

# Monoclonal Antibody and Antibody Drug Conjugate Separation by Polymer Based Reversed Phase Chromatography



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## INTRODUCTION

Recombinant monoclonal antibodies (mAbs) and antibody drug conjugates (ADC) have become two very important segments of protein drug therapeutics. There are several analytical methods to characterize the protein therapeutics such as size exclusion chromatography for size variant study, ion exchange chromatography for charge variants separation. Reversed phase chromatography has advantages in analyzing the intact proteins and protein digests because of its mass spectrometry compatible volatile mobile phases. However there are many challenges to analyze the intact mAb and ADC proteins based on their hydrophobicity by reversed phase chromatography, due to the high molecular weight of mAbs/ADCs and secondary interaction with the reversed phase column resins. Here we present mAb and ADC intact protein separation using a polymer based reversed phase chromatography (Proteomix® reversed phase). Proteomix®RP resins are based on highly cross-linked polystyrene/divinylbenzene (PS/DVB) resins with very narrow particle size and pore size distributions. With the high cross-linking structure, Proteomix®RP resins provide excellent chemical and physical stability, as well as high rigidity to tolerate high temperature. Its large pore size up to 1000 Å allows for the high resolution and maximizing the separation efficiency of intact mAb and ADC. MAb Herceptin and its corresponding ADCs are successfully separated on the Proteomix®RP-1000 with protein peaks indicating different hydrophobicity. Temperature effect is also discussed on the separation and protein recovery. With small particle size and short column, Proteomix®RP reversed phase chromatography provides high throughput and fast analysis of mAb, ADC and other protein therapeutics with online mass spec determination.

## EXPERIMENTAL

**Columns:** Proteomix® RP-1000 (5 µm, 2.1 x 50 mm)  
Proteomix® RP-1000 (5 µm, 4.6 x 100 mm)

**Samples:** monoclonal antibody (mAb), antibody drug conjugates (ADC), reduced mAb, reduced ADC

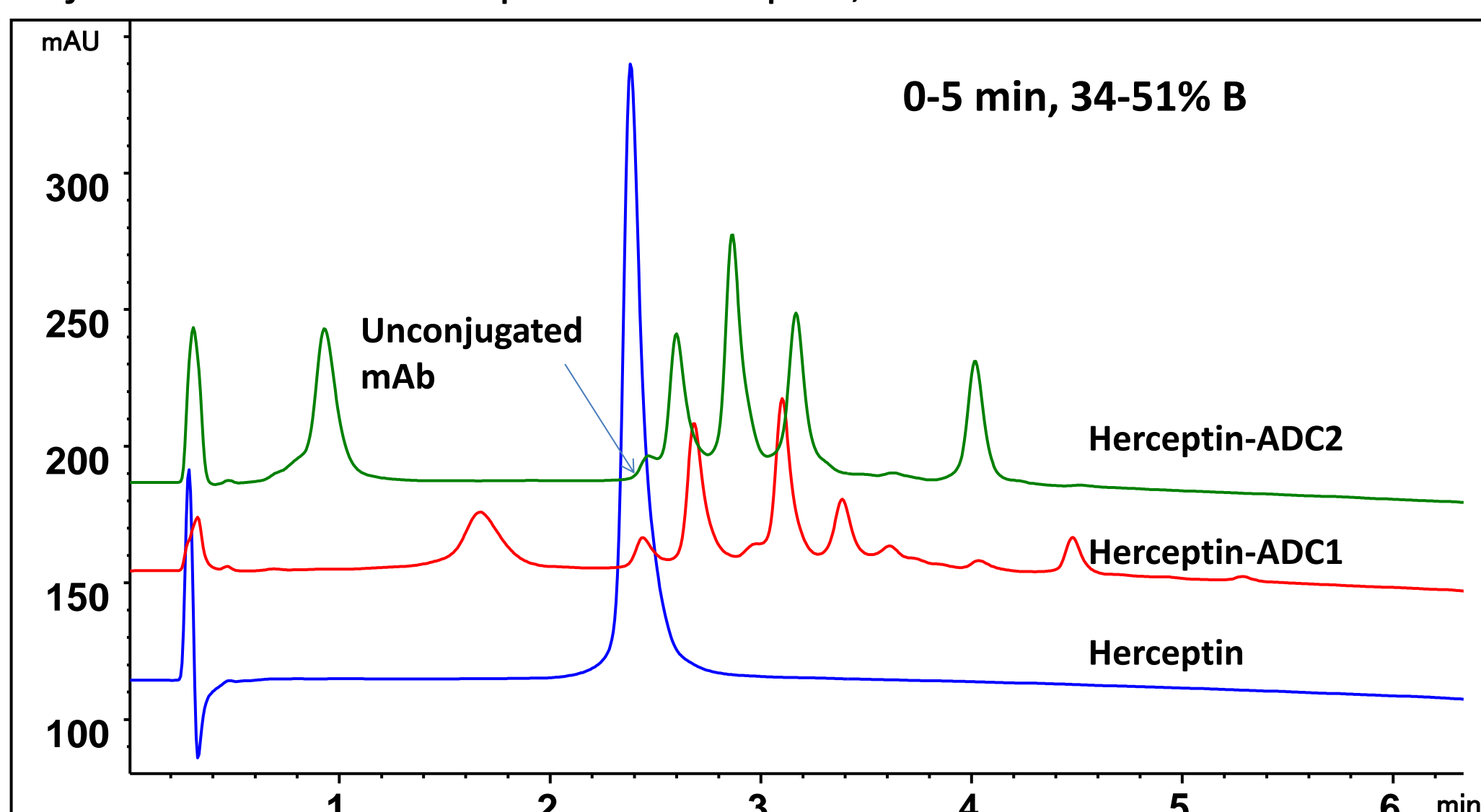
**Running condition:** see detail result section

## CONCLUSION

- 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.

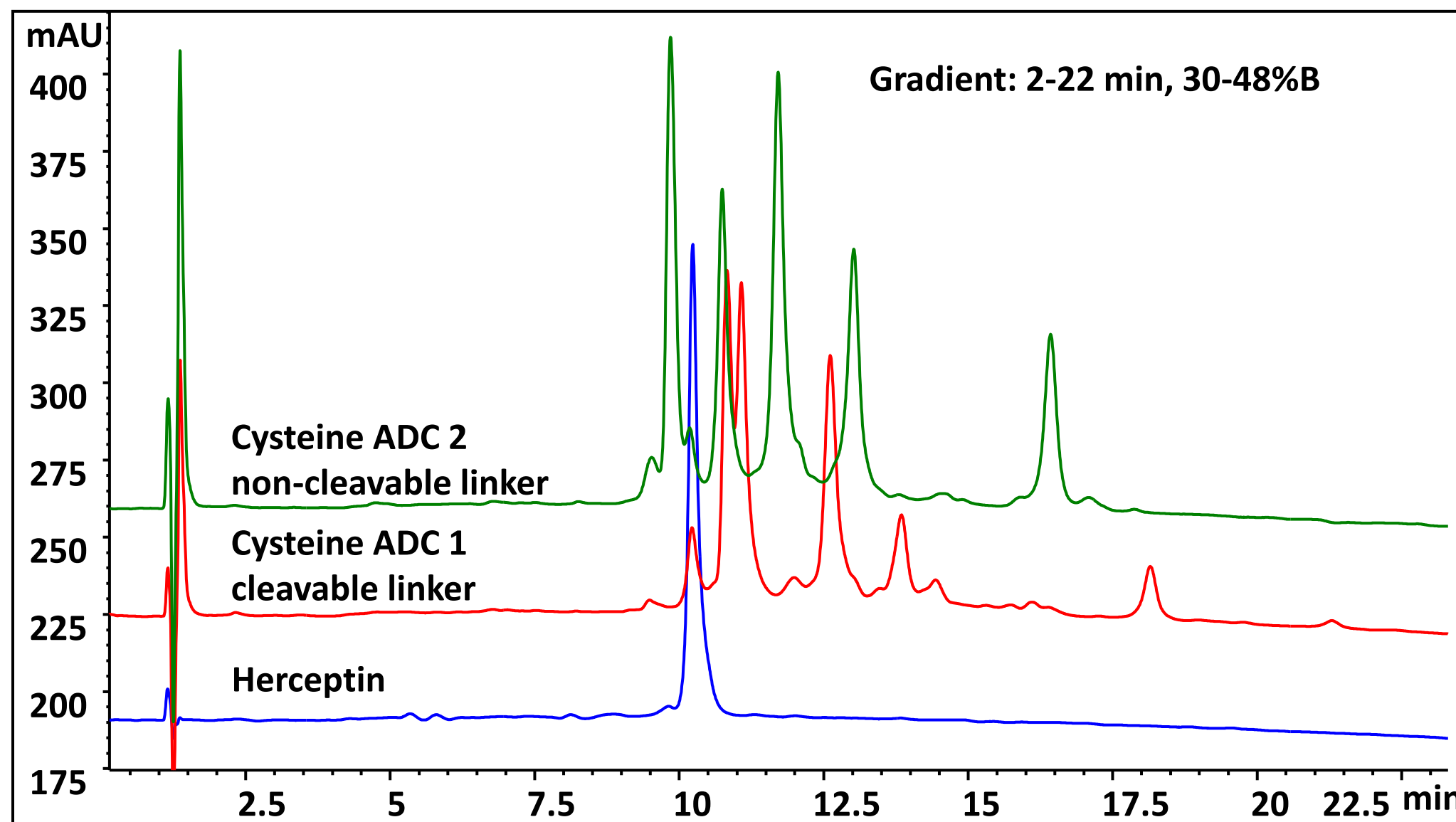
## HERCEPTIN/ADC1/ADC2 SEPARATION-SMALL SIZE

Column: Proteomix® RP-1000 (5 µm, 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 µL for Herceptin, 1 mL for ADC 1 and ADC 2



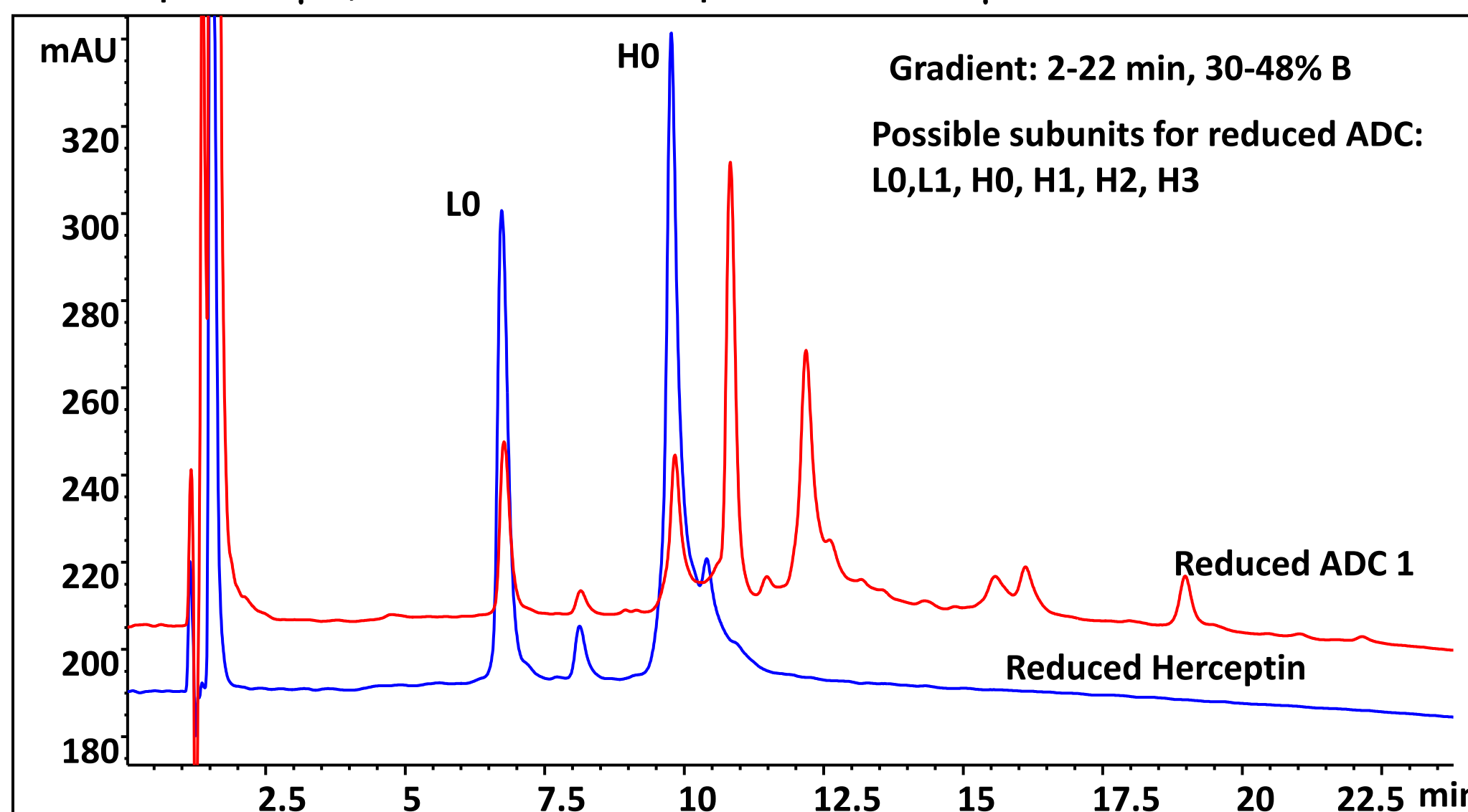
## MAB HERCEPTIN AND ITS ADCS SEPARATION

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



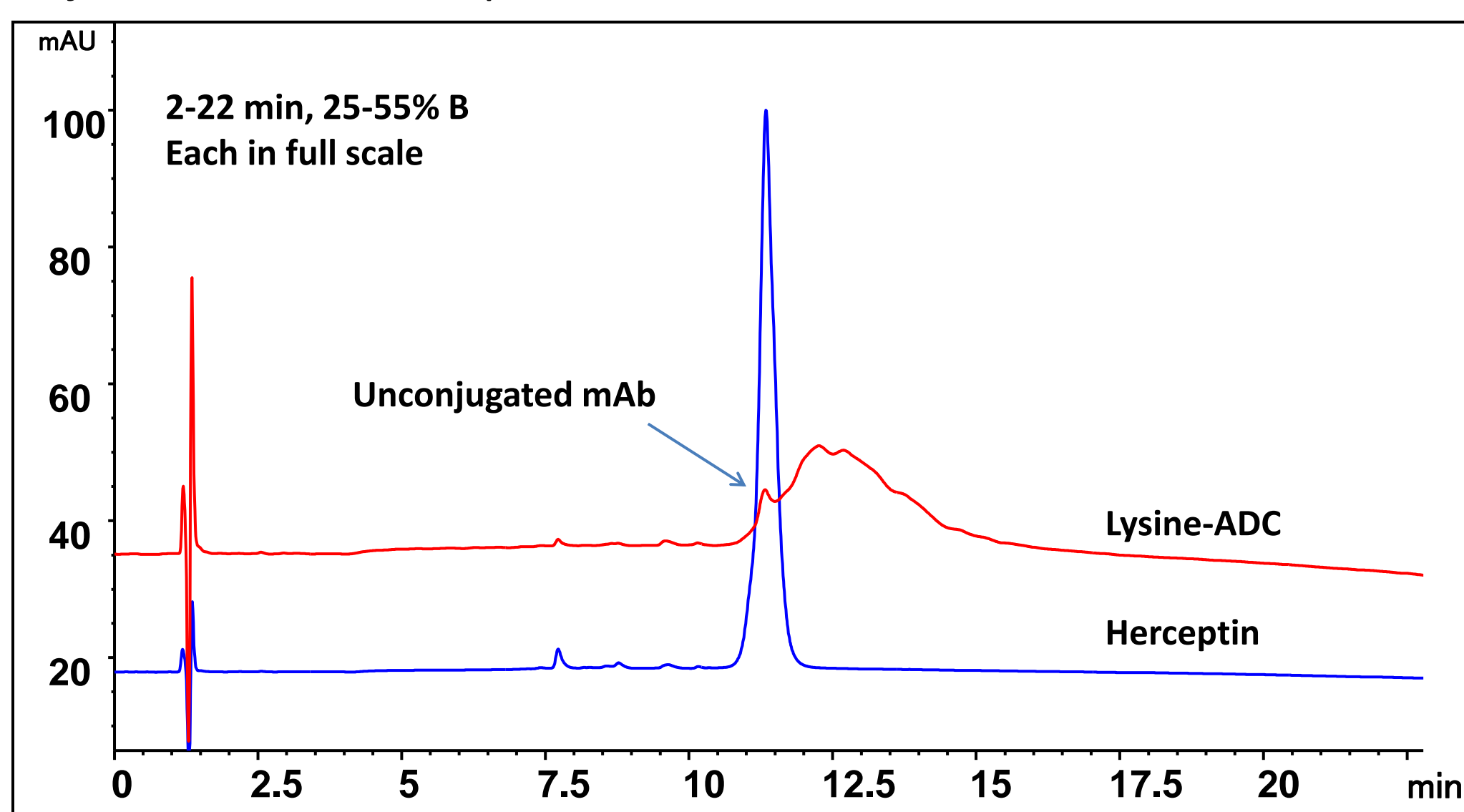
## MAB/ADC FRAGMENTS: REDUCED MAB HERCEPTIN AND CYSTEINE ADC 1

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



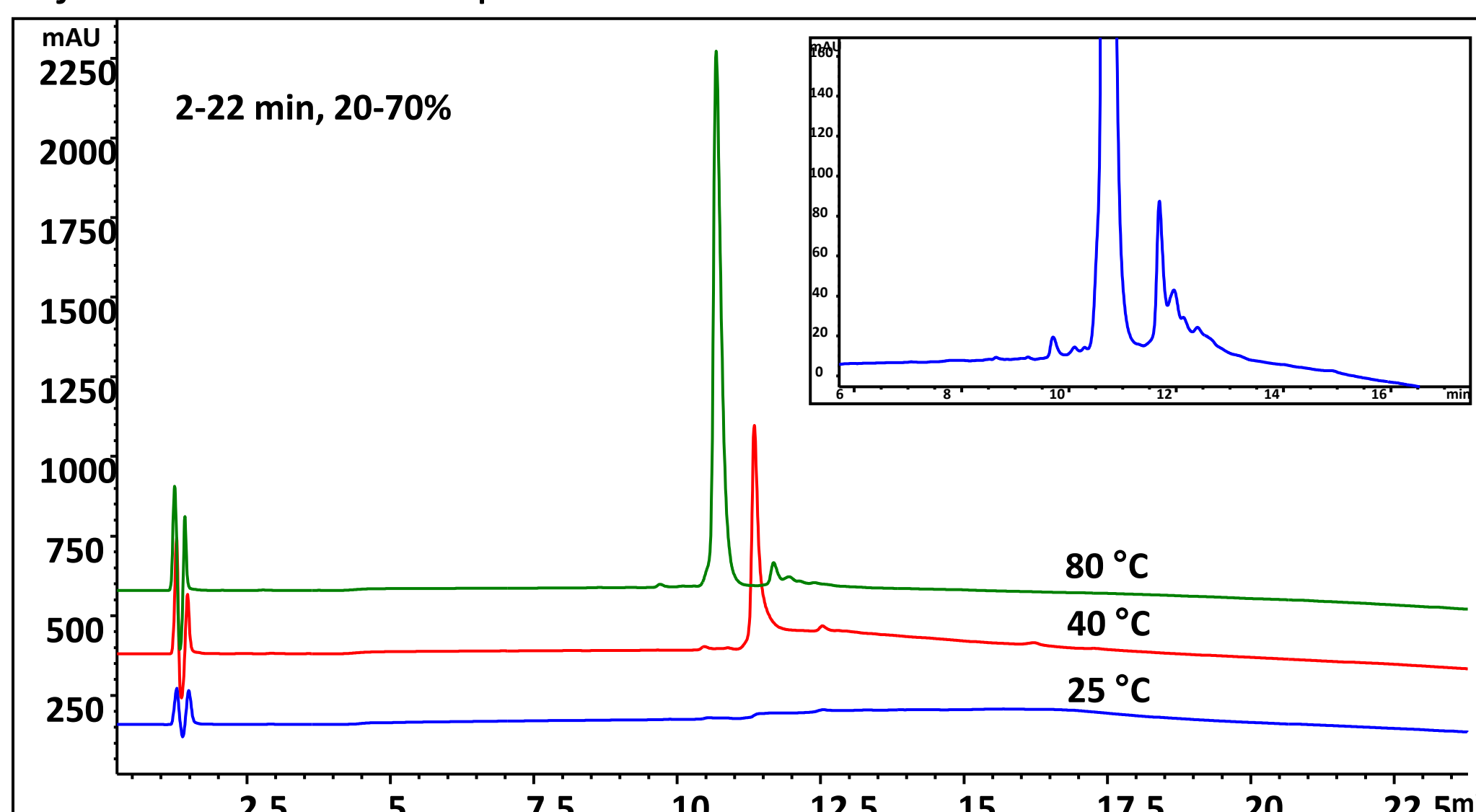
## HERCEPTIN AND ITS LYSINE ADC SEPARATION

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



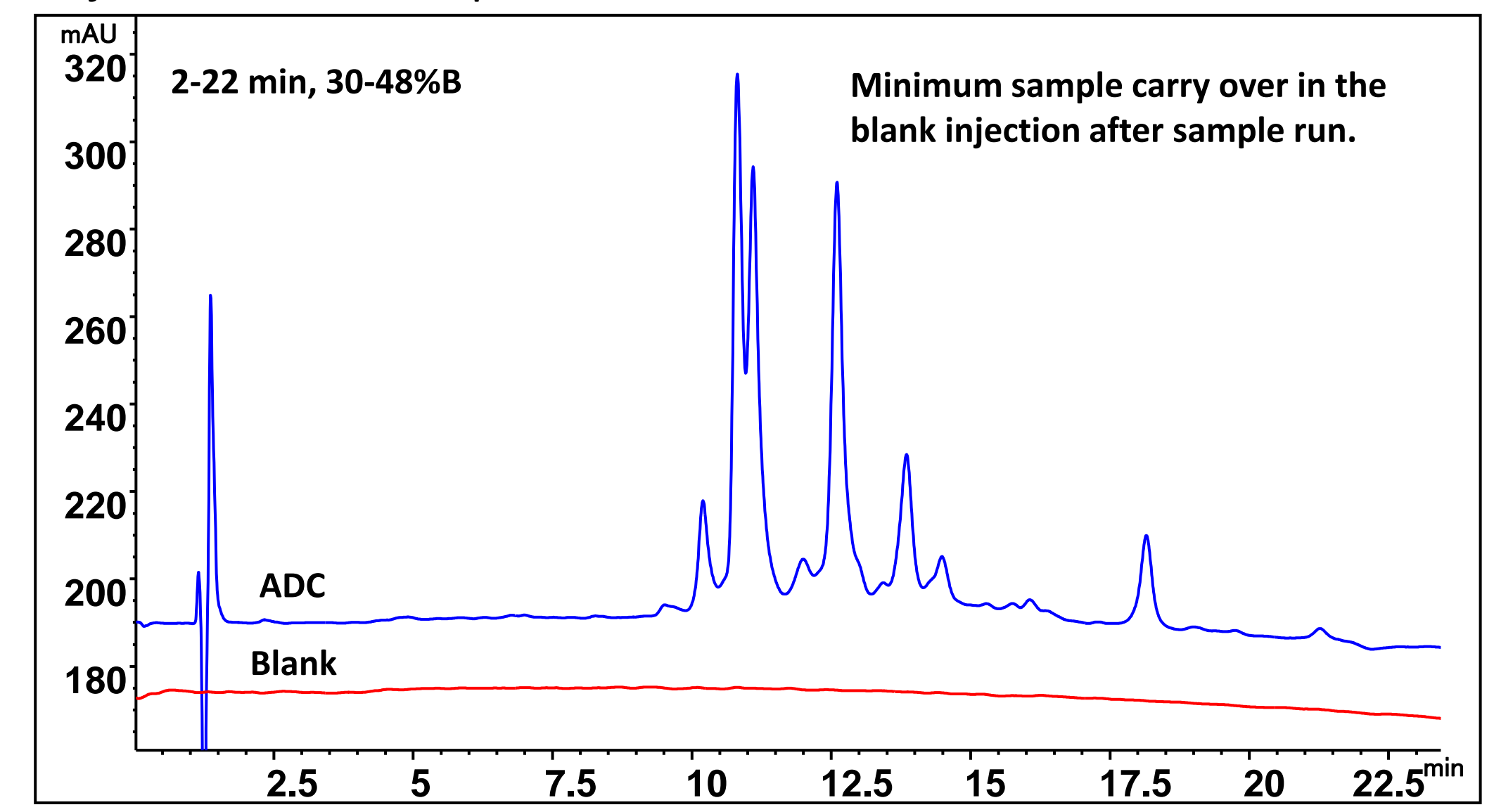
## TEMPERATURE EFFECT: MAB SEPARATION WITH 25°C / 40°C / 80°C

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



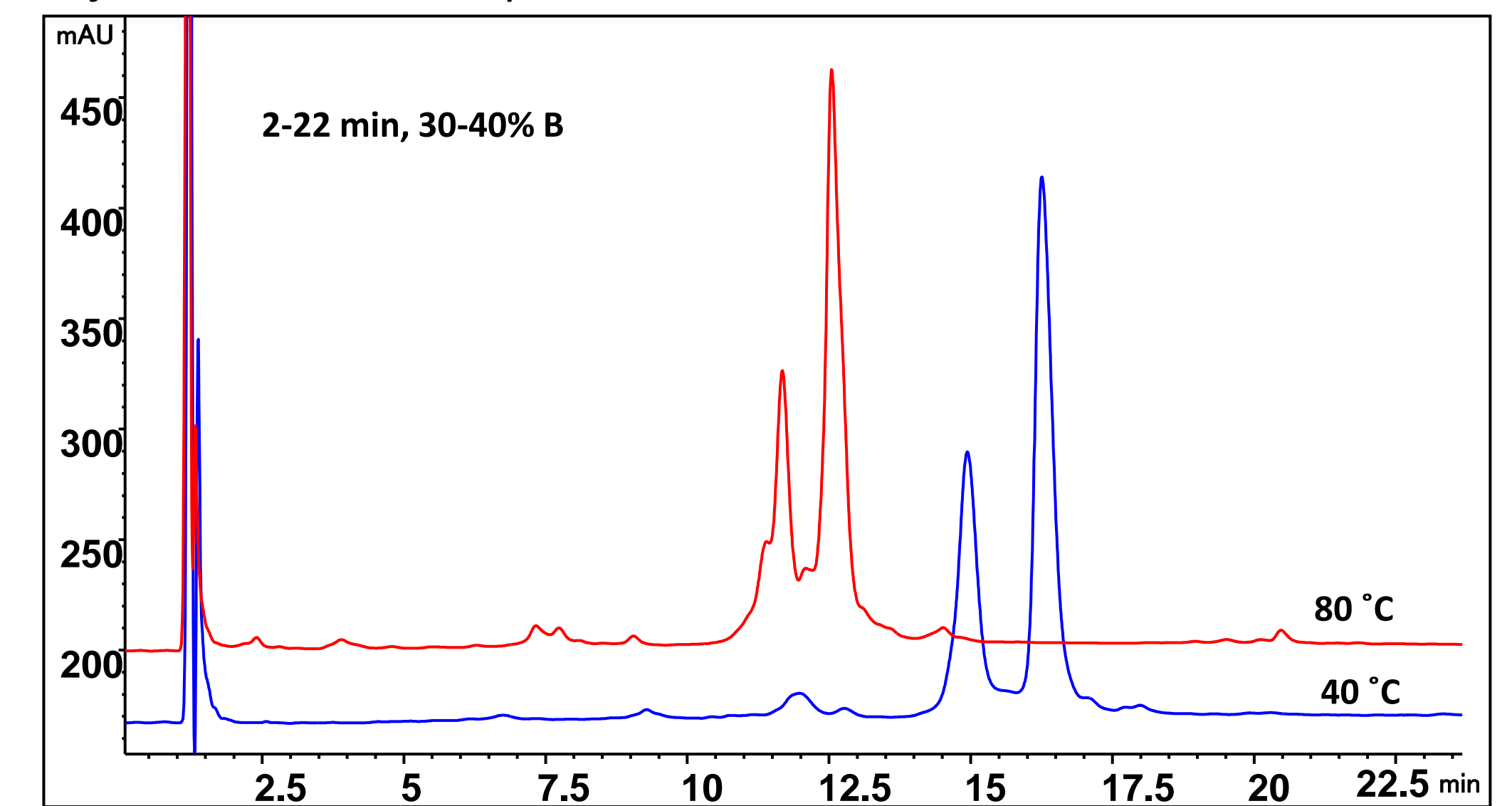
## MINIMUM SAMPLE CARRY OVER

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



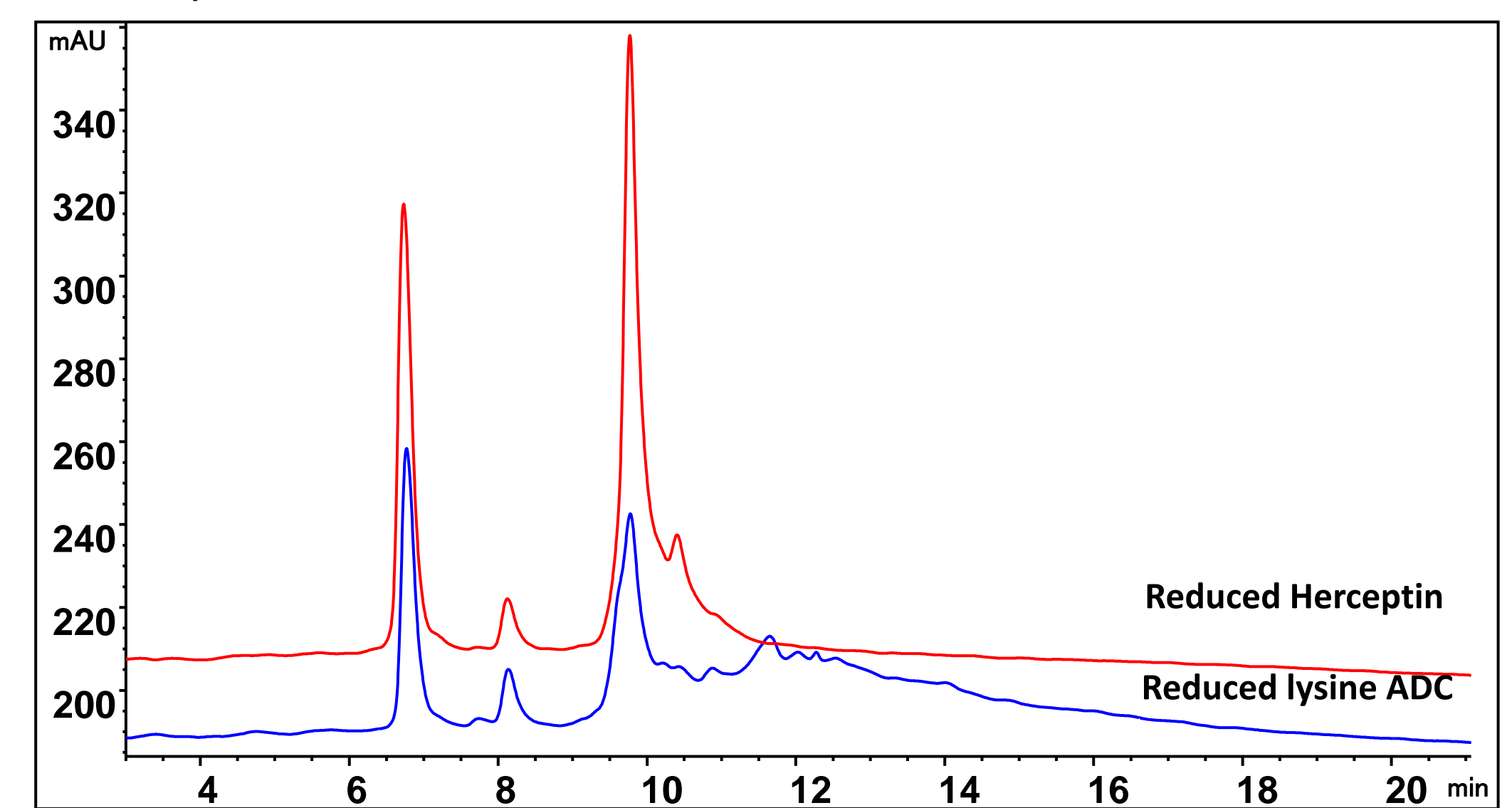
## MAB FRAGMENT: FAB/FC SEPARATION WITH 40 °C AND 80 °C

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 HERCEPTIN AND LYSINE ADC 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: Herceptin and lysine ADC 2 mg/mL reduced with 20 mM DTT, incubated at 65 °C for 20 minute; Injection volume: Reduced herceptin 2 µL, Reduced lysine ADC 5 µL



## TEMPERATURE EFFECT: HERCEPTIN CYSTEINE ADC 2 SEPARATION

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: 25, 80 °C;  
Sample: Herceptin Cysteine ADC 2 1 mg/mL diluted in 0.1% TFA;  
Injection volume: 20 µL

