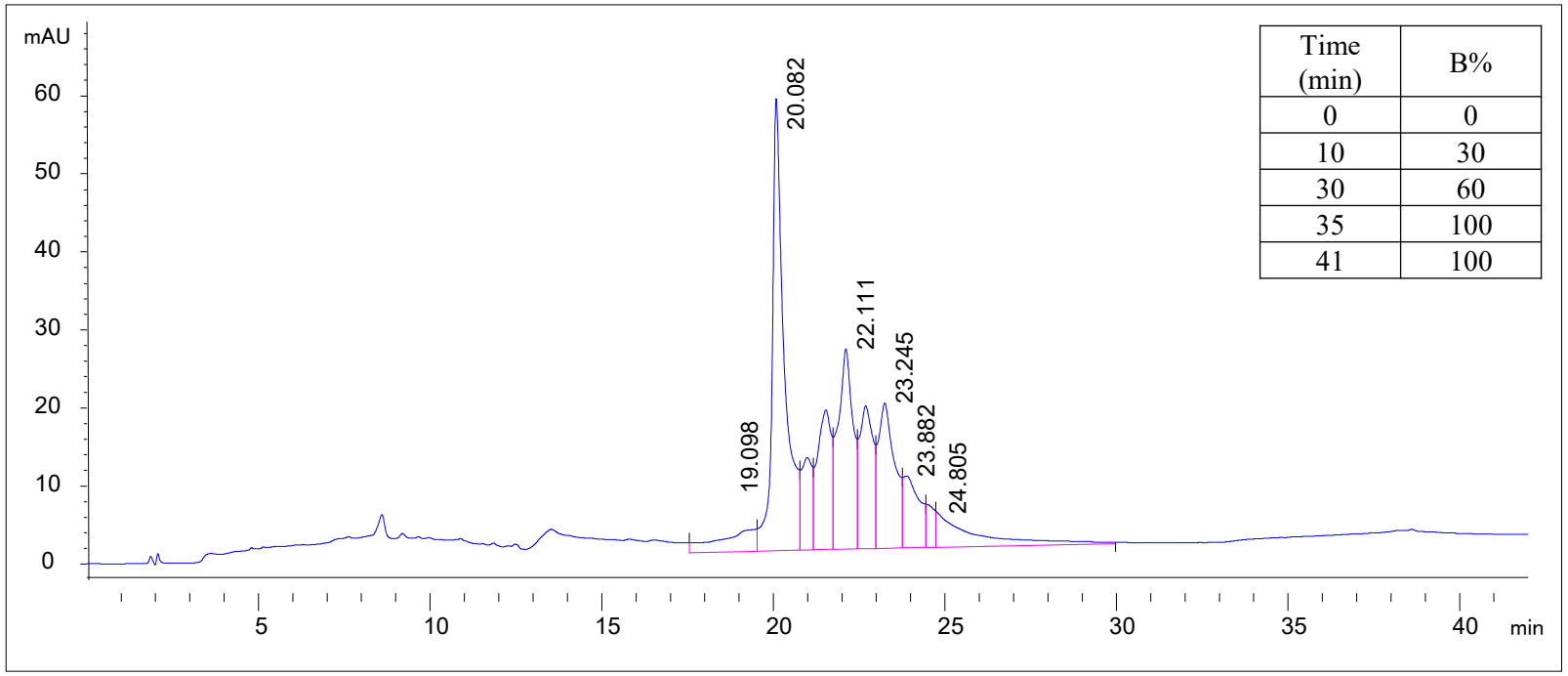
Column: Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 403NP5-4625; **Mobile Phase:** *A:* 20mM Tris, pH 8.0, *B:* 20mM Tris, 500mM NaCl, pH 8.0; **Flow Rate:** 0.8 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 6 µL; **Sample:** 4.5 mg/mL; **Column Pressure:** 170 bar



#	min	Area	Height	Width	Tailing	%	
1	23.048	112.9	2.1	0.6937	1.721	2.379	BV F
2	23.797	1345.9	72.6	0.2602	0.539	28.372	VV
3	24.48	242	13.9	0.2432	1.289	5.102	VV
4	24.901	461.4	20.9	0.3296	1.618	9.727	VV
5	25.332	716.5	30.3	0.3237	1.086	15.105	VV
6	25.78	505.7	24.6	0.2921	0.885	10.660	VV
7	26.192	636.5	25.4	0.3439	0.609	13.418	VV
8	26.677	250.7	12.1	0.3018	0.394	5.286	VV
9	27.155	159	7.6	0.2968	1.216	3.351	VV B
10	27.368	313	5.9	0.8814	4.89E-2	6.599	VB B

Fc Fusion Protein-1 on Proteomix SAX-NP5 – Salt Gradient 2

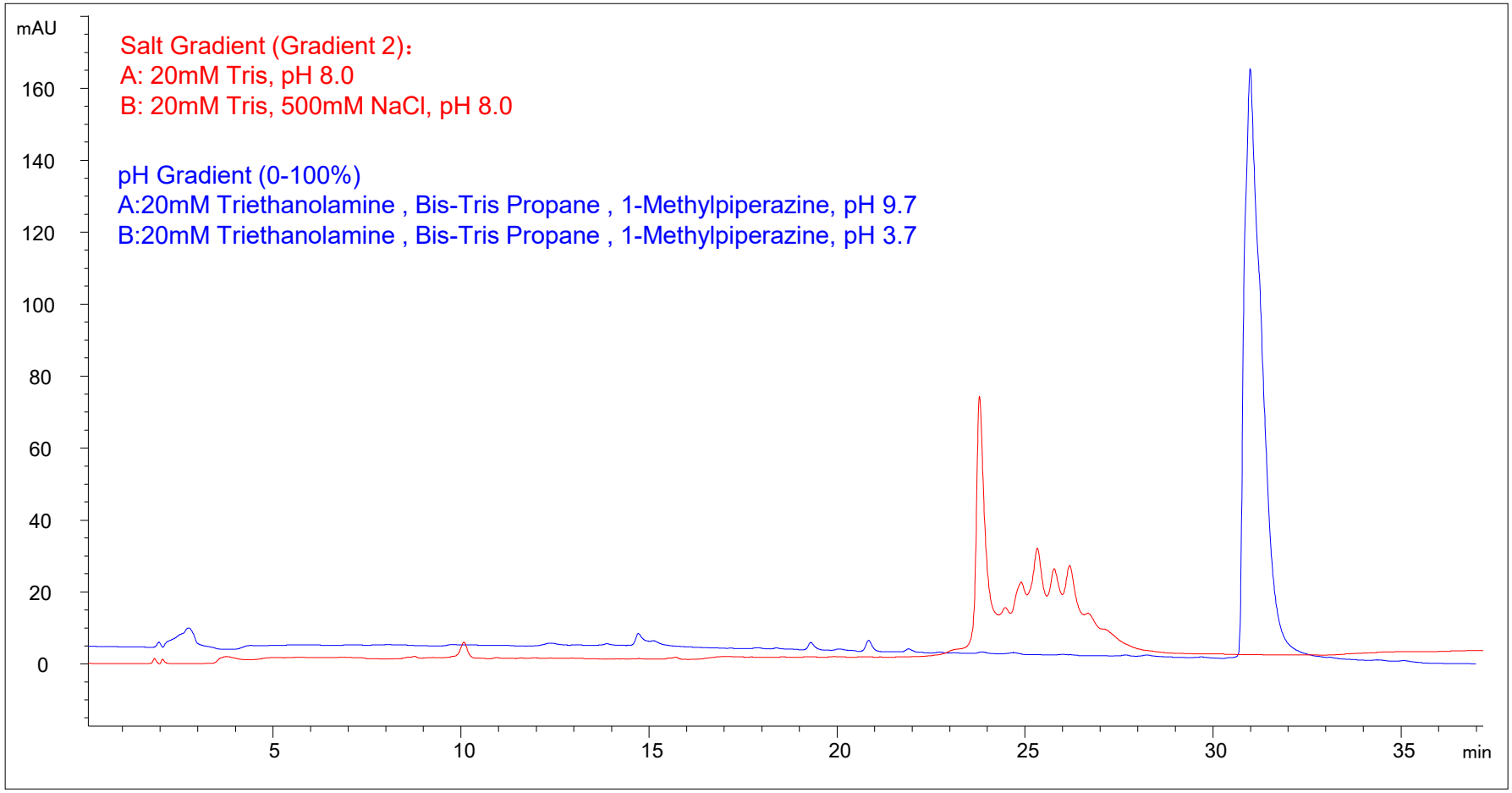
Column: Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 403NP5-4625; **Mobile Phase:** *A:* 20mM Tris, pH 8.0, *B:* 20mM Tris, 500mM NaCl, pH 8.0; **Flow Rate:** 0.8 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 6 µL; **Sample:** 4.5 mg/mL; **Column Pressure:**170 bar



Time (min)	B%
0	0
10	30
30	60
35	100
41	100

Fc Fusion Protein-1
Salt Gradient vs pH Gradient

Column: Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 403NP5-4625; **Flow Rate:** 0.8 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 6 µL; **Sample:** 4.5 mg/mL; **Column Pressure:** 170 bar



Fc Fusion Protein 2

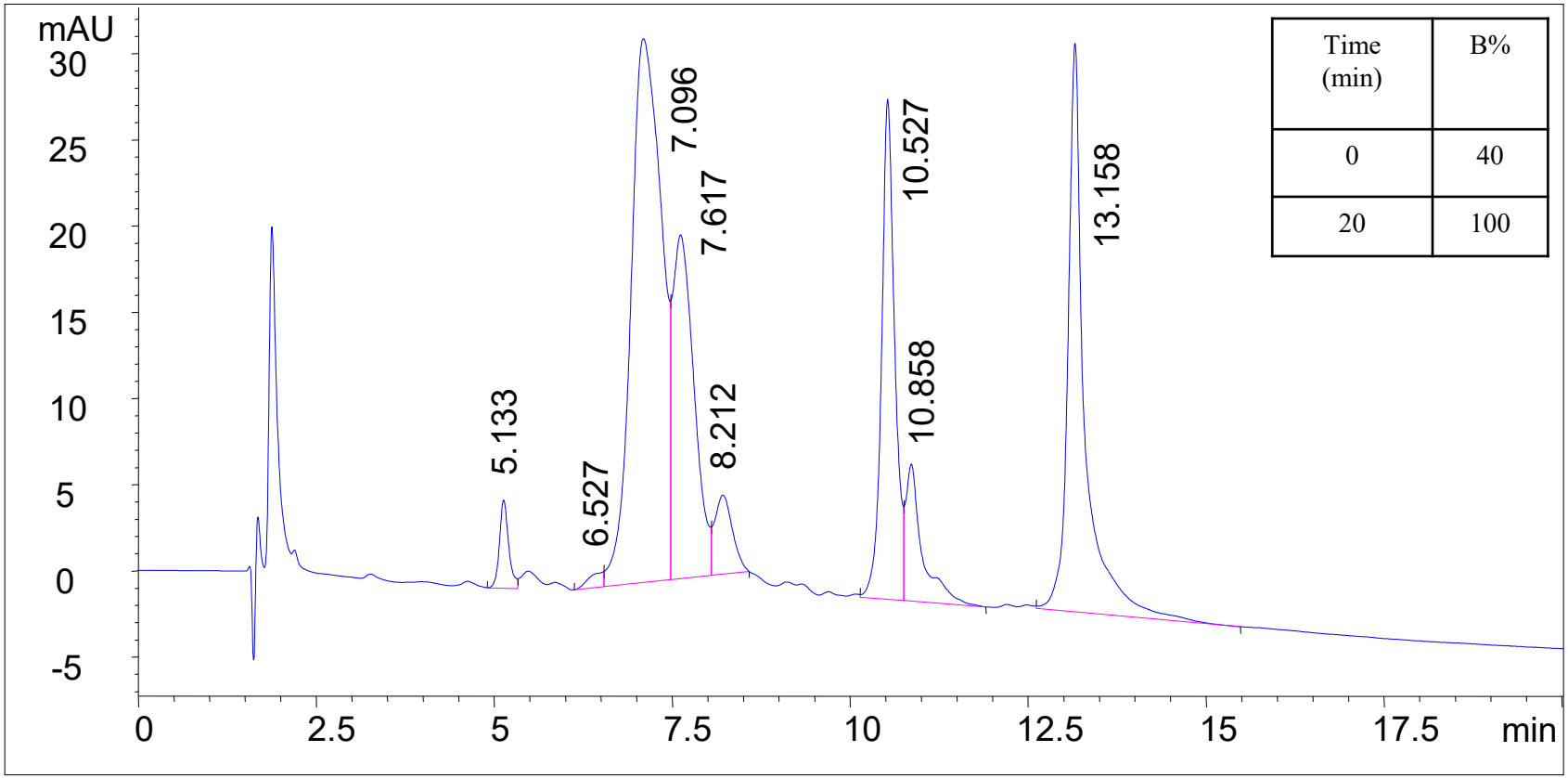
pH Gradient

Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm

Part Number: 403NP5-4625

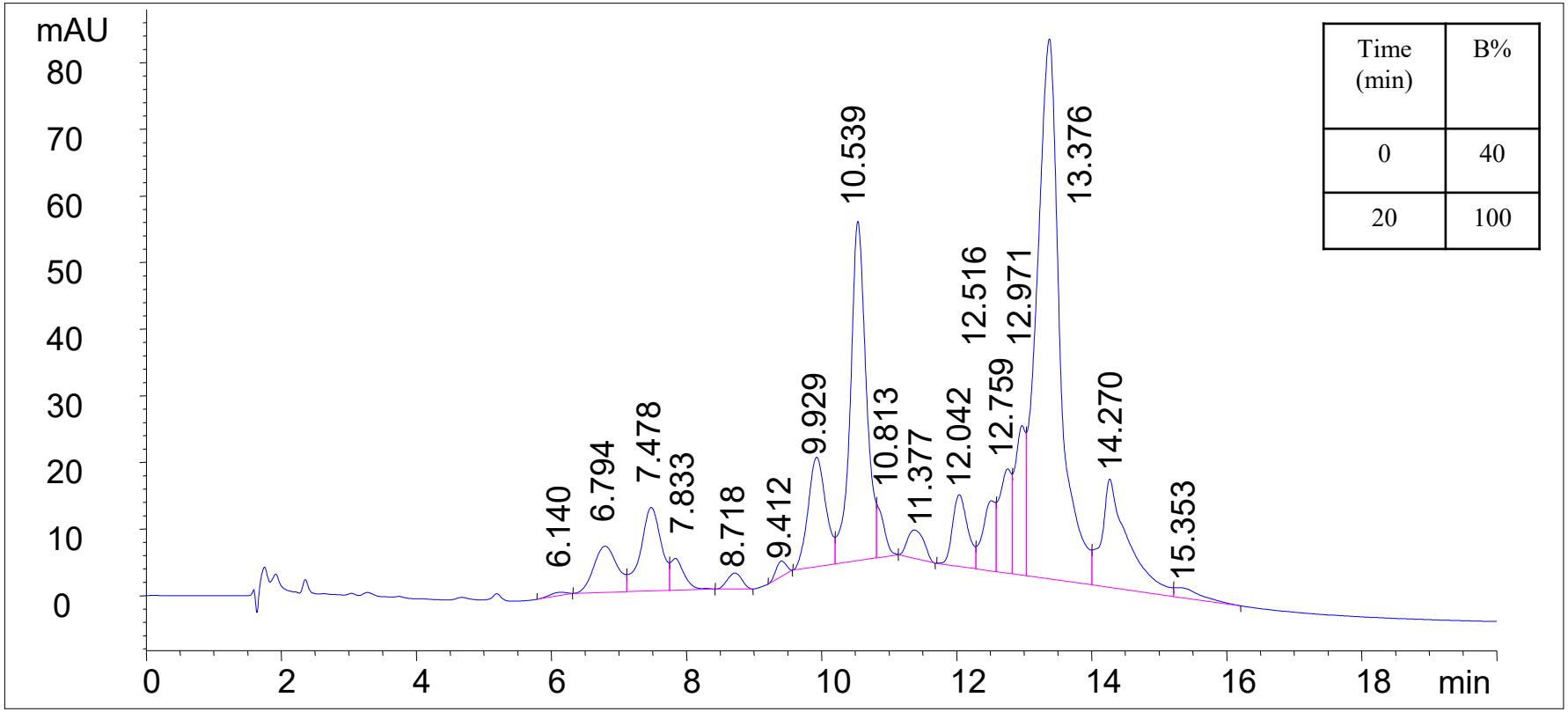
Fc Fusion Protein Bio-Innovator on Proteomix SAX – pH Gradient

Column: Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 403NP5-4625; **Mobile Phase:** *A:* 20 mM Bis-Tris Propane + 20 mM triethanolamine + 20 mM piperazine + 20 mM N-methylpiperazine, pH 9.0 by HCl, *B:* A, pH 3.4 by HCl; **Flow Rate:** 1.0 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 10 µL; **Sample:** Bio-Innovator (3 mg/mL); **Pressure:** 178-174 bar



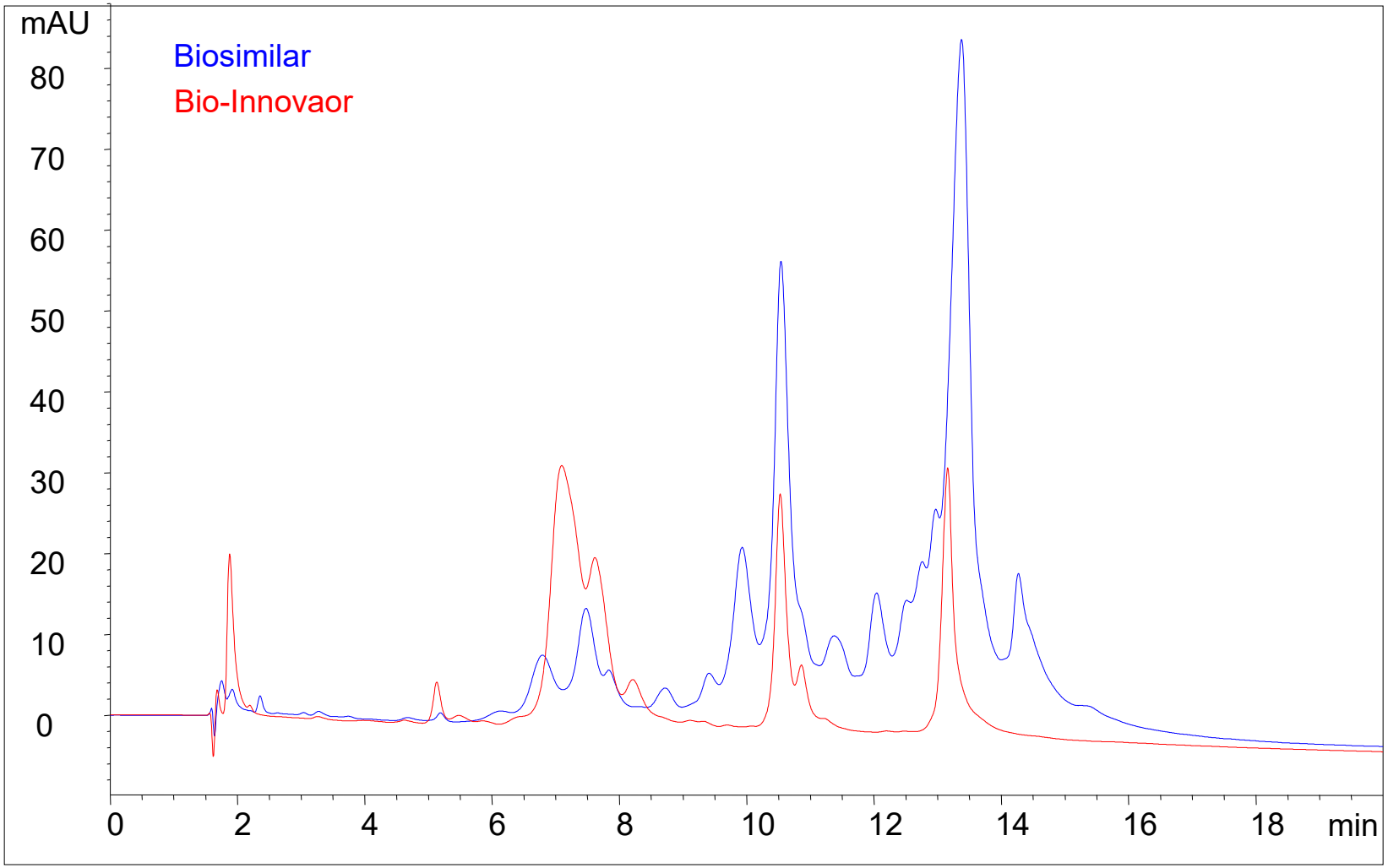
Fc Fusion Protein-2 Biosimilar on Proteomix SAX – pH Gradient

Column: Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 403NP5-4625; **Mobile Phase:** *A:* 20 mM Bis-Tris Propane + 20 mM triethanolamine + 20 mM piperazine + 20 mM N-methylpiperazine, pH 9.0 by HCl, *B:* A, pH 3.4 by HCl; **Flow Rate:** 1.0 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 20 µL; **Sample:** Biosimilar (1-3 mg/mL); **Pressure:** 178-174 bar



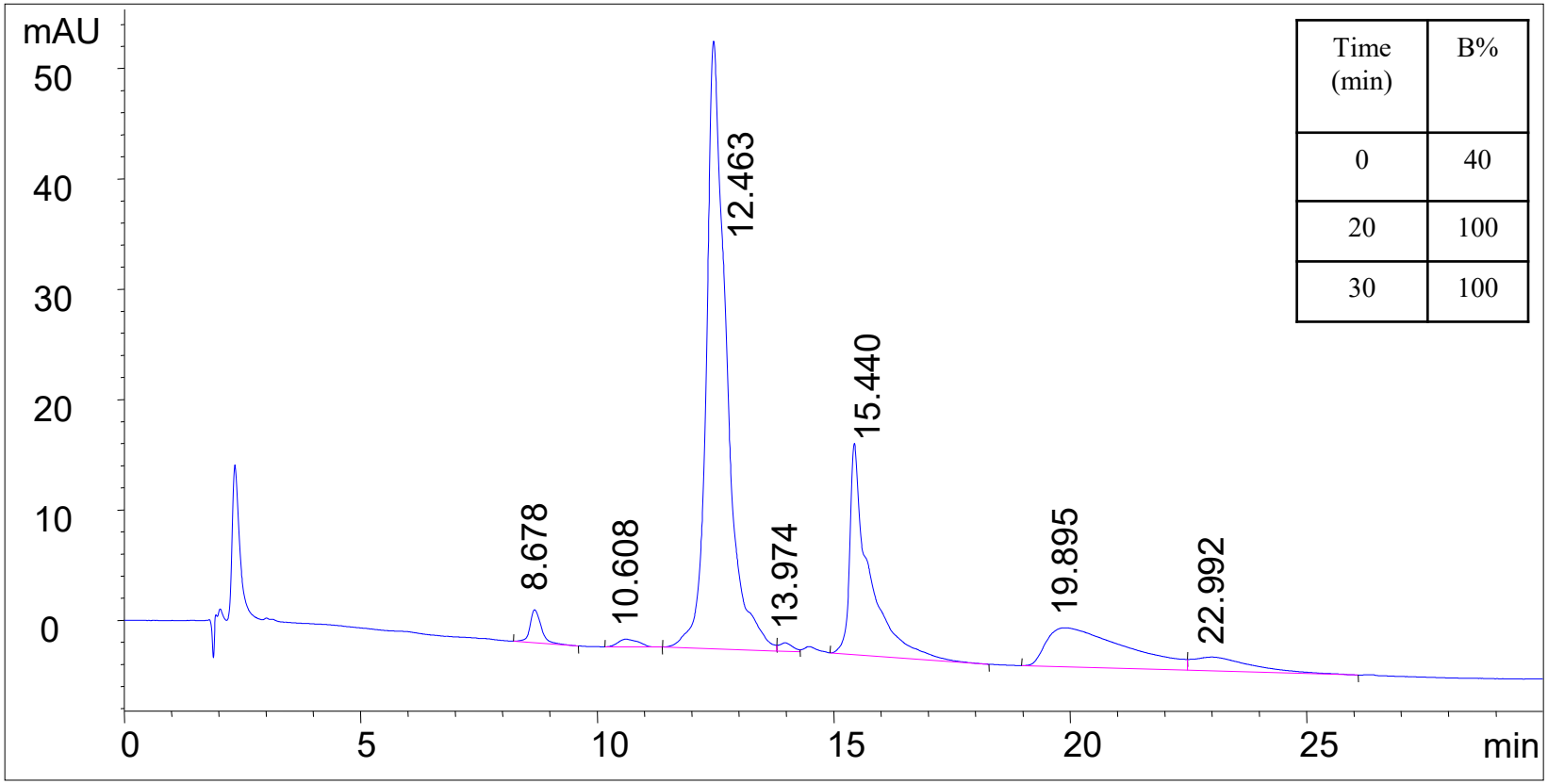
Time (min)	B%
0	40
20	100

Proteomix SAX
pH Gradient Overlay Signals



Fc Fusion Protein-2 Bio-Innovator on Proteomix WAX – pH Gradient

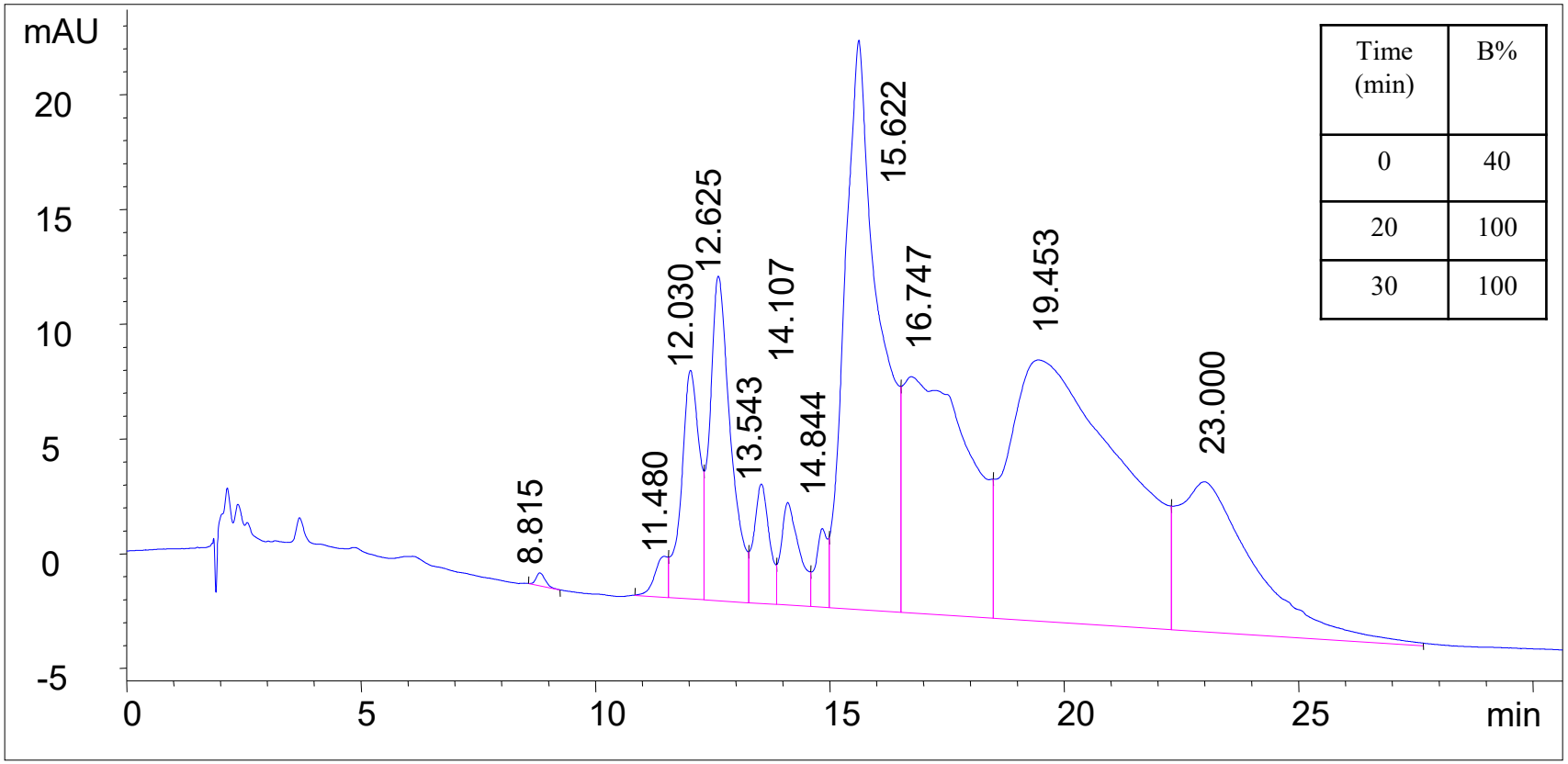
Column: Proteomix WAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 404NP5-4625; **Mobile Phase:** *A:* 20 mM Bis-Tris Propane + 20 mM triethanolamine + 20 mM piperazine + 20 mM N-methylpiperazine, pH 9.0 by HCl, *B:* A, pH 3.4 by HCl; **Flow Rate:** 1.0 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 10 µL; **Sample:** Bio-Innovator (3 mg/mL)



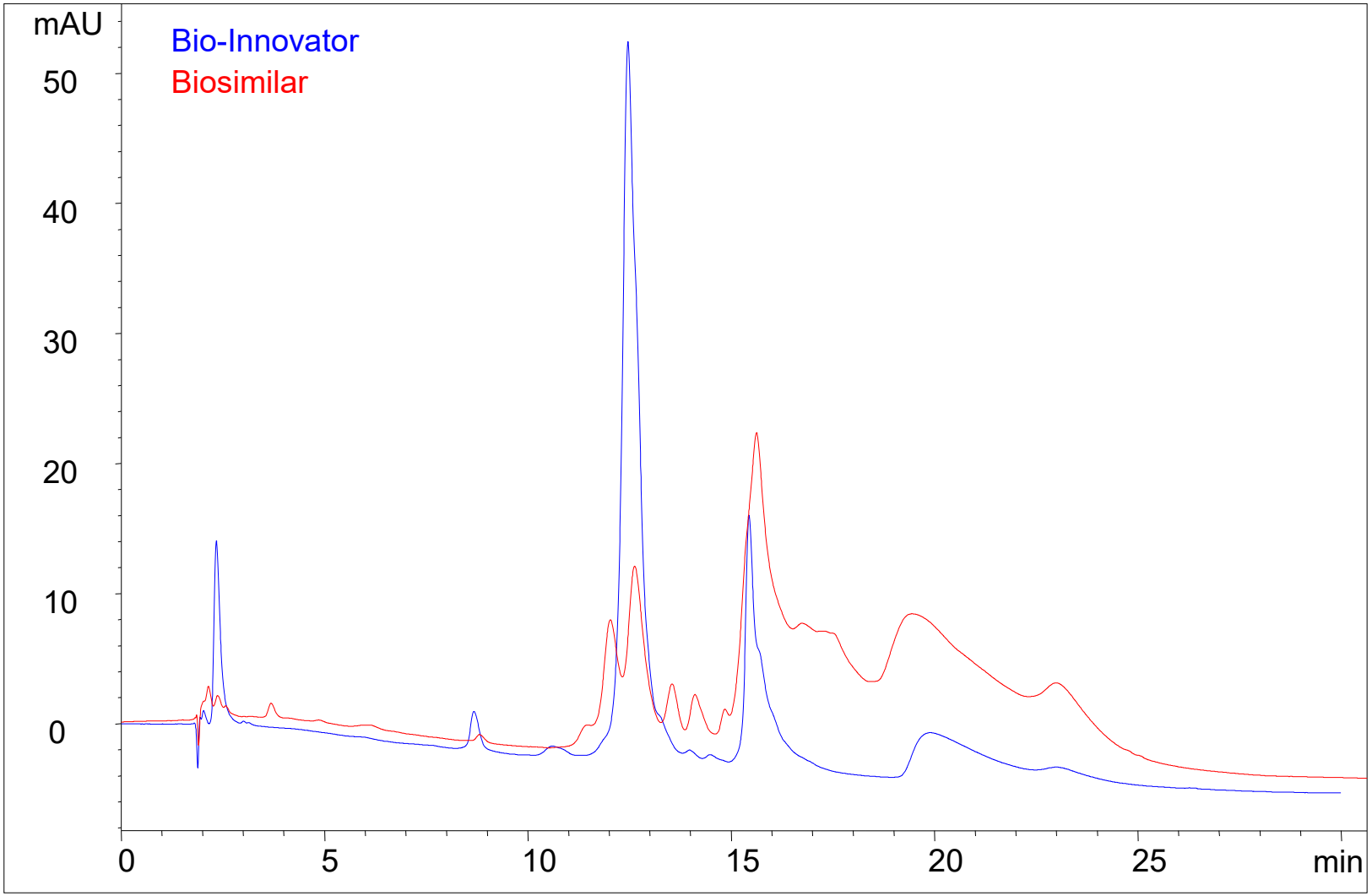
Time (min)	B%
0	40
20	100
30	100

Fc Fusion Protein-2 Biosimilar on Proteomix WAX – pH Gradient

Column: Proteomix WAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 404NP5-4625; **Mobile Phase:** *A:* 20 mM Bis-Tris Propane + 20 mM triethanolamine + 20 mM piperazine + 20 mM N-methylpiperazine, pH 9.0 by HCl, *B:* A, pH 3.4 by HCl; **Flow Rate:** 1.0 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 20 µL; **Sample:** Biosimilar (1-3 mg/mL); **Pressure:** 1117-113 bar



Proteomix WAX – pH Gradient Overlay Signals



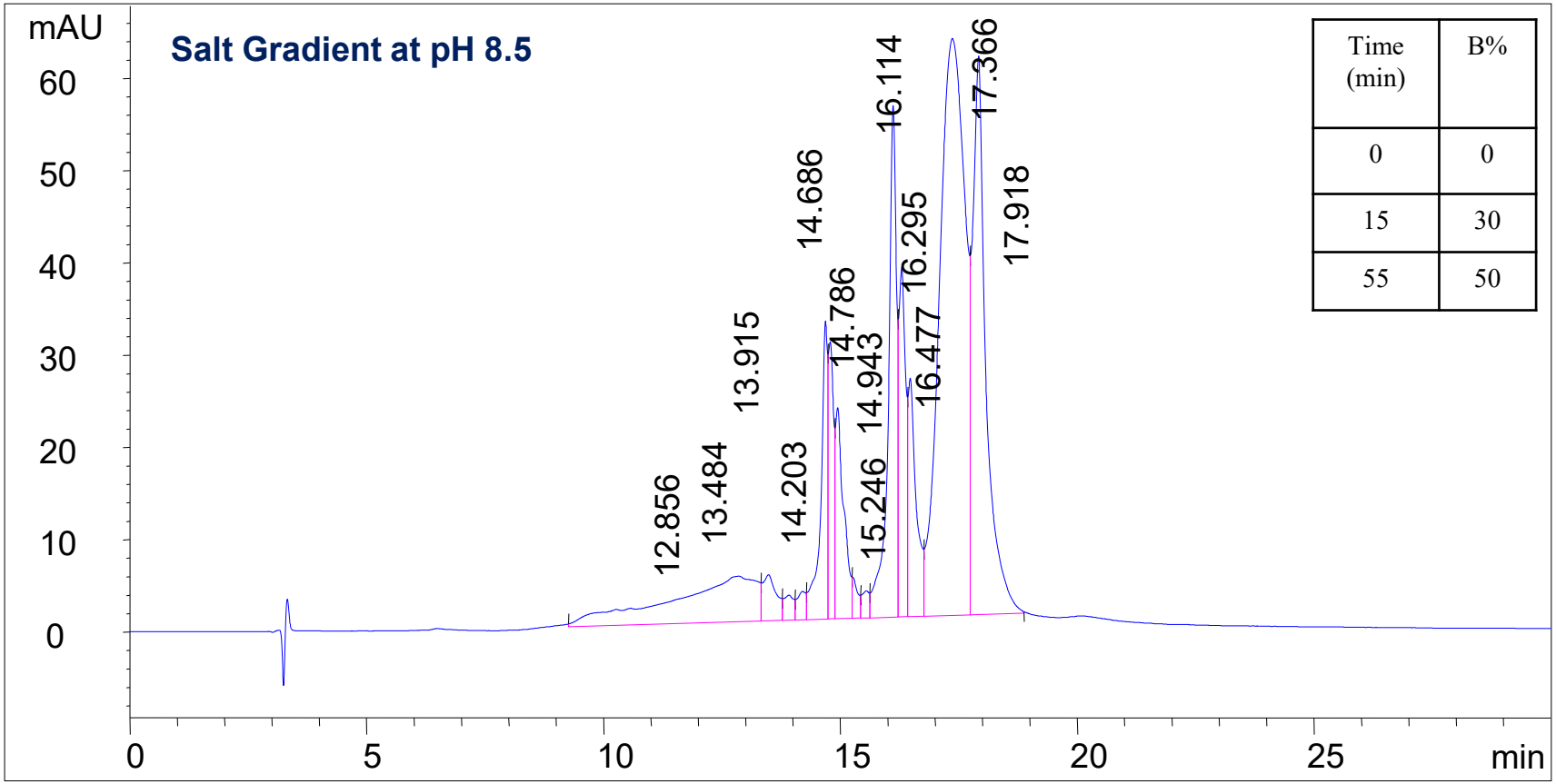
Fc Fusion Protein 2

Salt Gradient

Proteomix SAX-NP5, 5 µm, 4.6 × 250 mm
Part Number: 403NP5-4625

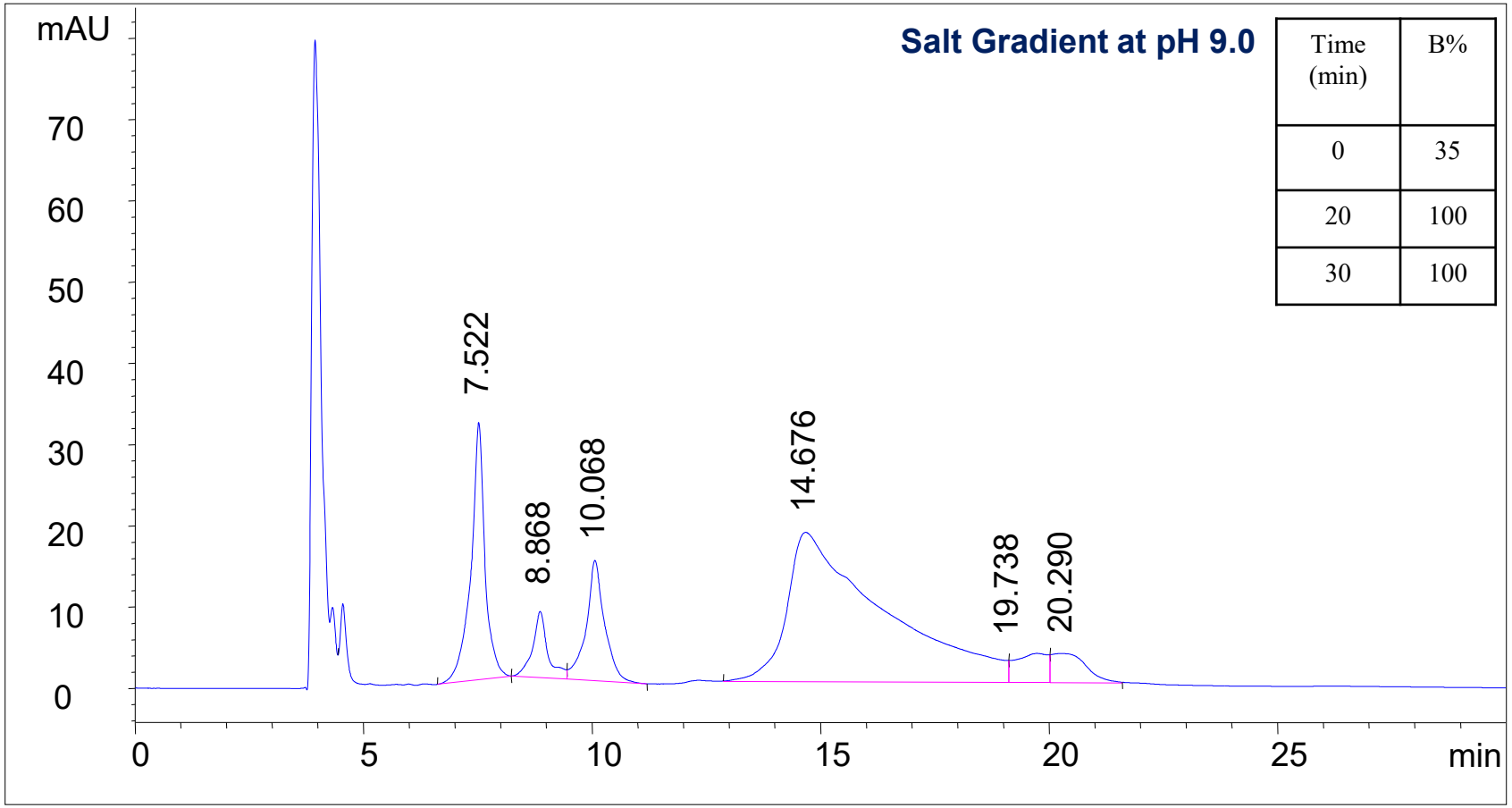
Fc Fusion Protein-2 Bio-Innovator on Proteomix SAX

Column: Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 403NP5-4625; **Mobile Phase:** *A:* 50 mM Tris-HCl, pH 8.5, *B:* A+ 1M NaCl, pH 8.5; **Flow Rate:** 0.5 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 ℃; **Injection Volume:** 10 µL; **Sample:** Bio-Innovator (3 mg/mL)



Fc Fusion Protein-2 Bio-Innovator on Proteomix WAX

Column: Proteomix WAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 404NP5-4625; **Mobile Phase:** *A:* 50 mM Tris-HCl, pH 9.0, *B:* A+ 1M NaCl, pH 9.0; **Flow Rate:** 0.5 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 10 µL; **Sample:** Bio-Innovator (3 mg/mL)

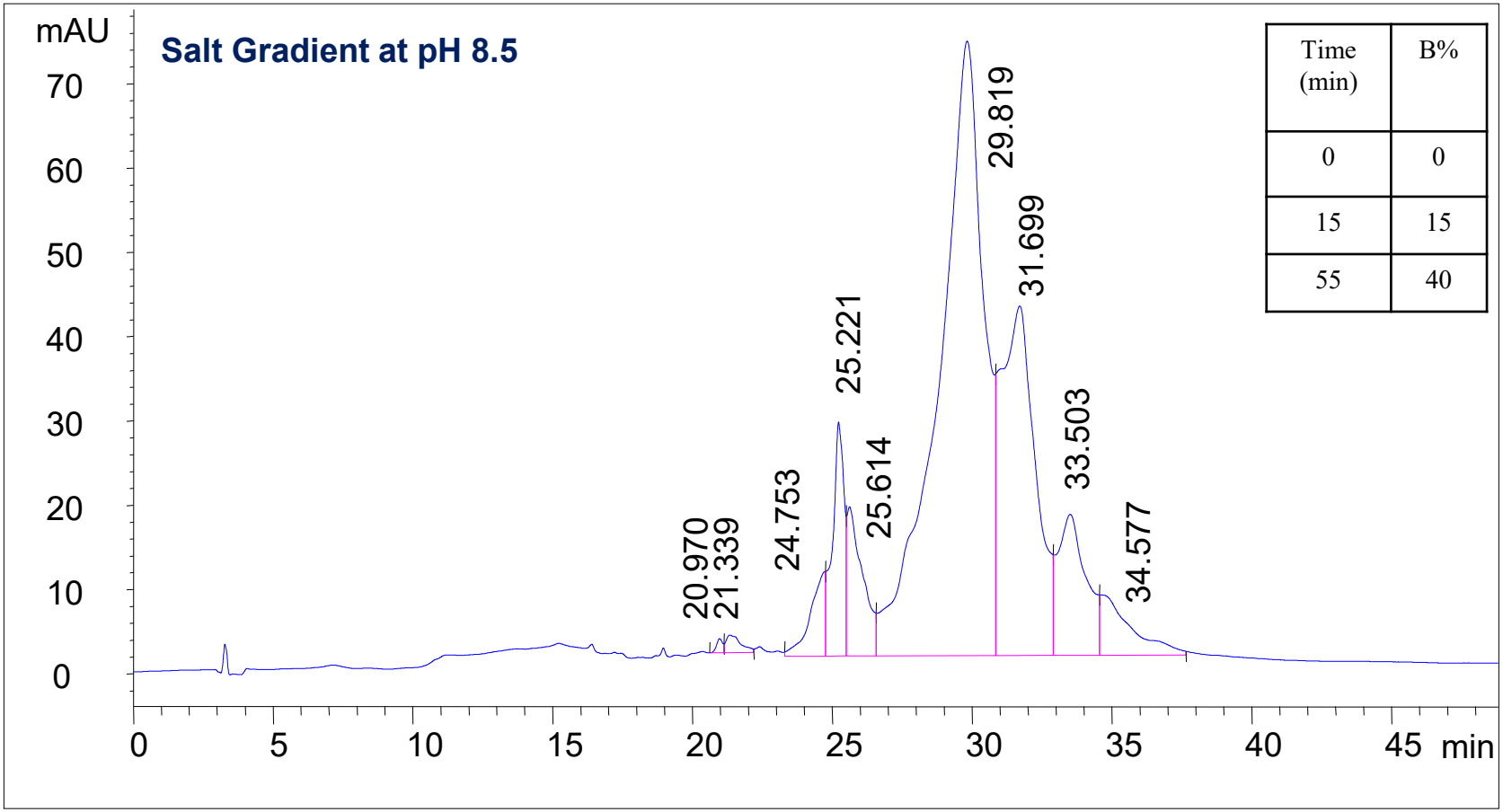


Salt Gradient at pH 9.0

Time (min)	B%
0	35
20	100
30	100

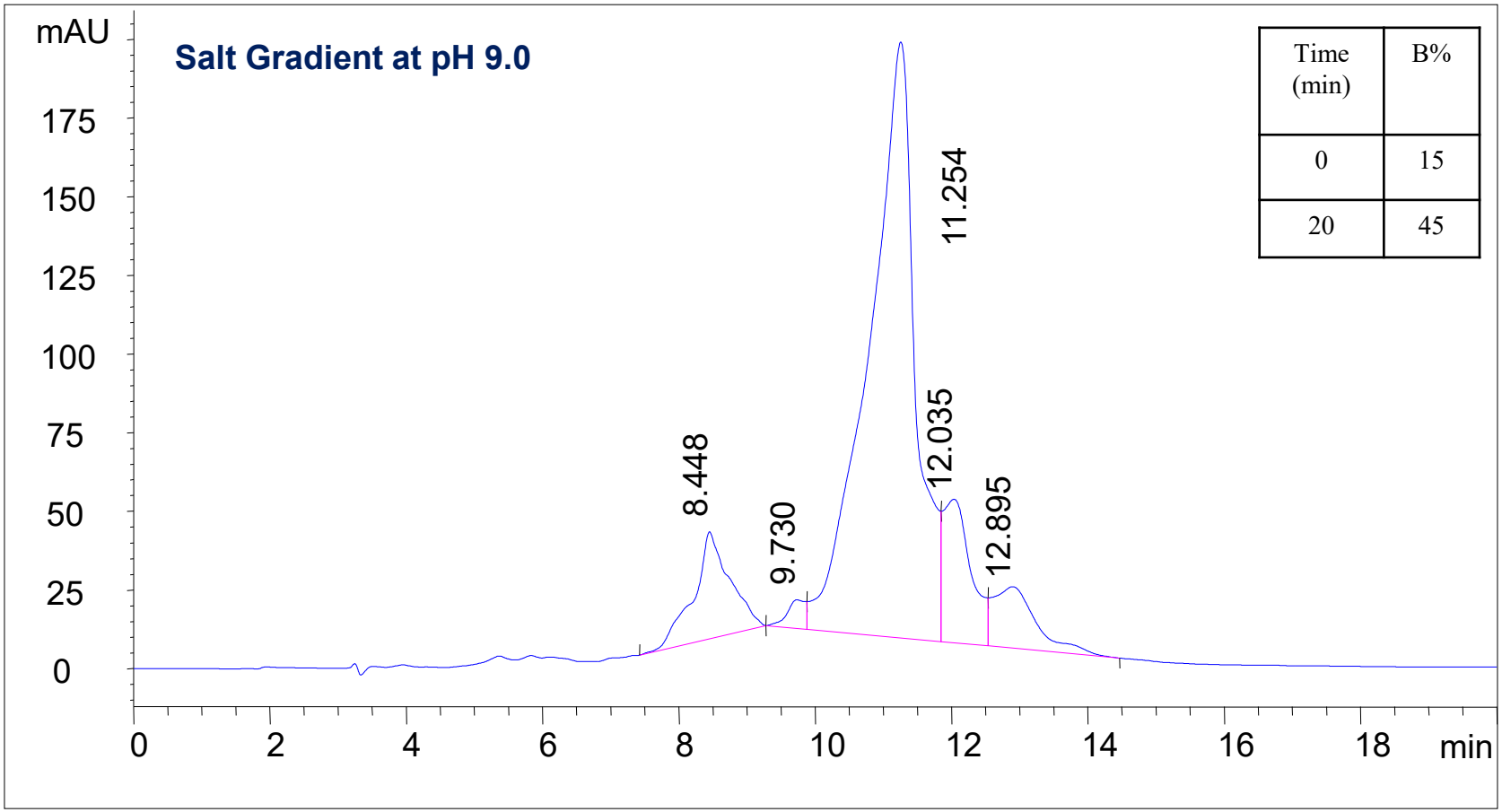
Fc Fusion Protein-2 Biosimilar on Proteomix SAX

Column: Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 403NP5-4625; **Mobile Phase:** *A:* 50 mM Tris-HCl, pH 8.5, *B:* A+ 1M NaCl, pH 8.5; **Flow Rate:** 0.5 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 ℃; **Injection Volume:** 20 µL; **Sample:** Biosimilar (1-3 mg/mL)



Fc Fusion Protein-2 Biosimilar on Proteomix SAX

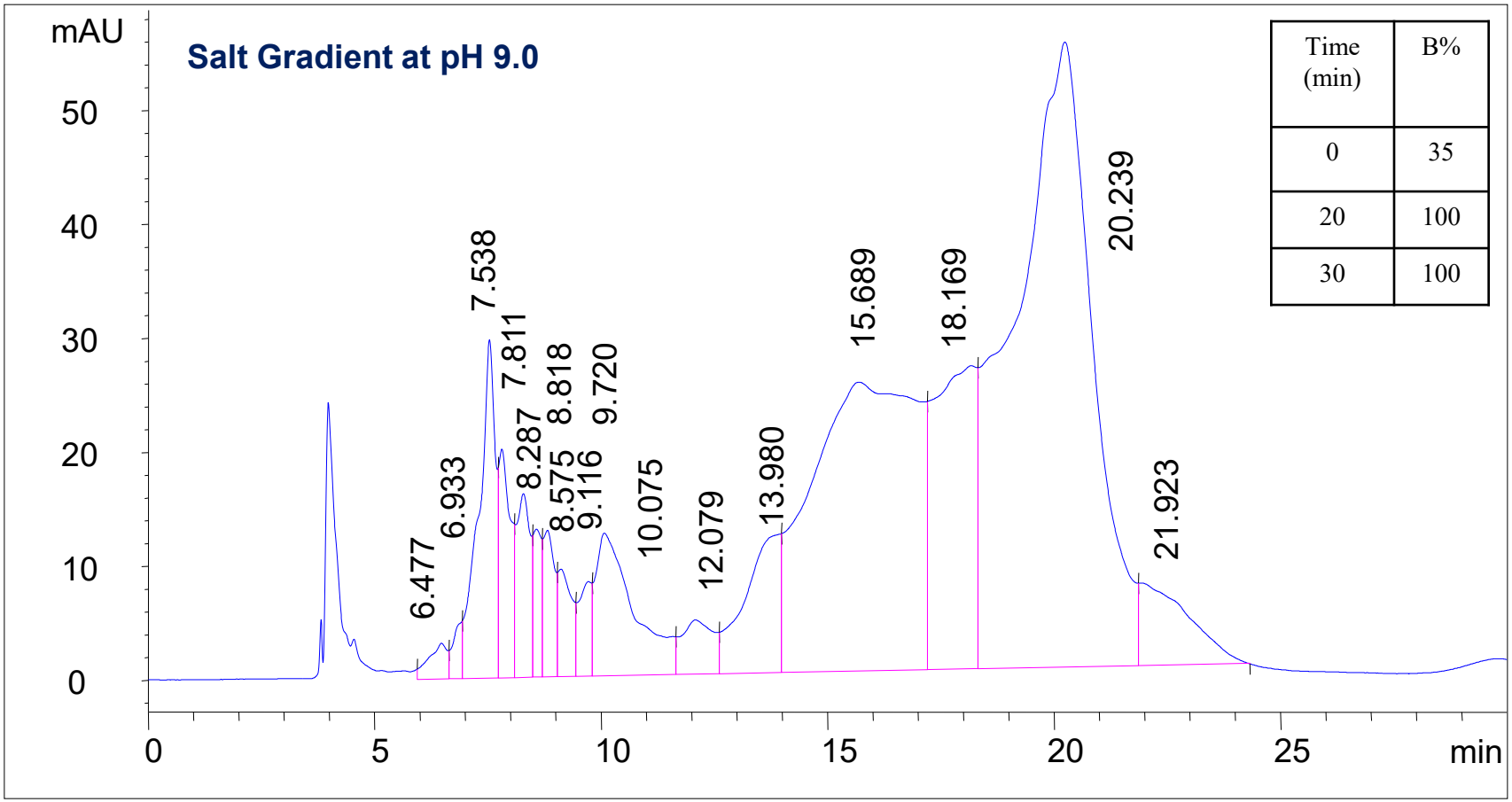
Column: Proteomix SAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 403NP5-4625; **Mobile Phase:** *A:* 50 mM Tris-HCl, pH 9.0; *B:* A+ 1M NaCl, pH 9.0; **Flow Rate:** 0.5 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 ℃; **Injection Volume:** 20 µL; **Sample:** Biosimilar (1-3 mg/mL)



Time (min)	B%
0	15
20	45

Fc Fusion Protein-2 Biosimilar on Proteomix WAX

Column: Proteomix WAX-NP5, 5 µm, 4.6 x 250 mm; **Part Number:** 404NP5-4625; **Mobile Phase:** *A:* 50 mM Tris-HCl, pH 9.0, *B:* A+ 1M NaCl, pH 9.0; **Flow Rate:** 0.5 mL/min; **Detector:** UV 280 nm; **Column Temperature:** 30 °C; **Injection Volume:** 20 µL; **Sample:** Biosimilar (1-3 mg/mL); **Pressure:** 52-49 bar



Time (min)	B%
0	35
20	100
30	100

WAX- Salt Gradient at pH 9 – Overlay Signals