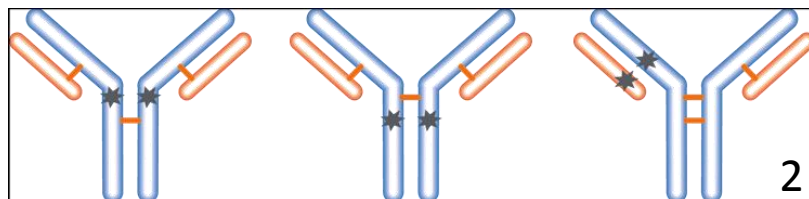


Intact and reduced mAb/ADC fragment separation on reversed phase chromatography with mass spec detection

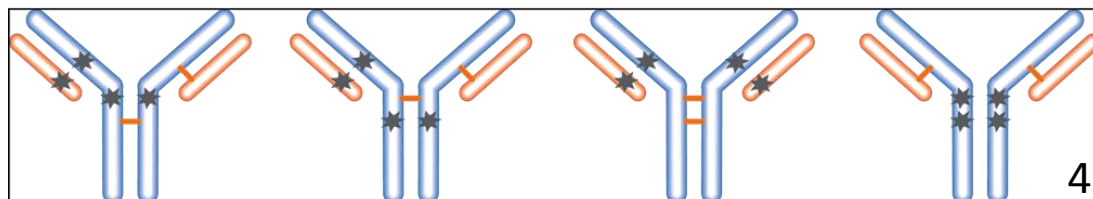
Proteomix® RP-1000



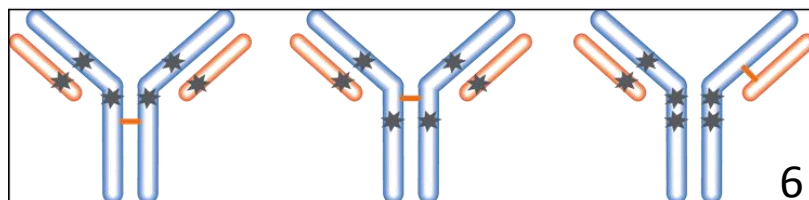
Possible Different cysteine ADC components under acidic/denaturing condition



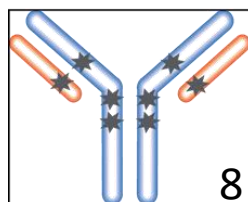
Intact 2, L1 and H1HL



L1, H2H1L, H1H1, LH2



L1, H2H2, H3, LH2



L1, H3

Non reduced: Intact 2, L1, H1HL, H2H1L, H1H1, LH2, H2H2, H3

Fully reduced: L0, H0, L1, H1, H2, H3

Herceptin: L0 = 23,439 Da, H0 = 50,620 Da

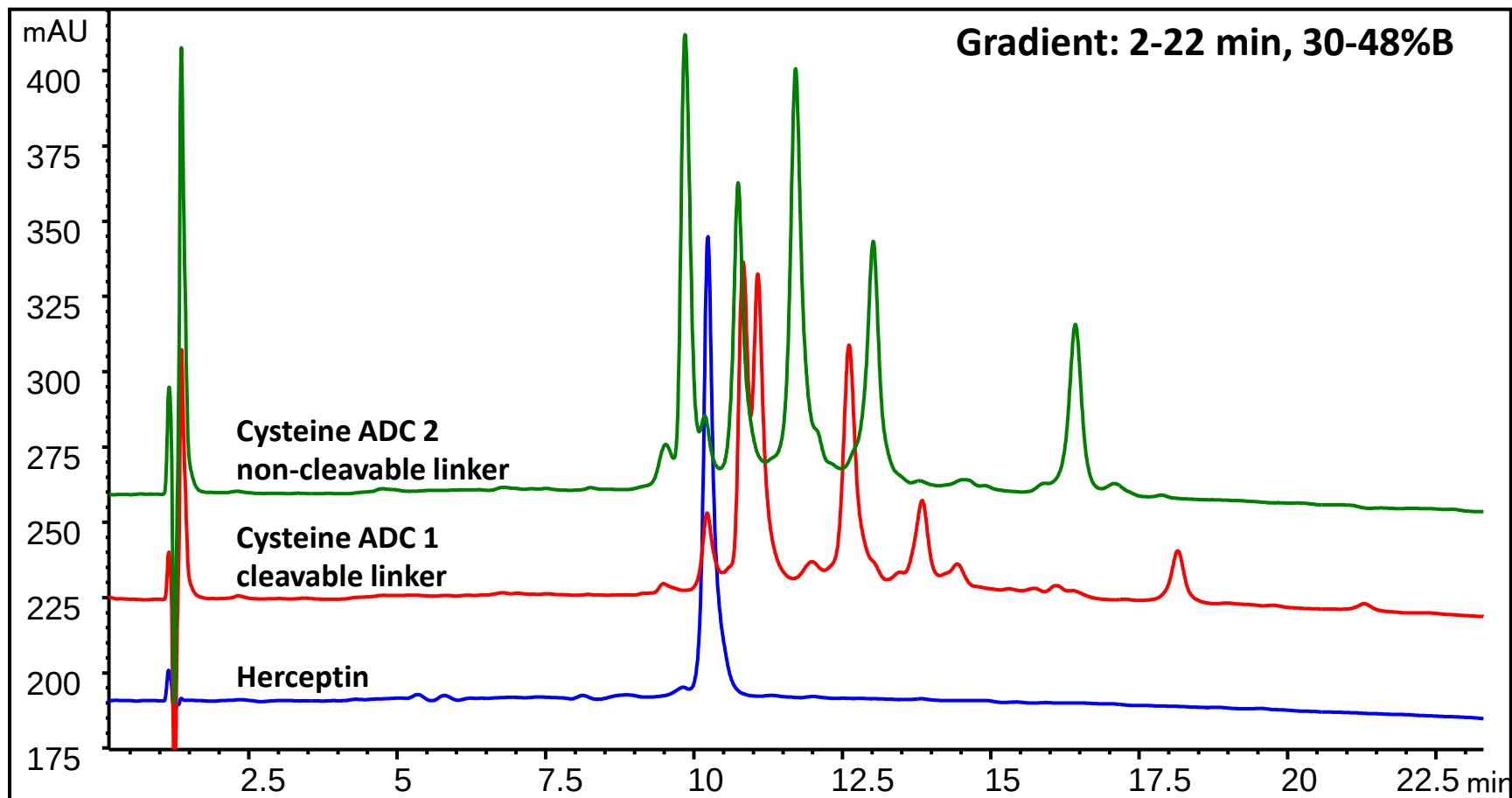
MAb Herceptin and its ADCs Separation on Proteomix[®] RP-1000

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 ADCs 1 mg/mL diluted in water; Injection volume: 10 μ L



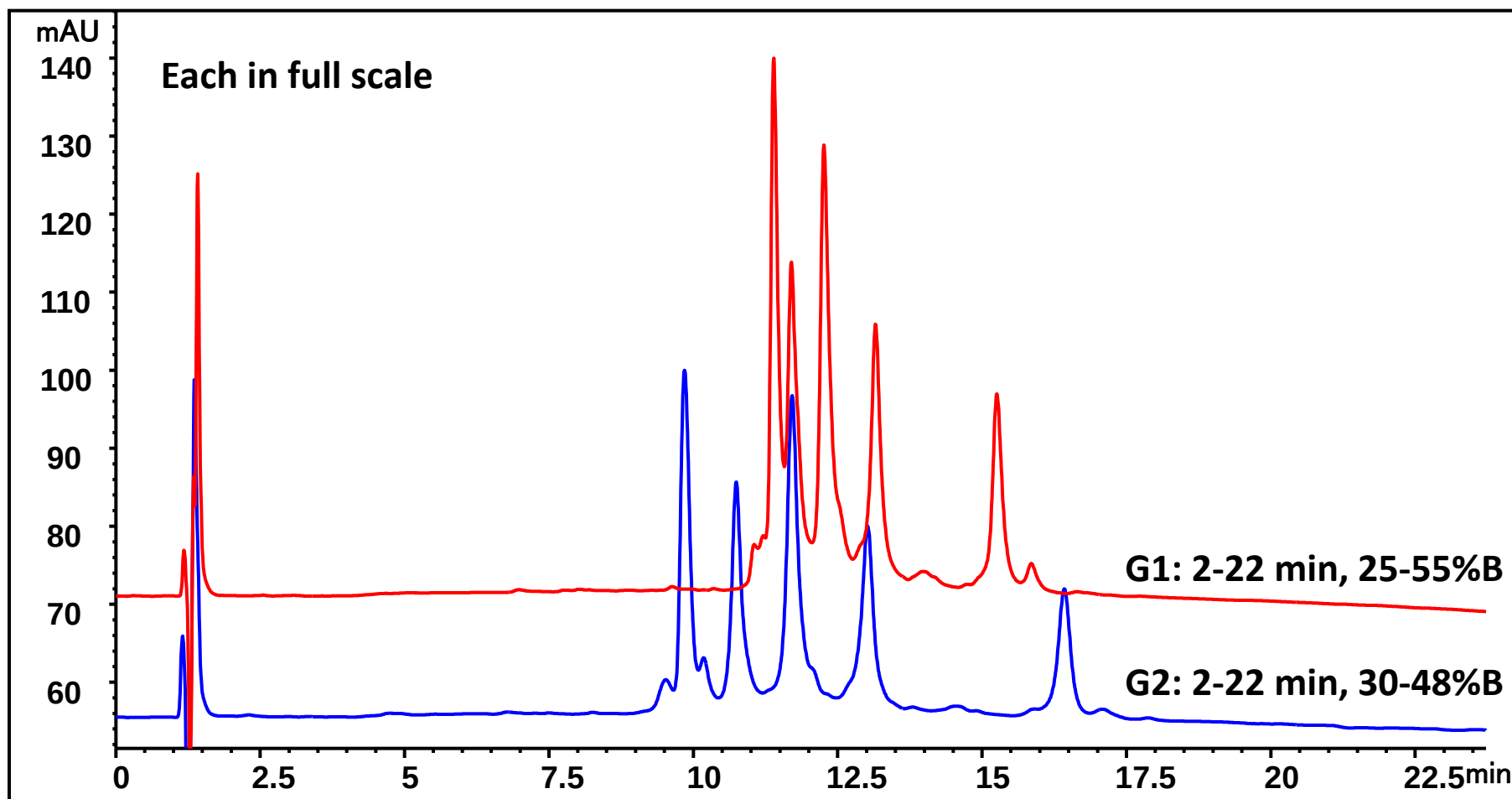
Herceptin Cysteine ADC2 Separation on Proteomix[®] RP-1000

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



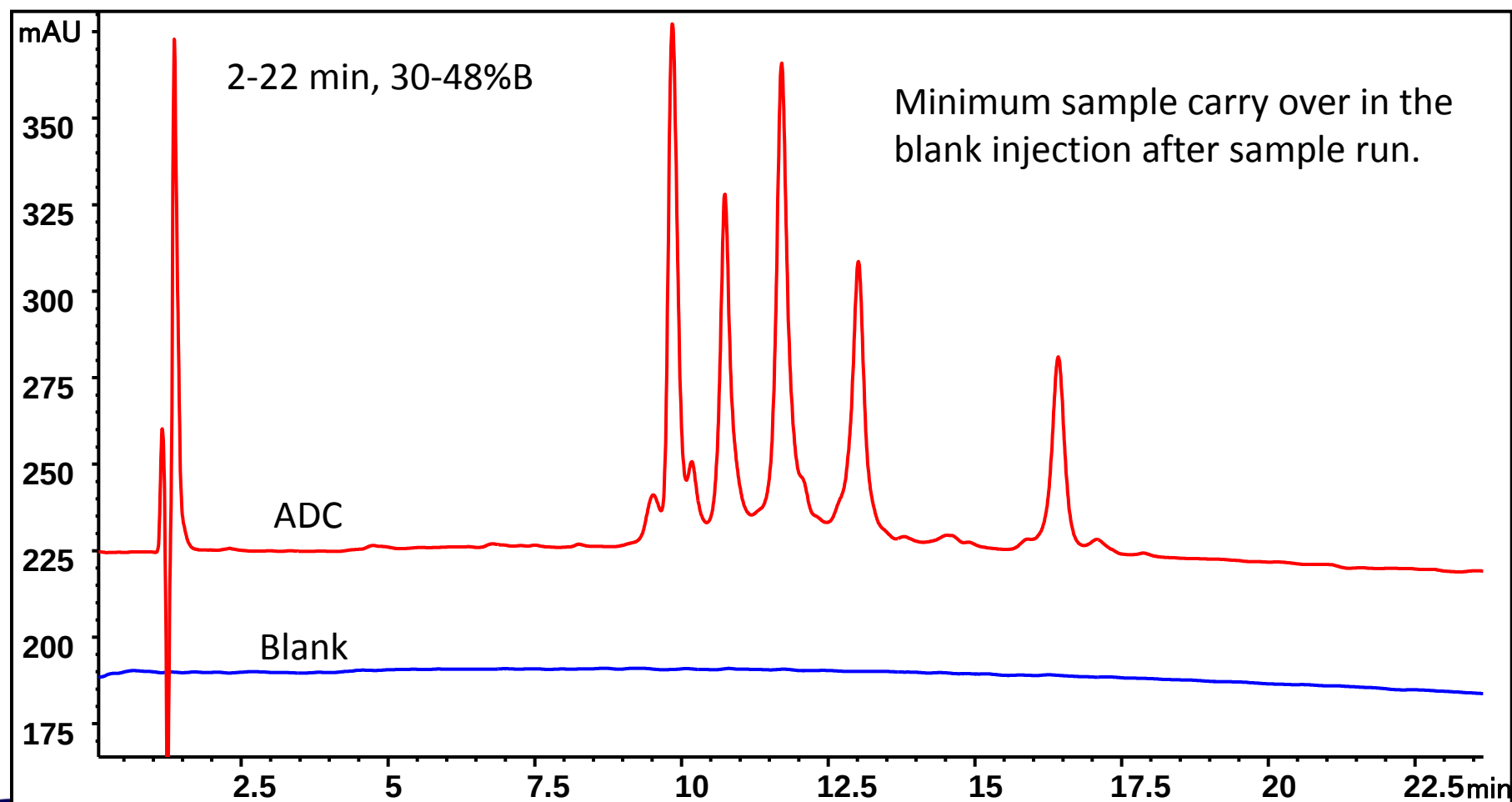
Herceptin Cysteine ADC2/Blank Separation on Proteomix® RP-1000

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: Cysteine ADC 2 1 mg/mL diluted in water; Injection volume: 8 μL



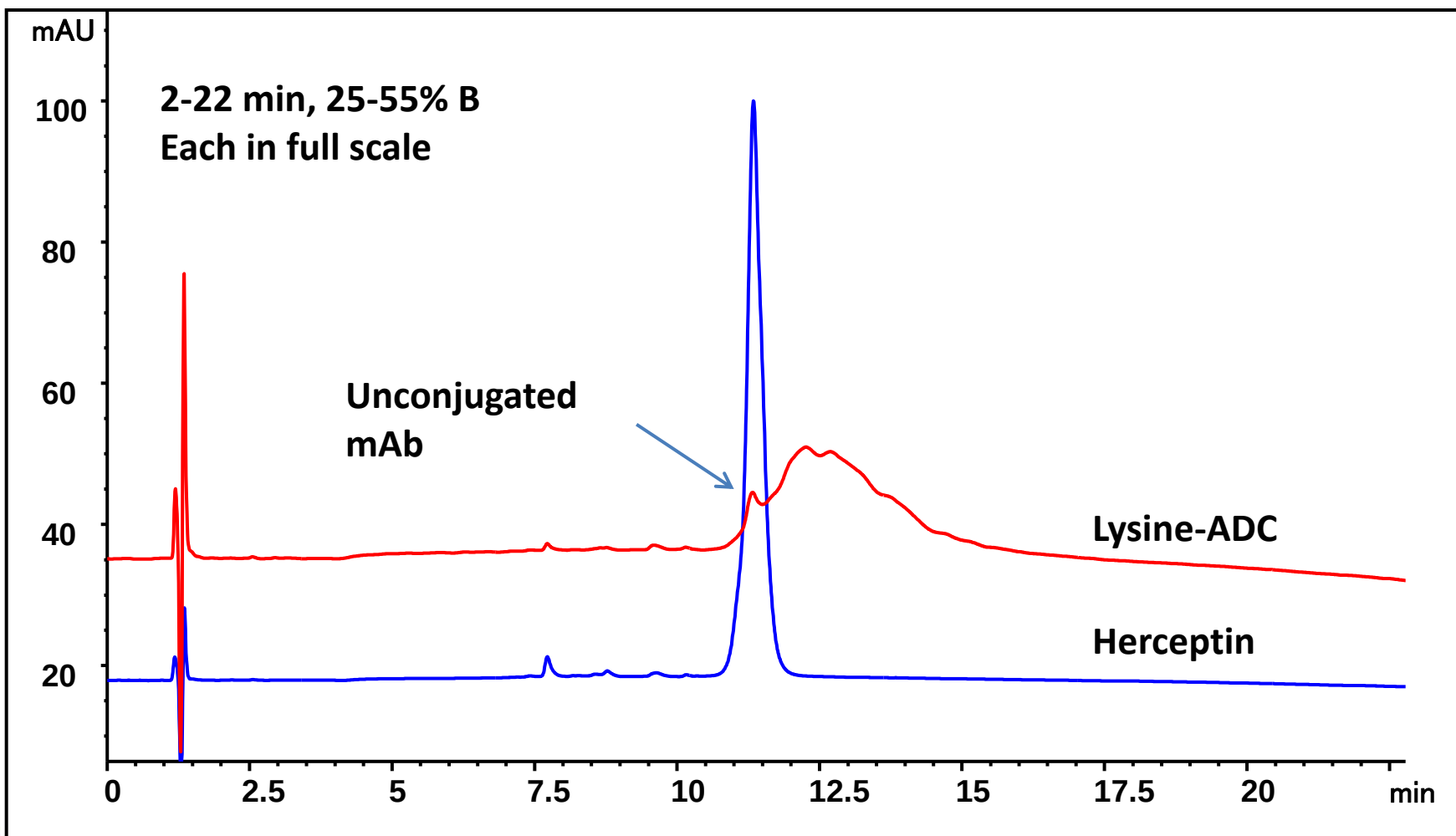
Herceptin and its lysine ADC separation **Proteomix® RP-1000**

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 1 mg/mL diluted in 0.1% TFA; Injection volume: 10 μ L



Herceptin Cysteine ADC2 Separation-2.1 x 50 mm

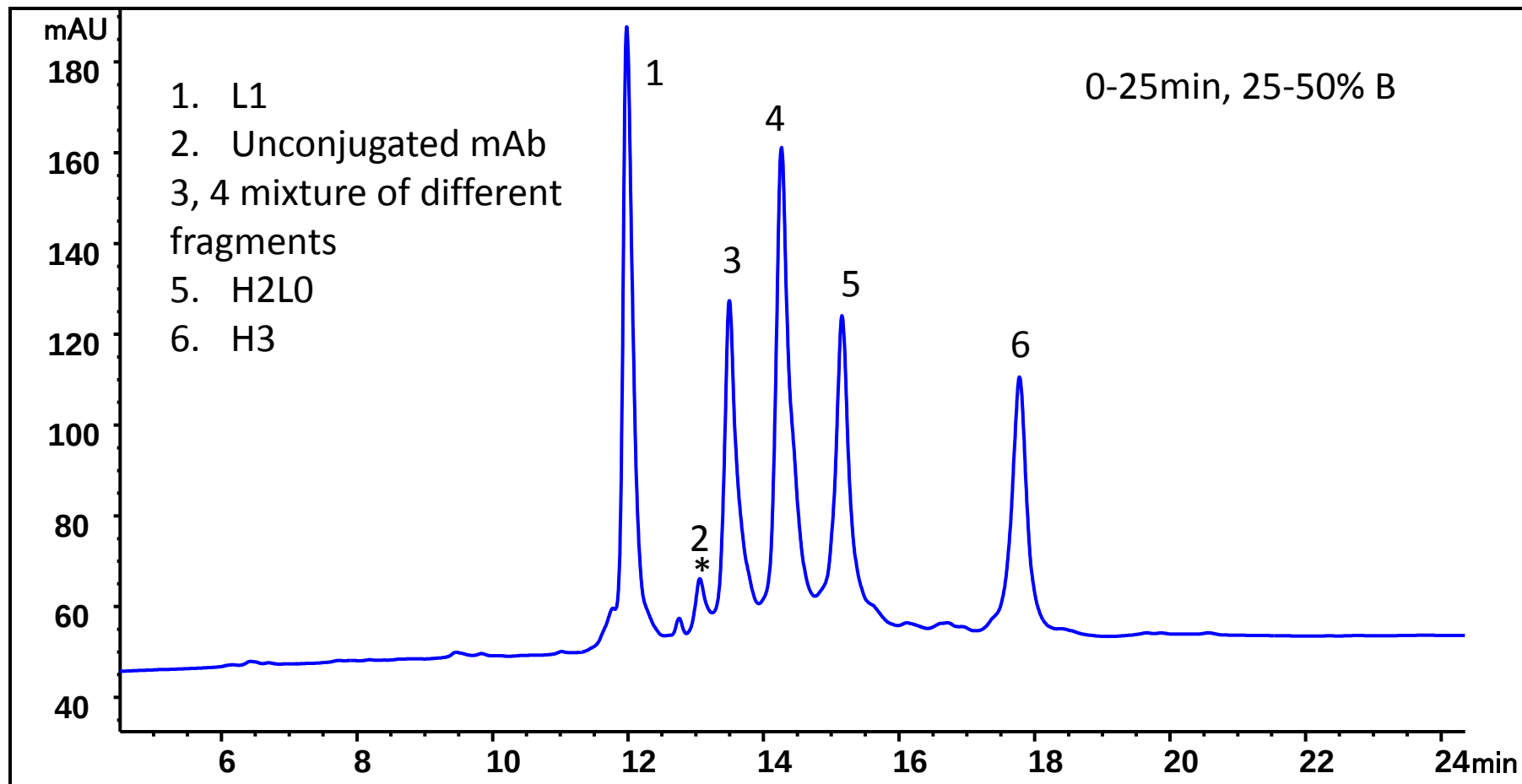
mass spec analysis

Column: **Proteomix® RP-1000** (5 μ m, 1000 Å, 2.1 x 50 mm)

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

Flow rate: 0.4 mL/min; Detector: UV 210 nm; Column temperature: 80 °C; Column pressure: 45 bar;

Sample: ADC 2 diluted in water; Injection volume: 3 μ L cysteine ADC 2



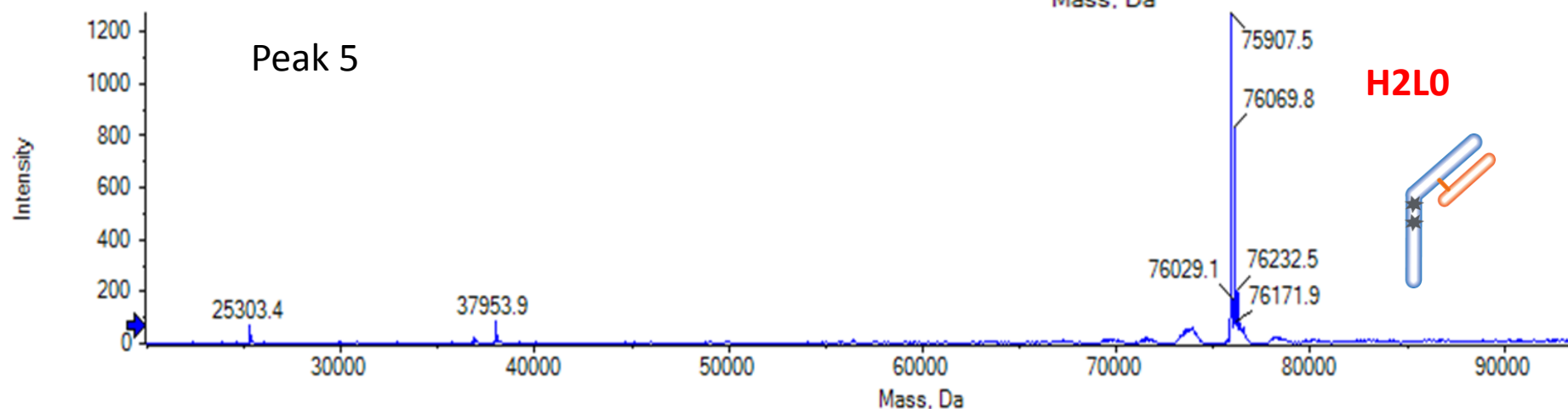
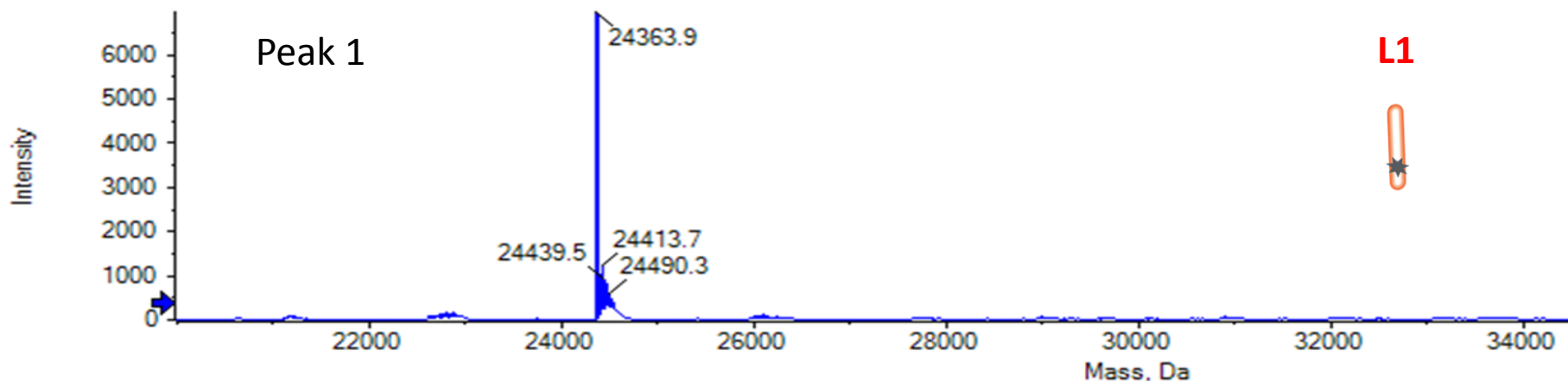
Herceptin Cysteine ADC2 Separation-2.1 x 50 mm

Column: **Proteomix® RP-1000** (5 μ m, 1000 Å, 2.1 x 50 mm)

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

Flow rate: 0.4 mL/min; Detector: UV 210 nm; Column temperature: 80 °C; Column pressure: 70 bar;

Sample: ADC 2 diluted in water; Injection volume: 3 μ L cysteine ADC 2



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

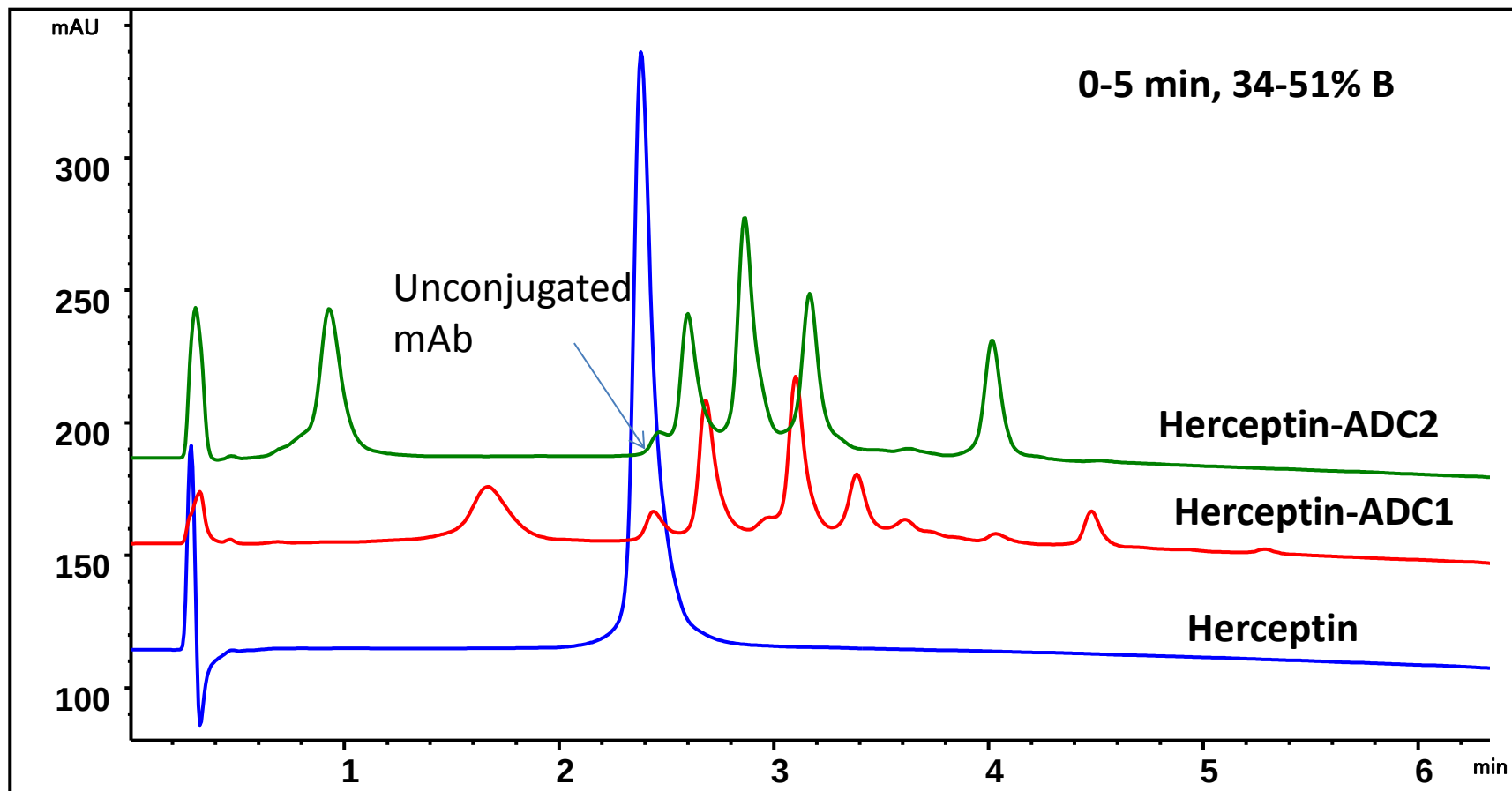
0.6 mL/min –fast analysis

Column: **Proteomix® RP-1000** (5 μ m, 1000 Å, 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 μ L for Herceptin, 1 μ L for ADC 1 and ADC 2



MAb/ADC Fragment Separation on Reversed Phase Proteomix RP-1000

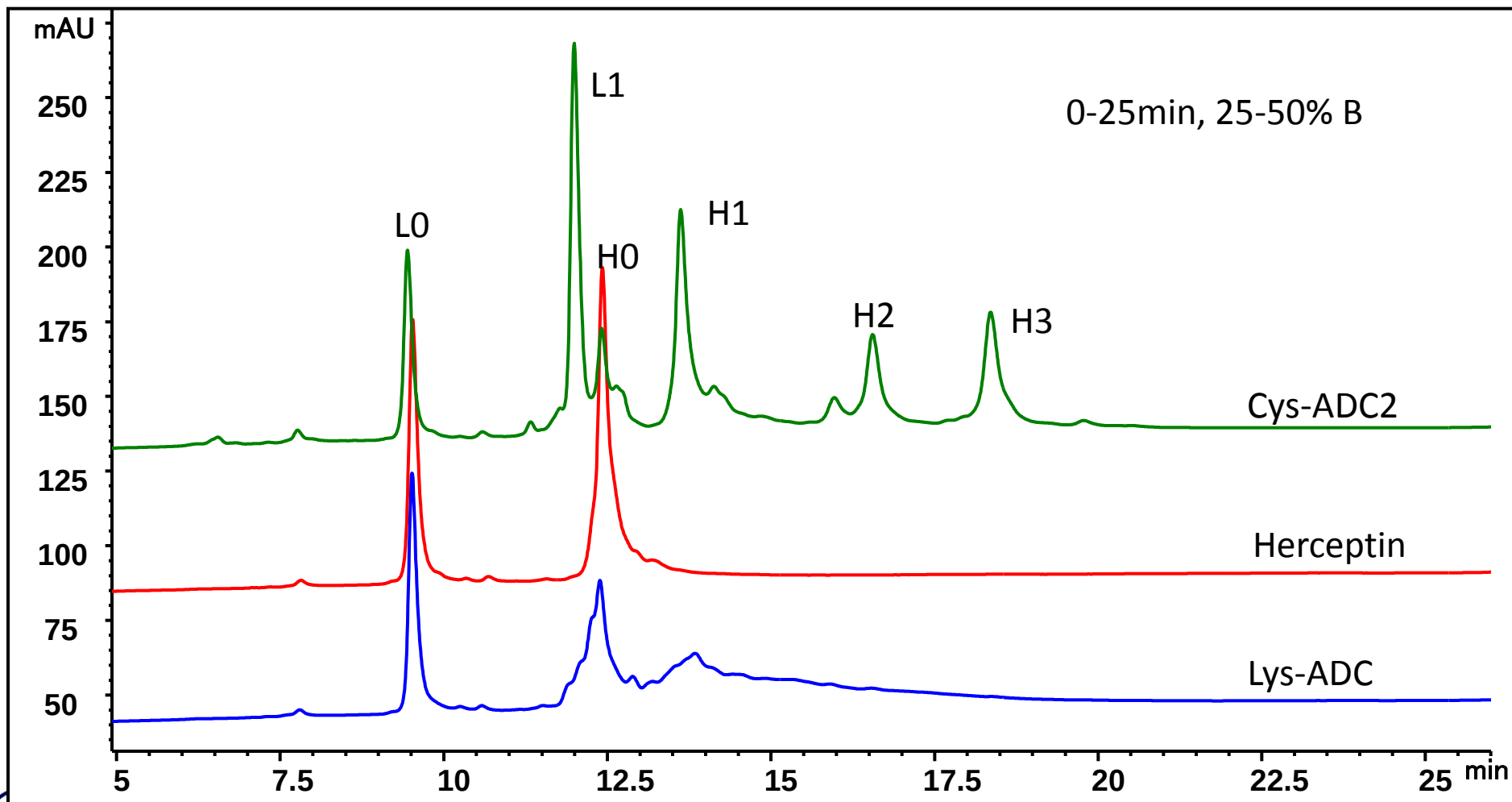
Reduced-Herceptin vs. Herceptin-Lys, Cys ADCs

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

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

Flow rate: 0.4 mL/min; Detector: UV 214 nm; Column temperature: 80 °C; Column pressure: 45 bar;

Injection: 1 μL for DTT reduced Herceptin (3 mg/mL), 3 μL Lys-ADC and Cys-ADC (1mg/mL each)



Cysteine ADC DAR analysis on Proteomix[®] HIC

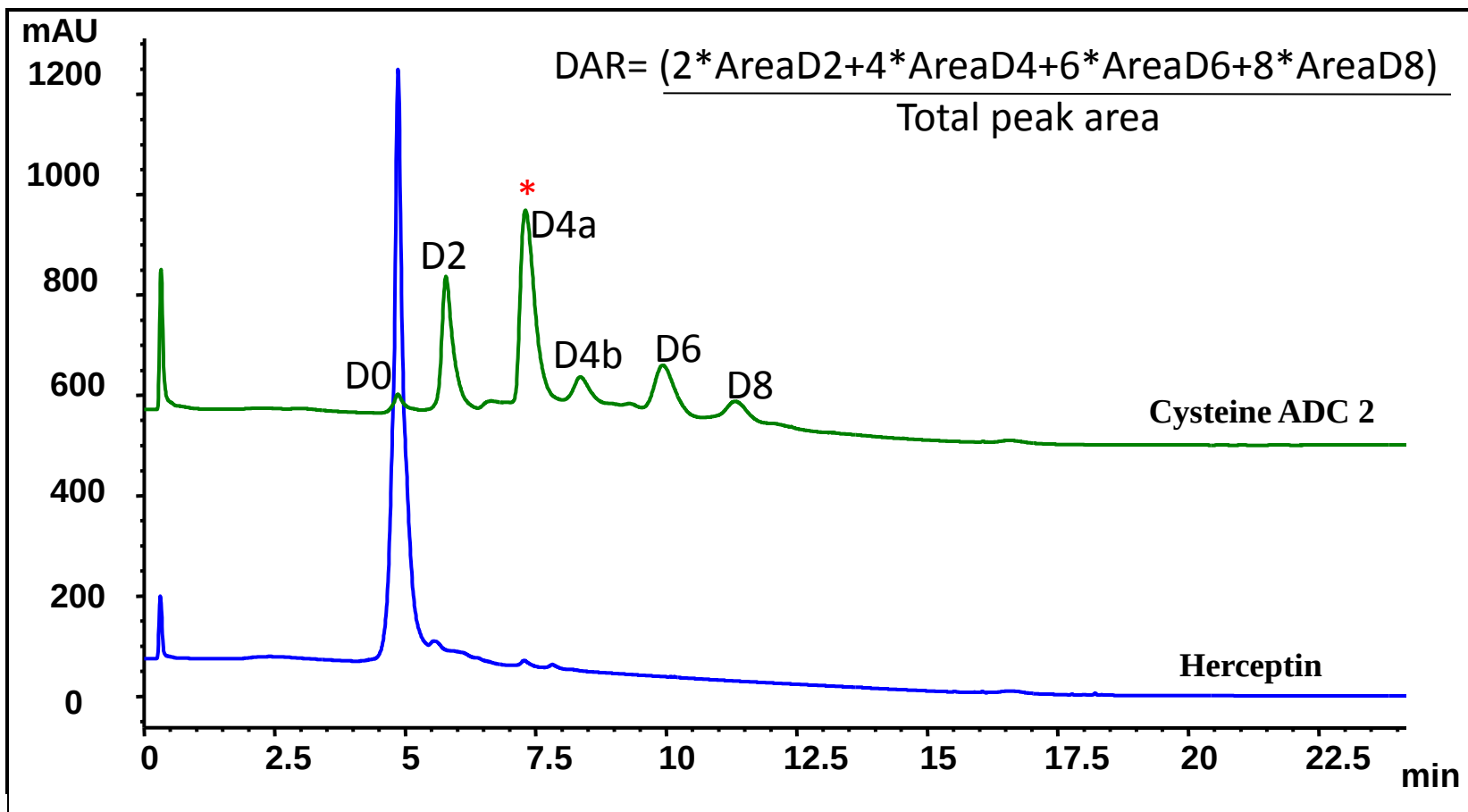
Column: Proteomix[®] HICBu-NP5 (5 µm, 4.6 x 35 mm)

Mobile phase: A: 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0,

B: 0.025 M sodium phosphate pH 7.0, C : 100% IPA

Flow rate: 0.8 mL/min, Detector: UV 214 nm, Column temperature: 25 °C

Sample: Herceptin ADC2, 1 mg/mL in 25 mM sodium phosphate, Injection: 10 µL,



ADC HIC fraction 4a-DTT reduced fragment separation

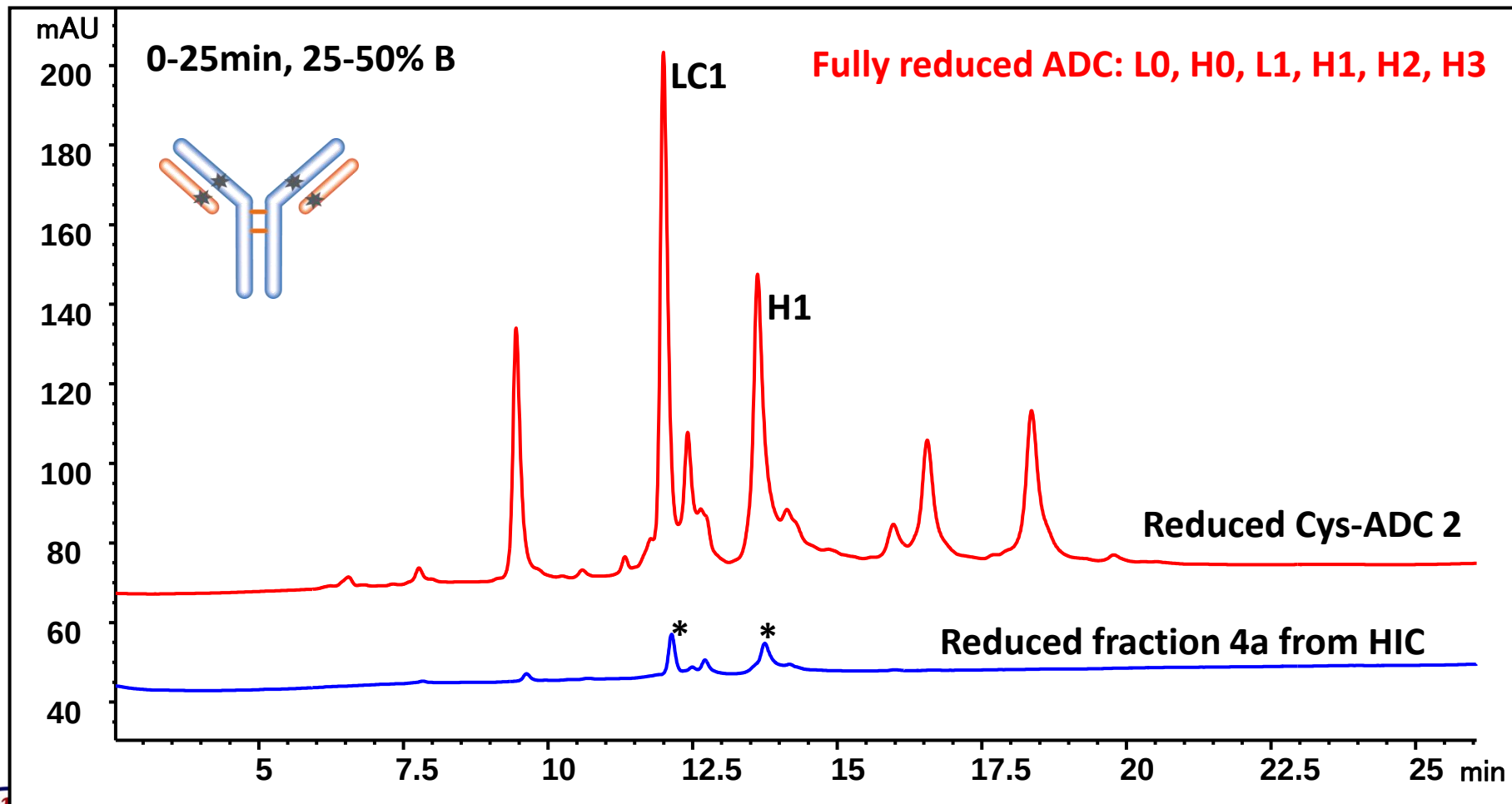
Reversed phase Proteomix RP-1000

Column: **Proteomix® RP-1000** (5 μ m, 1000 Å, 2.1 x 50 mm)

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

Flow rate: 0.4 mL/min; Detector: UV 214 nm; Column temperature: 80 °C; Column pressure: 45 bar;

Injection: 30 μ L for cysteine ADC separated on HIC, fraction 4a concentrated to 45 μ L, reduced with 20 mM DTT



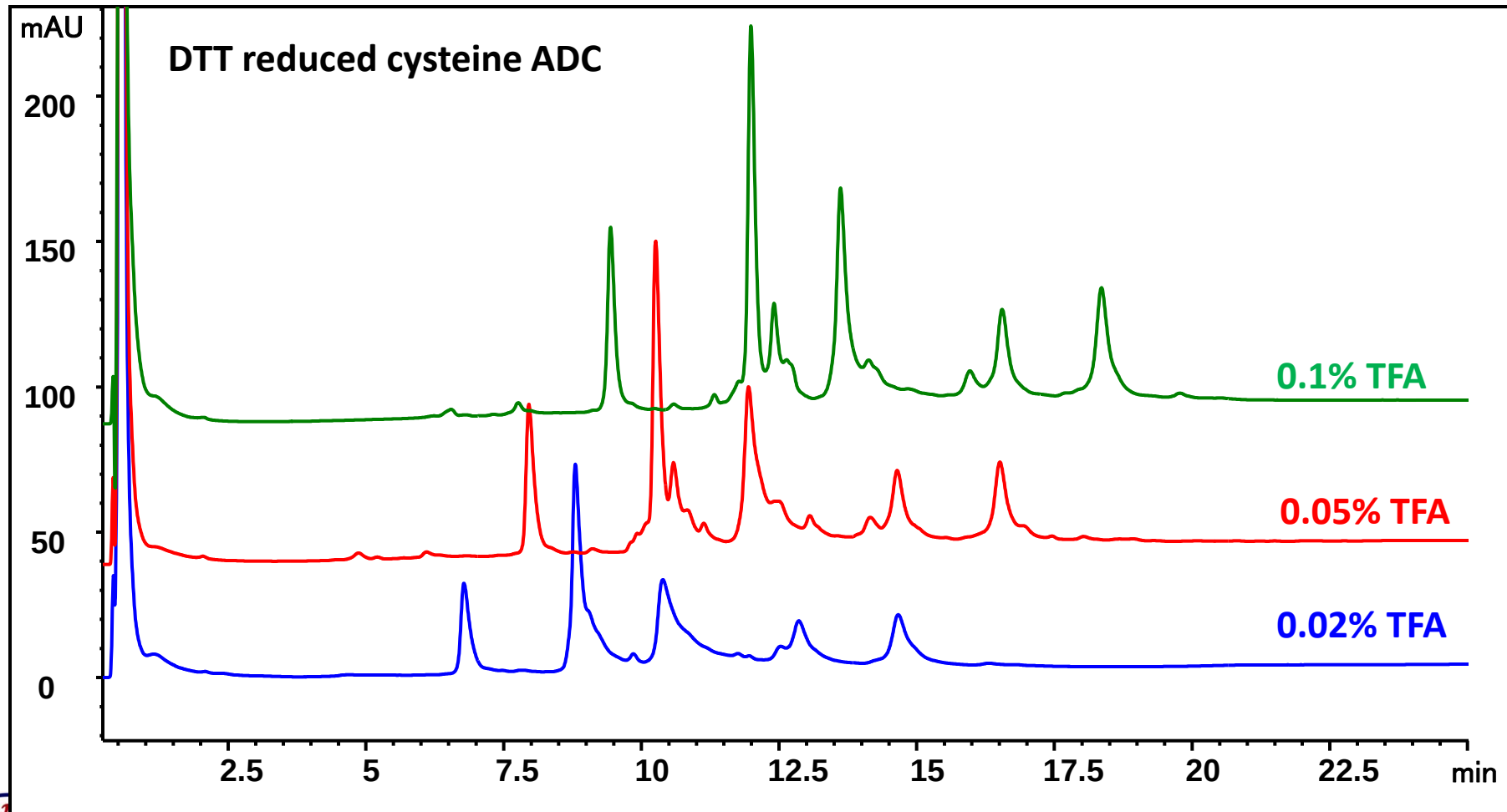
TFA effect on the ADC fragments separation

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

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

Flow rate: 0.4 mL/min; Detector: UV 214 nm; Column temperature: 80 $^{\circ}\text{C}$; Column pressure: 45 bar;

Injection: 3 μL for DTT reduced Herceptin cysteine ADC 0.1 and 0.05% TFA runs, 2 μL for 0.02% TFA)



Column Temperature effect

Proteomix[®] RP-1000

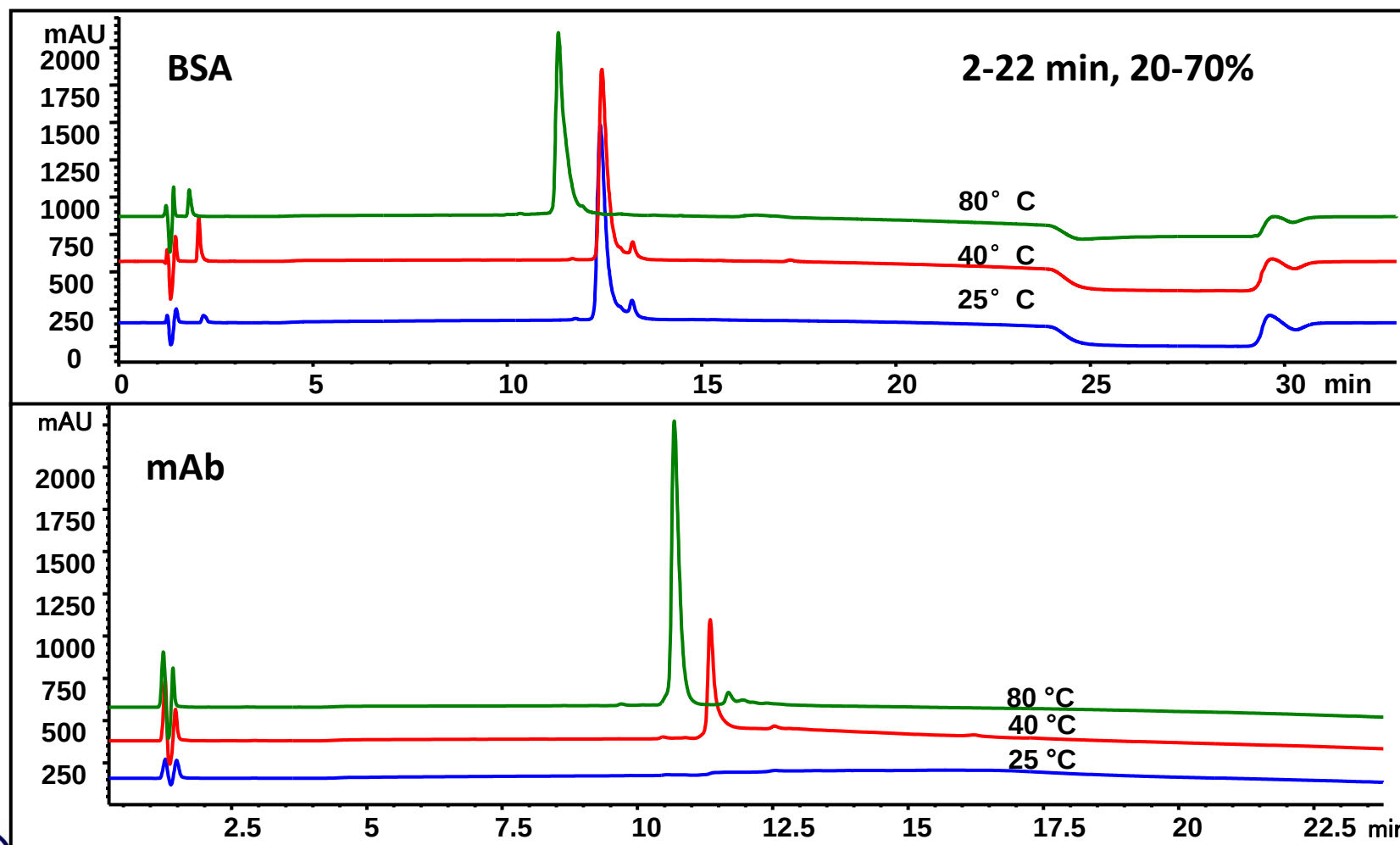


BSA and mAb separation with 25°C / 40°C / 80°C

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: BSA and mAb 1mg/mL; Injection volume: 20 µL



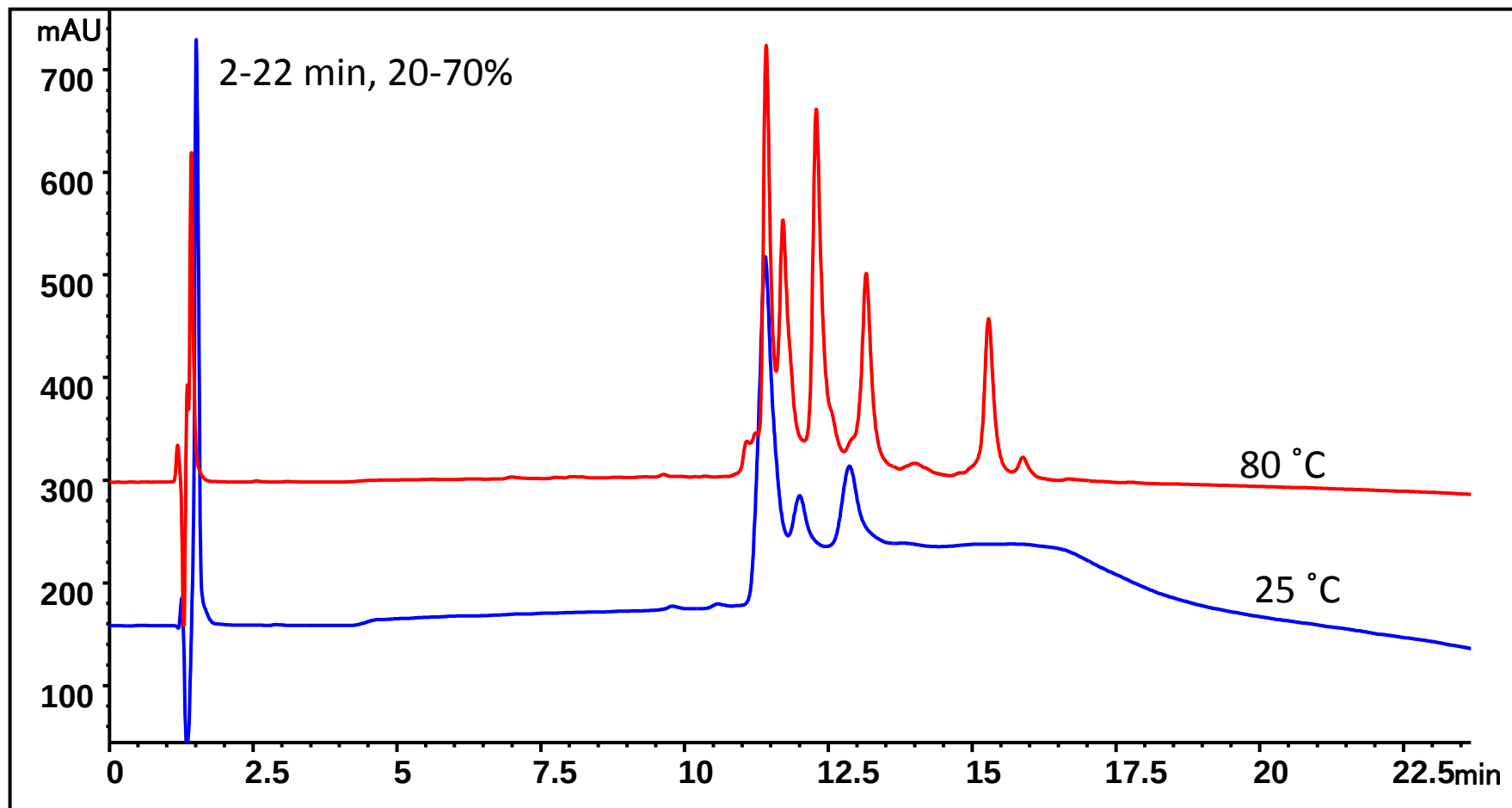
Herceptin Cysteine ADC 2 Separation

Column: **Proteomix® RP-1000** (5 μm , 1000 $\text{\AA}$, 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: 25, 80 $^{\circ}\text{C}$;

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



Product Information

Column	Part number
Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm)	465950-4610
Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 50 mm)	465950-2105
Proteomix® HIC Bu-NP5 (5 µm, 4.6 x 35 mm)	413NP5-4603