

# HPLC and LC-MS Method Development and Performance Monitoring of oligonucleotides with oligo standard-6

## Analysis of Oligonucleotides

- Recently, oligonucleotides (Oligos) have proven their importance in diagnostic and therapeutic applications.
- Currently, 15 oligonucleotide drugs have been approved for the treatment of a range of rare diseases in various countries.
- These approved oligonucleotide drugs commonly are around 20 nucleotides or slightly longer with a molecular weight of about 6-9 kDa.
- There are many established techniques to analyze and characterize oligonucleotides, including capillary gel electrophoresis (CGE), ion exchange chromatography (IEX), and ion pair reversed phase liquid chromatography (IP-RPLC).
- We are developing a Performance Standard mix of oligos that would help in the verification of the system suitability for the conditions employed in the oligonucleotide analysis.

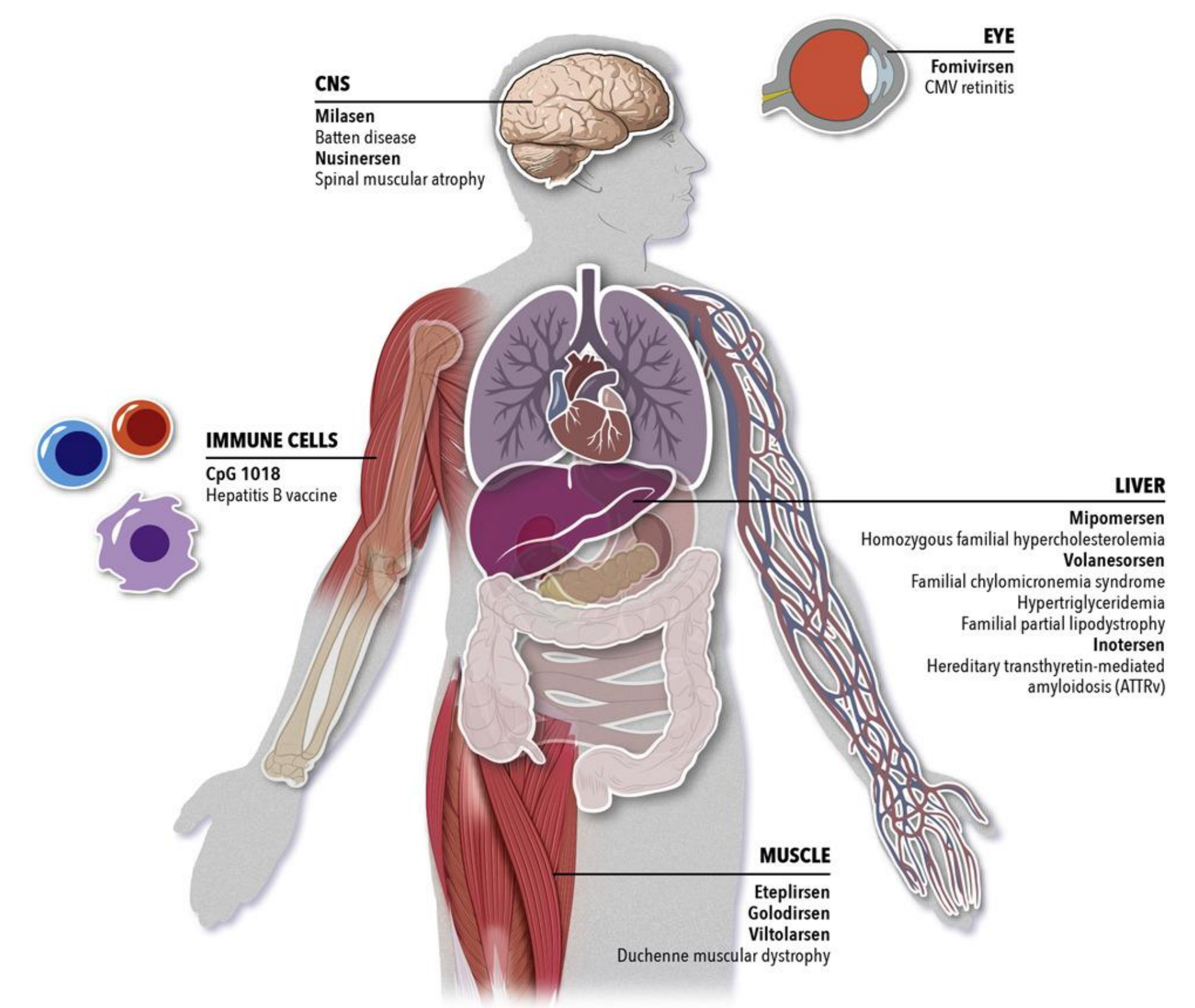
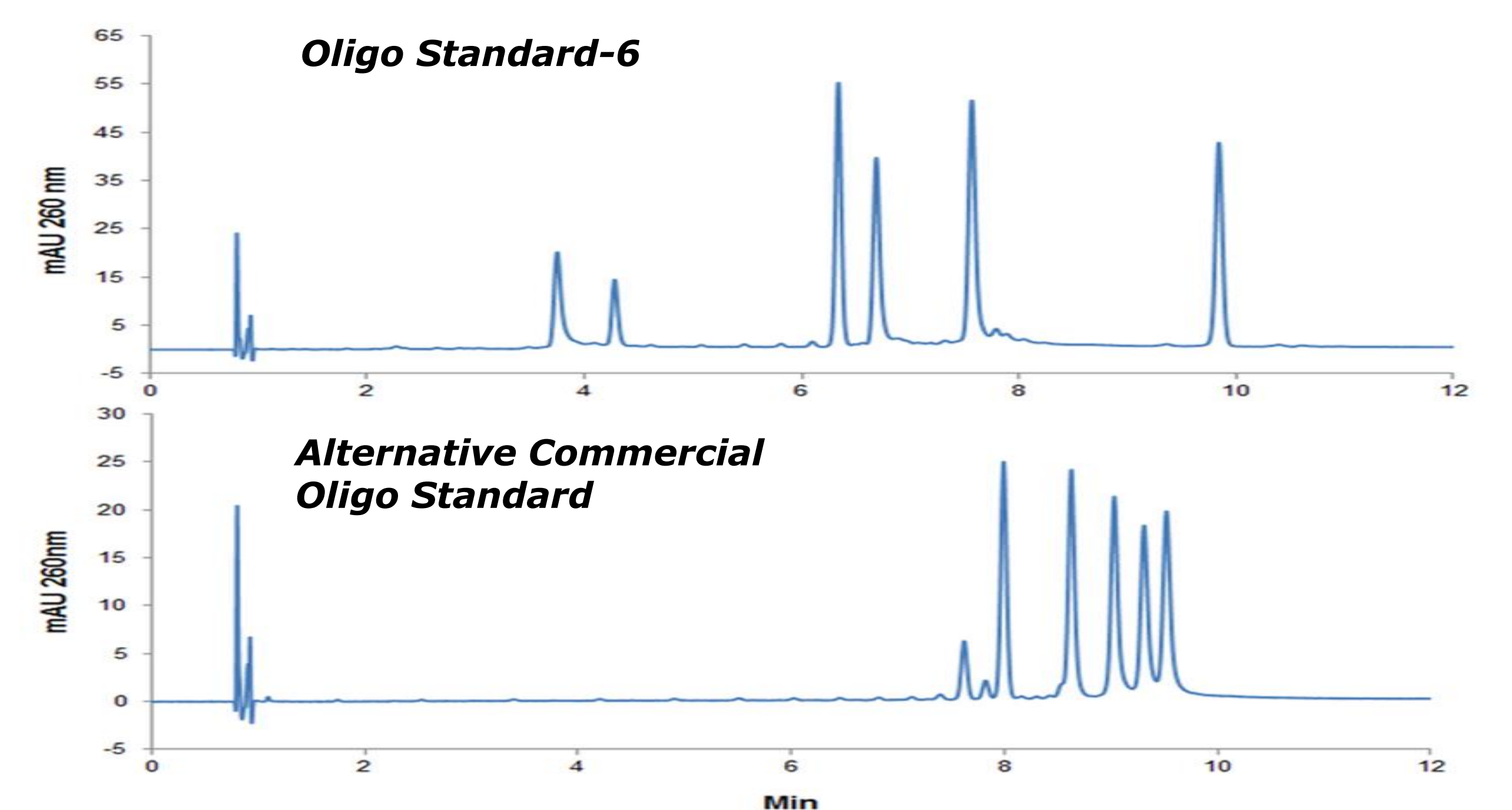


Image: Clinical Applications of Single-Stranded Oligonucleotides: Current Landscape of Approved and In-Development Therapeutics, *Molecular Therapy*, 2021, 540-554.

## Oligonucleotides Performance Standard-6 Mix: Design

- Generally, liquid chromatography of Oligos is challenging due to the similarity of oligonucleotide structures, very polar characteristics, presence of truncated and/or modified Oligos, ease of self-association into a variety of conformations, and affinity for certain surfaces.
- Most of the standards for HPLC columns are based on small organic molecules or poly-T sequences at different sizes, which do not always highlight variations impacting the behaviors of oligonucleotides.
- The Supelco Oligo Standard-6 Performance mix was designed for HPLC/UPLC and/or LC-MS, with the aim of providing a relevant and versatile solution for the analysis of oligonucleotides in various conditions.

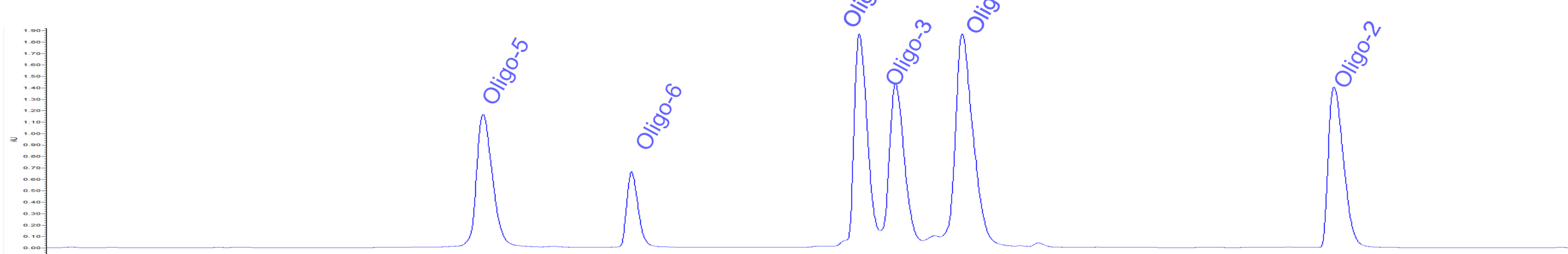


IP-RPLC: LC-UV Oligo performance standard separation on BIOshell A160 Peptide C18 2.7 $\mu$ m, 150 x 2.0 mm column. Mobile phase A: 95:5 50 mM TEAA/acetonitrile, B: 85:15 50 mM TEAA/acetonitrile.

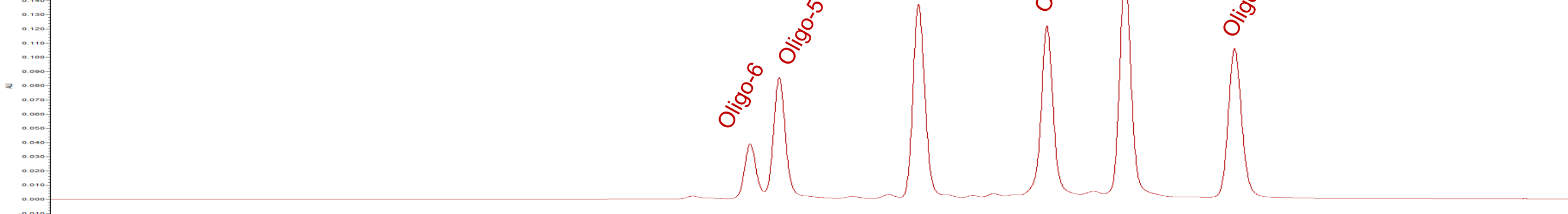
## Oligonucleotides Performance Standard-6 Mix: Applications

### Comparing & maintaining HPLC Systems

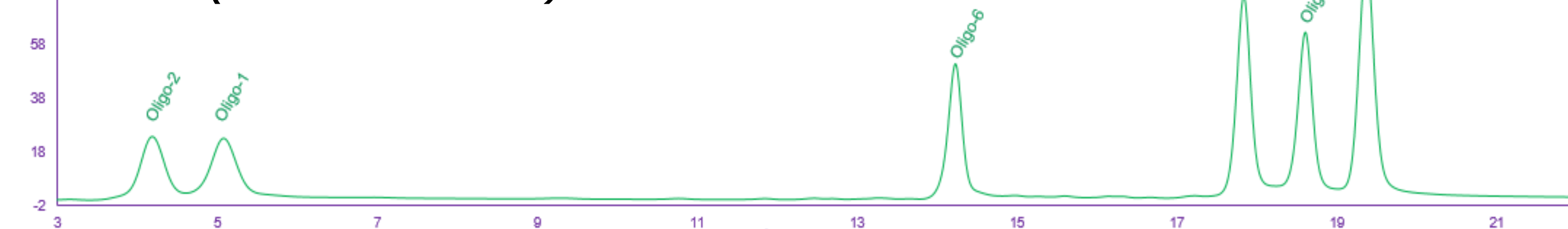
**BIOshell A160 PeptideC18 2.7 $\mu$ m- 150x2mm**  
**IP-RP-HPLC**



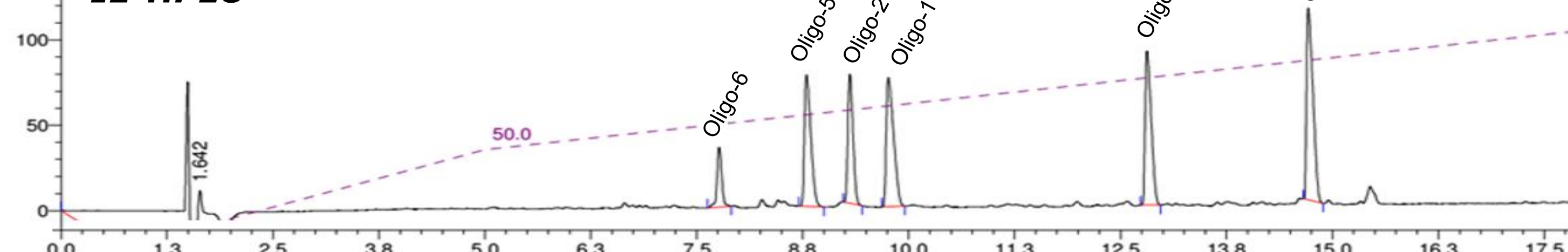
**Polymeric C18 300Å 3 $\mu$ m- 50x2.1mm**  
**IP-RP-HPLC**



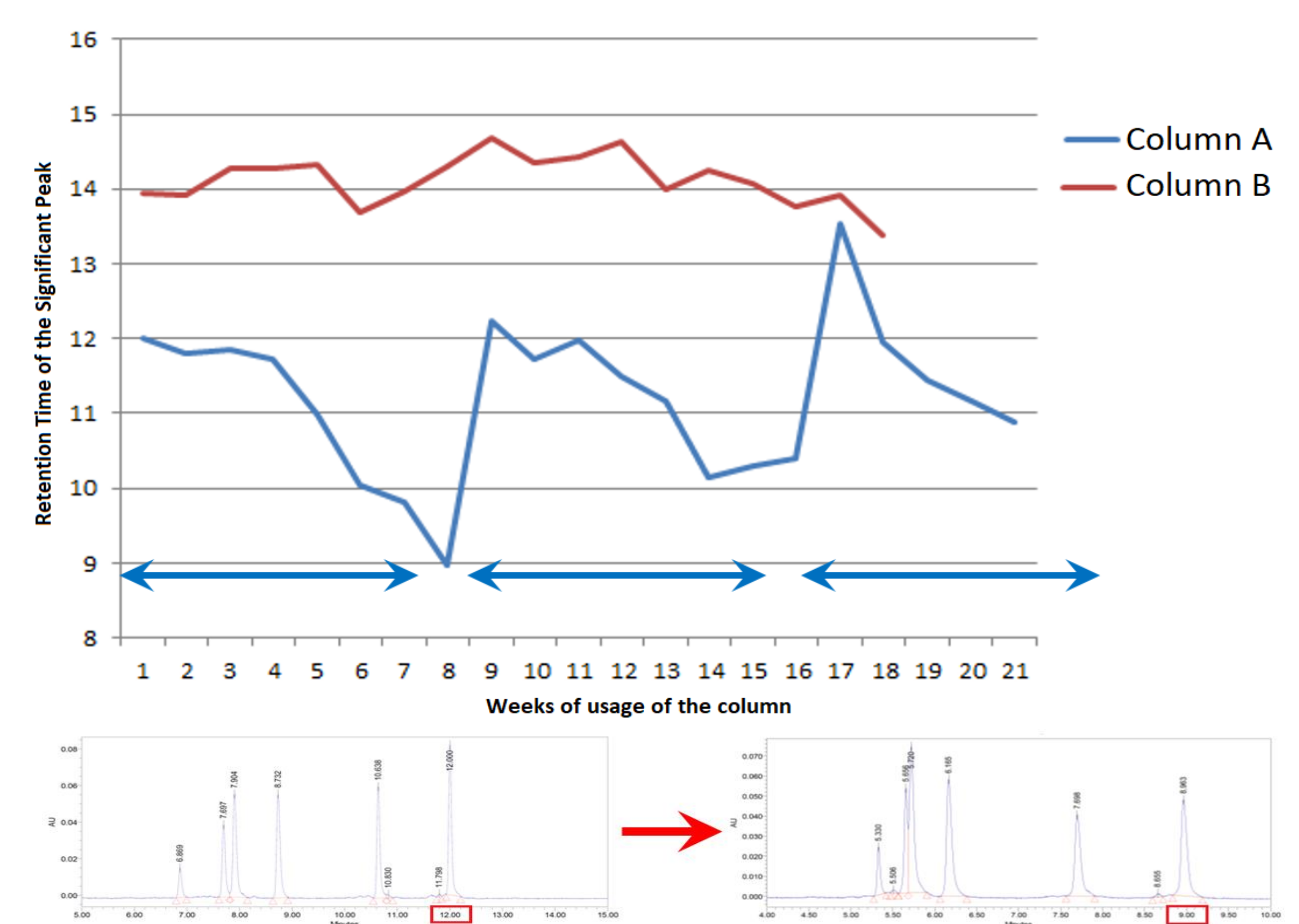
**SeQuant ZIC-HILIC 200Å 3.5 $\mu$ m- 150x2.1mm**  
**HILIC (Ammonium formate)**



**Anion exchange column, 8 $\mu$ m-250x4mm**  
**IE-HPLC**



### Testing HPLC Columns Lifespan



- Monitoring of the life span of 2 different IE-HPLC columns used in QC with Oligo Standard-6 mix.

- The performance is associated with a diminution of the Retention times (RT). When the last peak RT elutes at 9-10 min, the column was not fit for purpose anymore.

- This testing system allowed to quantify the degradation of the columns and show column B to be much superior to column A