

Intact and reduced mAb/ADC, mAb fragment separation on reversed phase chromatography

Proteomix[®] RP-1000



Highlights

- MAb Herceptin and its corresponding ADCs are successfully separated on the Proteomix[®] RP-1000 with protein peaks indicating different hydrophobicity with minimum sample carry over.
- Fragments from reduced mAb/ADC and Fab/Fc can be analyzed on the same column with high resolution.
- With small particle size and short column, Proteomix[®] RP-1000 reversed-phase chromatography provides high throughput and fast analysis of mAb, ADC and other protein therapeutics with online mass spec determination capability.



Proteomix[®] RP Technical Specifications

Resin Matrix:	Spherical, highly cross-linked PS/DVB
Pore Size:	500 and 1000 Å
Particle Size:	5 and 10 µm
Phase Structure:	Phenyl and substituted phenyl group
Separation Mechanism:	Hydrophobic interaction
pH Stability:	1-14
Mobile Phase Compatibility:	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, methanol, or THF

Column PN#	Particle Size	Pore Size	Column Size
465950-4605	5 µm	1000 Å	4.6 x 50 mm
465950-4610	5 µm	1000 Å	4.6 x 100 mm
465950-2105	5 µm	1000 Å	2.1 x 50 mm



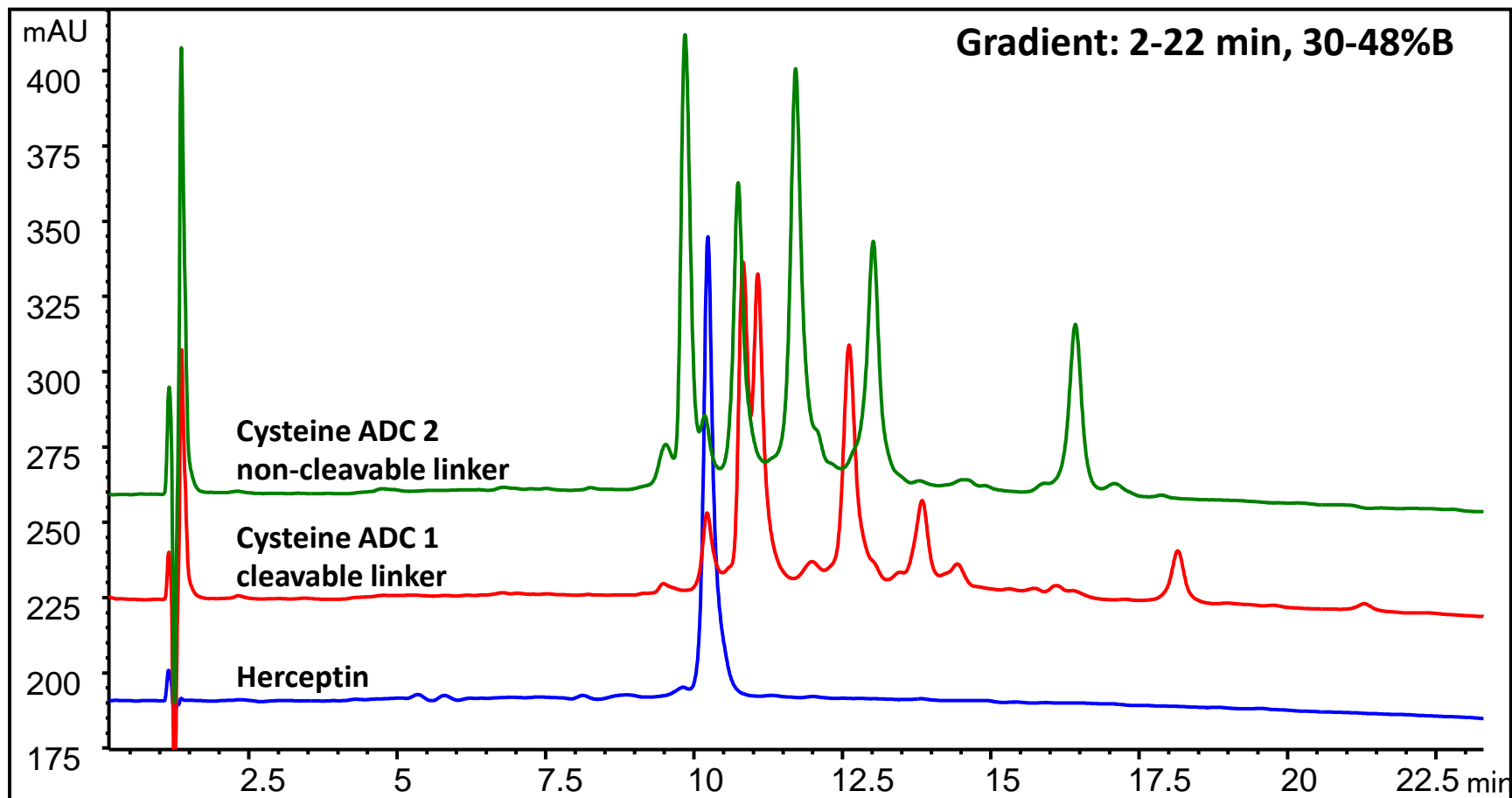
MAb Herceptin and its ADCs Separation- 4.6 x 100 mm

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 ° C;

Sample: Herceptin and ADCs 1 mg/mL diluted in water; Injection volume: 10 µL



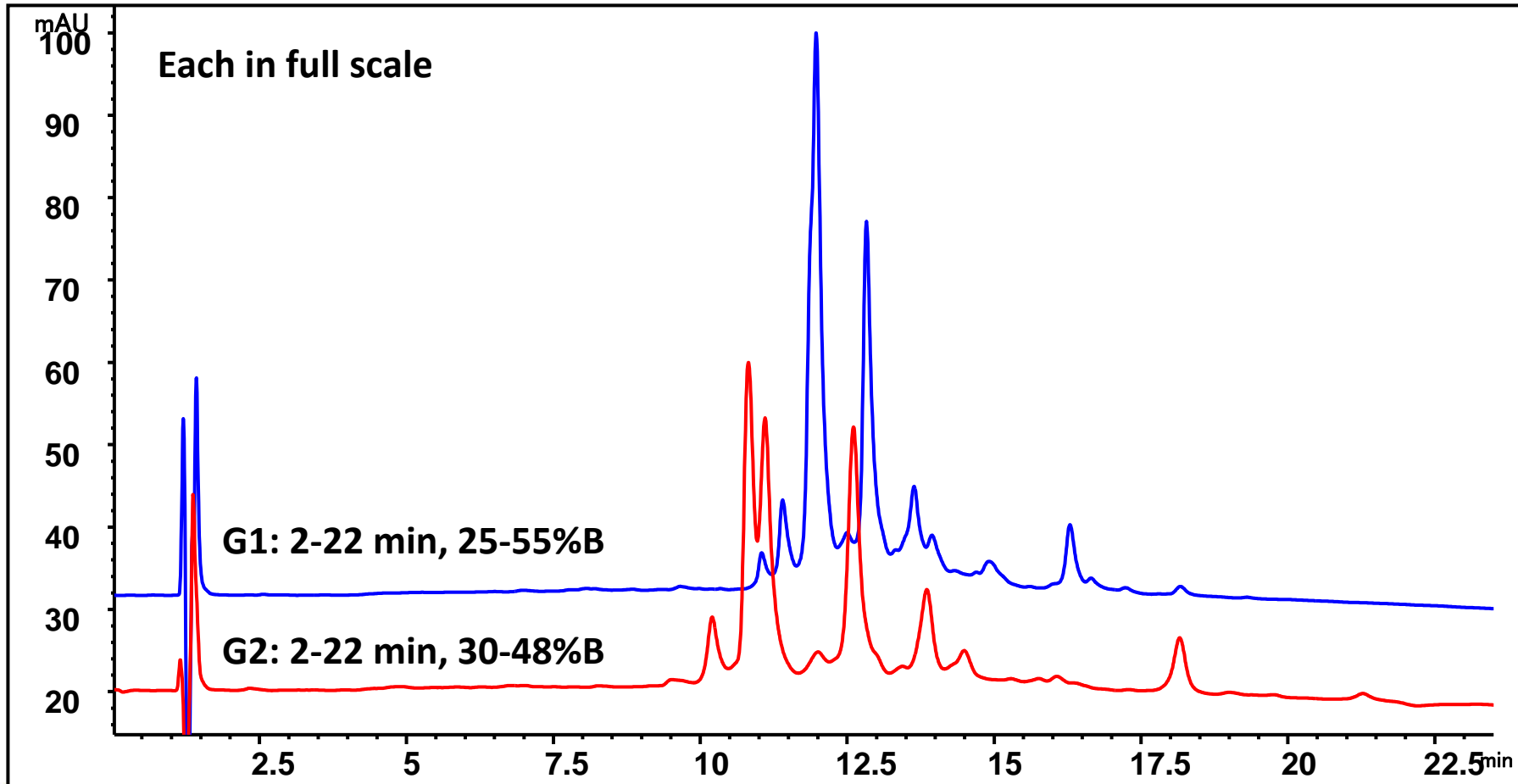
Herceptin Cysteine ADC1 Separation on **Proteomix[®] RP-1000** gradient optimization

Column: **Proteomix[®] RP-1000** (5 μm , 1000 \AA , 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 $^{\circ}$ C;

Sample: Cysteine ADC 1 1 mg/mL diluted in water; Injection volume: 10 μL for gradient 1, 5 μL for gradient 2



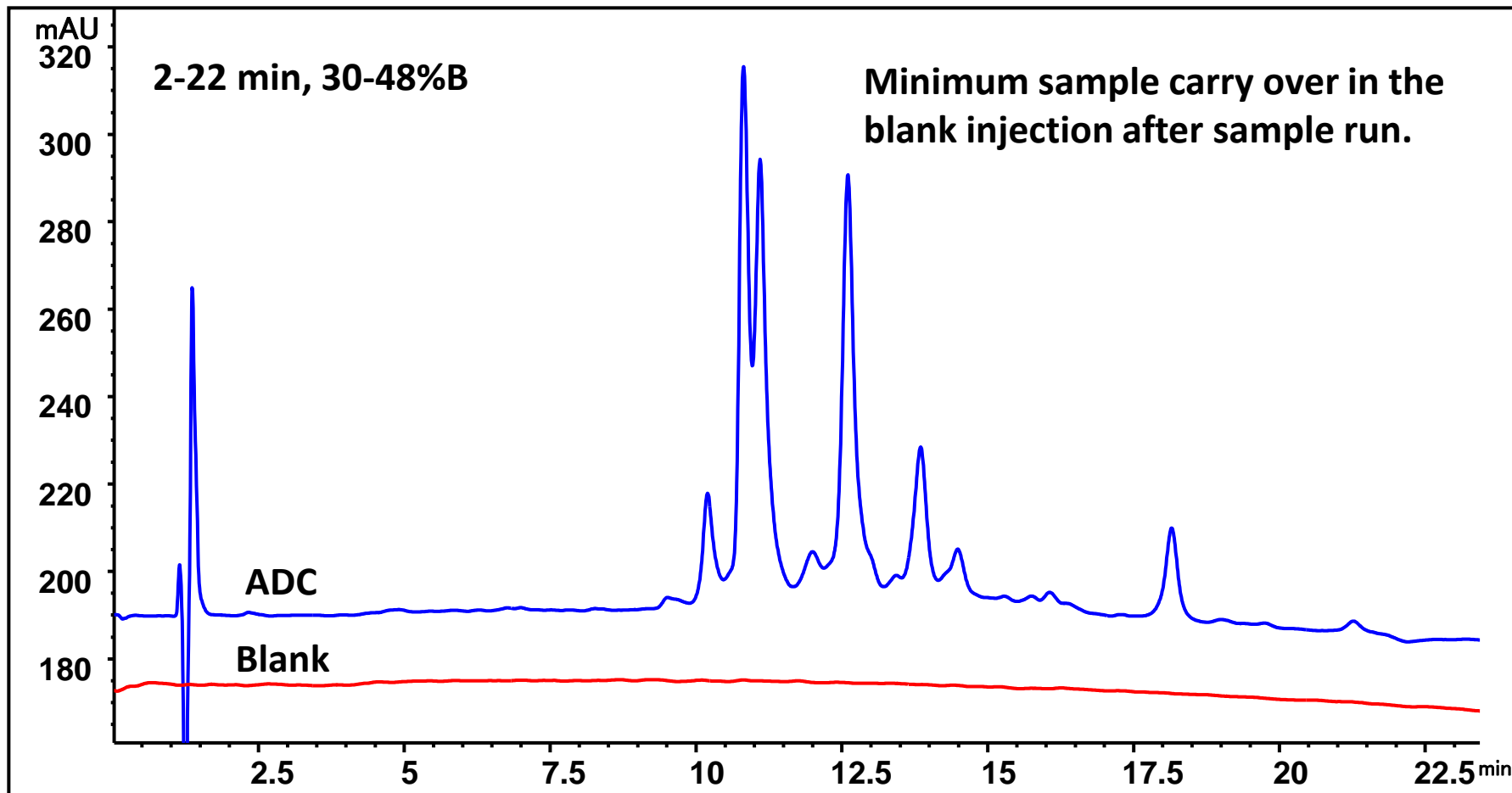
Minimum sample carry over

Column: **Proteomix[®] RP-1000** (5 μ m, 1000 Å , 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 ° C;

Sample: Cysteine ADC 1 mg/mL diluted in water; Injection volume: 8 μ L



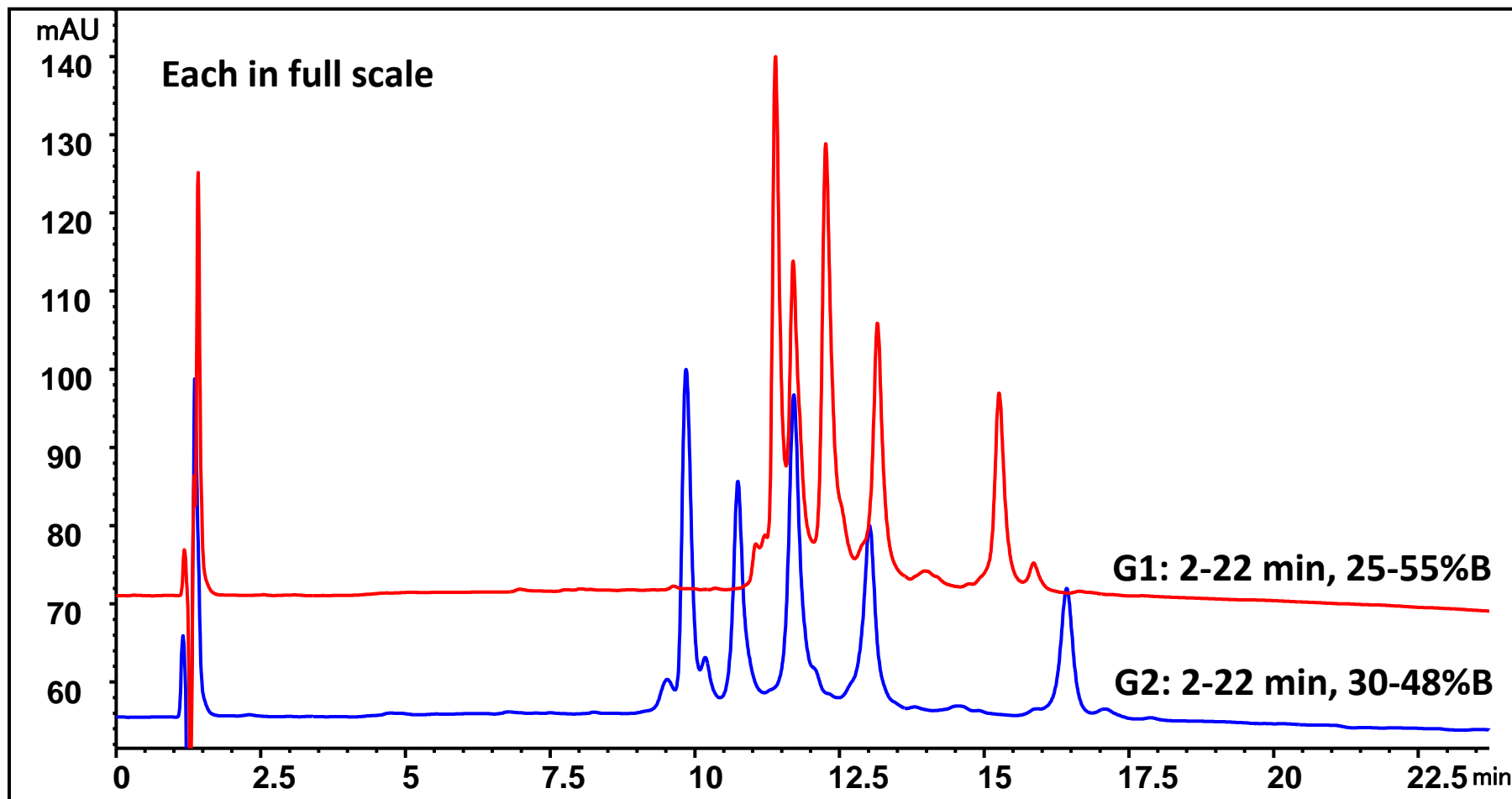
Herceptin Cysteine ADC2 Separation on Proteomix[®] RP-1000

Column: Proteomix[®] RP-1000 (5 μm , 1000 \AA , 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 $^{\circ}\text{C}$;

Sample: Cysteine ADC 2 1 mg/mL diluted in water; Injection volume: 10 μL for gradient 1, 8 μL for gradient 2



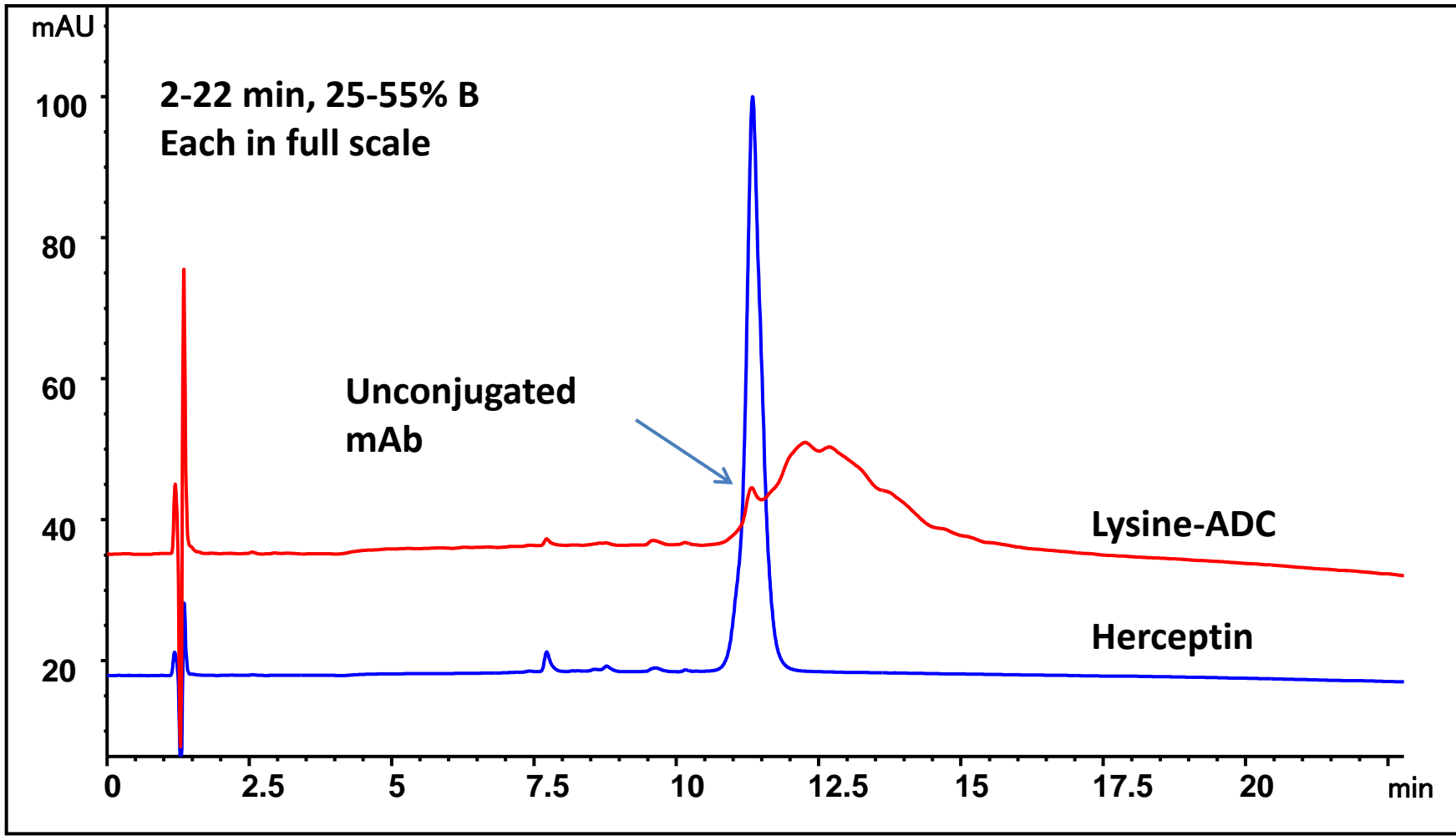
Herceptin and its lysine ADC separation

Column: **Proteomix[®] RP-1000** (5 μm , 1000 \AA , 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 $^{\circ}$ C;

Sample: Herceptin and lysine ADC 1 mg/mL diluted in 0.1% TFA; Injection volume: 10 μL



MAb/ADC fragments: Reduced mAb Herceptin and Cysteine ADC 1

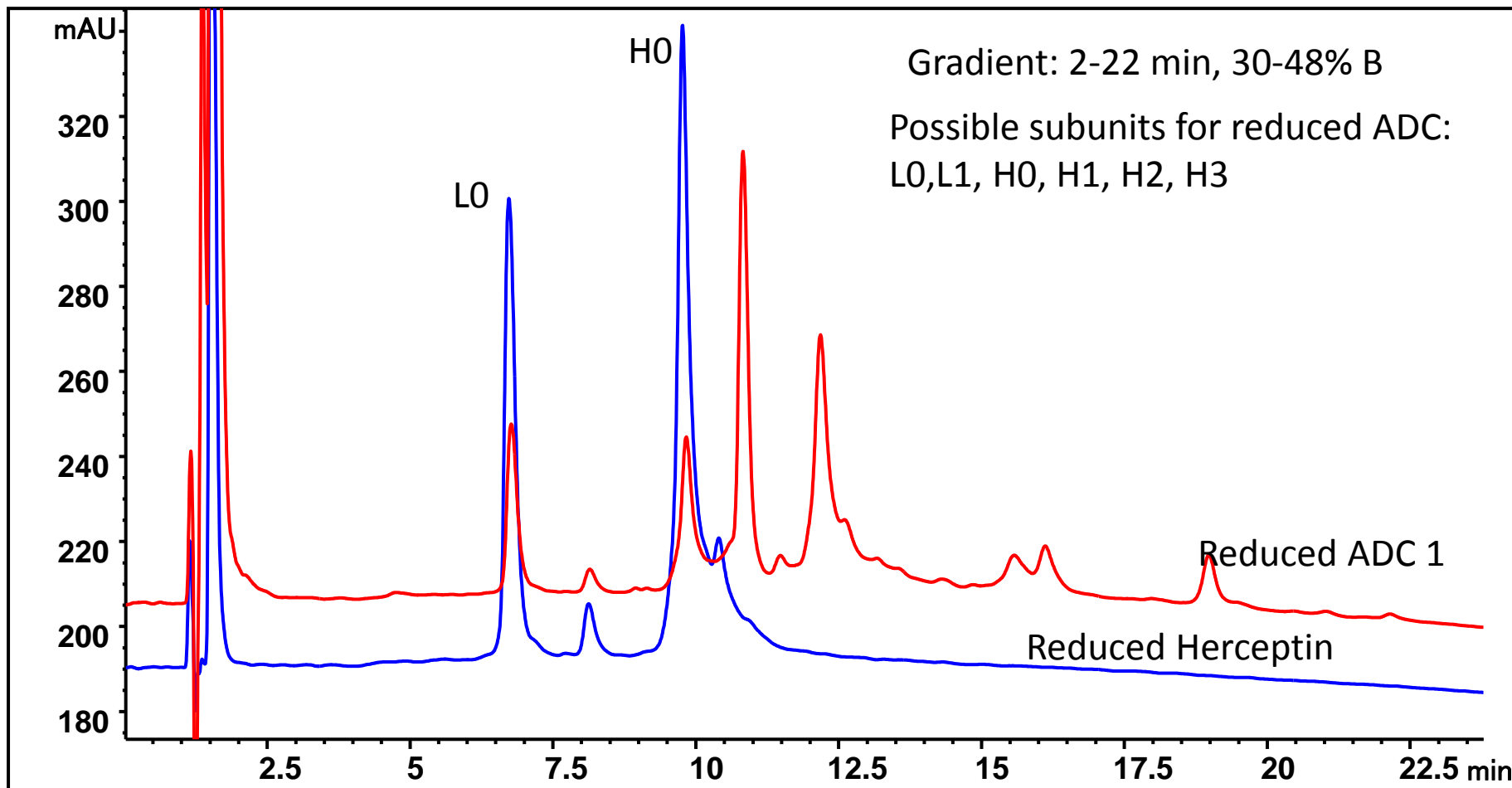
Column: Proteomix® RP-1000 (5 μm, 1000 Å, 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 ° C;

Sample: Herceptin and ADC1 2 mg/mL reduced with 20 mM DTT, incubated at 65 ° C for 20 minute;

Injection volume: Reduced herceptin 2 μL , Reduced herceptin ADC 1 5 μL



Intact and reduced Cysteine ADC 1 Separation on Proteomix[®] RP-1000

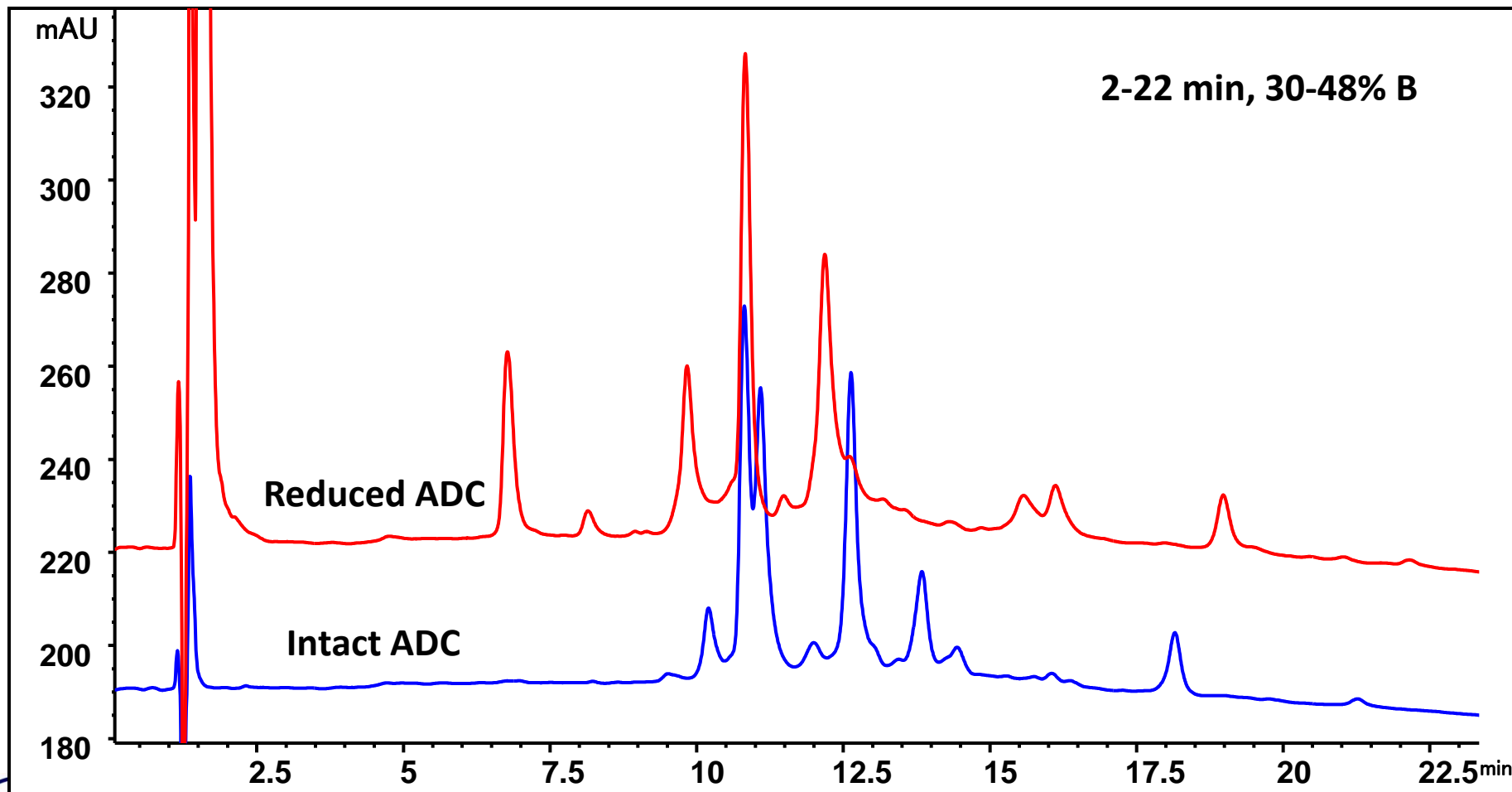
Column: Proteomix[®] RP-1000 (5 μ m, 1000 Å, 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 ° C;

Sample: Herceptin and ADC1 2 mg/mL reduced with 20 mM DTT, incubated at 65 ° C for 20 minute;

Injection volume: Intact ADC1 5 μ L , Reduced herceptin ADC 1 10 μ L



Reduced Herceptin and lysine ADC Separation on Proteomix[®] RP-1000

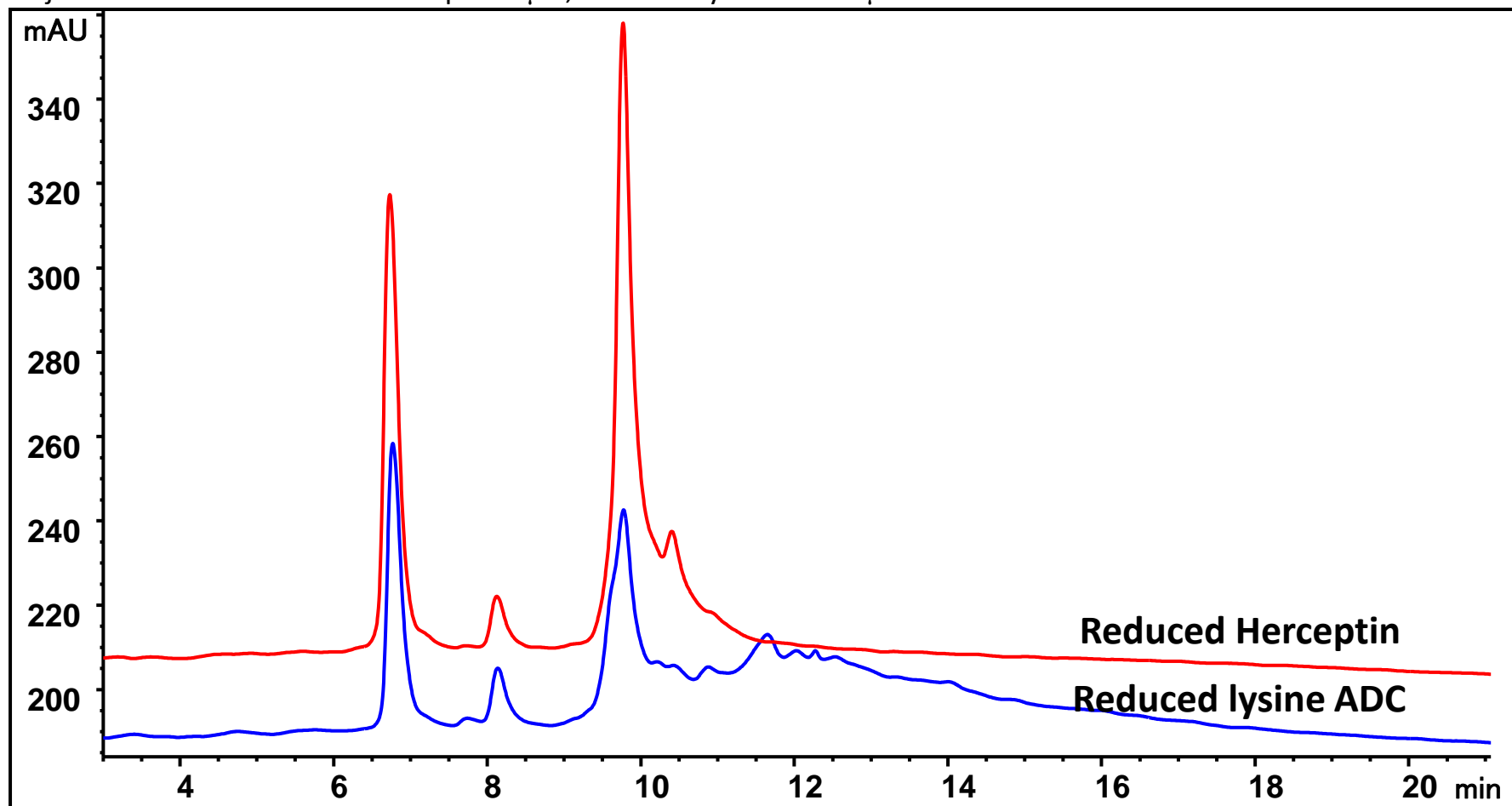
Column: Proteomix[®] RP-1000 (5 μ m, 1000 Å, 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 ° C;

Sample: Herceptin and lysine ADC 2 mg/mL reduced with 20 mM DTT, incubated at 65 ° C for 20 minute;

Injection volume: Reduced herceptin 2 μ L , Reduced lysine ADC 5 μ L



Column size 2.1 x 50 mm

Proteomix[®] RP-1000



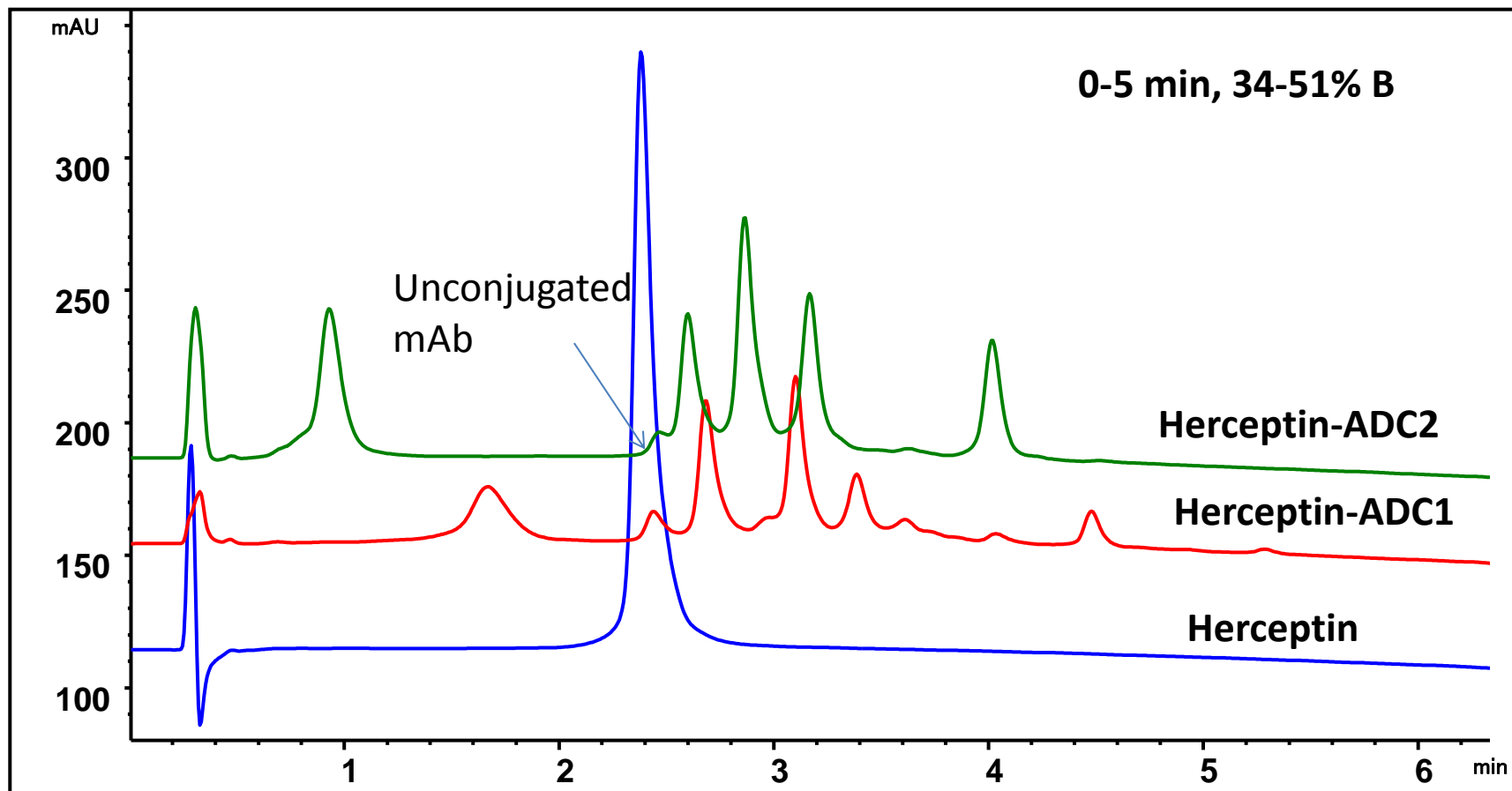
Herceptin/ADC1/ADC2 Separation- small size 2.1 x 50 mm

Column: **Proteomix® RP-1000** (5 μm , 1000 \AA , 2.1 x 50 mm)

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 0.6 mL/min; Detector: UV 210 nm; Column temperature: 80 °C; Column pressure: 70 bar;

Sample: Herceptin, ADC 1 and ADC 2 diluted in water; Injection volume: 0.5 μL for Herceptin, 1 μL for ADC 1 and ADC 2



Bio-C4 vs. Proteomix[®] RP-1000

Silica based vs. polymer based



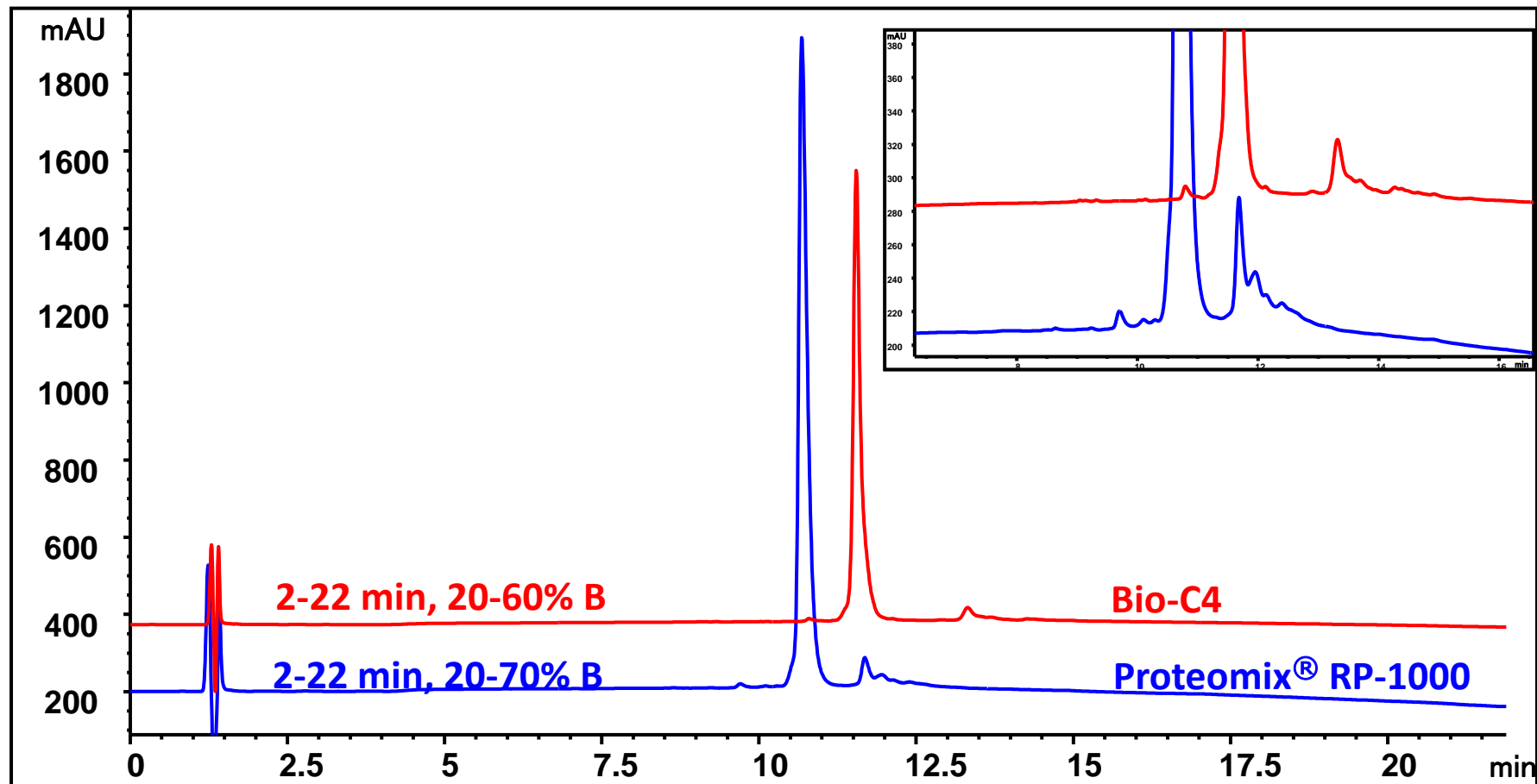
mAb 321 Separation

Column: **Proteomix® RP-1000** (5 μm, 1000 Å, 4.6 x 100 mm); Bio-C4 (5 μm, 300 Å, 4.6 x 100 mm)

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 °C;

Sample: mAb 321 1 mg/mL diluted in 0.1% TFA; Injection volume: 20 μL



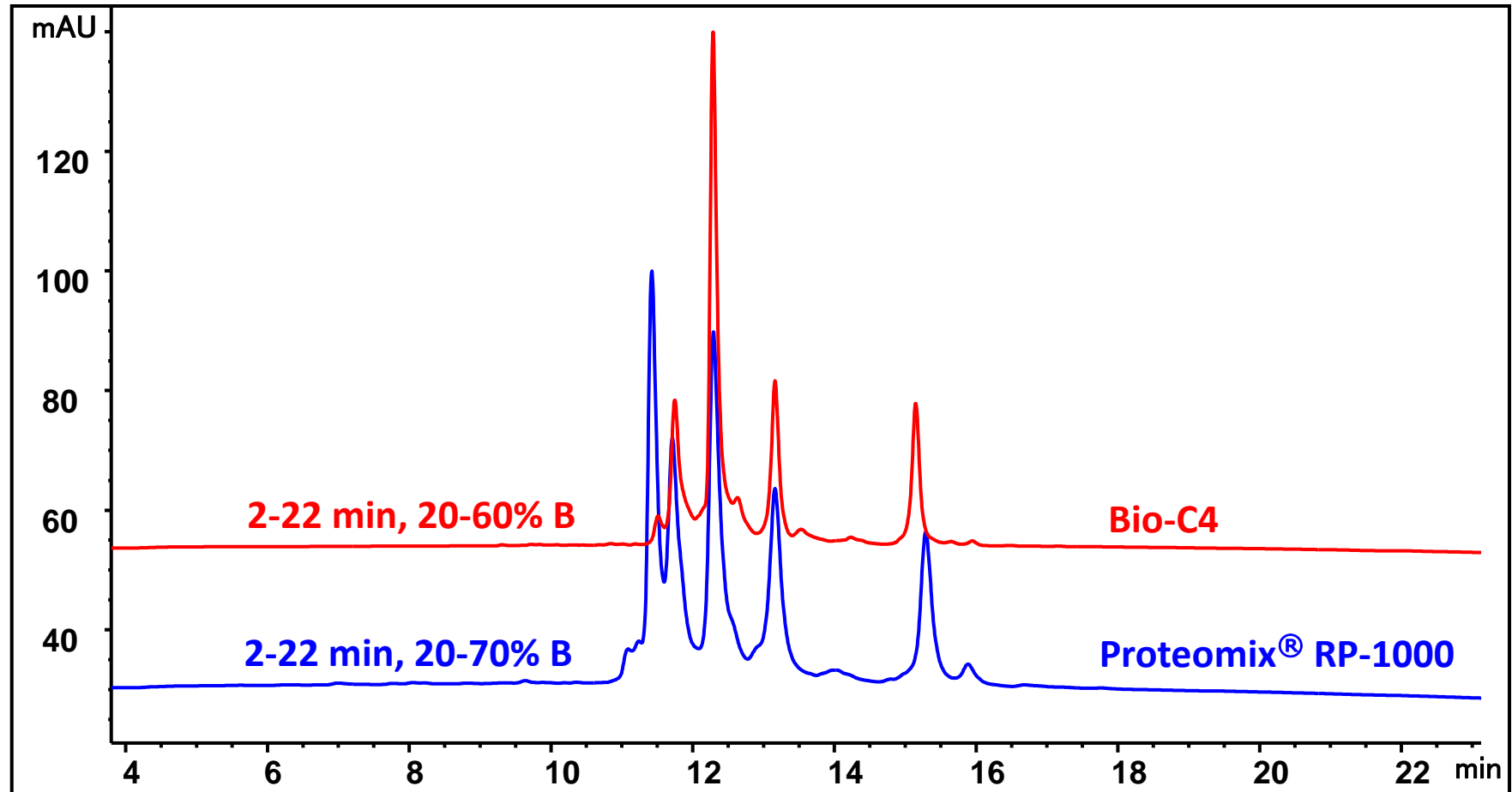
Herceptin cysteine ADC 2 Separation

Column: **Proteomix® RP-1000** (5 μm , 1000 \AA , 4.6 x 100 mm); Bio-C4 (5 μm , 300 \AA , 4.6 x 100 mm)

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 $^{\circ}\text{C}$;

Sample: ADC diluted in 0.1% TFA; Injection volume: 15 μL



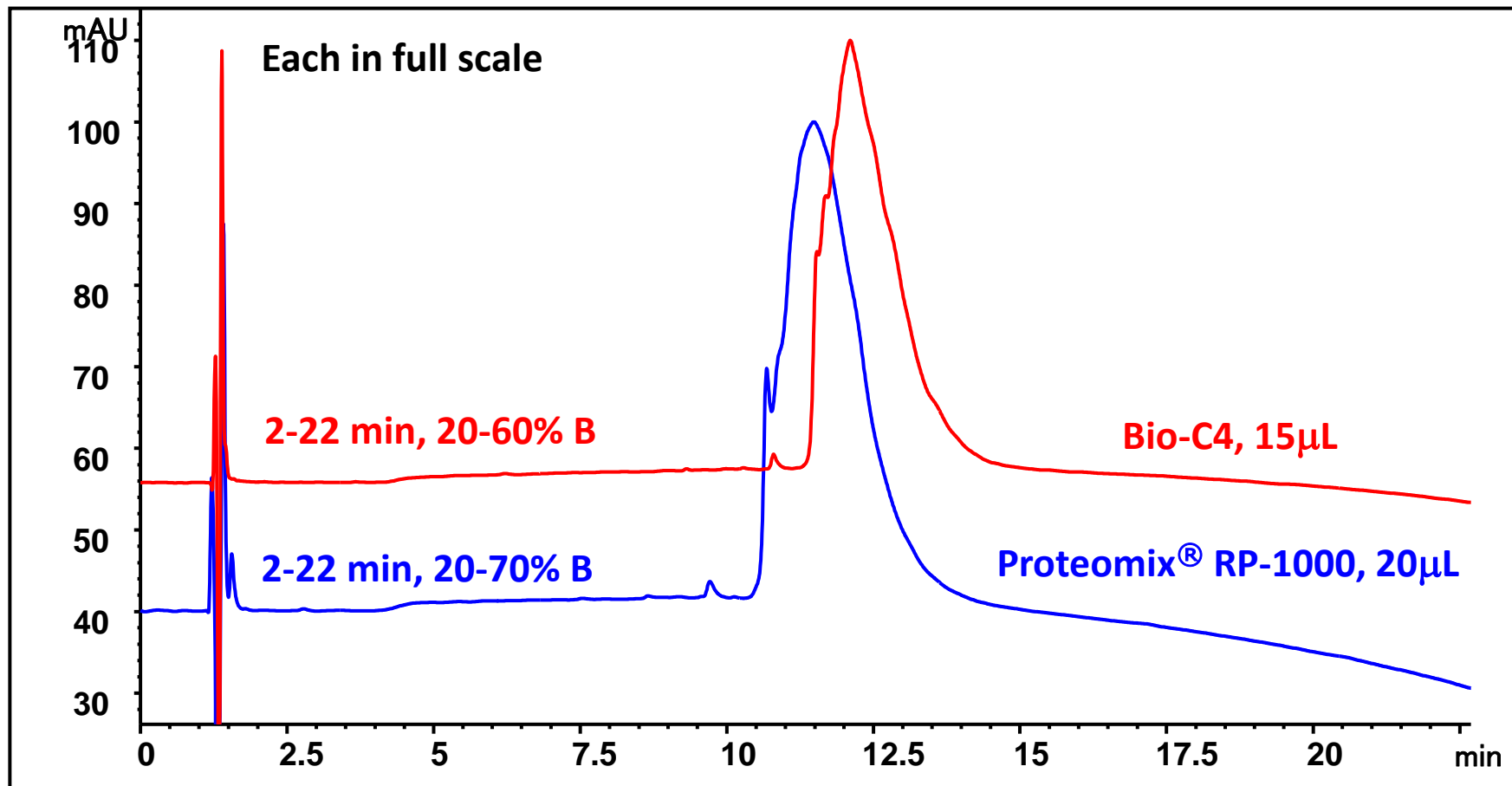
Herceptin-ADC (lysine conjugation) Separation

Column: **Proteomix® RP-1000** (5 μm , 1000 \AA , 4.6 x 100 mm); Bio-C4 (5 μm , 300 \AA , 4.6 x 100 mm)

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 $^{\circ}\text{C}$;

Sample: ADC diluted in 0.1% TFA; Injection volume: 15 μL



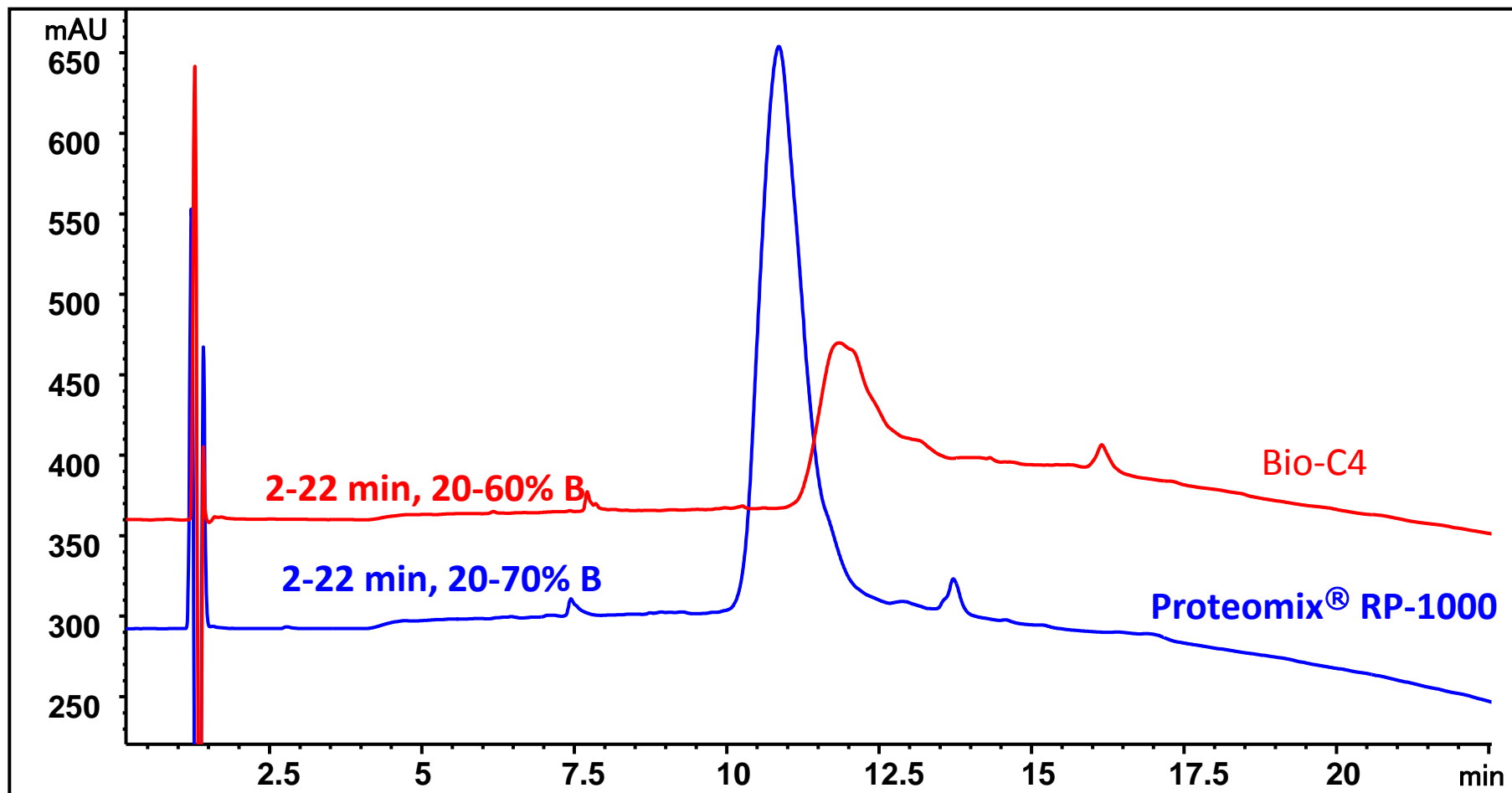
γ -globulin 150 kD Separation

Column: **Proteomix[®] RP-1000** (5 μ m, 1000 Å, 4.6 x 100 mm); Bio-C4 (5 μ m, 300 Å, 4.6 x 100 mm)

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 °C;

Sample: γ -globulin 1 mg/mL diluted in 0.1% TFA; Injection volume: 20 μ L



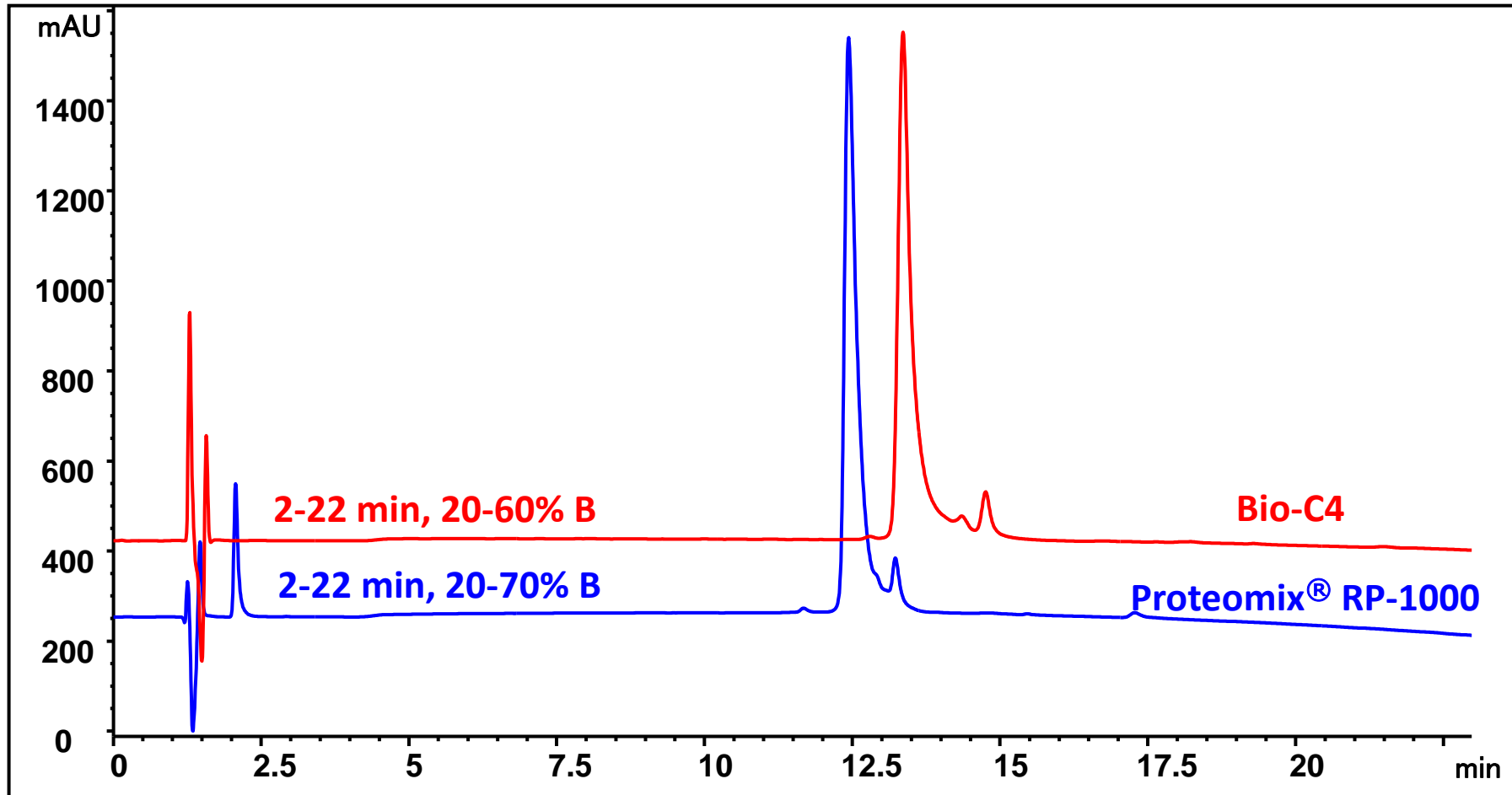
BSA 66 kD Separation

Column: **Proteomix® RP-1000** (5 μm , 1000 \AA , 4.6 x 100 mm); Bio-C4 (5 μm , 300 \AA , 4.6 x 100 mm)

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 40 $^{\circ}\text{C}$;

Sample: BSA 1 mg/mL diluted in 0.1% TFA; Injection volume: 20 μL



Temperature effect

Proteomix[®] RP-1000

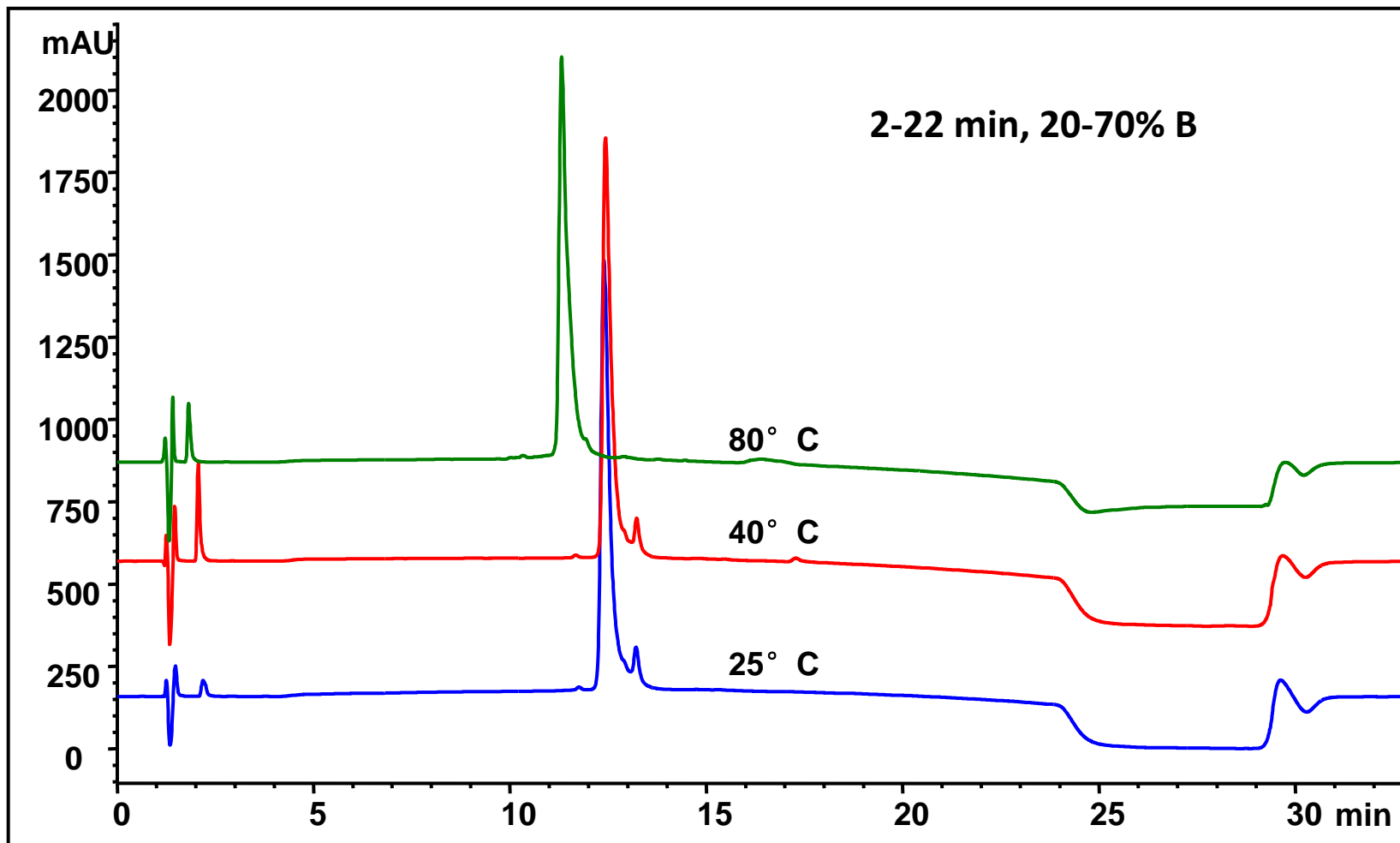


BSA separation with 25°C / 40°C / 80°C

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Sample: BSA 1mg/mL; Injection volume: 20 µL

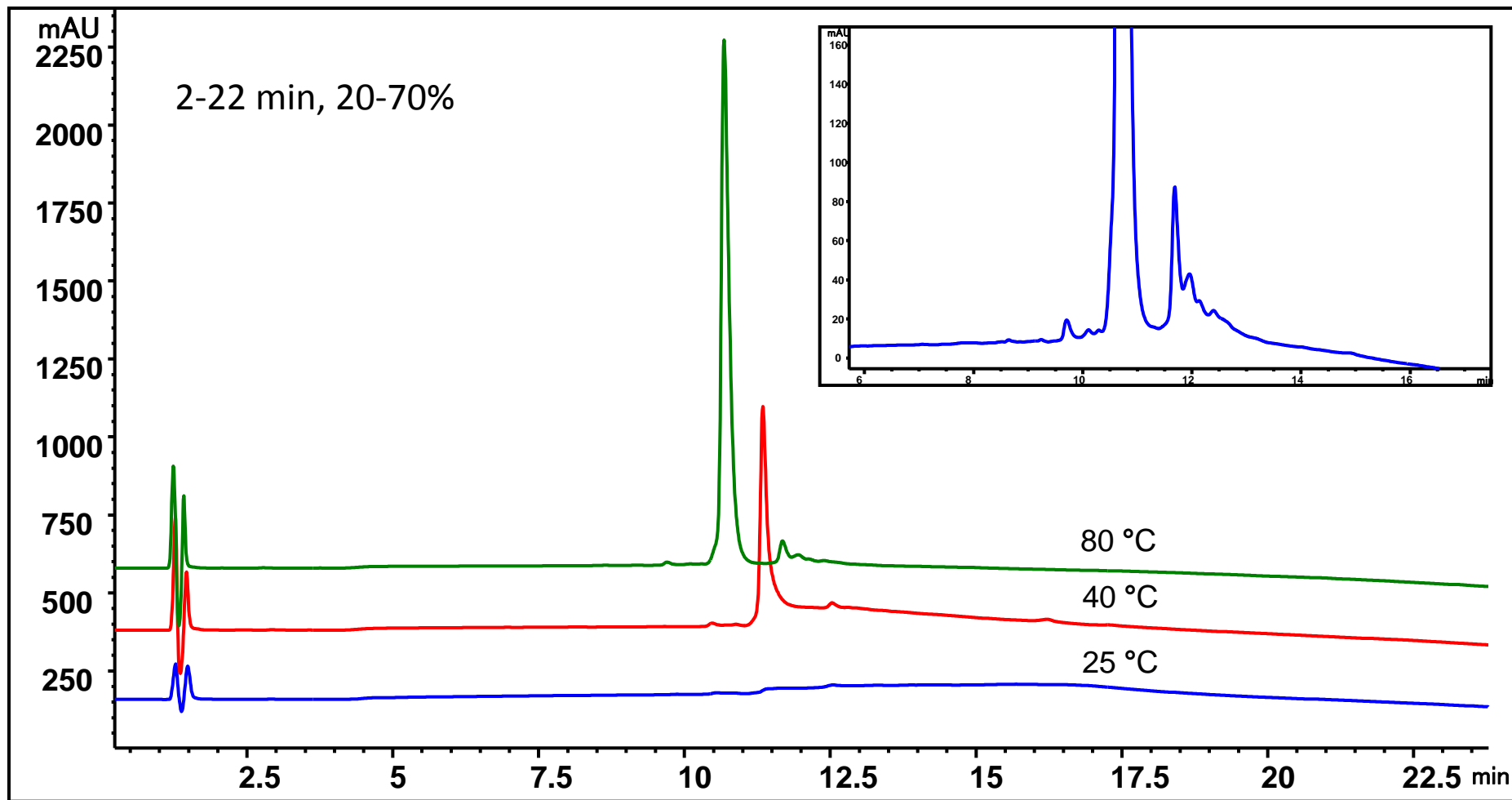


Temperature effect: mAb separation with 25°C / 40°C / 80°C

Column: Proteomix® RP-1000 (5 μm, 1000 Å, 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Sample: mAb 1mg/mL; Injection volume: 20 μL



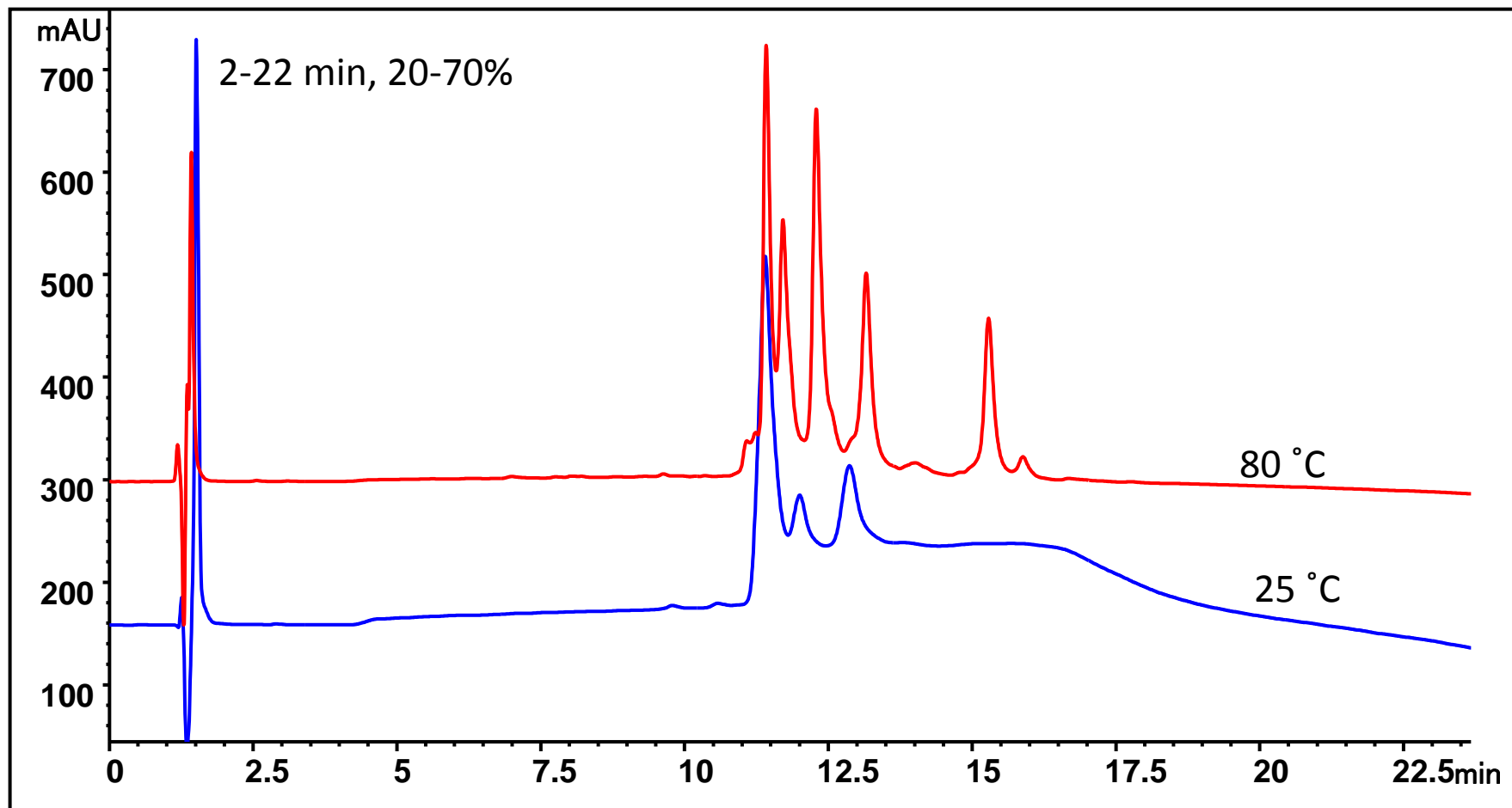
Temperature effect: Herceptin Cysteine ADC 2 Separation

Column: Proteomix[®] RP-1000 (5 μ m, 1000 Å, 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 25, 80 °C;

Sample: Herceptin Cysteine ADC 2 1 mg/mL diluted in 0.1% TFA; Injection volume: 20 μ L

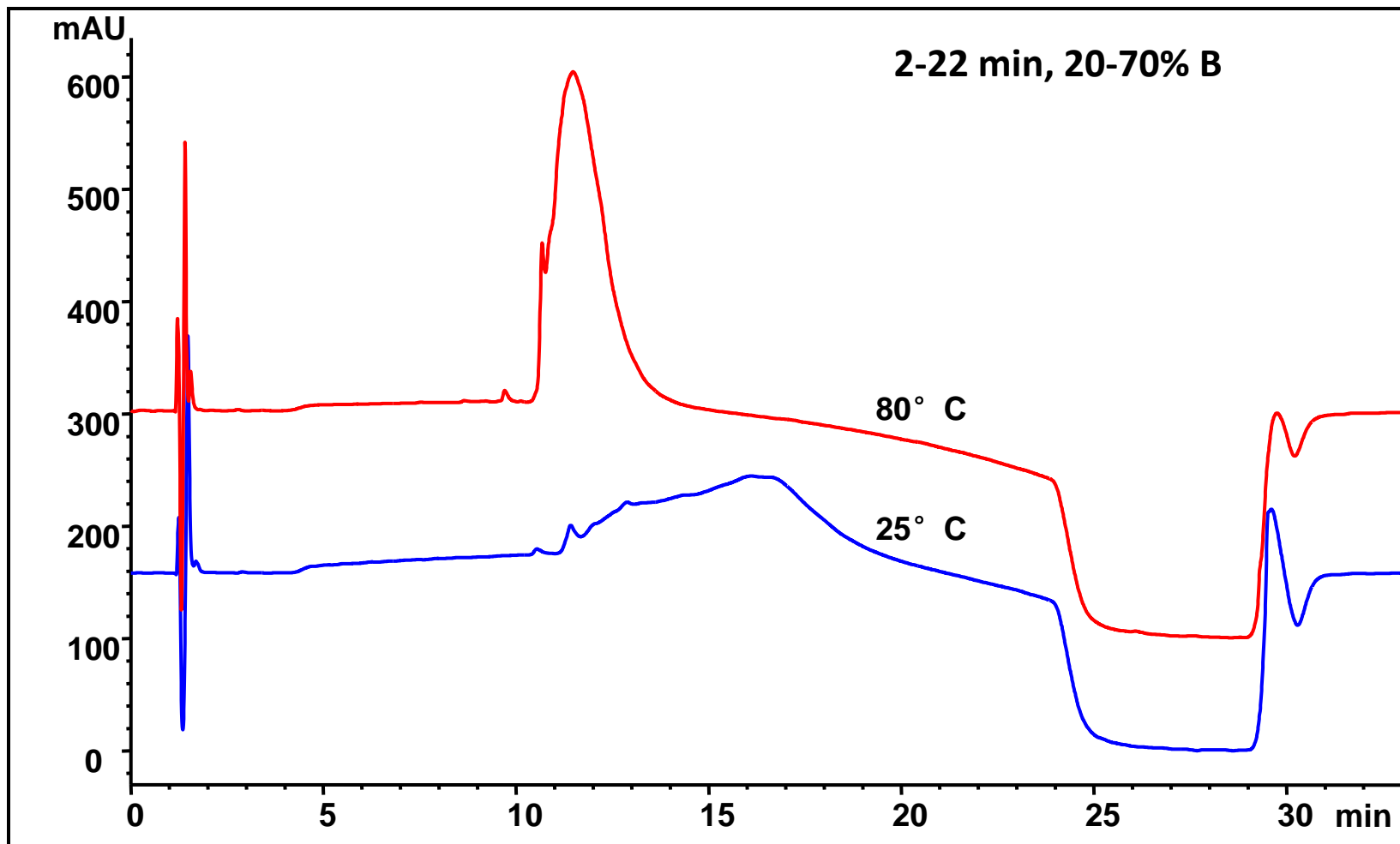


Herceptin-lysine ADC separation on **Proteomix® RP-1000** with 25°C /80°C

Column: **Proteomix® RP-1000** (5 µm, 1000 Å, 4.6 x 100 mm)

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Sample: Lysine ADC 1mg/mL; Injection volume: 20 µL



mAb fragment

Proteomix[®] RP-1000



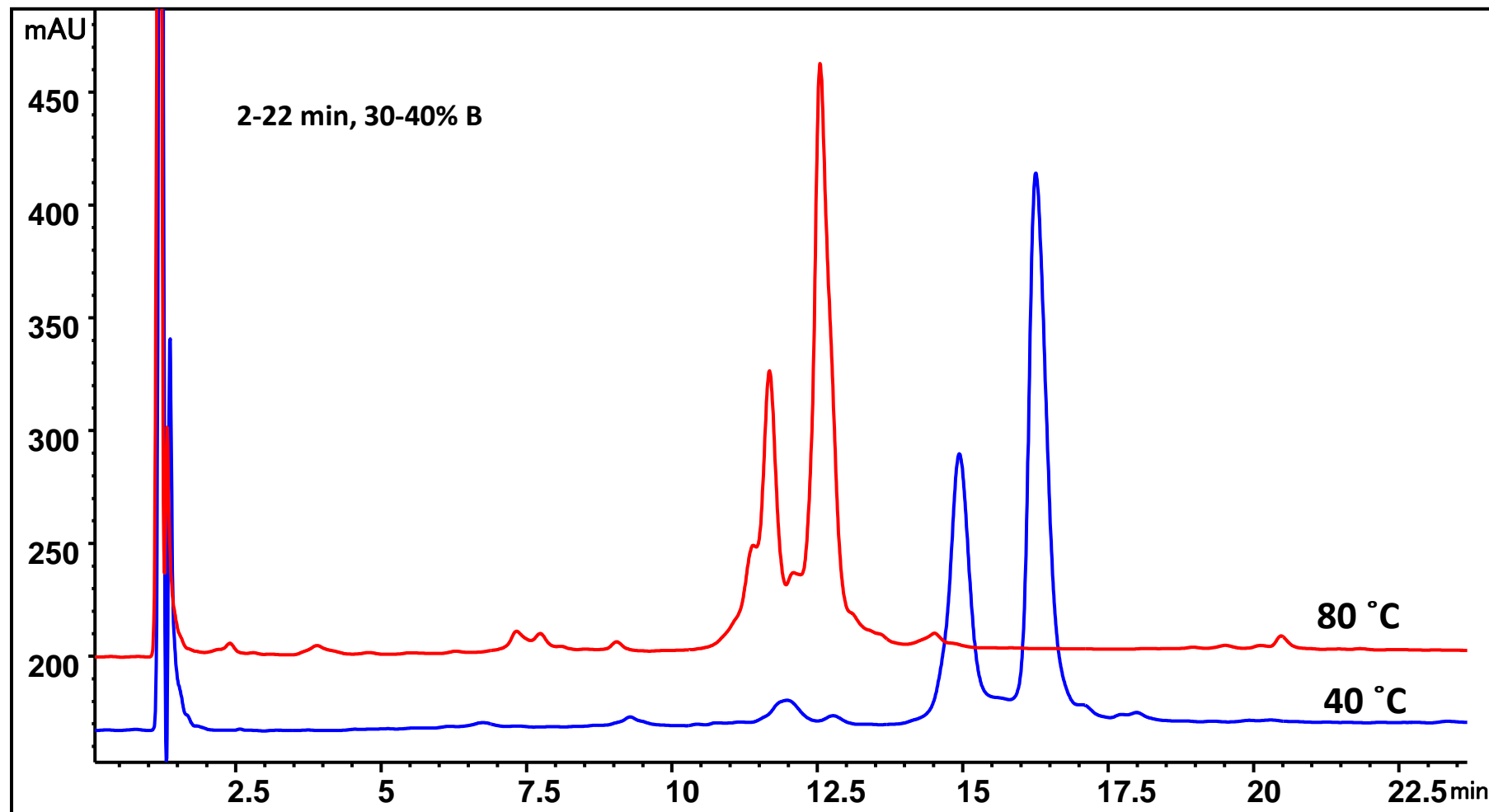
Fab/Fc separation on : Proteomix[®] RP-1000 with 40 °C and 80 °C

Column: Proteomix[®] RP-1000 (5 μm, 1000 Å, 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 40, 80 °C;

Sample: mAb (digested by papain), 1 mg/mL diluted in 0.1% TFA; Injection volume: 20 μL



Reduced mAb separation with 40 °C and 80 °C

Column: : **Proteomix® RP-1000** (5 μm , 1000 \AA , 4.6 x 100 mm);

Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;

Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 40, 80 ° C;

Sample: mAb reduced with 20 mM DTT at 65 ° C for 20 minutes, 1 mg/mL diluted in 0.1% TFA; Injection volume: 20 μL

