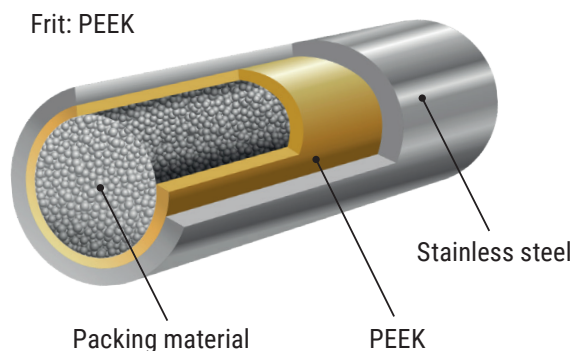


LC-HRMS analysis of the antisense oligonucleotide Mipomersen (Kynamro®)

Antisense oligonucleotides (ASOs) have an outstanding therapeutic potential, since they are used for the therapy of genetic diseases. Due to the specific binding of an ASO to the complementary sequence of the RNA of a target protein, its formation is suppressed. The ASO Mipomersen (commercially available as Kynamro®) was developed to treat homozygous familial hypercholesterolemia by inhibiting the production of the low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) and therefore reducing their concentration in blood.

In this application note, Mipomersen is used as a model for the analysis of ASOs using LC-HRMS according to Y. Sun et al. [1]. They worked with a YMC-TriartC8 PEEK-lined column which is an excellent choice for oligonucleotide analysis.

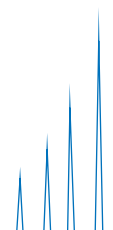
Its biocompatible hardware consists of a stainless steel body which has an inner layer of PEEK and is equipped with PEEK frits. Because of its special design, this hardware type is not just usable for high pressure, but it guarantees excellent peak shapes, high sensitivity and no carry-over effects.

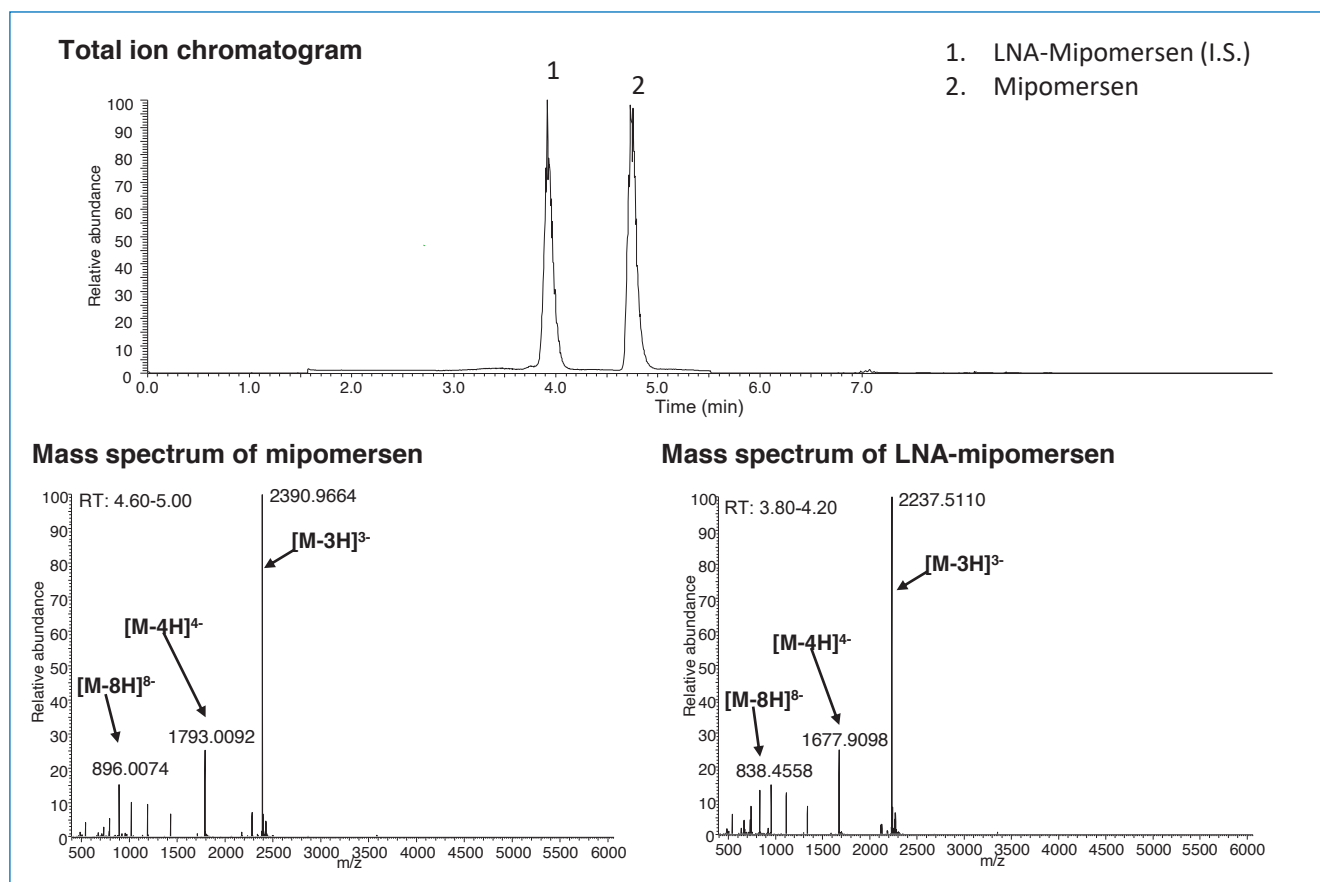


Column:	YMC-Triart C8 PEEK-lined (1.9 μ m, 12 nm) ¹ 100 x 2.1 mm
Part No.:	ID TO12SP9-10Q1PTP
Eluent:	A) water/triethylamine/HFIP ² (100/0.4/2; triethylamine 28.0mM, HFIP 135.8mM) B) methanol/triethylamine/HFIP (100/0.4/2)
Gradient:	[Sample separation step] 10–40%B (0–5.0 min) [Column wash steps] 40–70 %B (5.0–5.1 min), 70 %B (5.1–7.0 min), 70–10 %B (7.0–7.1 min), 10 %B (7.1–8.0 min), 10–90 %B (8.0–8.1 min), 90 %B (8.1–9.0 min), 90–10 %B (9.0–9.1 min), 10 %B (9.1–10.0 min), 10–90 %B (10.0–10.1 min), 90 %B (10.1–11.0 min), 90–10 %B (11.0–11.1 min)
Flow rate:	0.3 mL/min
Temperature:	50 °C
Injection:	10 μ L (1000 ng/mL)
System :	LC) Vanquish Binary Pump H system HRMS) Orbitrap HRMS Q Exactive Plus

*1 Prewash the column prior to the first use with water/methanol/phosphoric acid (70/30/0.1) for 1 hour

*2 1,1,1,3,3,3-hexafluoro-2-propanol





Courtesy of Y. Sun, National Institute of Health Sciences

Reference:

- [1] Y. Sun et al, Development of a bioanalytical method for an antisense therapeutic using high-resolution mass spectrometry, *Bioanalysis*, 2020 NOV 26, doi: 10.4155/bio-2020-0225.