Analysis of intact proteins by MicroLC-MS

The demand for therapeutic proteins is growing. It has become even more important to strictly monitor the amounts and purity of protein during production and for greater characterisation for clinical uses. Often only small sample amounts are available. Therefore, the use of MicroLC columns and systems is essential in order to detect even very small amounts present. Protein standards are an important tool for testing LC-MS system suitability and intact protein mass measurement.
In this application the protein mix MSRT2 from Sigma-Aldrich was analysed using an YMC-Triart Bio C4 capillary column.

The MSRT2 standard includes the following proteins, which were detected:

- Ribonuclease B (1)
- Lysozyme (2)
- Insulin (3)
- Transferrin (4)
- Bovine serum albumin (5)
- Trypsin inhibitor (6)
- β-Lactoglubolin A (7)
- Lactate dehydrogenase (8)

This application shows that the measurement of protein mixtures is possible using MicroLC-MS.

Table 1: chromatographic conditions

Column:	YMC-Triart Bio C4 (3 µm, 30 nm) 100 x 0.3 mm ID with 1/16" endfittings
Part No.:	TB30S03-10H0AU
Eluent:	A) water + 0.1 % FA B) acetonitrile + 0.1 % FA
Gradient:	20 % B (0–2.5 min), 20 %–60 % B (2.5–11 min), 60 %–100 % B (11–12 min), 100 % B (12–13 min), 20 % B (13–15 min)
Flow rate:	15 µL/min
Temperature:	50 °C
Detection:	Shimadzu LCMS-9030 QTOF
Injection:	0.1 µL
Sample:	MSRT2 standard proteins à 100 µg (Sigma-Aldrich), resuspended in 500 µL 0.1 % FA in water; concentration 0.2 µg/µL
LC system:	Shimadzu Nexera Mikros

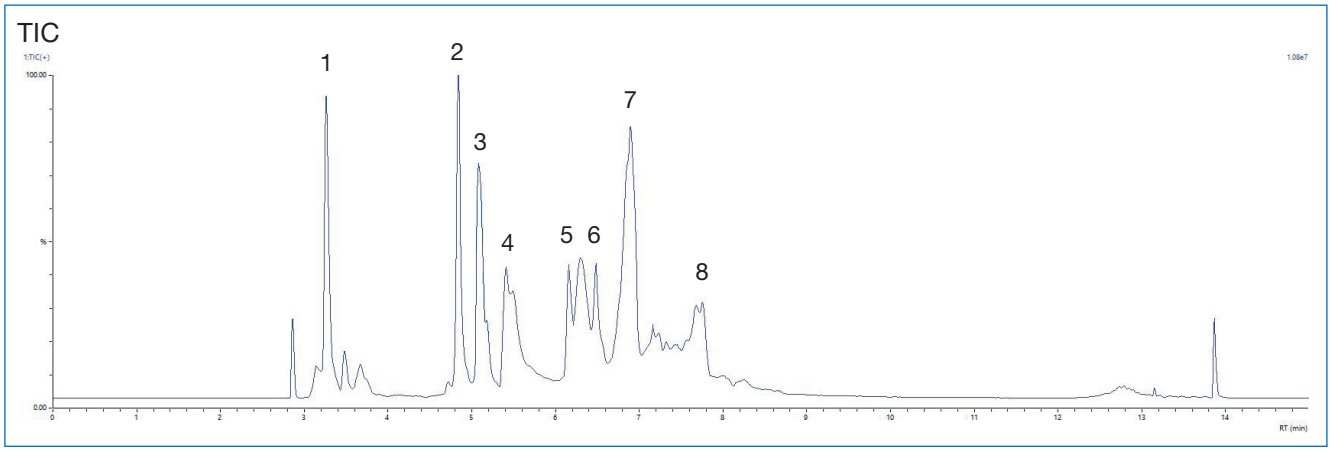

Figure 1: TIC of the MSRT2 protein standard using YMC-Triart Bio C4.

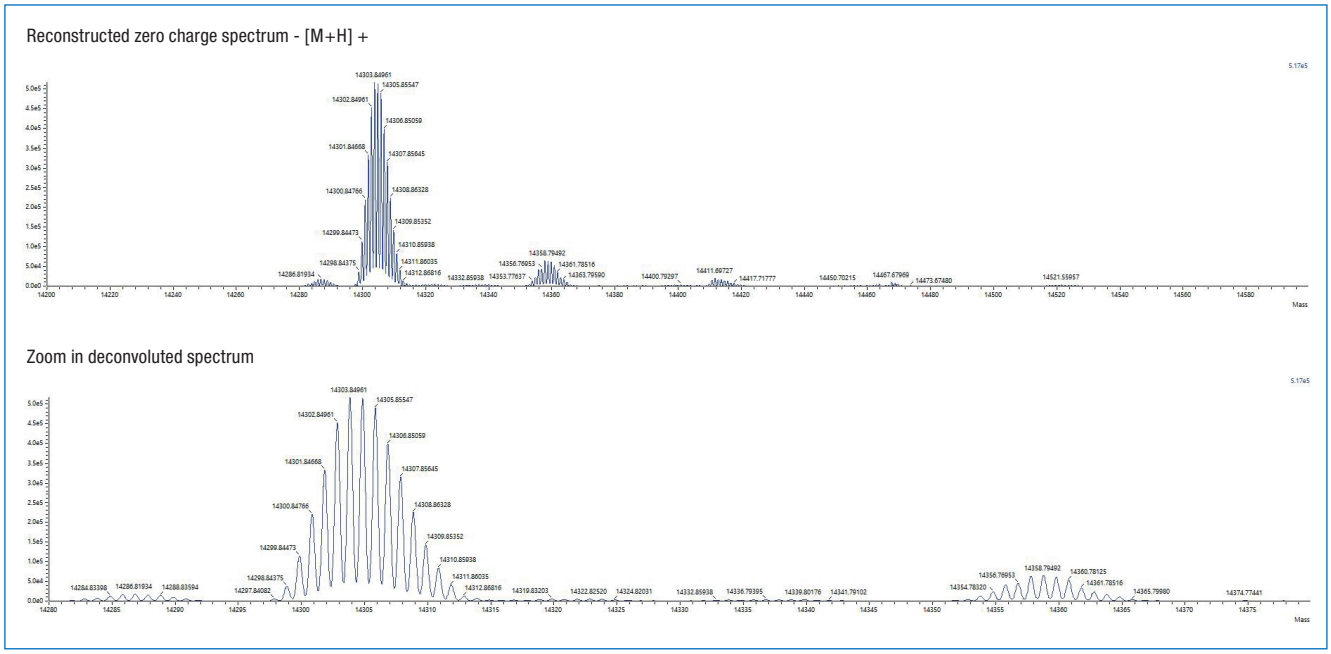

Figure 2: Deconvoluted spectrum of lysozyme (2) at the retention time of 4.85 min.

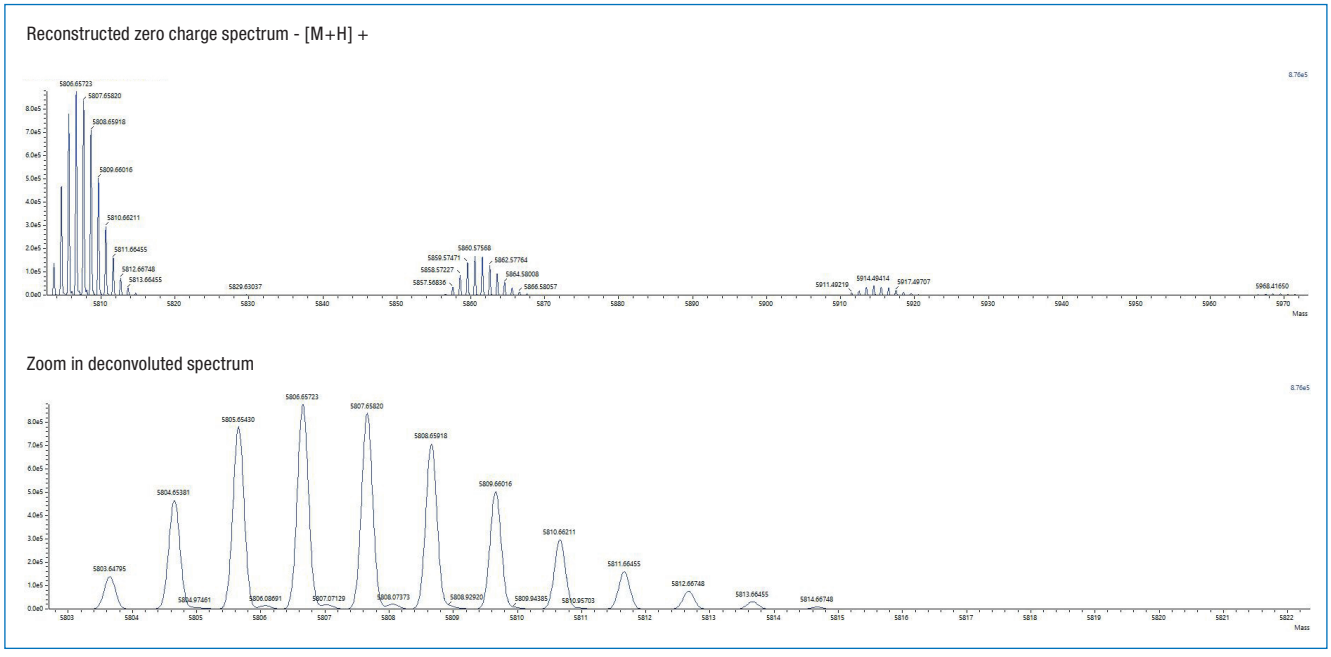

Figure 3: Deconvoluted spectrum of insulin (3) at the retention time of 5.1 min.