

# E. Coli lysate separation

SEC, IEX-SAX, Reversed-Phase



# E. Coli lysate separation

Size exclusion chromatography

Zenix<sup>®</sup> SEC-150 (3  $\mu\text{m}$ , 150  $\text{\AA}$ , 7.8 x 300 mm);

Zenix<sup>®</sup> SEC-300 (3  $\mu\text{m}$ , 300  $\text{\AA}$ , 7.8 x 300 mm);

Two columns in tandem



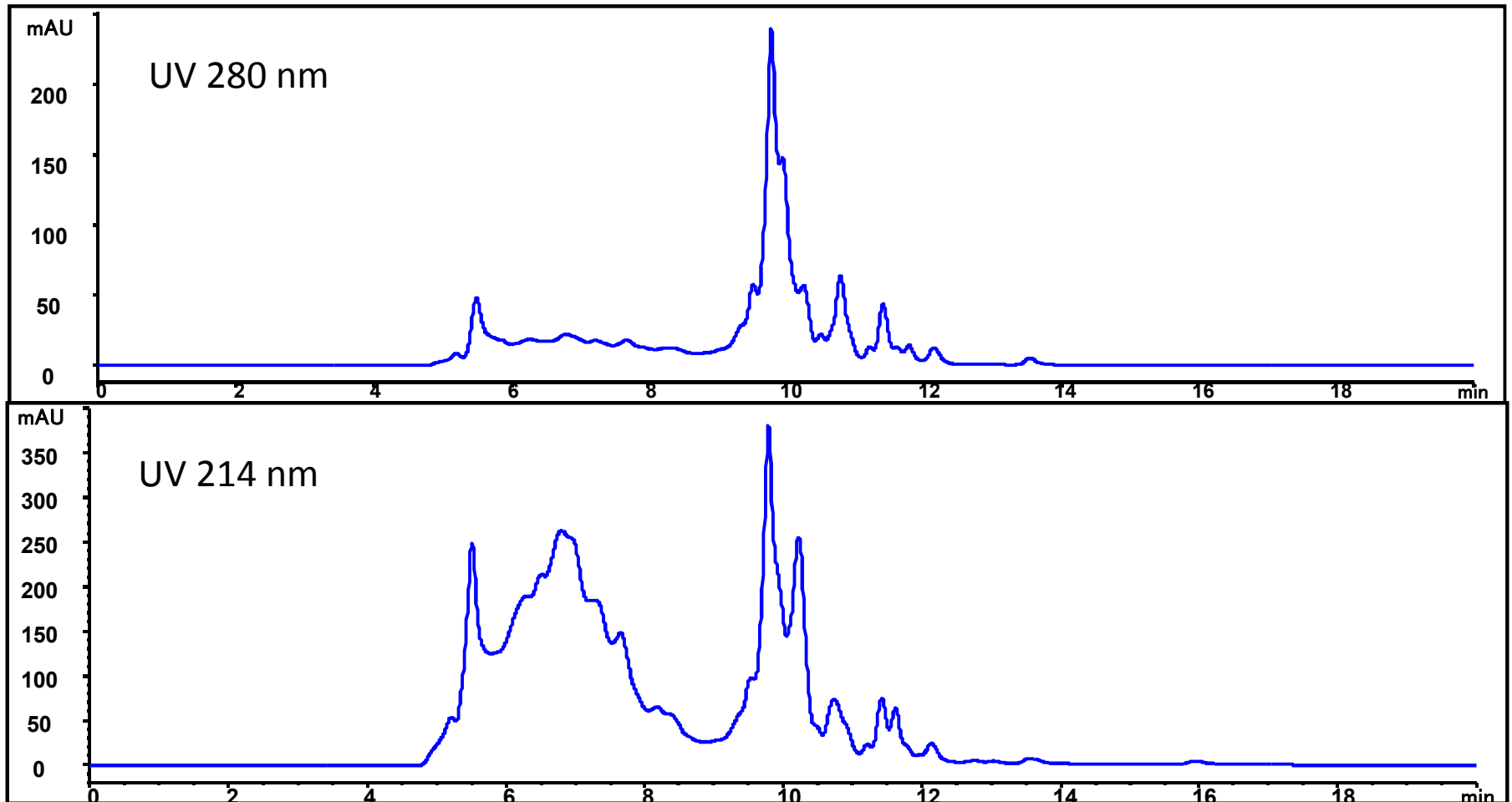
## Separation of E.coli lysate *Bio-rad* #163-2110 Zenix SEC-150

Column: Zenix<sup>®</sup> SEC-150 (3  $\mu$ m, 150  $\text{\AA}$ , 7.8 x 300 mm);

Mobile phase: 150mM Sodium Phosphate Buffer, pH=7.0

Flow rate: 1 mL/min; Detector: UV 280, 214 nm; Column temperature: 25  $^{\circ}$ C ;

Sample: 2.7 mg/mL Bio-rad E. coli lysate; Injection volume: 10  $\mu$ L



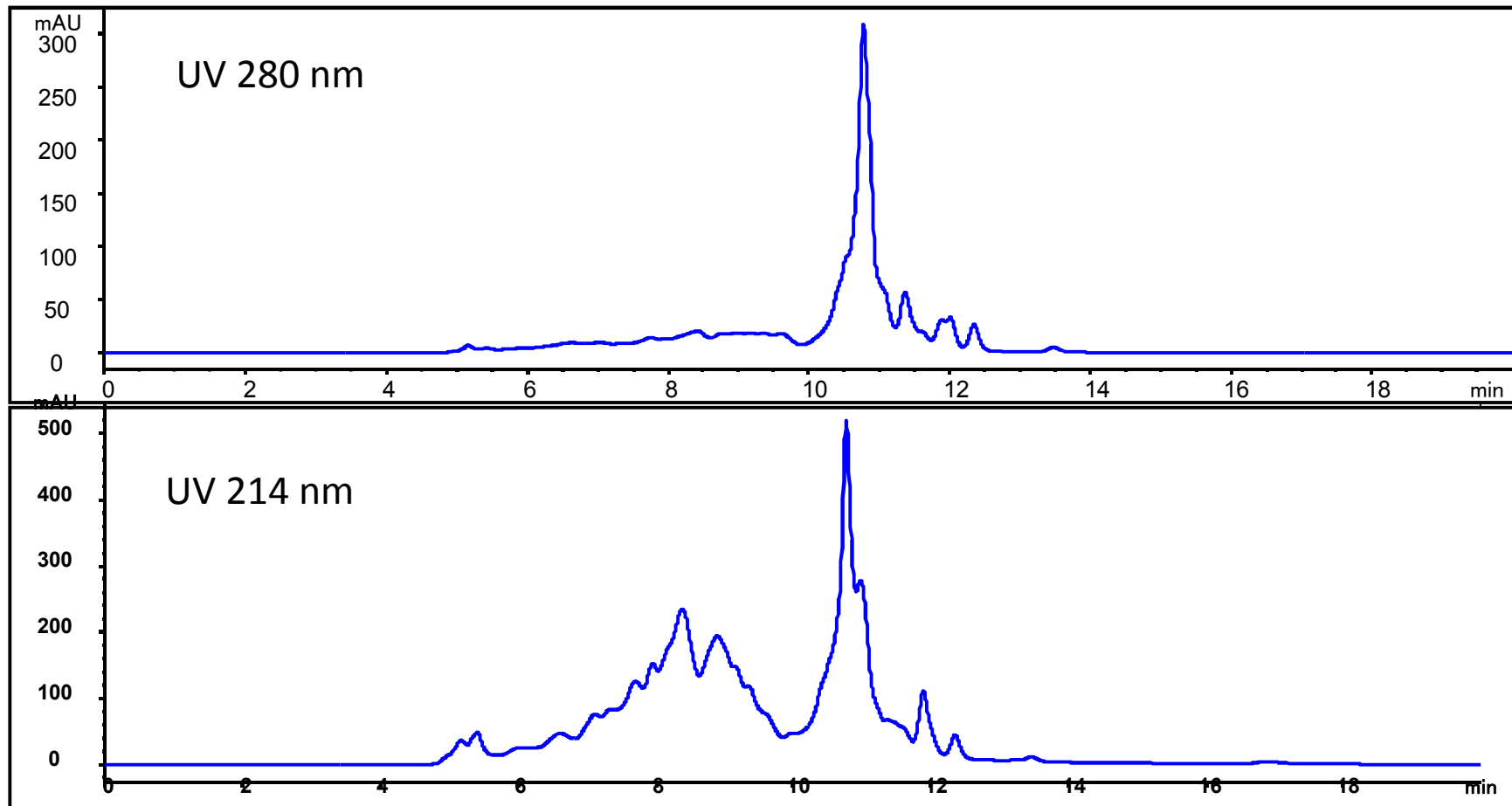
## Separation of E.coli lysate *Bio-rad* #163-2110 Zenix SEC-300

Column: Zenix<sup>®</sup> SEC-300 (3  $\mu\text{m}$ , 300  $\text{\AA}$ , 7.8  $\times$  300 mm);

Mobile phase: 150mM Sodium Phosphate Buffer, pH=7.0

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25  $^{\circ}\text{C}$  ;

Sample: 2.7 mg/mL Bio-rad E. coli lysate; Injection volume: 10  $\mu\text{L}$



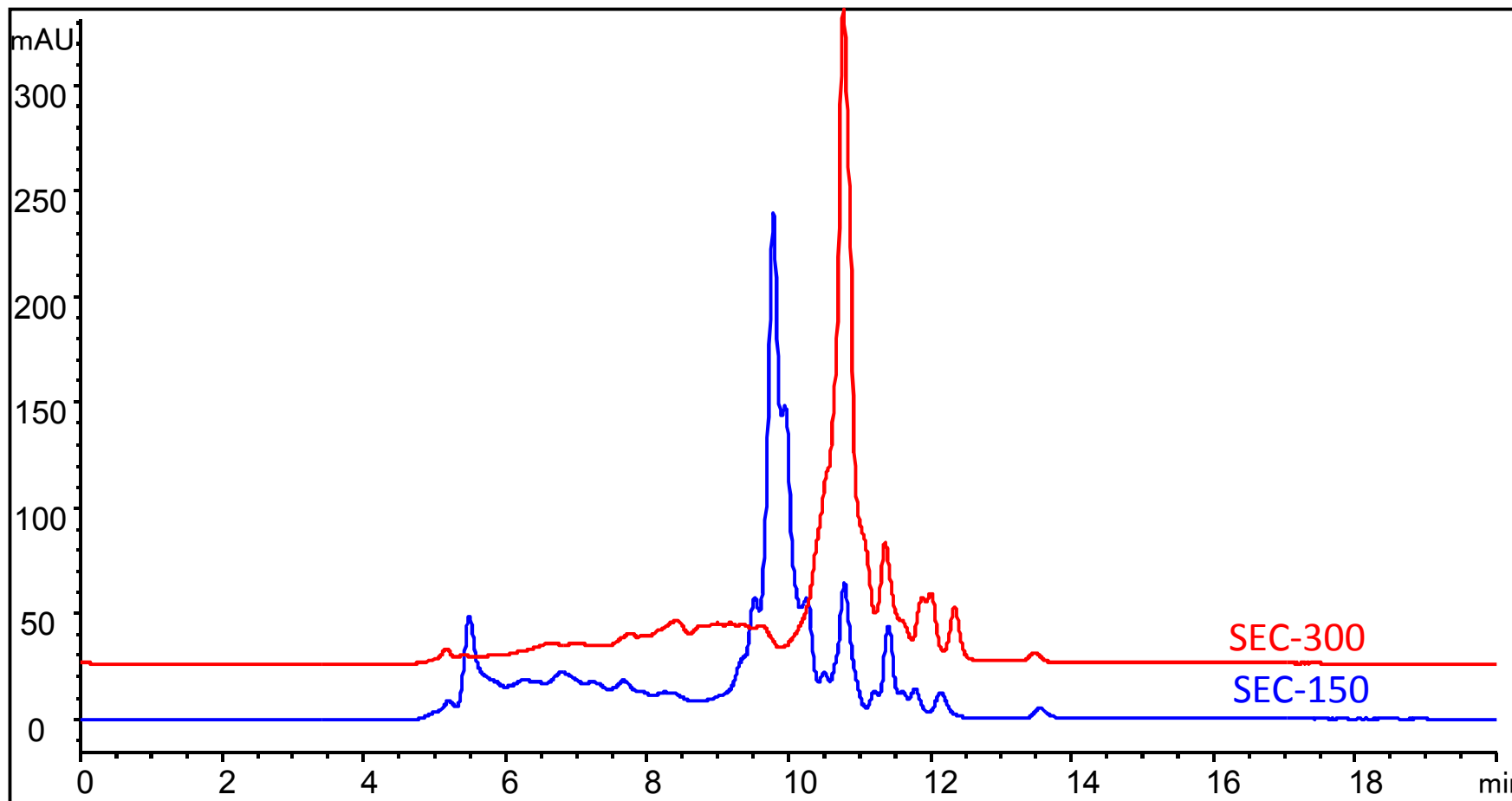
## Overlay of SEC-150 and SEC-300 for E. coli lysate separation-UV280 nm

Column: Zenix<sup>®</sup> SEC-150 (3  $\mu$ m, 150  $\text{\AA}$ , 7.8  $\times$  300 mm); Zenix<sup>®</sup> SEC-300 (3  $\mu$ m, 300  $\text{\AA}$ , 7.8  $\times$  300 mm);

Mobile phase: 150mM Sodium Phosphate Buffer, pH=7.0

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25  $^{\circ}$ C ;

Sample: 2.7 mg/mL Bio-rad E. coli lysate; Injection volume: 10  $\mu$ L



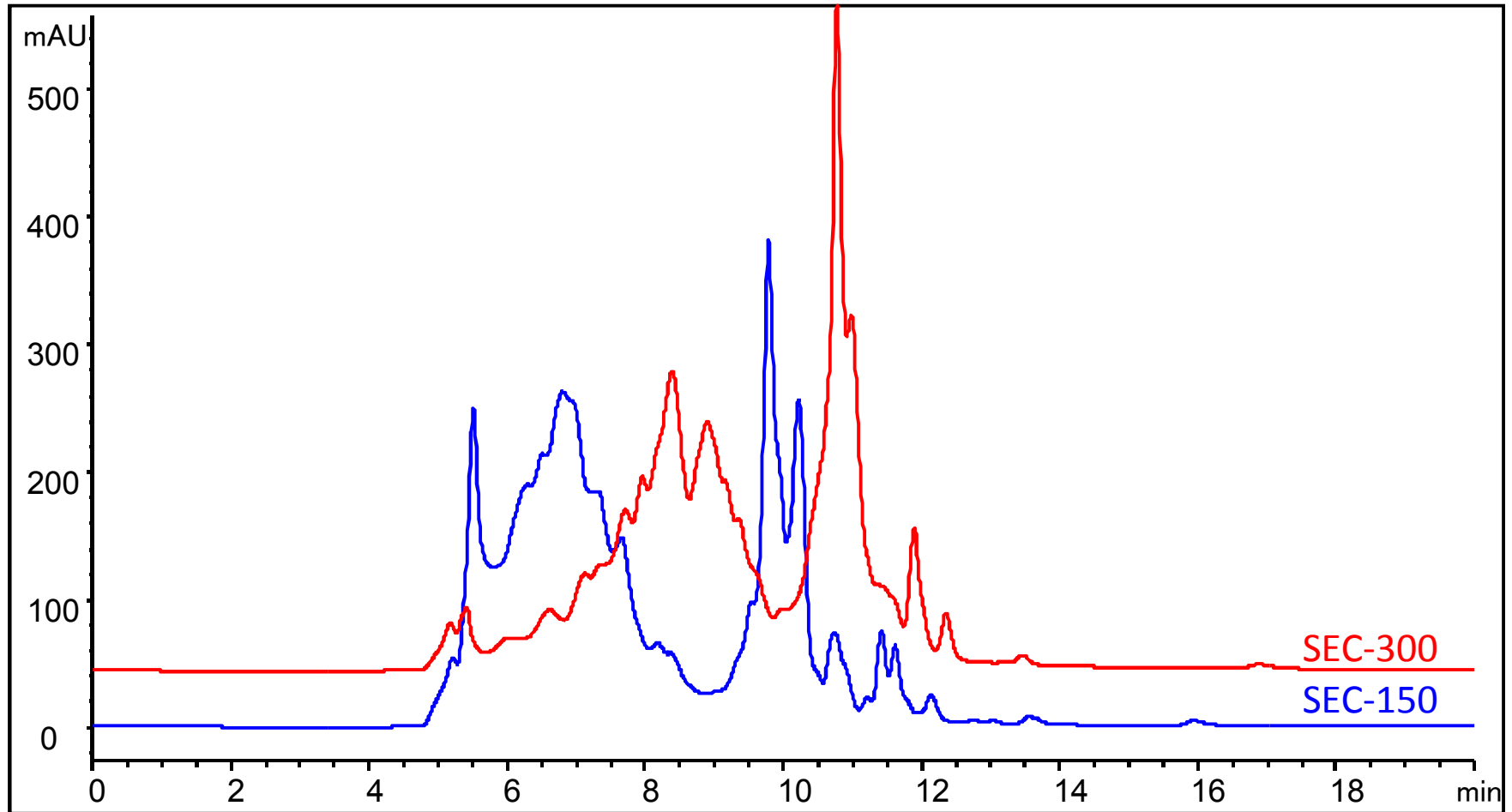
## Overlay of SEC-150 and SEC-300 for E. coli lysate separation-UV214 nm

Column: Zenix<sup>®</sup> SEC-150 (3  $\mu$ m, 150  $\text{\AA}$ , 7.8 x 300 mm); Zenix<sup>®</sup> SEC-300 (3  $\mu$ m, 300  $\text{\AA}$ , 7.8 x 300 mm);

Mobile phase: 150 mM Sodium Phosphate Buffer, pH=7.0

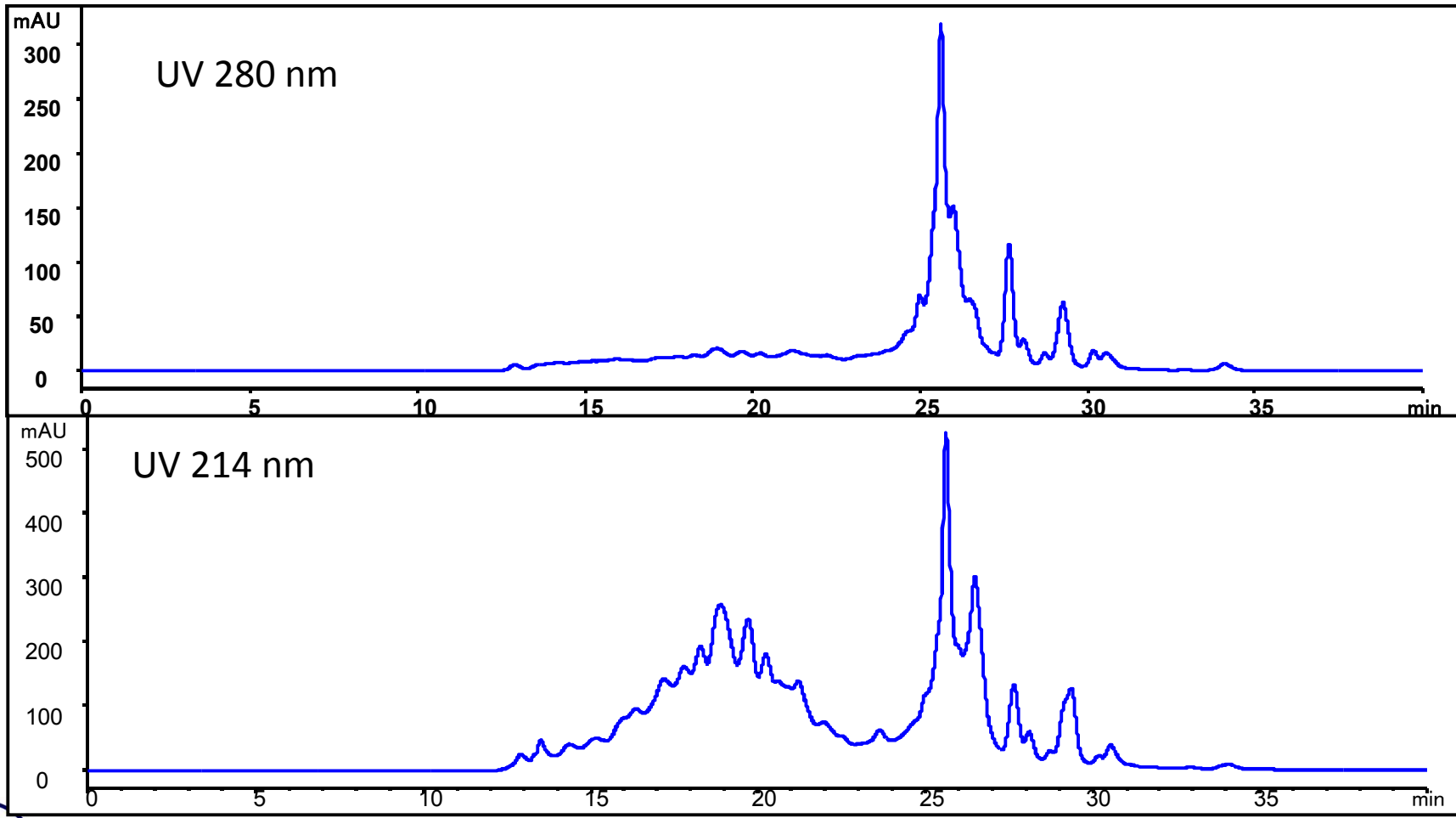
Flow rate: 1 mL/min; Detector: UV 214 nm; Column temperature: 25  $^{\circ}$ C ;

Sample: 2.7 mg/mL Bio-rad E. coli lysate; Injection volume: 10  $\mu$ L



## SEC-150 and SEC-300 in tandem for E. coli lysate separation

Column: Zenix<sup>®</sup> SEC-150 (3  $\mu$ m, 150  $\text{\AA}$ , 7.8 x 300 mm) and Zenix<sup>®</sup> SEC-300 (3  $\mu$ m, 300  $\text{\AA}$ , 7.8 x 300 mm);  
Mobile phase: 150 mM Sodium Phosphate Buffer, pH=7.0  
Flow rate: 1 mL/min; Detector: UV 280 nm, 214 nm; Column temperature: 25  $^{\circ}$ C ;  
Sample: 2.7 mg/mL Bio-rad E. coli lysate; Injection volume: 20  $\mu$ L



# SEC-150 and SEC-300 in tandem for E. coli lysate separation-UV214 nm <sup>ZY1002</sup>

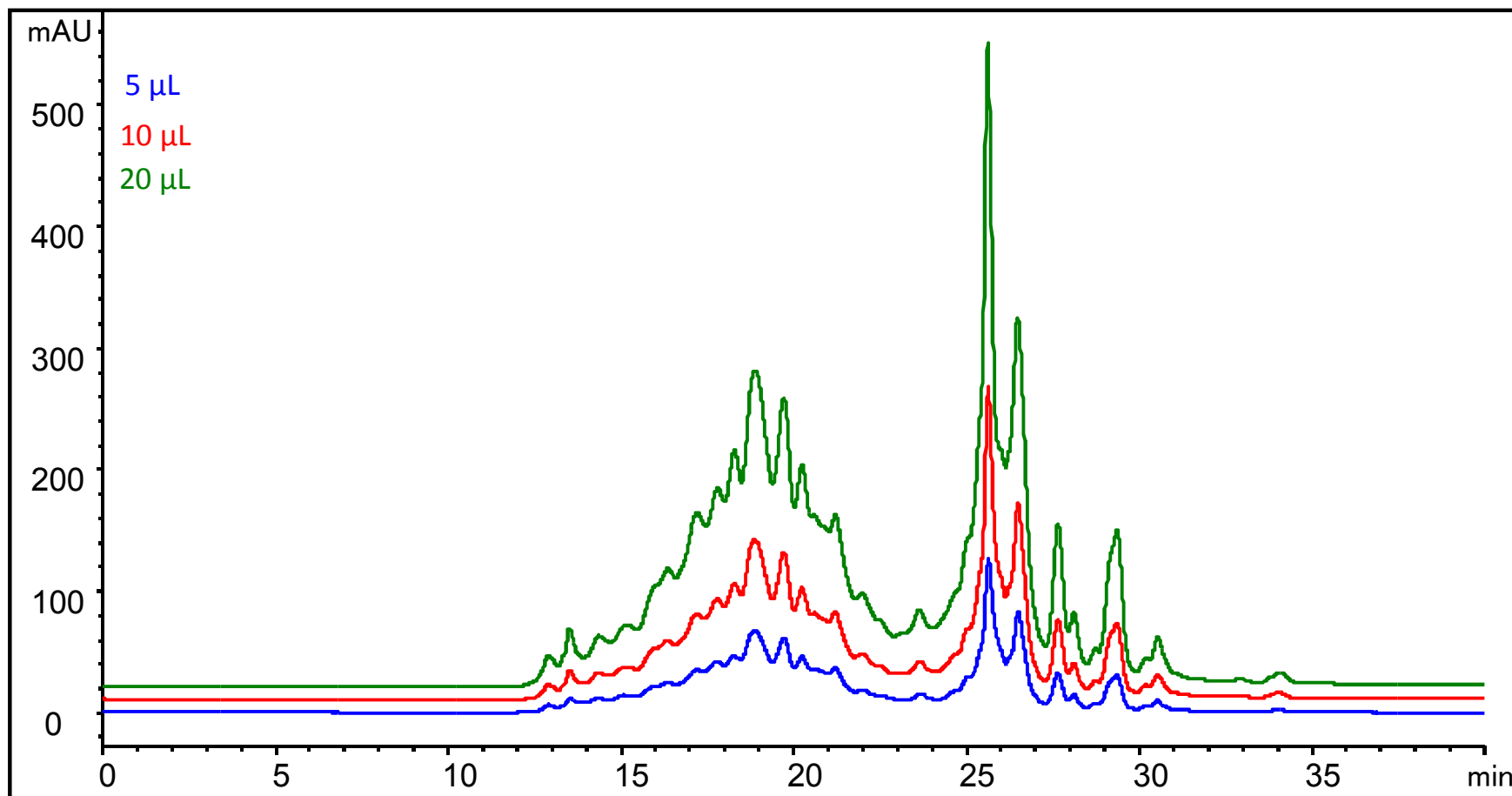
## Loading test

Column: Zenix<sup>®</sup> SEC-150 (3  $\mu$ m, 150  $\text{\AA}$ , 7.8 x 300 mm) and Zenix<sup>®</sup> SEC-300 (3  $\mu$ m, 300  $\text{\AA}$ , 7.8 x 300 mm);

Mobile phase: 150 mM Sodium Phosphate Buffer, pH=7.0

Flow rate: 0.8 mL/min; Detector: UV 214 nm; Column temperature: 25  $^{\circ}$ C ;

Sample: 2.7 mg/mL Bio-rad E. coli lysate; Injection volume: 5, 10, 20  $\mu$ L





# SEC-150 and SEC-300 in tandem for E. coli lysate separation-UV280 nm <sup>ZY1002</sup>

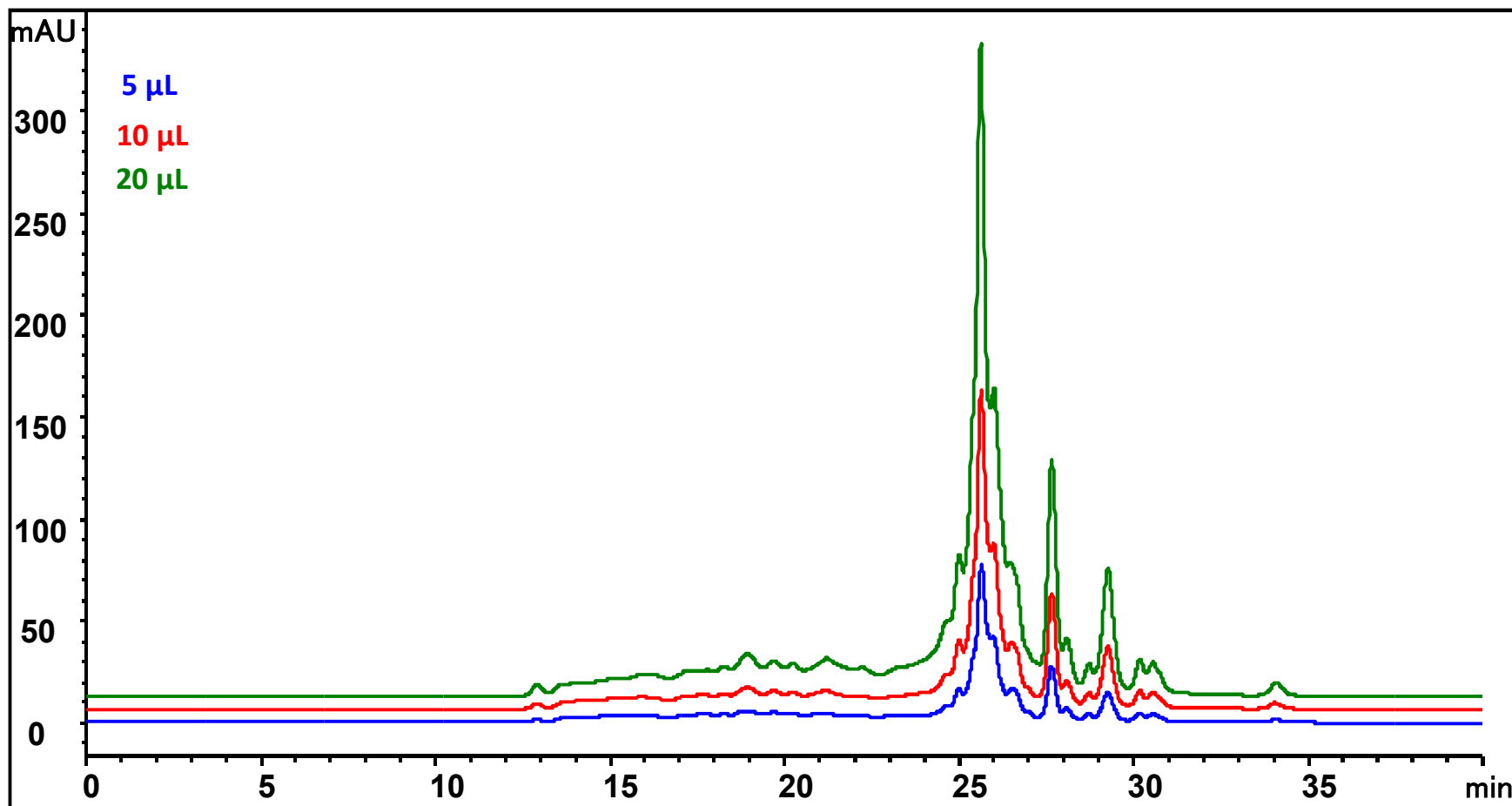
## Loading test

Column: Zenix<sup>®</sup> SEC-150 (3  $\mu$ m, 150  $\text{\AA}$ , 7.8 x 300 mm) and Zenix<sup>®</sup> SEC-300 (3  $\mu$ m, 300  $\text{\AA}$ , 7.8 x 300 mm);

Mobile phase: 150 mM Sodium Phosphate Buffer, pH=7.0

Flow rate: 0.8 mL/min; Detector: UV 280 nm; Column temperature: 25  $^{\circ}$ C ;

Sample: 2.7 mg/mL Bio-rad E. coli lysate; Injection volume: 5, 10, 20  $\mu$ L



# E. Coli lysate separation

Ion exchange chromatography

Proteomix<sup>®</sup> SAX-NP3 (3  $\mu$ m, 4.6 x 50 mm)



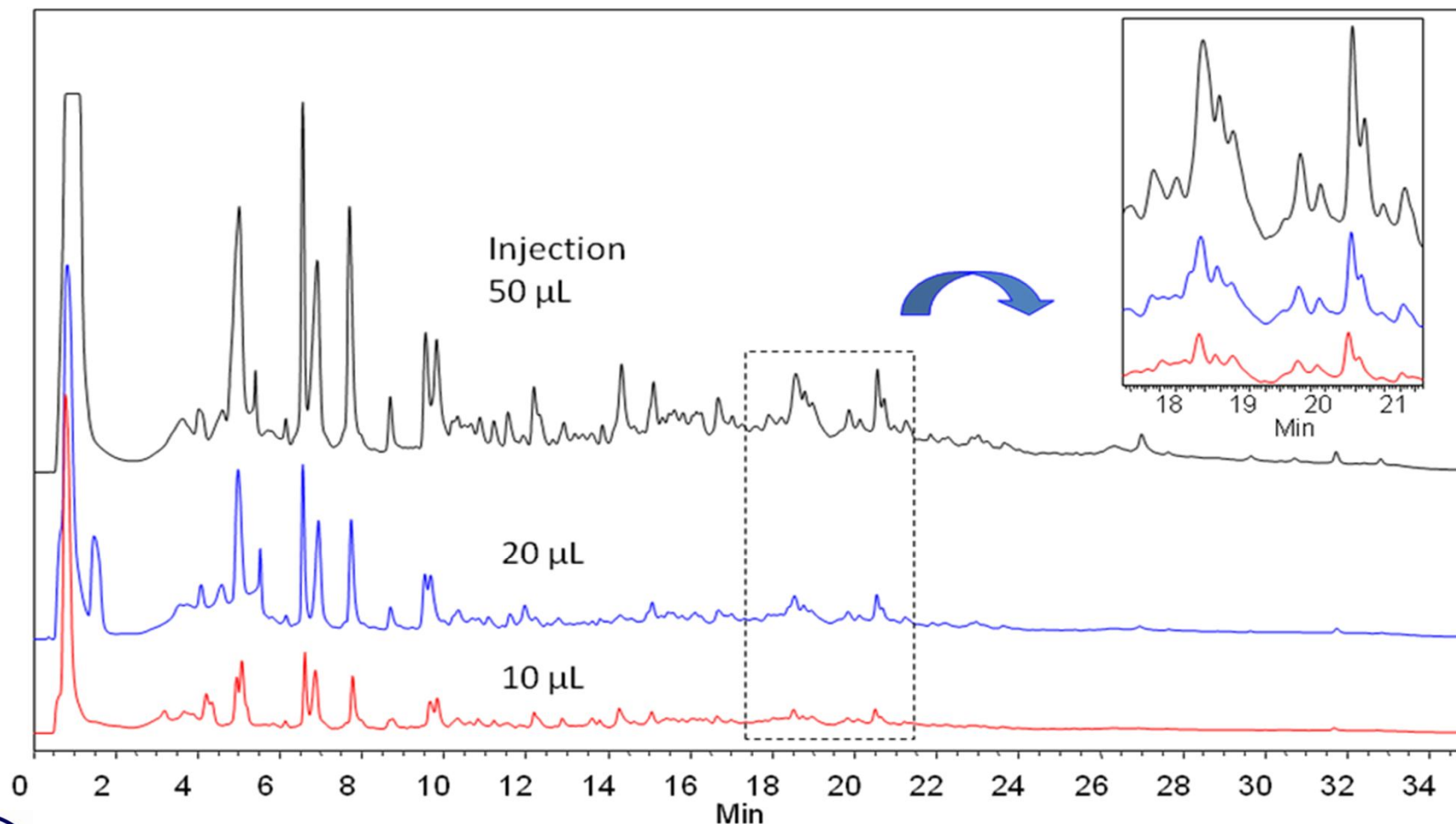
## Separation of *E. coli* lysate with Proteomix SAX columns

Column: Proteomix® SAX-NP3 (3  $\mu$ m, 4.6 x 50 mm);

Mobile phase: A: 20 mM Tris, pH 9.0; B: A + 0.5 M NaCl; Gradient: 0-100% B (30 min)

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: ambient;

Sample: 2.5 mg/mL Custom source *E. coli* lysate; Injection volume: 10, 20, 50  $\mu$ L



# E. Coli lysate separation

Reversed phase chromatography  
PolyRP-1000 (5  $\mu\text{m}$ , 1000  $\text{\AA}$ , 4.6 x 100 mm)



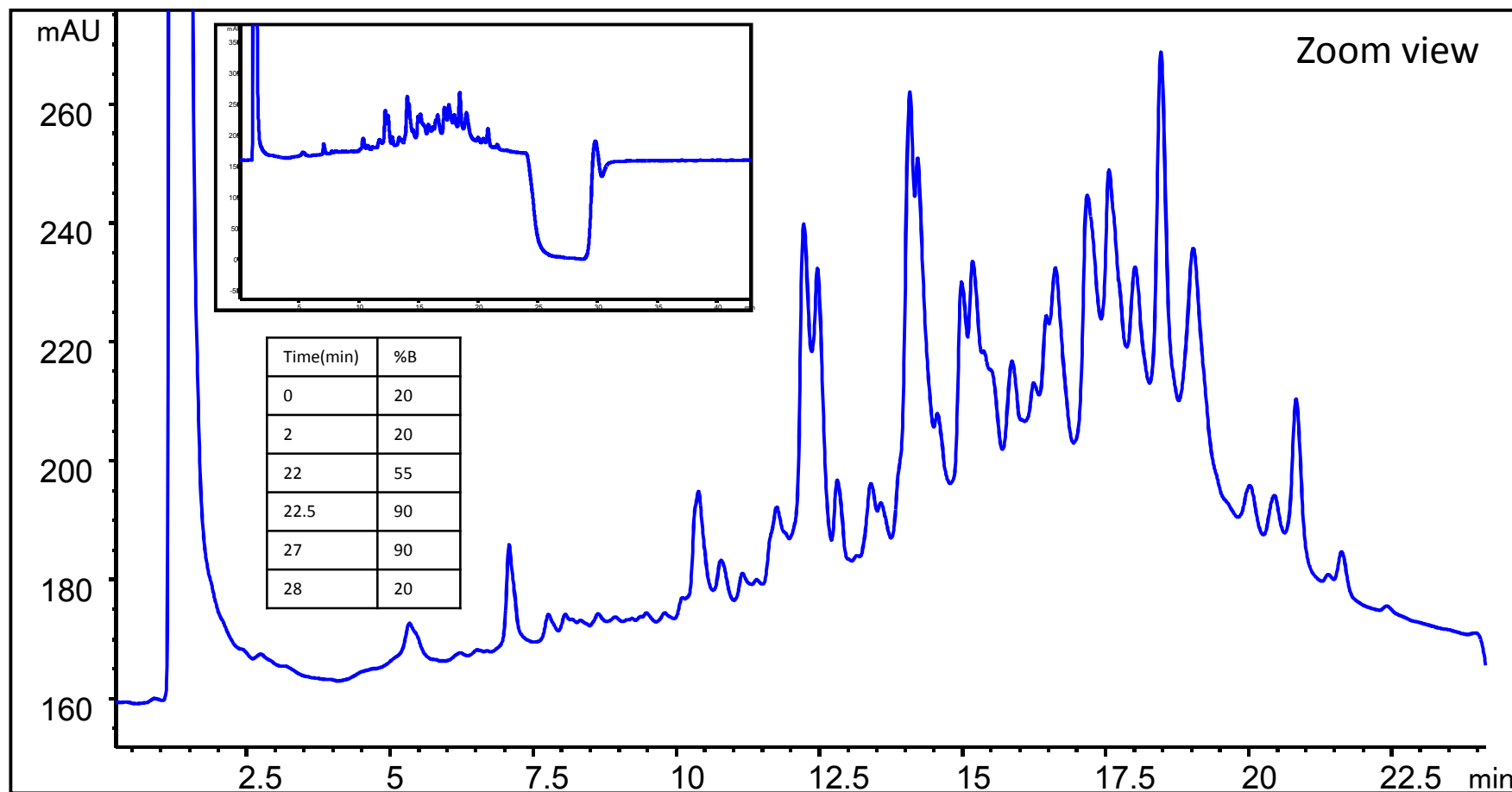
## E. Coli lysate separation on PolyRP-1000

Column: PolyRP-1000 (5  $\mu\text{m}$ , 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: 80  $^{\circ}\text{C}$ ;

Sample: Bio-rad E. coli lysate diluted in 0.1% TFA, filter before injection (1.3 mg/mL) ; Injection volume: 30  $\mu\text{L}$



## Bio-rad E. Coli lysate separation on PolyRP-1000

Column: PolyRP-1000 (5  $\mu\text{m}$ , 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: 80  $^{\circ}\text{C}$ ;

Sample: See chromatogram

