WHITE PAPER

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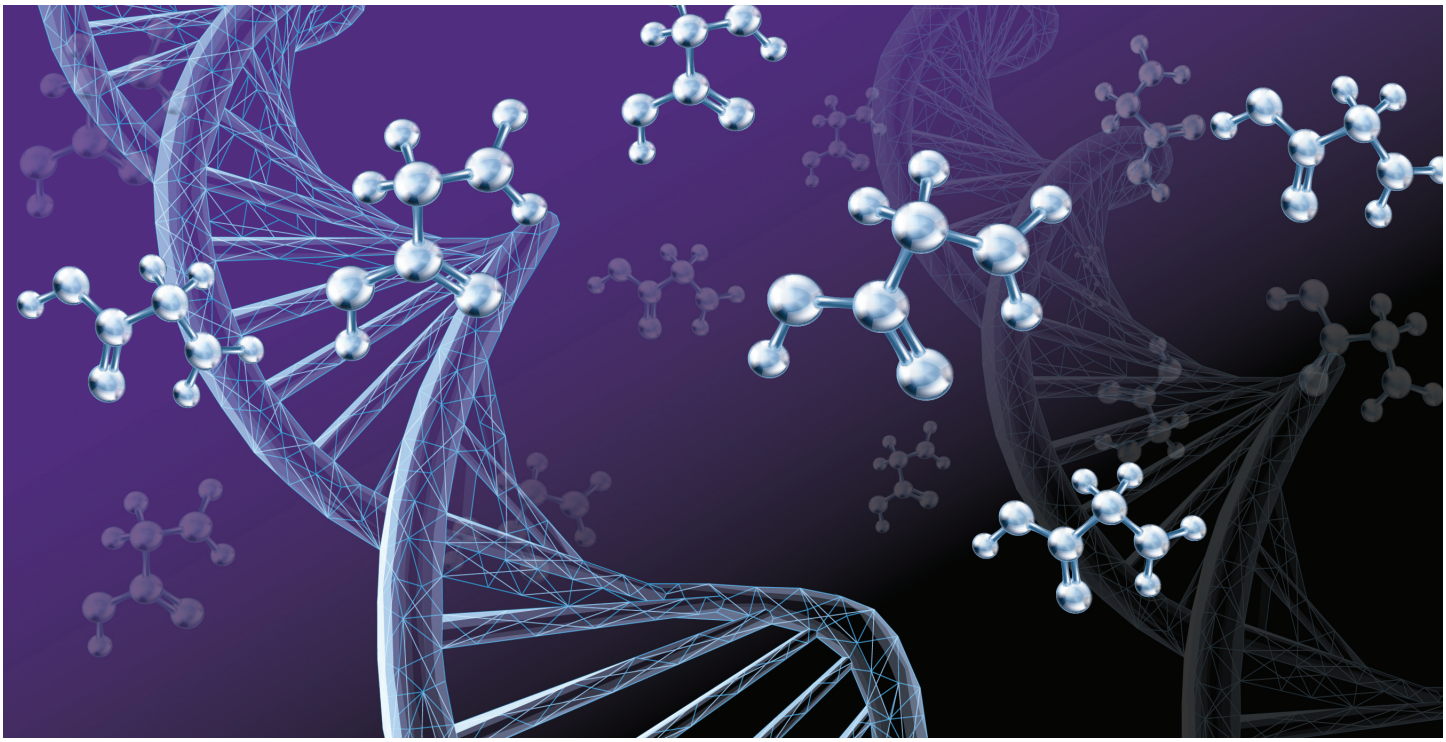
Diverse Analytical Approaches for Complex Oligonucleotides

Oligonucleotide therapies are novel therapeutics which have only been approved for use in the past two decades. Development in this sector is progressing at an increasingly accelerated rate. However, experience in manufacture, analysis, regulatory requirements, and dossier submittal is not widespread. This is partly due to the complex nature of the analytical methods for these drugs which necessitates a high degree of equipment and analyst specialization. Improvements in drug delivery, target specificity, drug stability, manufacturing capacity, analysis complexity and robust regulatory guidelines are required to unlock the true potential of oligonucleotides. This white paper highlights how a lab with experience in oligonucleotide testing can successfully work with challenging compounds and navigate regulatory requirements.

Oligonucleotides are a relatively new class of drugs, composed of natural and synthetic nucleotides. These nucleotide molecules are comprised of either single stranded RNA/DNA (e.g. antisense oligonucleotide (ASO), aptamers, CpG oligodeoxynucleotides) or double stranded RNA (e.g. small interfering RNA (siRNA) and microRNA (miRNA)). Due to their comparative ease of synthesis, cost effectiveness and wide range of applications, ASOs, siRNA and miRNA have become the leading commercial and developmental oligonucleotide therapies. These molecules achieve therapeutic effects through RNA interference, degradation or splice-modulating pathways. Oligonucleotides, originally designed to treat rare genetic diseases, are now being developed to treat a wide range of common conditions including neurodegenerative disorders, respiratory illnesses, cancers, diabetes and hemophilia.[1] The effectiveness and ability to treat previously "undruggable" conditions has greatly increased the demand for novel oligonucleotide therapies. Consequently, the ability to accurately analyze and characterize these products in a cost-effective and timely manner has become of utmost importance.



Biggest Trends in Oligonucleotides

Initially designed for targeting of rare genetic diseases, the oligonucleotide focus has shifted recently to large populations of patients affected by oncology, cardiovascular, hepatic, metabolic and infectious diseases. Traditionally, the most common technique employed for oligonucleotide identification, purity and impurity profiling, was ion paring reversed-phase high performance liquid chromatography ultraviolet spectroscopy (IPRP-HPLC-UV). With the advent of these increased applications, demands for timely and cost-effective laboratory testing techniques have also risen. Recently, these techniques are being coupled with powerful liquid chromatography mass spectrometry (LC-MS) and high resolution mass spectrometry (HR-MS) platforms to enable more sensitive, accurate and timely analyses. These platforms allow for UV and MS detectors to be used in tandem with a single HPLC. Additionally, the HR-MS platform possesses the ability to characterize the individual nucleobases that comprise the oligonucleotide molecule, making it an indispensable tool for development, identification and quality control.

In addition to the testing of the drug product, there has also been a shift in delivery modes and devices used for therapeutic products. The move from traditional glass vials to prefilled syringes (PFSs) and prefilled pens (PFPs) to assist in personal patient administration has created a large demand for device testing capabilities. The ability to access the relevant device parameters such as needle guard lock out, needle safe distance, needle guard override, injection force and break loose glide force have become vital criteria for manufacturers, as well as a strict regulatory requirement.

Key Challenges Oligonucleotide Drug Developers Face

In this burgeoning market, there are a few key challenges oligonucleotide drug developers face. Due to the novel nature of oligonucleotide therapies and their rapid expansion, there is a lack of dedicated regulatory guidelines, and they are fully or partially excluded from the scope of ICH Q3A[2]/B[3], ICH Q6A[4]/B[5] and ICH M7[6] guidelines. This creates challenges for companies attempting to develop strategies for chemistry, manufacturing and controls (CMCs) that will be acceptable to regulatory agencies. Additionally, the lack of consensus between the various regulatory agencies (U.S. Food and Drug Administration, European Medicines Agency, Medicines and Healthcare products Regulatory Agency (MHRA), etc.) regarding oligonucleotide requirements for manufacture, quality control, impurity/purity thresholds, clinical trials and subsequent registration and approval, increases the complexity of these processes.

Another challenge is the lack of industry experience in performing complex analytical methods to support analysis, development and testing. In particular, LC-UV, LC-MS and HR-MS methods require a high degree of equipment and analyst specialization. These methods - when applied to impurity profiling - can generate substantial quantities of complex data, often requiring highly trained analysts to correctly integrate the resulting profiles. Furthermore, the quantity of data generated frequently requires exportation from chromatographic software to external statistical programs to produce reportable results. To date, no oligonucleotide specific software is available to achieve this. Therefore, in scenarios where LC-UV and MS systems are performed in tandem, this issue can be compounded. This complex and time-consuming process is one of the main challenges for oligonucleotide analytical laboratories, as it greatly impacts the time and cost of method validation, transfer and routine analysis, which consequently has a downstream effect on regulatory data submissions.

A further challenge for oligonucleotide laboratories is the ability to rapidly onboard specialized instrumentation required for changing customer and regulatory requirements. Extended lead times for acquisition of MS and HR-MS systems and protracted installation, operational and performance qualifications (IQs, OQs, PQs) can all produce delayed project timelines.

Applications of Oligonucleotide-based Therapies Across Therapeutic Areas and Indications

Given the wide variety of tissues that can be targeted, oligonucleotide-based therapies can potentially be applied to all therapeutic areas across many indications. Of the first oligonucleotide therapies to reach market, 11 of the 13 targeted rare genetic diseases[7] that were predominately metabolic conditions for which limited treatment options were available. Following the success of these pioneering treatments, companies shifted their focus to non-genetic conditions with larger patient populations. The first of these non-genetic oligonucleotides to be approved for market in 2020 was Inclisiran, which targets metabolic and cardiovascular disorders.

Currently, oligonucleotide products are in various development and clinical stages for a spectrum of pathologies. Due to the large number of hepatic targets available, these are predominantly metabolic conditions. The second most targeted therapeutic area is oncology[1]; however, these treatments are still at the clinical trial phase. Muscular disorders, such as Duchenne muscular dystrophy, currently have oligonucleotide treatments on the market. Also, several ASO and siRNA therapies are currently being developed to treat infectious diseases such as hepatitis B. Other therapeutic areas, including dermatology, hematology, respiratory, endocrinology, neurology and immunology, are also being targeted by oligonucleotide therapeutics across various stages of development.

Although much has being achieved in the past 20 years, improvements in drug delivery, target specificity, drug stability, manufacturing capacity, analysis complexity, along with more robust regulatory guidelines are required to unlock the true potential of oligonucleotides as an effective treatment for an inexhaustible array of clinical conditions.

Developing the next generation of technologies

To develop the next generation of drug delivery technologies, nucleotide-based therapeutic companies need strong, collaborative partners.

- The novel nature of these therapies often results in a deficiency of specifically dedicated regulatory guidelines. Companies look for demonstrated success with these therapeutics, including experience in supporting regulatory submissions.
- Due to the industry shift in delivery modes and devices used for therapeutic products, they also need a partner that is able to perform device functionality analysis for auto-injectors, PFSs and PFPs, in house.
- Procurement and onboarding of equipment are important due to specialized equipment needs. Experience in adapting rapidly to changing client strategies and requirements greatly streamlines the onboarding process of new client programs, which significantly reduces time and cost in delivering oligonucleotide products to market.
- Additionally, customers wanting to market in the EU seek partners that have testing capabilities there, to save both time and cost during the delivery of product to market[8].

By leveraging partners' regulatory experience, device functionality expertise and equipment handling strategies, drug developers can address and satisfy the challenges for their predominant therapeutic areas.

Why PPD

The PPD™ laboratory services' GMP lab has collaborated with clients to bring multiple oligonucleotide products to market since 2015 and has supported the first FDA-approved siRNA therapy.

- **Track record of success** – The PPD laboratory services' GMP lab in Athlone, Ireland performed release testing for the first siRNA therapeutic to receive FDA and EMA approval in 2018. Since 2015, this lab has collaborated with clients to bring four oligonucleotide therapies to market, with one more currently in the process of submission. The team has successfully performed more than 80 complete/supplemental method validations/verifications, and conducted method transfers, method developments and photostability/forced-degradation studies.
- **Access to innovative technology** – PPD is part of Thermo Fisher Scientific's Clinical Research business, which provides a complete suite of services across the clinical development spectrum, as well as the development and production of the therapeutics. The GMP lab has quick access to procurement and onboarding of specialized equipment for oligonucleotides.

The team can pivot to changing customer strategies and requirements, greatly streamlining the onboarding process of complex nucleotide analysis. Rapidly adapting to the equipment and reagent requirements of our various customers is fundamental in reducing the cost and time in bringing products to market. In addition to equipment from multiple vendors in-house, the GMP lab in Athlone recently acquired a ThermoFisher Orbitrap Exploris™ 240 MS, a high resolution mass spectrometer which allows for the accurate characterization of oligonucleotide nucleobase sequences.

- **Device functionality analysis** – To meet the demand for device testing capabilities, the GMP lab in Athlone has the ability to perform device functionality analysis for auto-injectors, PFSs and PFPs, (i.e. needle guard lock out, needle safe distance, needle guard override, injection force, break loose glide force etc.) in-house.

Extensive oligonucleotide testing experience

The PPD laboratory services GMP lab has highly experienced staff with extensive testing experience, making it a complete solution center for all aspects of oligonucleotide testing – including both study and method experience.

- **Study Experience:** Single and double stranded oligonucleotides, drug substances, drug products, release, stability, confirmation, temperature cycling, transportation, degradation, photostability, method development, optimization, transfer, validation studies and data collation for dossier submissions.
- **Method Experience:** Osmolality, denaturing AX-HPLC, IPRP-HPLC, assay determination, and % nucleic acid encapsulation, device testing, assay of lipid components HPLC with evaporative light scattering detector (ELSD), sterility and bacterial endotoxins*, mass ID of siRNA LC-MS, particulate matter, particle size, container closure integrity, microflow imaging, ethanol residual solvent by GC, non-volatile leachables, elemental impurities, residual EDTA by HPLC, fluorometric assay of release of siRNA in lipid nanoparticles by dissolution, T-melt, high resolution mass spectrometry nucleotide characterization.

Locations to suit client's needs, globally

The PPD laboratory services GMP lab has two locations: Europe and the US. Both sites share the same SOPs, operating, data collection and quality systems. Products tested at the PPD laboratory services GMP lab meet EU requirements for marketing throughout the 26 EU member states. This greatly streamlines the submission process and reduces regulatory requirements. The teams in each site cooperate on multiple client studies. This allows for an increased knowledge base and support of expertise, equipment, testing capacity, data and QA review and approval, and provides flexibility and resource sharing to meet evolving client priorities.

The PPD laboratory services' GMP lab in Athlone, Ireland has extensive experience in method development, optimization, transfer and validation, and supports a wide range of studies, including:

- Release
- Stability
- Confirmation
- Temperature cycling
- Transportation
- Degradation
- Photostability

There has been significant progress in oligonucleotide therapies as novel therapeutics, yet there are challenges that need to be addressed, including the complex nature of analytical methods, a lack of regulatory guidelines, and a shortage of industry experience in manufacturing, analysis, and regulation. Improvements in drug delivery, target specificity, drug stability, manufacturing capacity, analysis complexity and regulatory guidelines are necessary to fully unlock the potential of oligonucleotides in various therapeutic areas. In order to develop the next generation of drug delivery technologies, it is important to collaborate with companies that have experience in regulatory, device functionality and equipment handling strategies.

Our GMP lab solutions provide reliability for oligonucleotide companies due to a successful track record, access to innovative technology, in-house device functionality analysis capabilities, and locations that cater to client needs. At the PPD laboratory services GMP lab in Athlone, Ireland, our professionals have extensive experience in method development, validation and supporting various studies, including release, stability and degradation testing. Our team is well-equipped to support customers in navigating the regulatory landscape and accelerating the delivery of oligonucleotide products to market via FDA, EMA and HPRA approval. Our GMP lab solutions provide the expertise, capabilities and global presence needed for oligonucleotide companies seeking to overcome challenges and bring their innovative therapies to patients efficiently and effectively.

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The PPD laboratory services' GMP lab in Athlone has experience in supporting costumer dossier submission and is a qualified GMP site with FDA, EMA and HPRA approval.

Learn how the PPD laboratory services' GMP Lab in Athlone provides a comprehensive solution for oligonucleotide services.

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