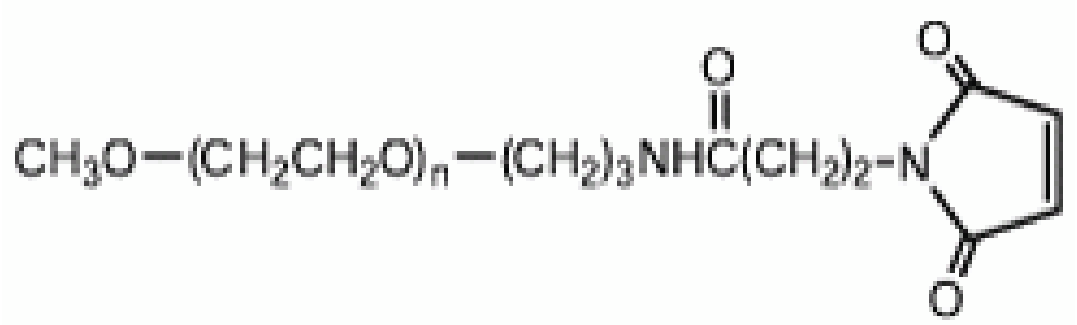
BSA pegylation

- BSA 5mg/mL, pI= 4.7, Sigma A7096, 65-70% free sulfyhydryl, cysteine 34 of BSA is not paired with another cysteine in the protein structure[1].
- PEG 10kD, 20kD, 30 kD 10mg/mL
- PEG:BSA=1.2:1 (molar ratio)
- Incubate at RT for overnight
- Columns:, Zenix SEC-300 and SRT-C SEC-500, Proteomix SAX

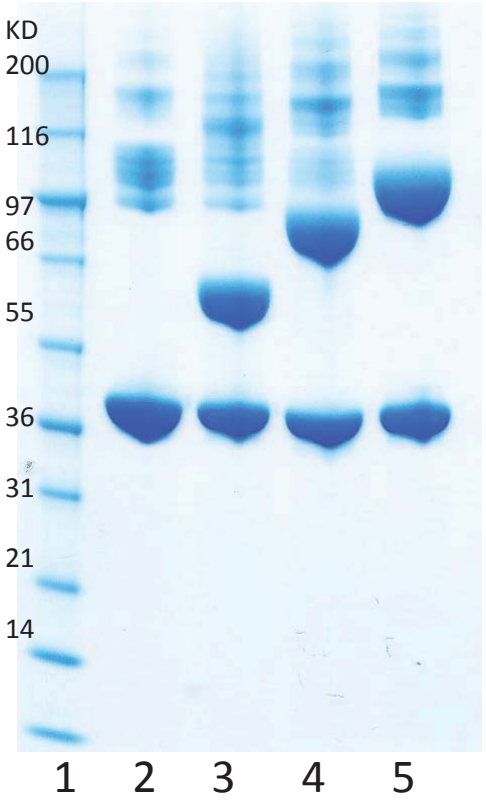
1. Journal of Chromatography A, 1147 (2007) 172-182

PEG

- PEG-10kD
- PEG-20kD
- PEG-30kD
- From NOF America, SUNBRIGHT® MA Series (Maleimide PEG), target group -SH



4-12% Bis-Tris SDS-PAGE-MOPS buffer

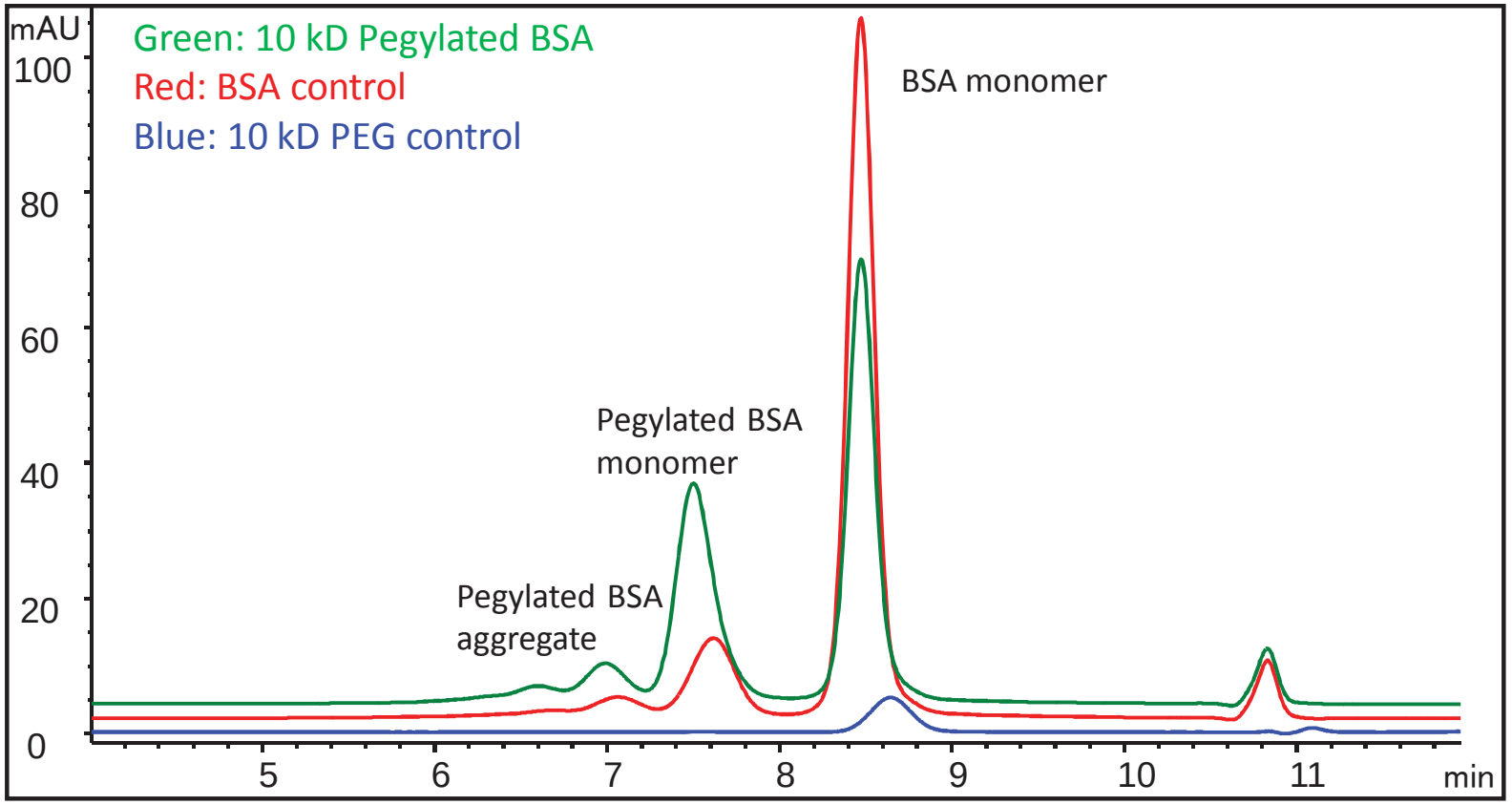


1. Marker
2. BSA control
3. BSA + 10 kD PEG
4. BSA + 20 kD PEG
5. BSA + 30 kD PEG

Due to the non-reducing condition, the MW of BSA samples appear lower which allows the identification of covalently linked aggregates.

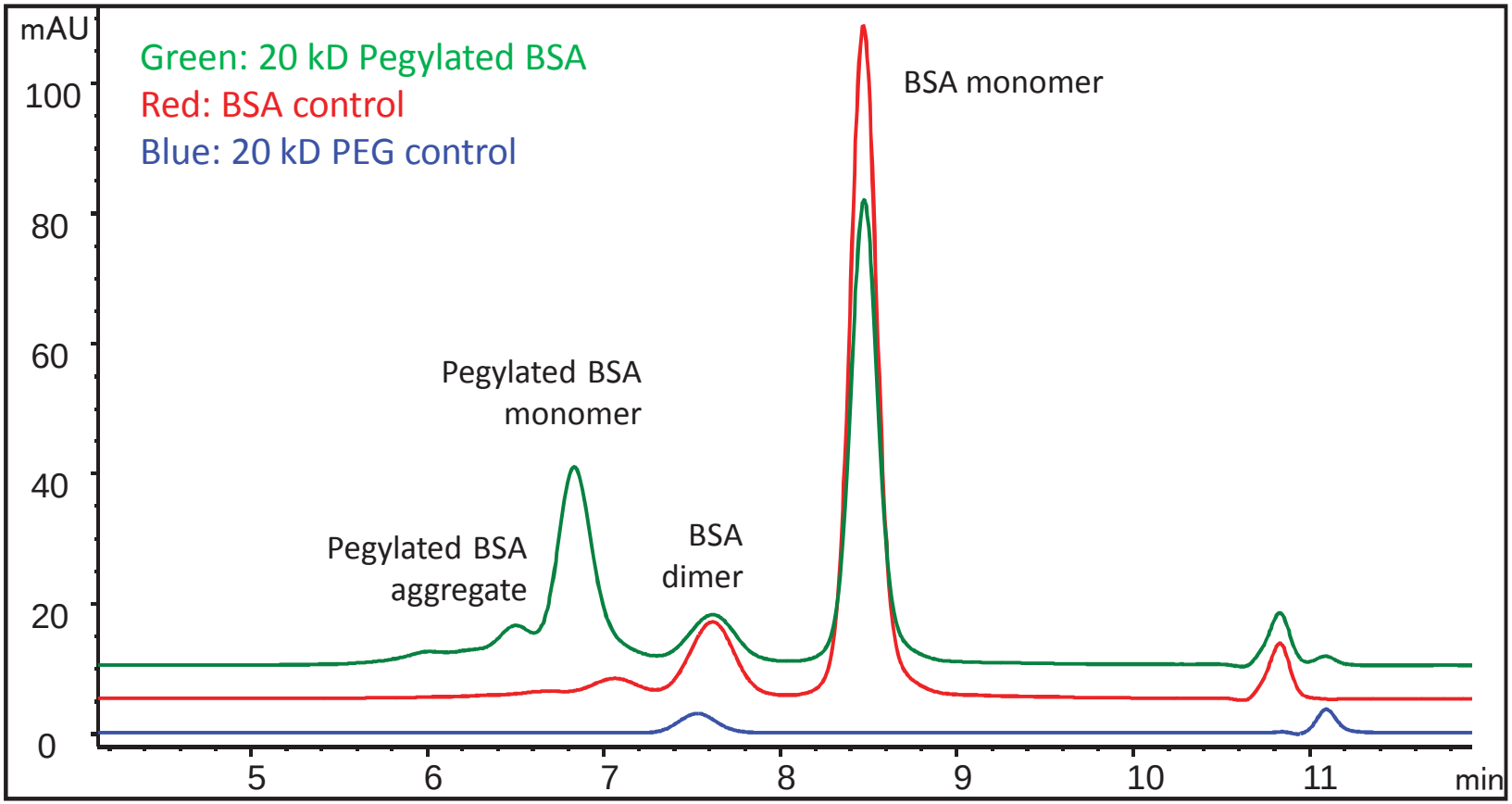
Pegylated BSA (10 kD PEG) Analysis on SEC-300 (7830)

Column: Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: BSA control (5 mg/mL in sodium acetate), 10 µL
PEG 10 kD, 10 mg/mL, 5 µL
10 kD pegylated BSA, 4.59 mg/mL, 11 µL



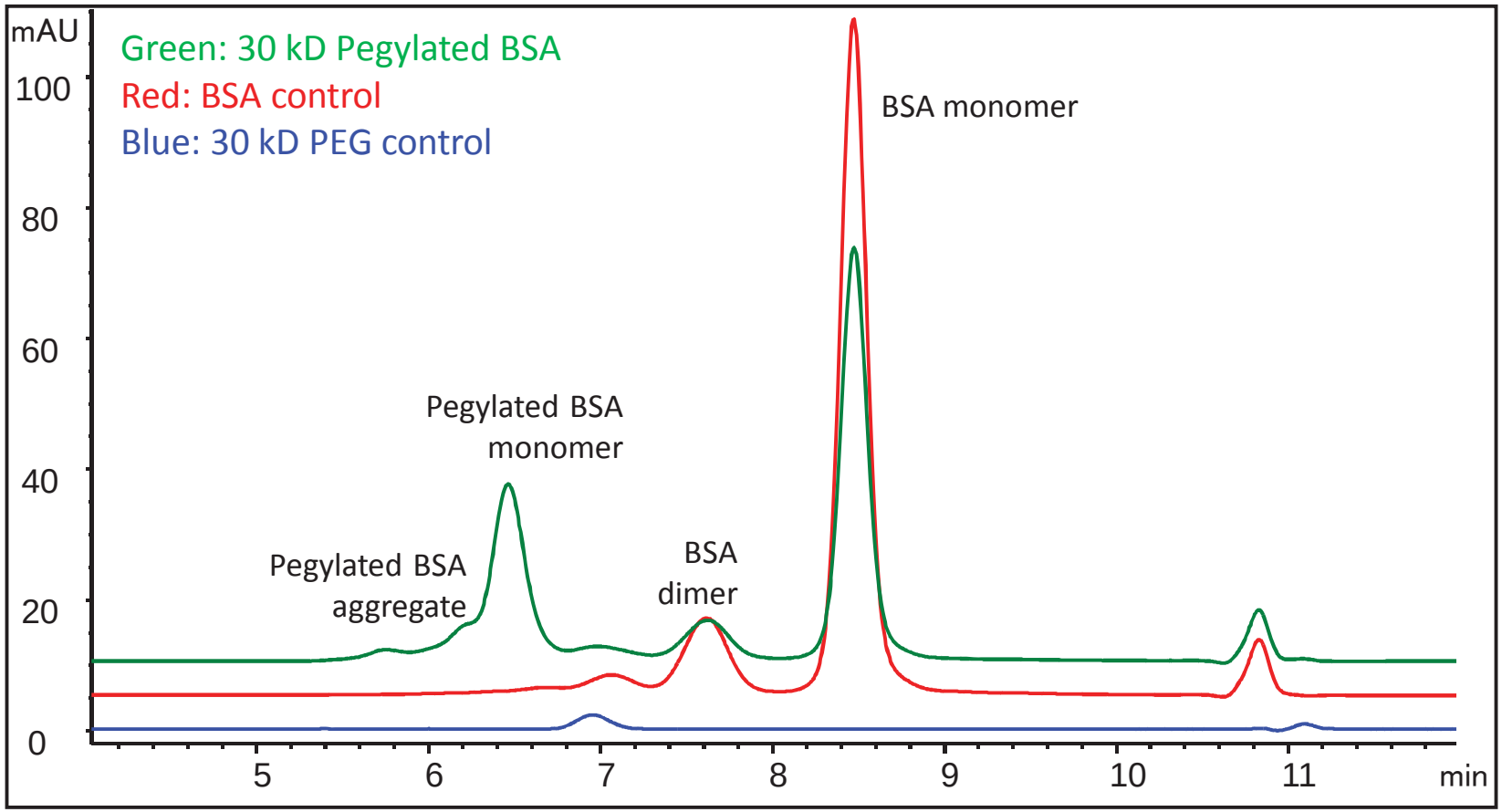
Pegylated BSA (20 kD PEG) Analysis on SEC-300 (7830)

Column: Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: BSA control (5 mg/mL in sodium acetate), 10 µL
PEG 20 kD, 10 mg/mL, 5 µL
20 kD pegylated BSA, 4.23 mg/mL, 12 µL



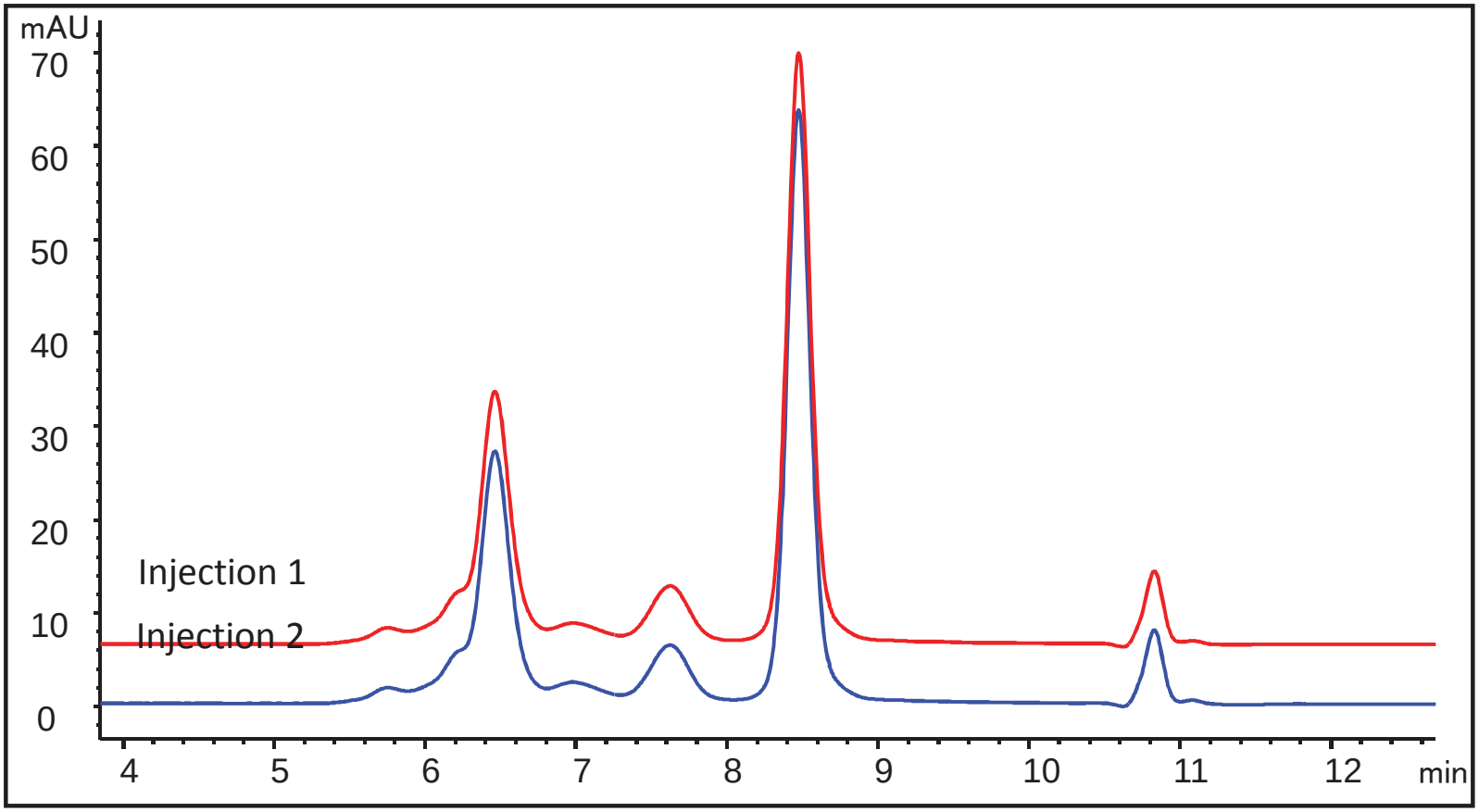
Pegylated BSA (30 kD PEG) Analysis on SEC-300 (7830)

Column: Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: BSA control (5 mg/mL in sodium acetate), 10 µL
PEG 30 kD, 10 mg/mL, 5 µL
30 kD pegylated BSA, 3.93 mg/mL, 12.7 µL



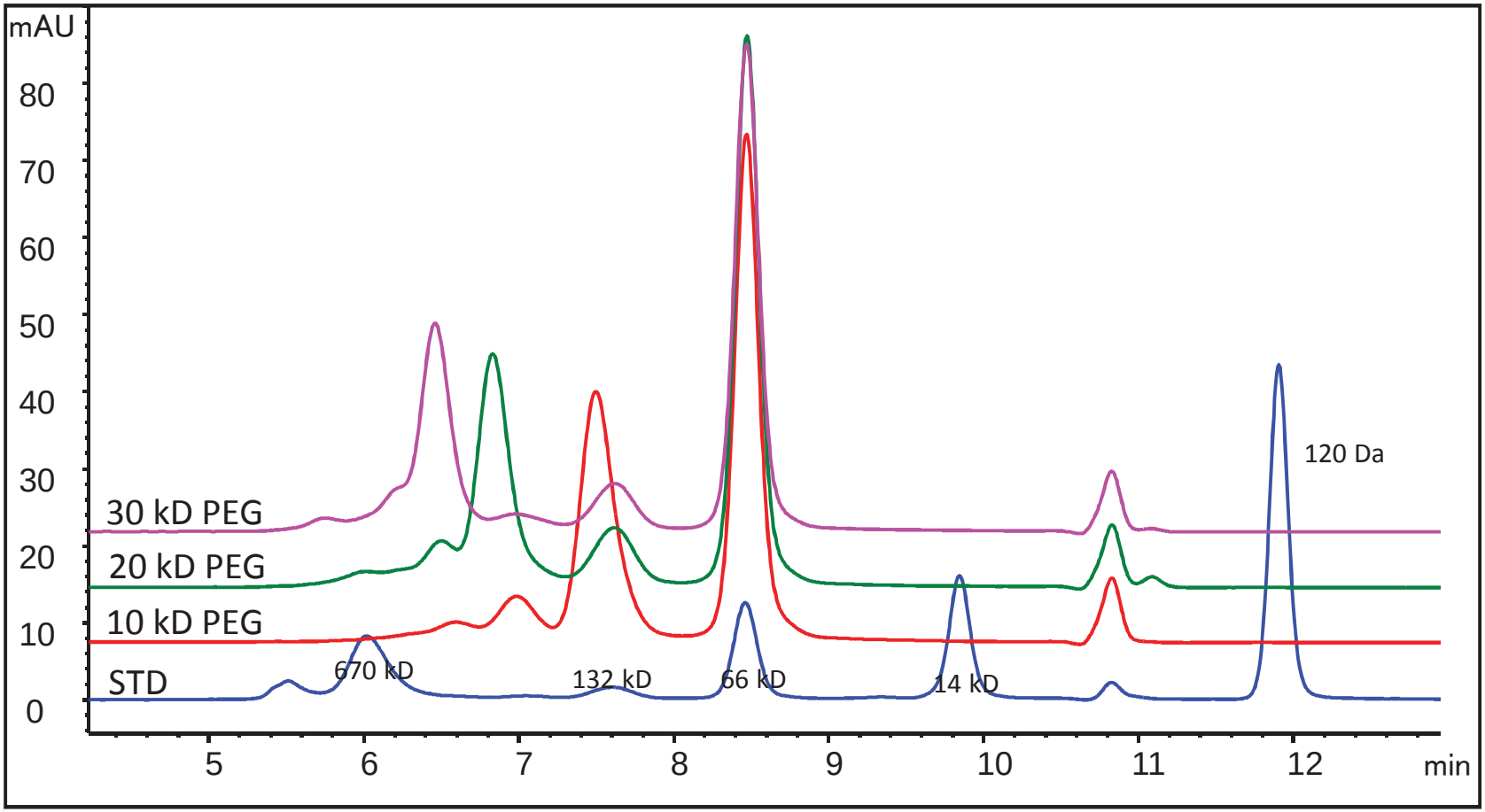
Duplicated injection- Pegylated BSA (30 kD PEG)

Column: Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: BSA control (5 mg/mL in sodium acetate), 10 µL
PEG 30 kD, 10 mg/mL, 5 µL
30 kD pegylated BSA, 3.93 mg/mL, 12.7 µL



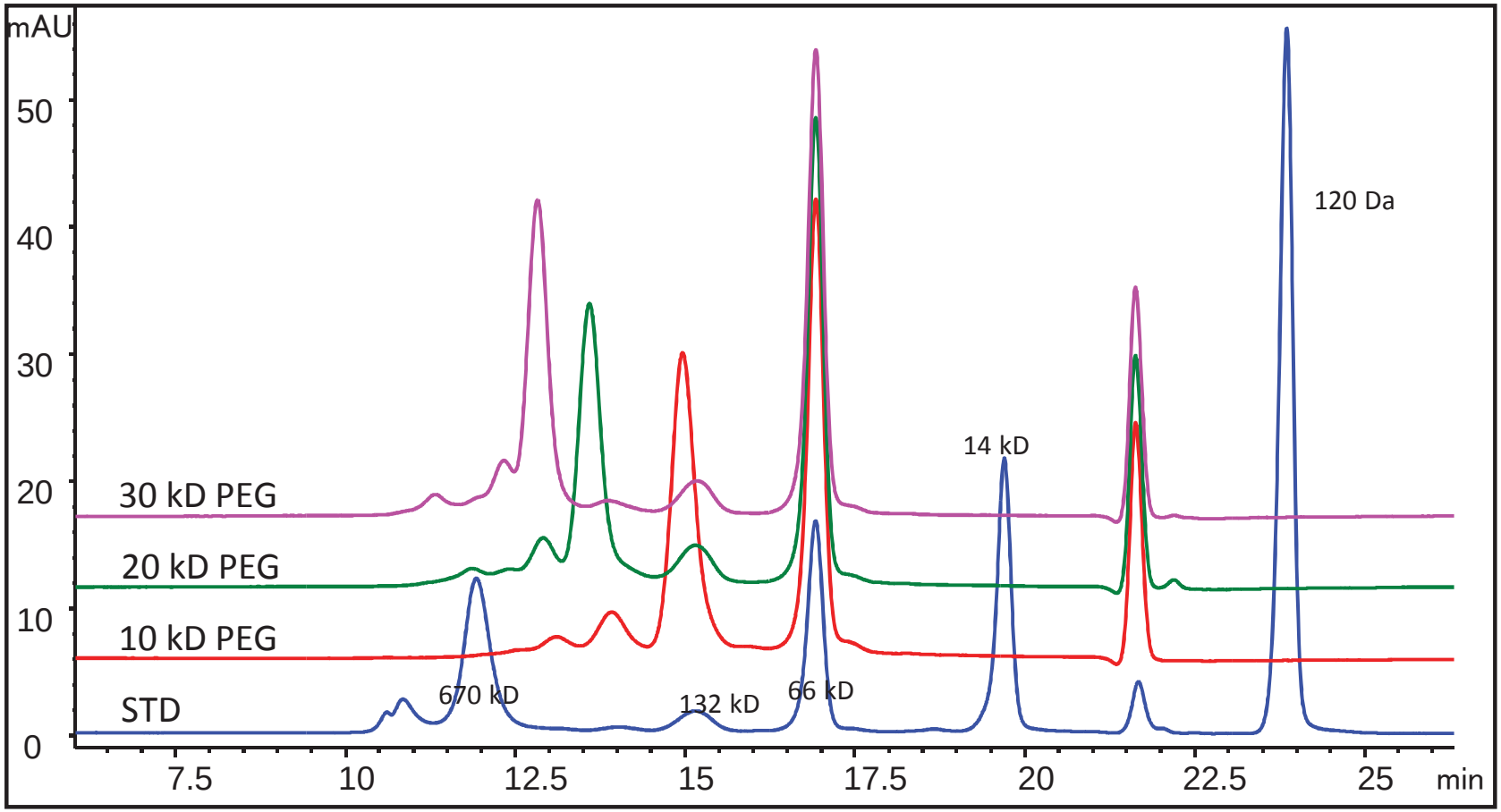
Pegylated BSA overlays with QC standard

Column: Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: Protein standards, Thyroglobulin (670 kD), BSA (66 kD), Ribonuclease A (14 kD), Uracil (120 Da)
10, 20 , 30 kD pegylated BSA, 50 µg each



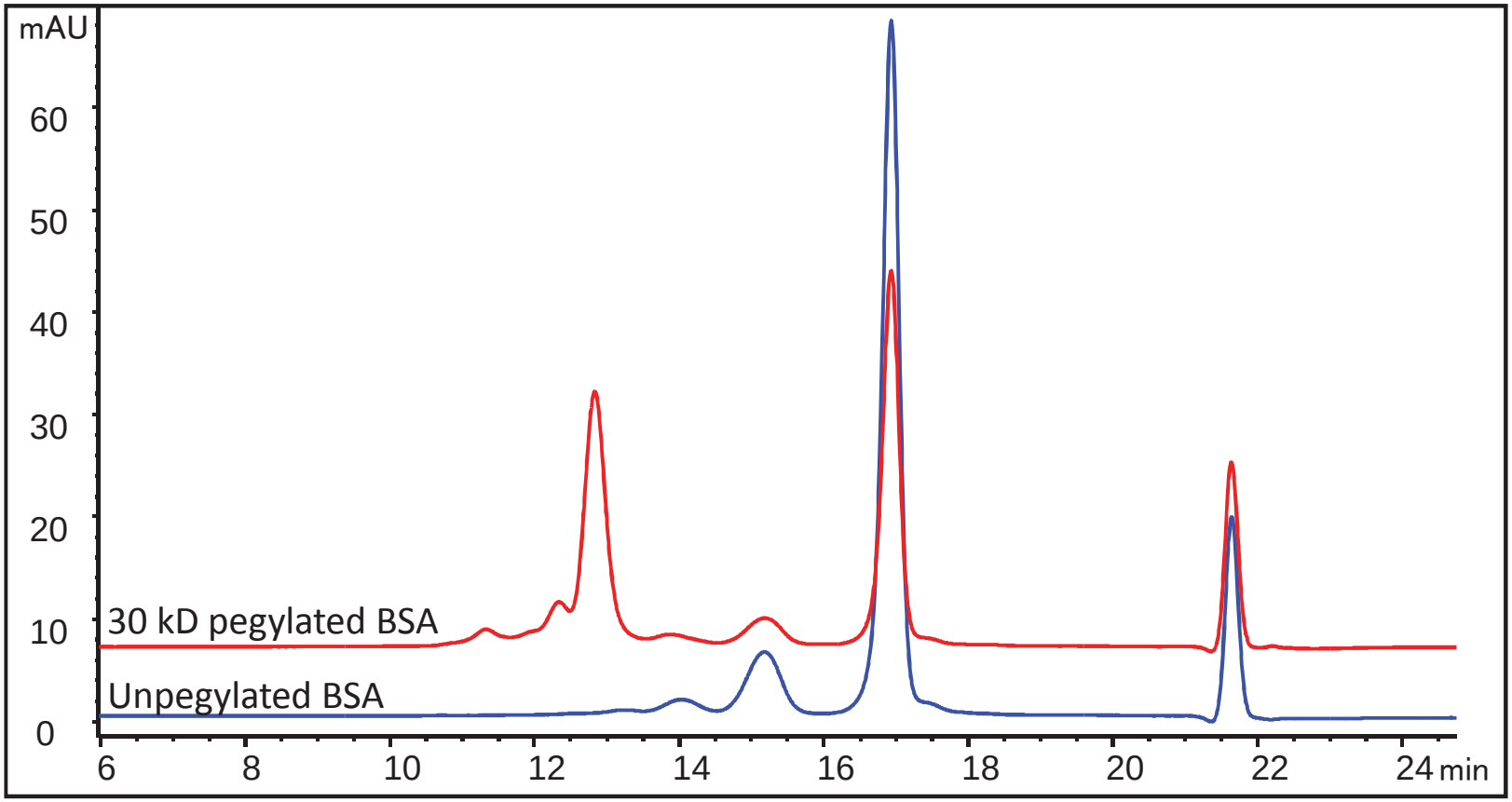
Pegylated BSA analysis with two Zenix in tandem

Column: 2 x Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm), SN 44909 + 32695
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: QC standards, 10, 20, 30 kD pegylated BSA, 50 µg each



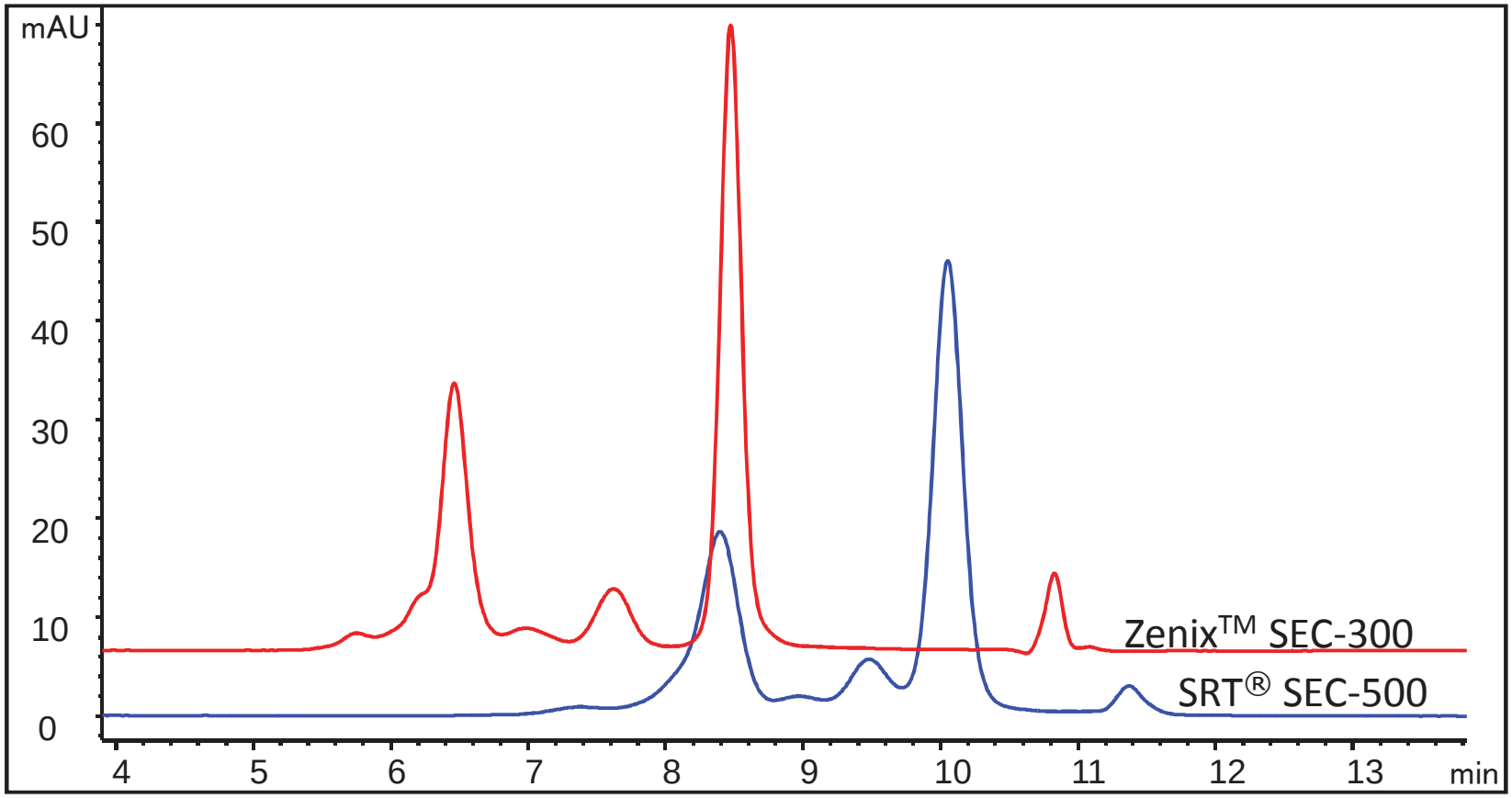
Pegylated (30 kD PEG) BSA analysis with two Zenix in tandem

Column: 2 x Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm), SN 44909 + 32695
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: BSA 50 µg, 30 kD pegylated BSA, 50 µg each



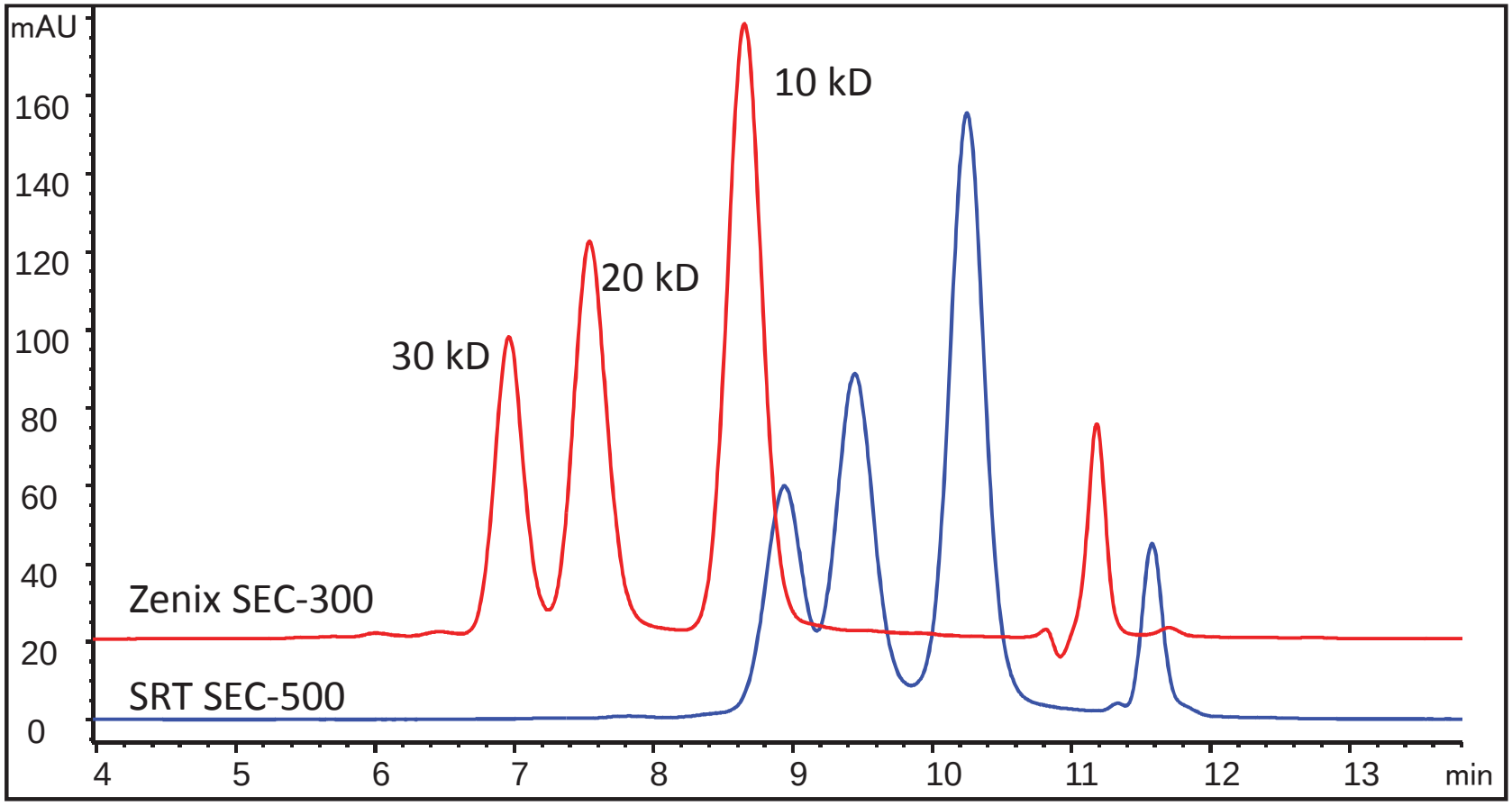
Pegylated (30 kD PEG) BSA analysis – Pore size difference

Column: Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm), SRT SEC-500 (5 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: 30 kD pegylated BSA, 50 µg



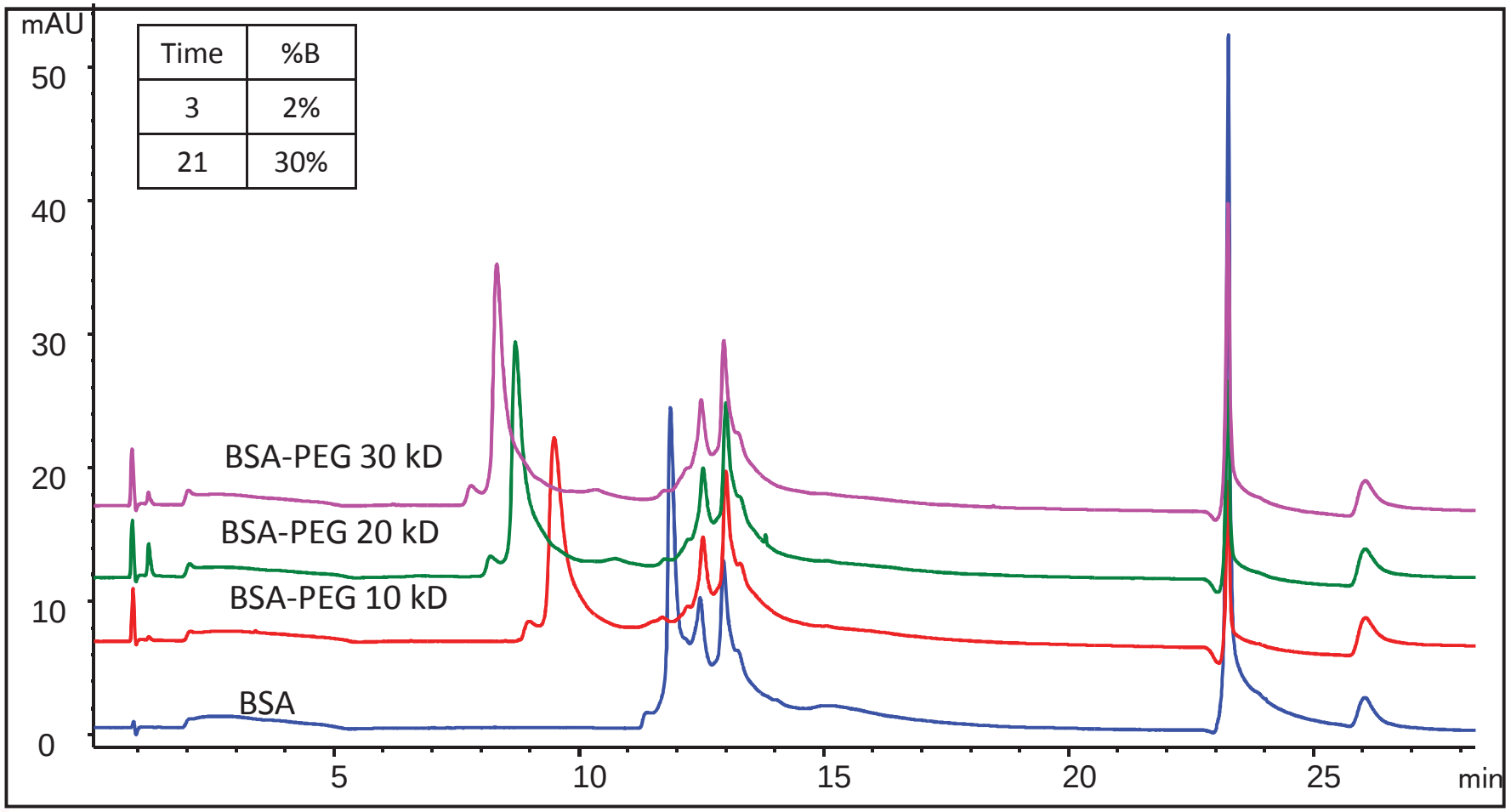
Pegylated (30 kD PEG) BSA analysis – Pore size difference

Column: Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm), SRT SEC-500 (5 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃;
Samples: 10, 20, 30 kD Maleimide PEG (sunbright from NOF), 50 µg



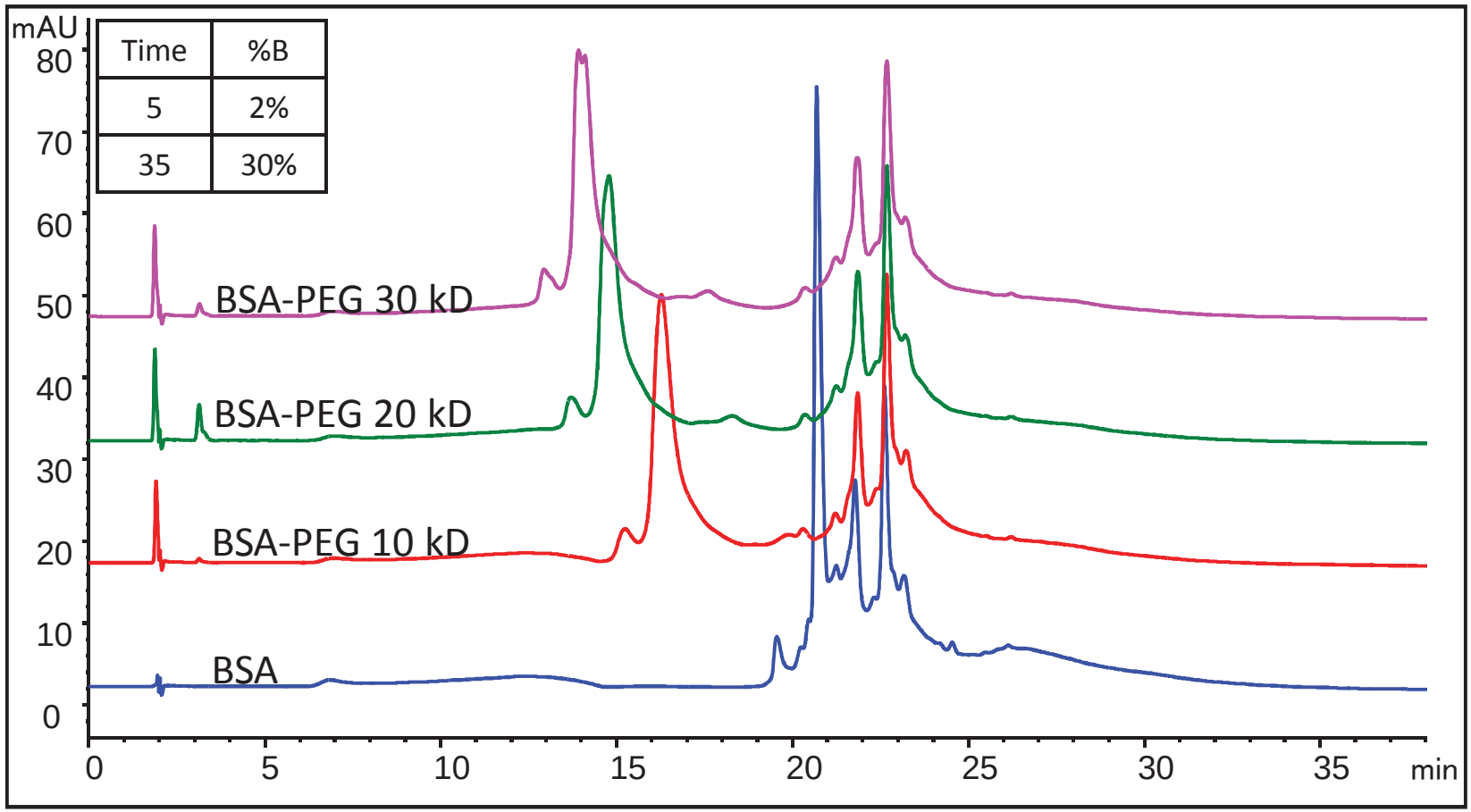
Proteomix® SAX – Pegylated BSA separation-4615

Column: Proteomix® SAX-NP5 (5 µm, 4.6 x 150 mm),
Mobile phase: A: Bis-Tris Propane, pH 7.0, B: A + 1 M NaCl, pH 7.0,
Flow rate: 0.8 mL/min, 170 bar; Detector: UV 280 nm, Column temperature: 25 °C,
Samples: **50 µg** each, Unconjugated BSA, PEG 10 kD, PEG 20 kD and PEG 30 kD conjugated BSA



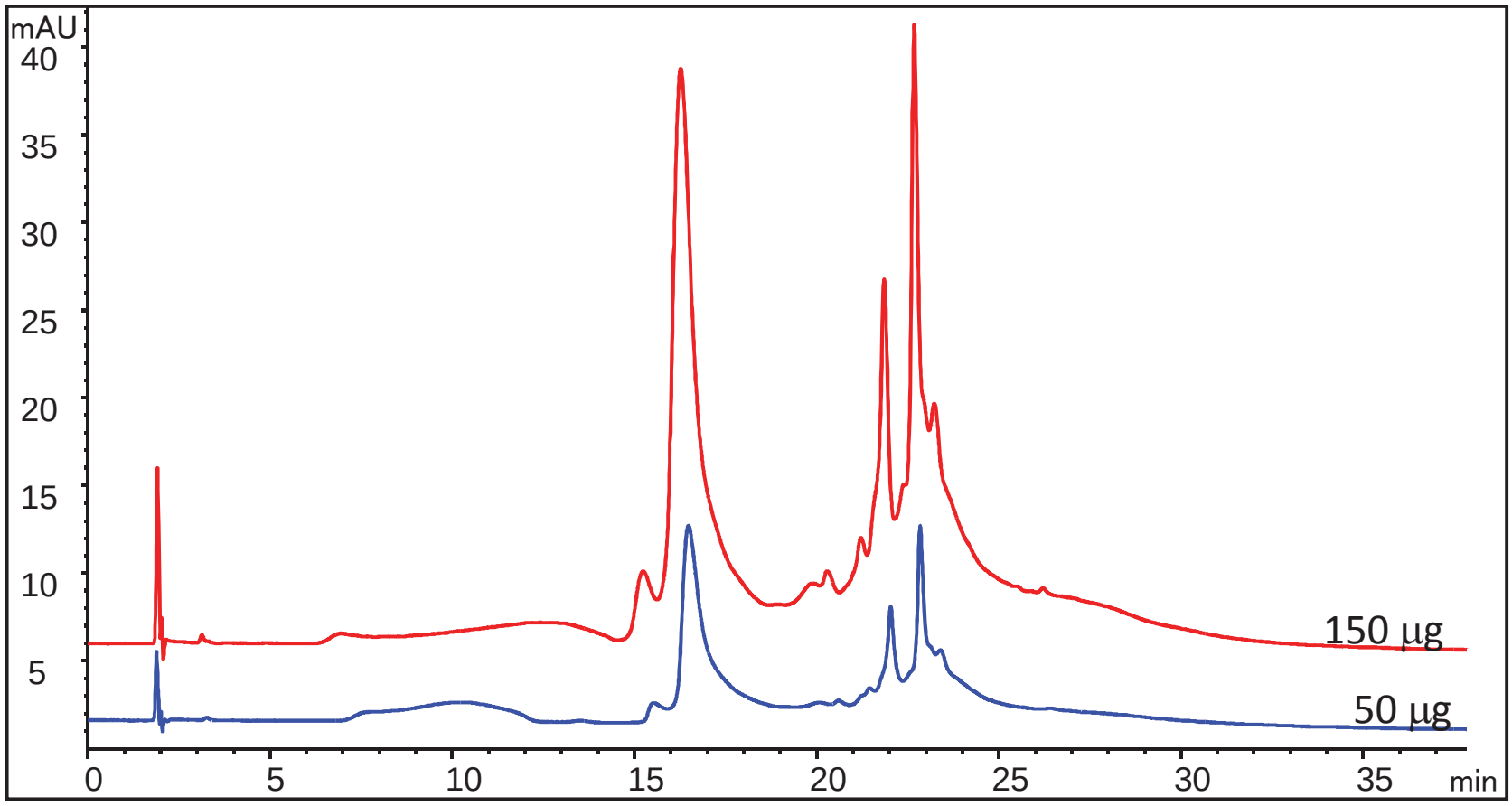
Proteomix® SAX – Pegylated BSA separation

Column: Proteomix® SAX-NP5 (5 µm, 4.6 x 250 mm),
Mobile phase: A: Bis-Tris Propane, pH 7.0, B: A + 1 M NaCl, pH 7.0,
Flow rate: 0.8 mL/min, 170 bar; Detector: UV 280 nm, Column temperature: 25 ℃,
Samples: **150 µg** each, Unconjugated BSA, PEG 10 kD, PEG 20 kD and PEG 30 kD conjugated BSA



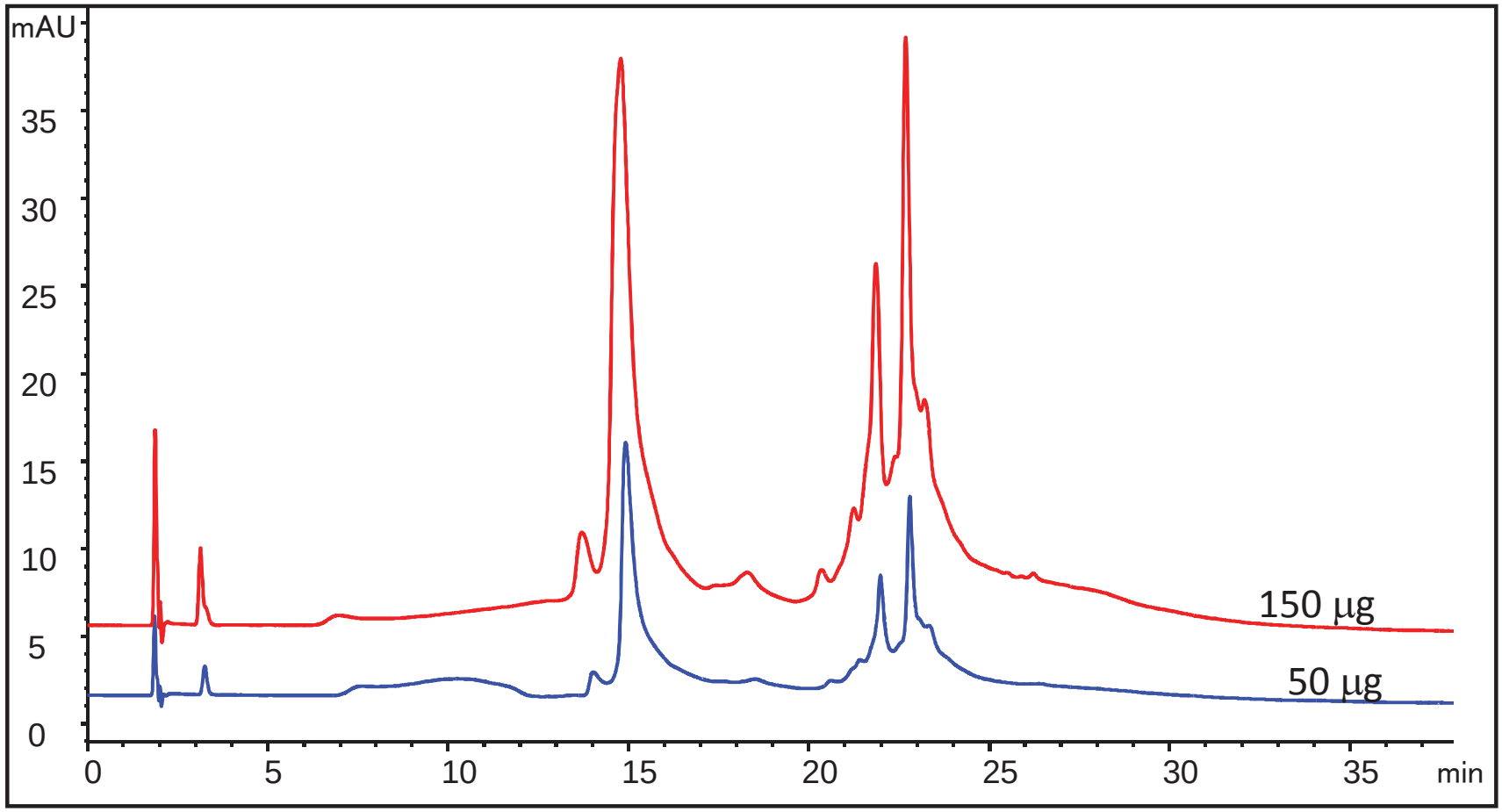
Proteomix® SAX – 10 kD Pegylated BSA Loading

Column: Proteomix® SAX-NP5 (5 µm, 4.6 x 250 mm),
Mobile phase: A: Bis-Tris Propane, pH 7.0, B: A + 1 M NaCl, pH 7.0,
Flow rate: 0.8 mL/min, 170 bar; Detector: UV 280 nm, Column temperature: 25 ℃,
Samples: **50, 150 µg** each, PEG 10 kD conjugated BSA



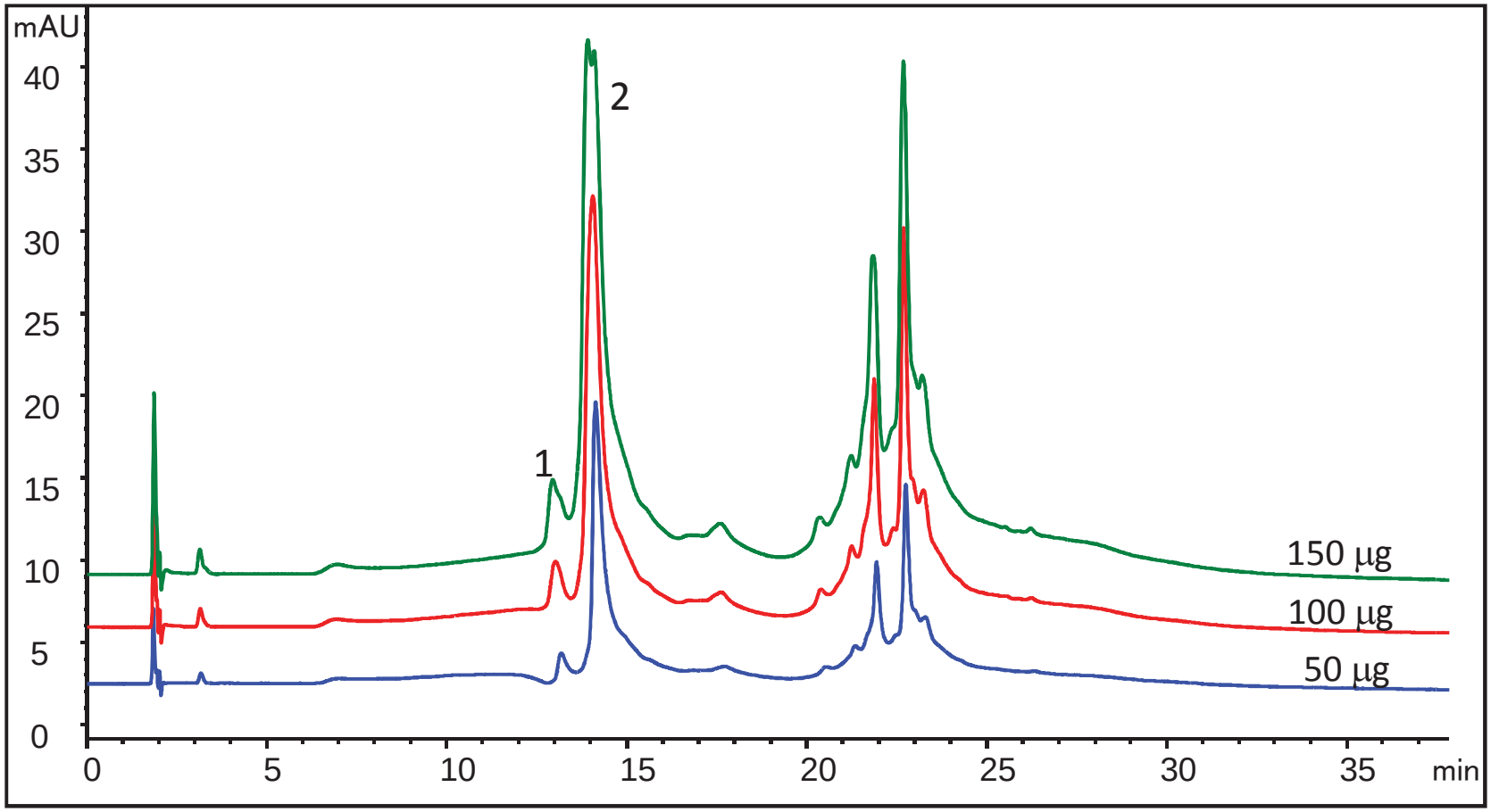
Proteomix® SAX – 20 kD Pegylated BSA Loading

Column: Proteomix® SAX-NP5 (5 µm, 4.6 x 250 mm),
Mobile phase: A: Bis-Tris Propane, pH 7.0, B: A + 1 M NaCl, pH 7.0,
Flow rate: 0.8 mL/min, 170 bar; Detector: UV 280 nm, Column temperature: 25 ℃,
Samples: **50, 150 µg** each, PEG 20 kD conjugated BSA



Proteomix® SAX – 30 kD Pegylated BSA Loading

Column: Proteomix® SAX-NP5 (5 µm, 4.6 x 250 mm),
Mobile phase: A: Bis-Tris Propane, pH 7.0, B: A + 1 M NaCl, pH 7.0,
Flow rate: 0.8 mL/min, 170 bar; Detector: UV 280 nm, Column temperature: 25 ℃,
Samples: **50, 100, 150 µg** each, PEG 30 kD conjugated BSA



SAX collected fractions on Zenix SEC-300 peak 1 and 2 from previous slide

Column: Zenix™ SEC-300, (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C;
Samples: 30 kD pegylated BSA fractionated on SAX, fraction 1 and 2 concentrated with a 3kD Amicon Ultra