SHIMADZU

DRUGS OF THE FUTURE
Optimizing Oligonucleotide Analysis

Oligonucleotide therapeutics are small synthetic nucleic acids that can be used to treat different types of diseases. They act by modulating either gene expression or protein function by binding specific genetic sequences or target proteins.[1,2] Due to their ability to tackle targets that were considered undruggable, oligonucleotides represent a key cornerstone of the future of personalized medicine. This infographic presents the different types of therapeutic oligonucleotides and highlights some innovative solutions for their analysis.

A growing market

Since the first approval of a therapeutic oligonucleotide in 1998,[3] the field has grown significantly and has become an emerging area in drug development.

13 therapeutic oligonucleotides obtained approval between 1998 and 2021.[1]

44 companies with therapeutic oligonucleotides on the market or in late clinical development.

130 clinical trials (phase II or III) involving 80 oligonucleotides.

Global market for oligonucleotide therapeutics[4]

Market forecast to grow at CAGR of

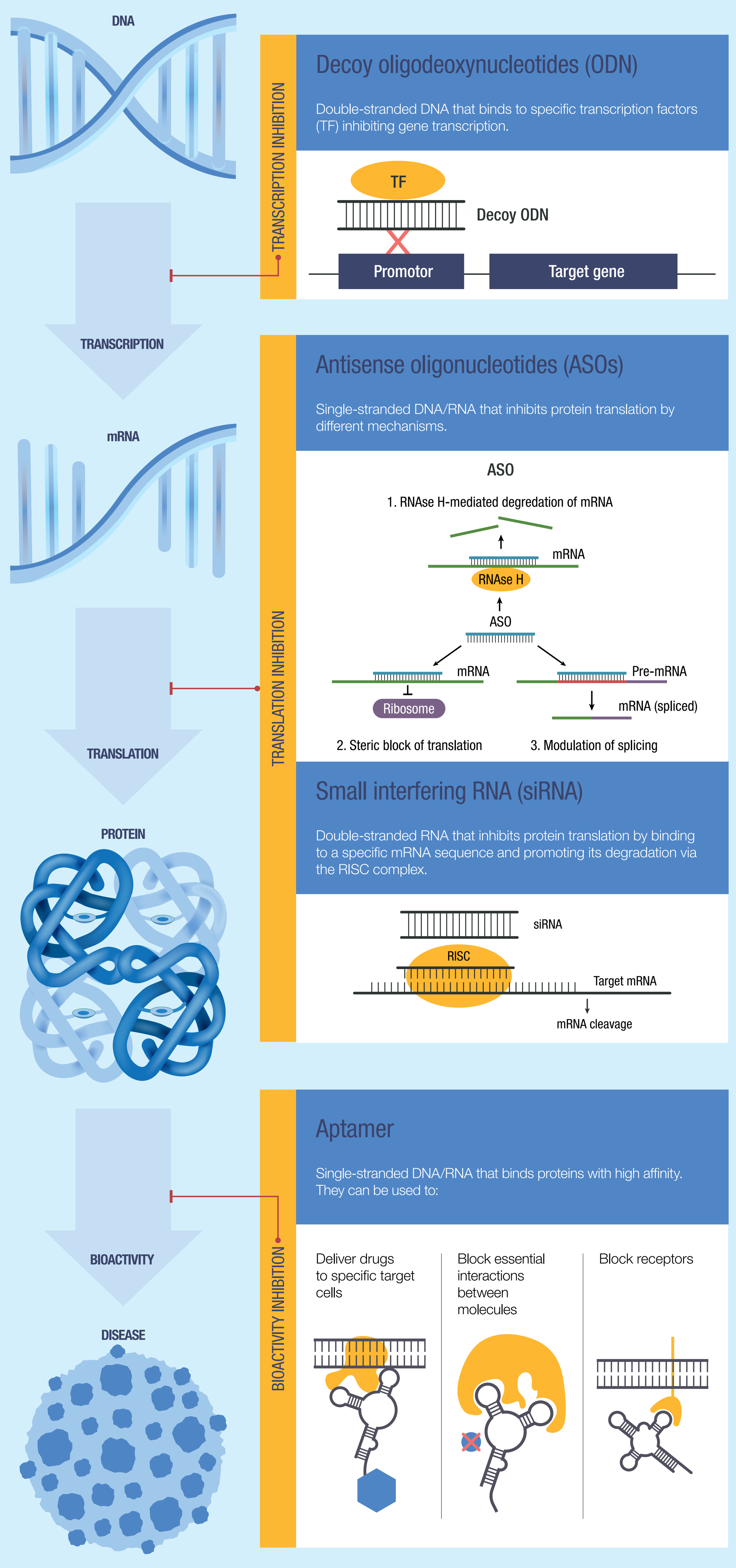
16.7%

USD **3.68** Billion — 2021

USD **10.97** Billion — 2028

Types of therapeutic oligonucleotides

Therapeutic oligonucleotides are short fragments of single- or double-stranded RNA or DNA that act on different stages of pathological gene expression.[1,5]



Tools for oligonucleotide manufacturing

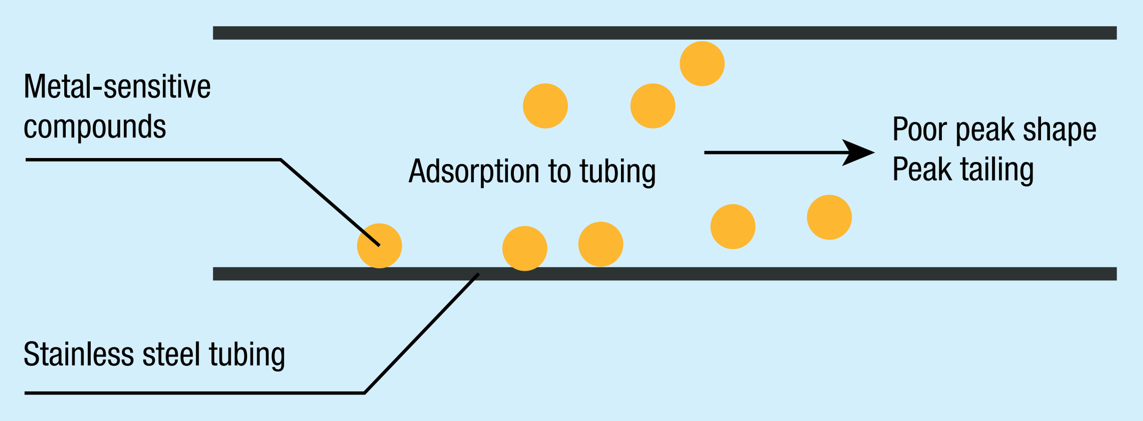
The manufacturing workflow for therapeutic oligonucleotides requires high resolution tools. High and ultrahigh performance liquid chromatography (HPLC and UHPLC) have proven to be effective for fast and efficient purification, analytical characterization and quality control.[6,7]

SYNTHESIS	CLEAVAGE AND DEPROTECTION	PURIFICATION	BUFFER EXCHANGE	ANALYSIS
Solid-supported synthesis using the phosphoramidite method. Computer-controlled and fully automated	Removal of solid support and collection of crude oligonucleotides in aqueous ammonia solution (ammonolysis)	Removal of impurities using: • Reversed phase HPLC • Anion exchange chromatography (AEXC) • Hydrophobic interaction chromatography (HILIC)	Removal of salts (elution buffer) and concentration Precipitation, tangential flow filtration (TFF), lyophilization	Measurement of purity, recovery, and identity using: • HPLC/UHPLC • Liquid chromatography and mass spectroscopy (LC-MS)

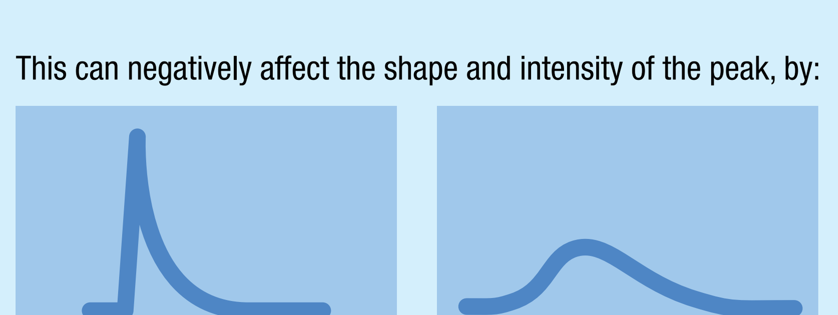
Challenges for HPLC systems

Stainless steel is commonly used in HPLC systems because it is pressure-proof and corrosion-resistant. However, since it is neither bio-inert nor bio-compatible, it can interact with compounds that contain phosphate groups. This can cause oligonucleotides to adsorb to the metal surface making results unreliable.

Metal adsorption represents a critical challenge when analyzing oligonucleotides as it decreases the efficiency of quality control processes and can affect drug efficiency and safety.

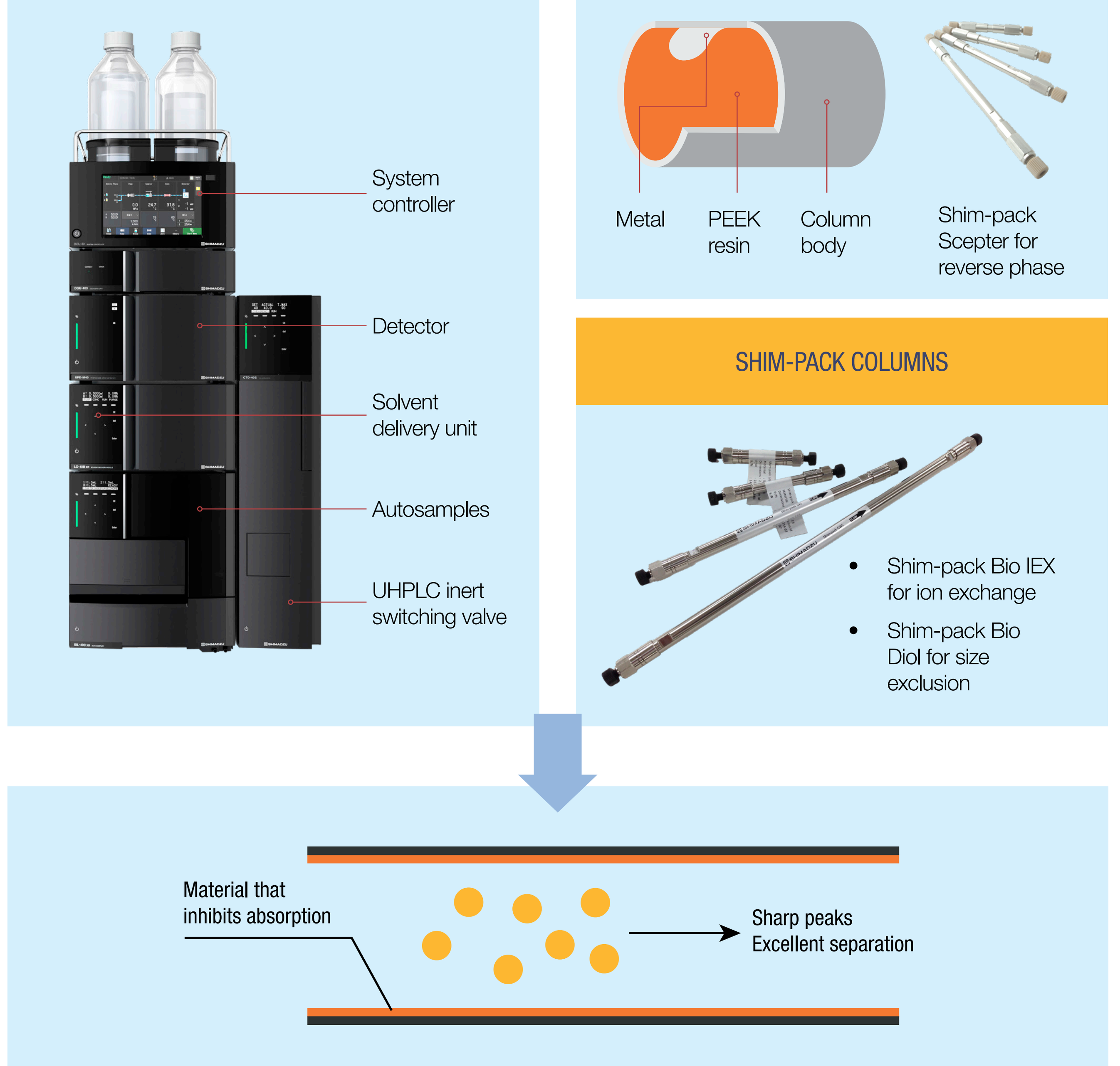


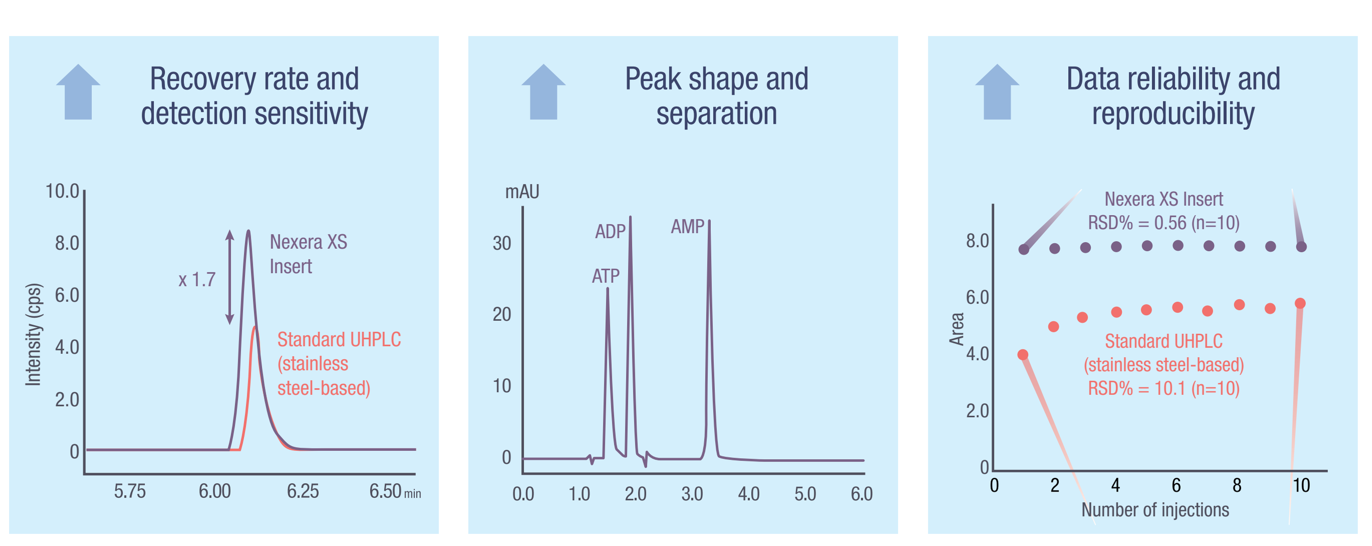
This can negatively affect the shape and intensity of the peak, by:



An innovative UHPLC solution

The Nexera XS inert system is a UHPLC system containing a metal-free flow path that can be combined with metal-free Shim pack columns. These tools allow reliable and accurate quantitation, even for low-concentration samples. In addition to the elevated pressure tolerance, the absence of wetted surfaces offers ultra-high resistance to corrosion and ensures complete inertness of the sample flow path.





Learn more about **innovative solutions for oligonucleotide analysis**

REFERENCES

1. Moumné L, Marie AC, Crouvezier N. Oligonucleotide therapeutics: from discovery and development to patentability. *Pharmaceutics*. 2022;14:260. doi: 10.3390/pharmaceutics14020260
2. Keefe A, Pai S, Ellington A. Aptamers as therapeutics. *Nat Rev Drug Discov*. 2010;9:537-550. doi: 10.1038/nrd3141
3. Roehr B. Fomivirsen approved for CMV retinitis. *J Int Assoc Physicians AIDS Care*. 1998;4:14-16.
4. Global oligonucleotide therapeutics market insights and forecast to 2028. *Market Growth Reports*. Available at: https://www.marketgrowthreports.com/global-oligonucleotide-therapeutics-market-20112037
5. Xiong H, Veedu RN, Diermeier SD. Recent advances in oligonucleotide therapeutics in oncology. *Int J Mol Sci*. 2021;22:3295. doi: 10.3390/ijms22073295
6. Studzińska S, Buszewski B. Evaluation of ultra high-performance liquid chromatography columns for the analysis of unmodified and antisense oligonucleotides. *Anal Bioanal Chem*. 2014;406:7127-7136. doi: 10.1007/s00216-014-7959-5
7. Andrews BI, Antia FD, Brueggemeier SB, et al. Sustainability challenges and opportunities in oligonucleotide manufacturing. *J Org Chem*. 2021;86:49-61. doi: 10.1021/acs.joc.0c02291