

Technical Notification 0012

Evaluation of Dr. Maisch Columns for Size Exclusion Chromatography (SEC)

In this Technical Notification, we introduce the sub-2 µm ReproSil SEC Product Line, specifically designed for UHPLC-SEC applications. The following four SEC columns feature the most widely used silica pore size (250 Å), available with different chemical modifications and hardware options.

Part Number (P/N)	Description	Modification	Hardware Type
r219.sec.s1546	ReproSil 200 SEC, 1.9 µm, 150 mm x 4.6 mm	PEG ¹⁾	Standard Stainless Steel
r219.sec.b1546	ReproSil 200 SEC, 1.9 µm, 150 mm x 4.6 mm	PEG ¹⁾	Bioinert Stainless Steel
r219.sec2.s1546	ReproSil 200 SEC-2, 1.9 µm, 150 mm x 4.6 mm	Diol	Standard Stainless Steel
r219.sec2.b1546	ReproSil 200 SEC-2, 1.9 µm, 150 mm x 4.6 mm	Diol	Bioinert Stainless Steel

1) PEG: polyethylene glycol.

This overview highlights their key advantages and features:

Why Use Bioinert Hardware for HPLC?

Bioinert HPLC hardware is essential for applications involving biological samples, biomolecules, or sensitive analytes. Conventional stainless steel can interact with certain compounds, leading to adsorption, degradation, or peak tailing. Bioinert materials eliminate these issues and improve analytical performance.

Key Benefits of Bioinert HPLC Hardware:

1. Prevention of Adsorption and Sample Loss
 - Proteins, peptides, and nucleic acids tend to adsorb onto metal surfaces, leading to inaccurate quantification and poor reproducibility.
2. Enhanced Sensitivity for Biopharmaceuticals
 - Biomolecules such as monoclonal antibodies (mAbs) or oligonucleotides require a contamination-free flow path.
 - Bioinert hardware ensures that the true concentration and purity of the analyte are measured without interference.
3. Improved Peak Shape and Reproducibility
 - Reduces peak tailing caused by interactions with reactive metal sites.
 - Delivers sharper peaks, better resolution, and more accurate quantification, especially in SEC chromatography.

Why Use Sub-2 µm Particles for Size Exclusion Chromatography (SEC)?

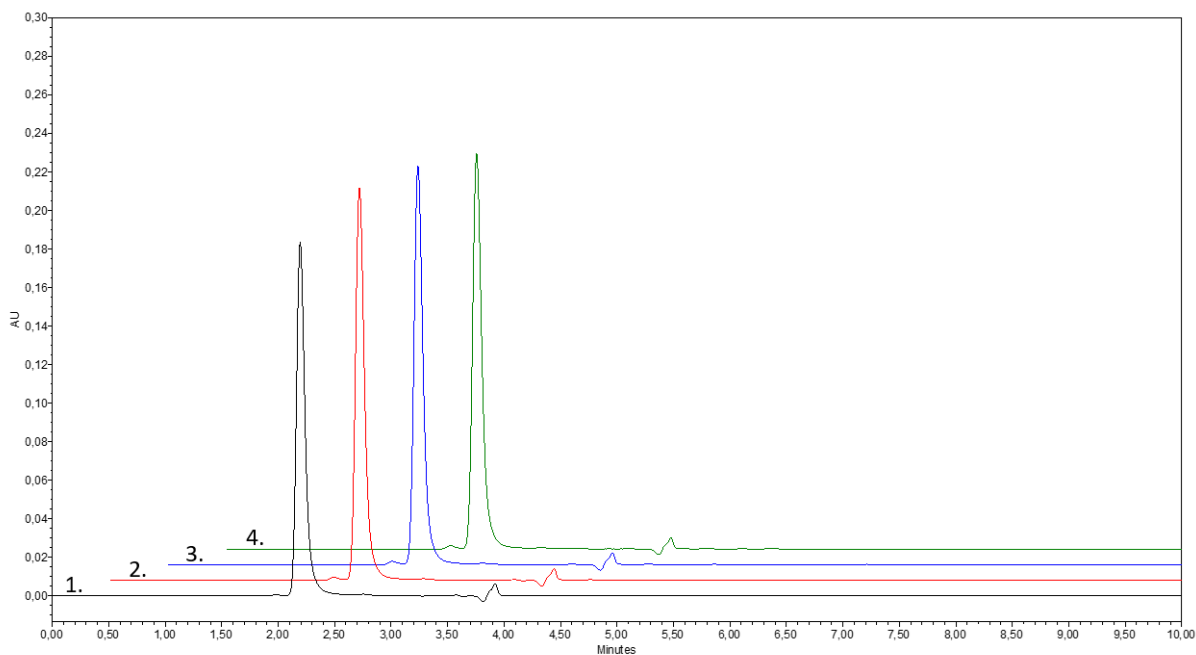
Size exclusion chromatography (SEC) traditionally uses larger particle sizes (3–10 µm) to separate biomolecules based on hydrodynamic size. However, sub-2 µm particles (e.g., 1.7 µm or 1.9 µm) offer significant advantages, particularly in UHPLC-SEC (Ultra-High-Performance SEC) applications.

Advantages of Sub-2 µm Particles:

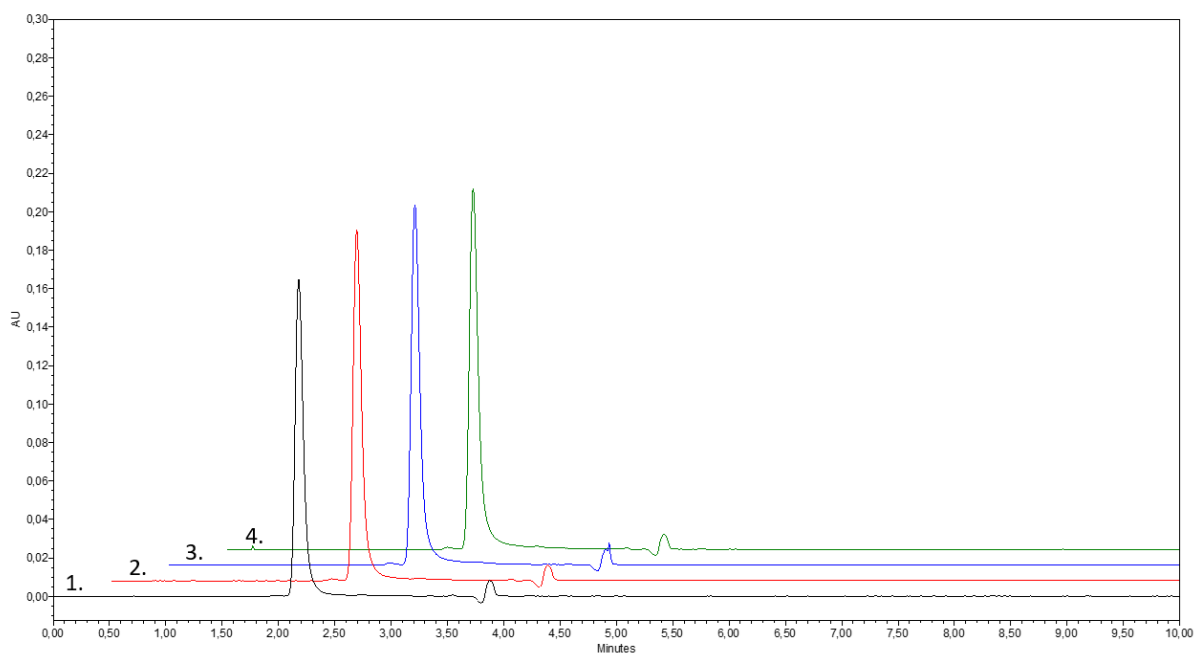
- Higher Resolution: Improved separation of closely sized biomolecules.
- Faster Analysis: Shorter diffusion paths allow for higher flow rates without compromising resolution.
- Increased Sensitivity: Reduced peak broadening enhances signal intensity and detection of low-abundance species.
- Optimized for UHPLC: Designed for high-pressure SEC applications, ensuring efficient and reproducible separations.

ID No.:	0012
Analytes:	Adalimumab and Impurities NOTE: Impurities include high molecular weight mass (HMWM), such as dimers, and low molecular weight mass (LMWM) fragments.
Material:	ReproSil 200 SEC, 1.9 µm
Column Dimension:	150 mm x 4.6 mm
Part No. (P/N):	r219.sec.s1546 (Standard Stainless Steel) r219.sec.b1546 (Bioinert Stainless Steel)
Elution Type:	Isocratic
Eluent A:	50mM KH ₂ PO ₄ +100 mM NaCl (pH = 6.8)
Flow Rate:	0.5 ml/min
Temperature:	35 °C
Injection:	20 µl of 0.4 mg/ml
Detection:	UV-Detection @280 nm

1a) r219.sec.s1546 (ReproSil 200 SEC, 1.9 μm , Standard Stainless Steel)

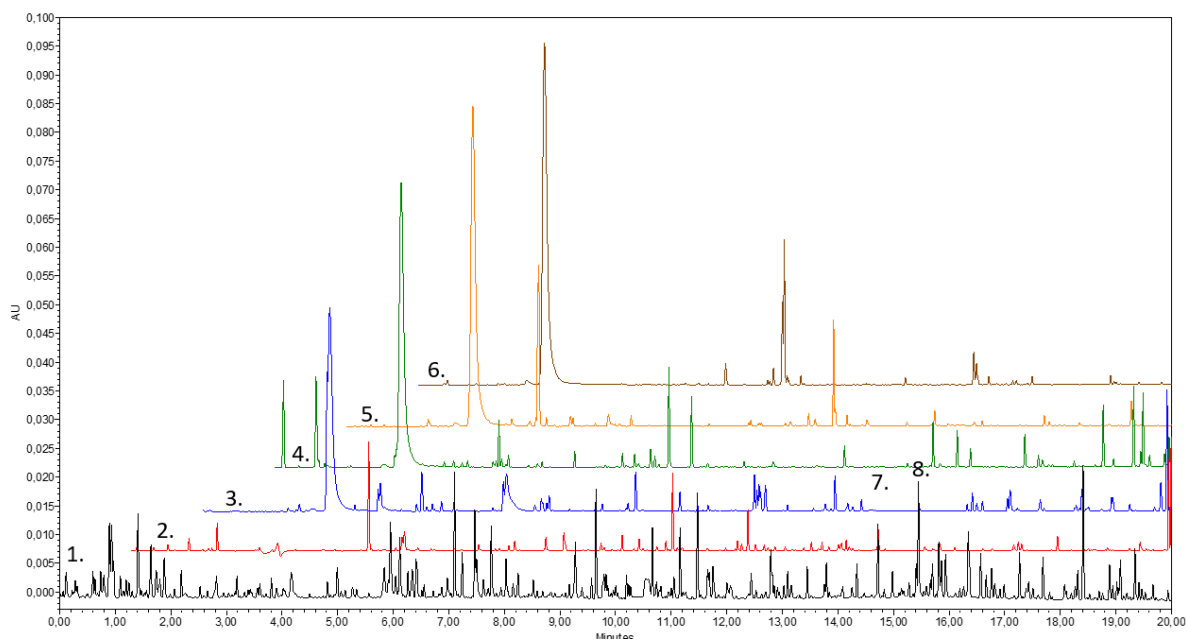


1b) r219.sec.b1546 (ReproSil 200 SEC, 1.9 μm , Bioinert Stainless Steel)

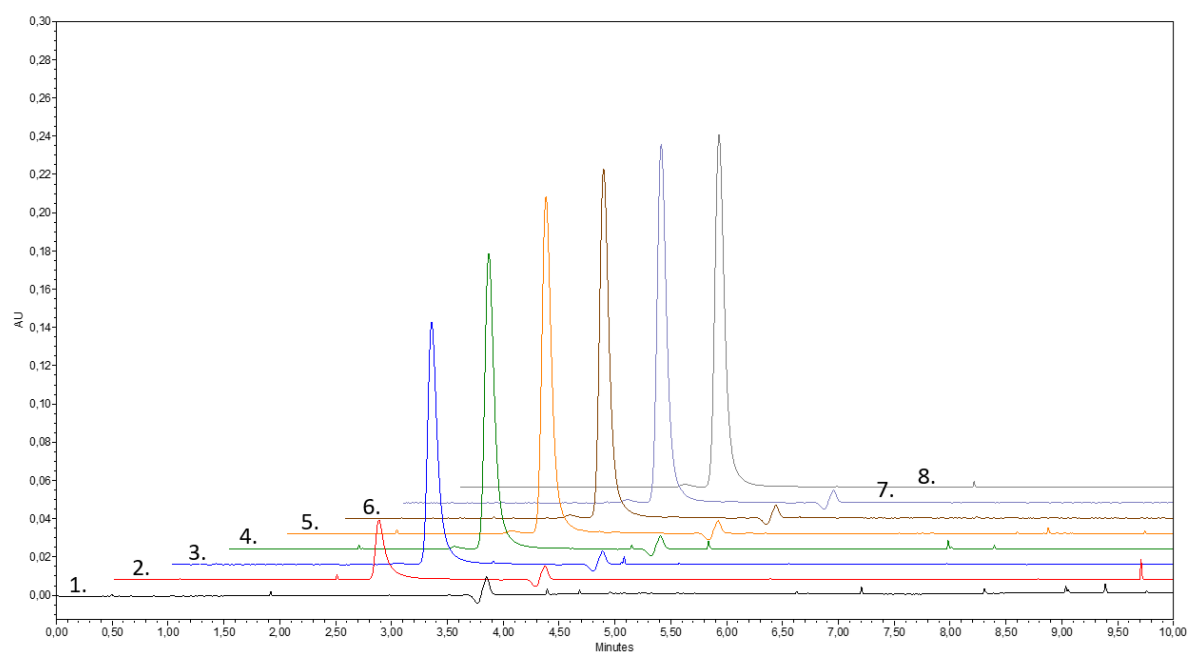


ID No.:	0012
Analytes:	Adalimumab and Impurities NOTE: Impurities include high molecular weight mass (HMWM), such as dimers, and low molecular weight mass (LMWM) fragments.
Materials:	ReproSil 200 SEC-2, 1.9 µm
Column Dimension:	150 mm x 4.6 mm
Part No. (P/N):	r219.sec2.s1546 (Standard Stainless Steel) r219.sec2.b1546 (Bioinert Stainless Steel)
Elution Type:	Isocratic
Eluent A:	50mM KH ₂ PO ₄ +100 mM NaCl (pH = 6.8)
Flow Rate:	0.5 ml/min
Temperature:	35 °C
Injection:	20 µl of 0.4 mg/ml
Detection:	UV-Detection @280 nm

2a) r219.sec2.s1546 (ReproSil 200 SEC-2, 1.9 μ m, Standard Stainless Steel)



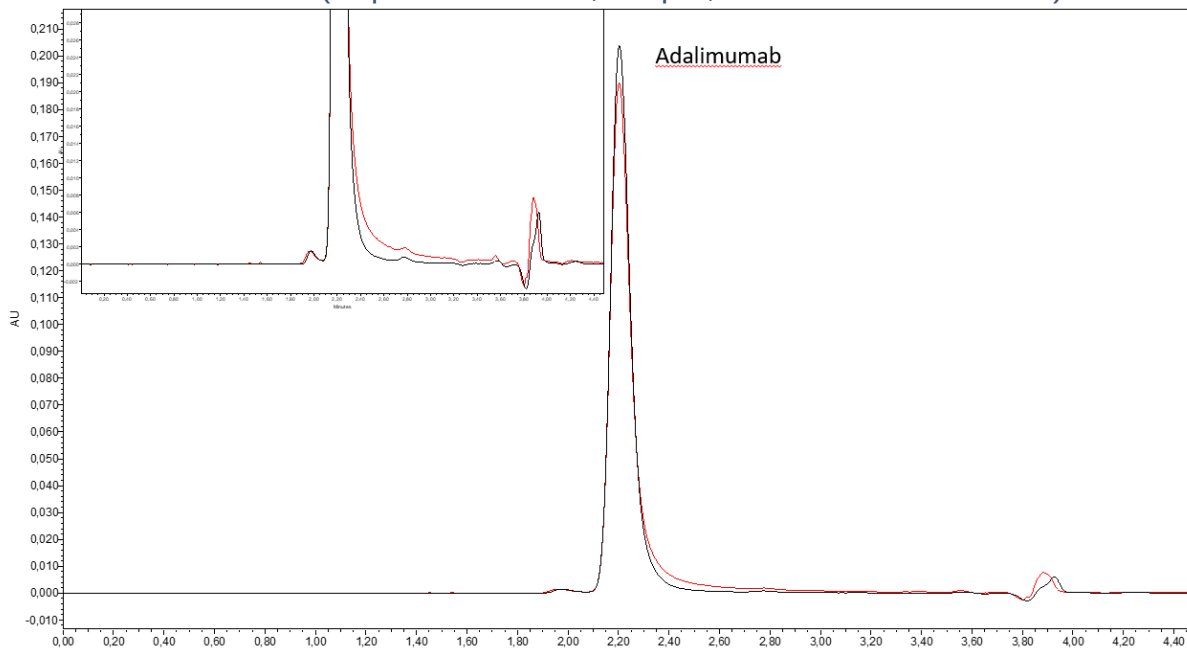
2b) r219.sec2.b1546 (ReproSil 200 SEC-2, 1.9 μ m, Bioinert Stainless Steel)



ID No.:	0012
Analytes:	Adalimumab and Impurities NOTE: Impurities include high molecular weight mass (HMWM), such as dimers, and low molecular weight mass (LMWM) fragments.
Materials:	ReproSil 200 SEC, 1.9 µm ReproSil 200 SEC-2, 1.9 µm
Column Dimension:	150 mm x 4.6 mm
Part No. (P/N):	r219.sec.s1546 (Standard Stainless Steel) r219.sec.b1546 (Bioinert Stainless Steel) r219.sec2.s1546 (Standard Stainless Steel) r219.sec2.b1546 (Bioinert Stainless Steel)
Elution Type:	Isocratic
Eluent A:	50mM KH ₂ PO ₄ +100 mM NaCl (pH = 6.8)
Flow Rate:	0.5 ml/min
Temperature:	35 °C
Injection:	20 µl of 0.4 mg/ml
Detection:	UV-Detection @280 nm

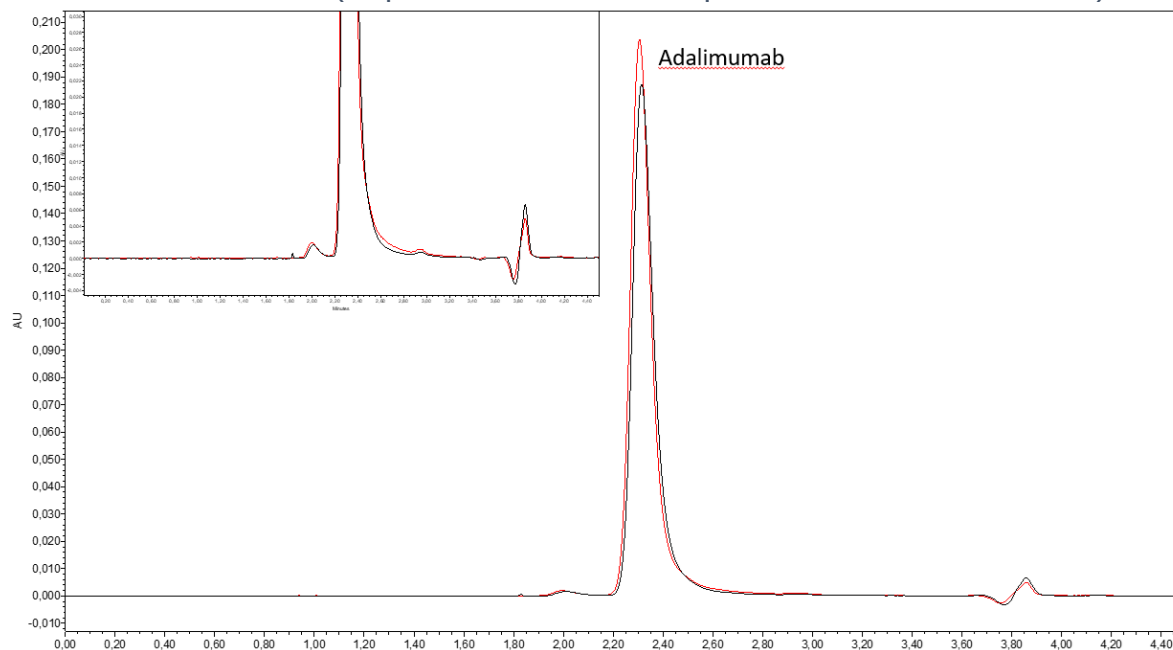
3a)

- r219.sec.s1546 (ReproSil 200 SEC, 1.9 µm, Standard Stainless Steel)
- r219.sec.b1546 (ReproSil 200 SEC, 1.9 µm, Bioinert Stainless Steel)



3b)

- r219.sec2.s1546 (ReproSil 200 SEC-2, 1.9 µm, Standard Stainless Steel)
- r219.sec2.b1546 (ReproSil 200 SEC-2, 1.9 µm, Bioinert Stainless Steel)



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Analytes:	1. Thyroglobulin	660 kDa
	2. IgG	150 kDa
	3. BSA	67 kDa
	4. Myoglobin	17 kDa
	5. Uracil	0.1 kDa

(= BEH200 SEC Protein Standard Mix, Waters, P/N 186006518)

Materials: ReproSil 200 SEC, 1.9 µm
ReproSil 200 SEC-2, 1.9 µm

Column Dimension: 150 mm x 4.6 mm

Part No. (P/N): r219.sec.s1546 (Standard Stainless Steel)
r219.sec.b1546 (Bioinert Stainless Steel)
r219.sec2.s1546 (Standard Stainless Steel)
r219.sec2.b1546 (Bioinert Stainless Steel)

Elution Type: Isocratic

Eluent A: 50mM KH₂PO₄ +500 mM NaCl (pH = 6.8)

Flow Rate: 0.5 ml/min

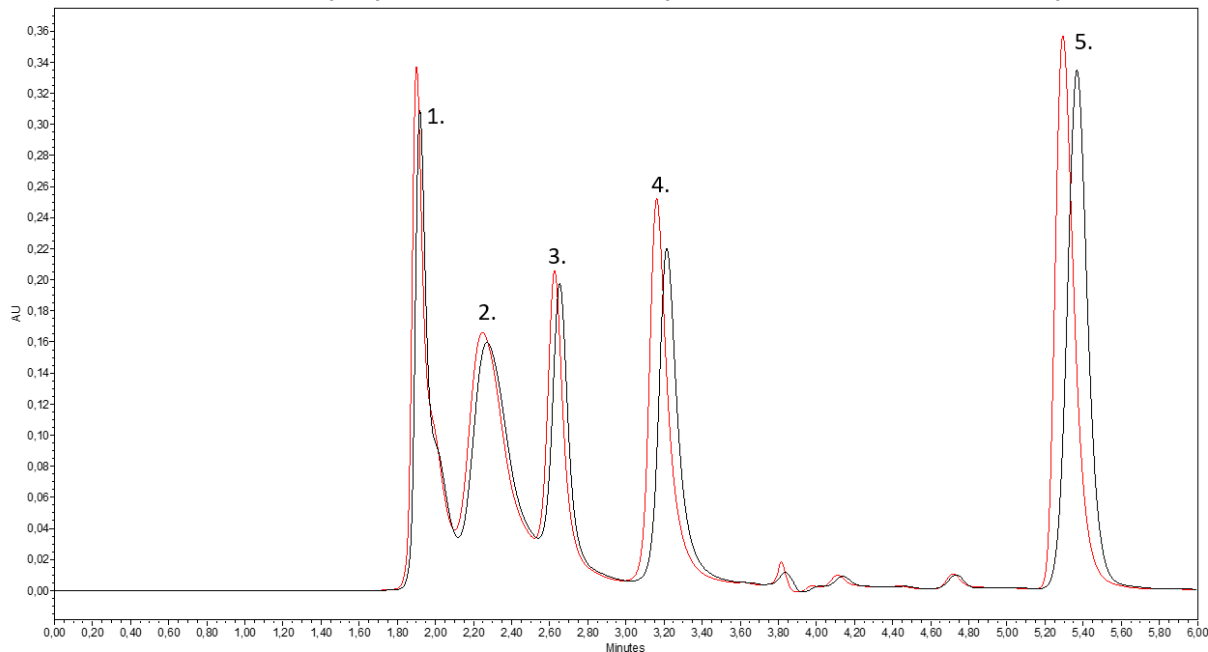
Temperature: 35 °C

Injection: 20 µl in Eluent A, concentrations see above.

Detection: UV-Detection @280 nm

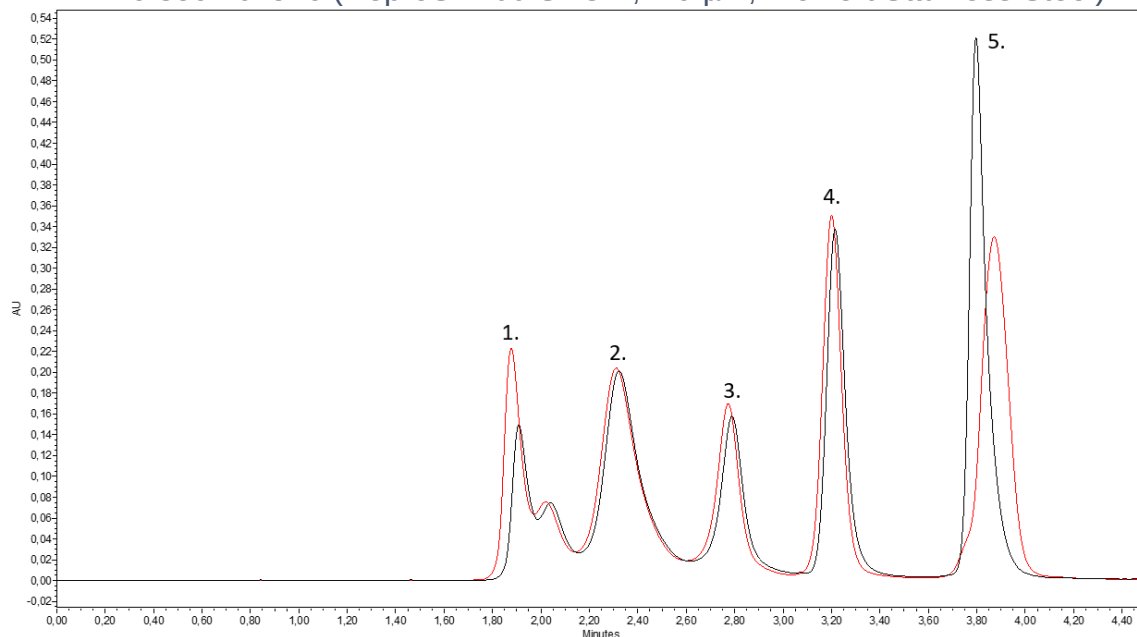
4a)

- r219.sec.s1546 (ReproSil 200 SEC, 1.9 μ m, Standard Stainless Steel)
- r219.sec.b1546 (ReproSil 200 SEC, 1.9 μ m, Bioinert Stainless Steel)



4b)

- r219.sec2.s1546 (ReproSil 200 SEC-2, 1.9 μ m, Standard Stainless Steel)
- r219.sec2.b1546 (ReproSil 200 SEC-2, 1.9 μ m, Bioinert Stainless Steel)



Data courtesy of Chromicent, Berlin (Germany).

Dr. Maisch products are available globally. For more information contact your Dr. Maisch representative at info@dr-maisch.com.