intertek
Total Quality. Assured.

OLIGONUCLEOTIDE THERAPEUTICS ANALYTICAL DEVELOPMENT SERVICES

CMC Development Support, Characterisation,
Stability and Quality Control Services

Oligonucleotide Analytical Development Services

Oligonucleotide development, registration and quality control requires robust and accurate analytical support to characterise the product in terms of identity, purity, activity and to assess stability.

Research into products incorporating deoxyribonucleic acid (DNA), sense/antisense oligonucleotides, interfering ribonucleic acid (RNAi)-based therapies and nucleic acid based aptamers has seen significant growth based on the potential that these type of molecules present as therapeutic agents.

The structural complexity of these products can present many challenges in both synthesis and analysis. This includes undesired backbone modifications, the quality of starting materials and random insertion/deletions which can result in improper sequence generation.

With a growing global development pipeline the safety, efficacy, purity, stability and activity of these products are a key area of concern for developers and regulatory authorities. Characterization methods that are specific, robust and quantitative are required to progress development, achieve successful regulatory compliance as well as to ensure the continued quality of therapeutic oligonucleotides.

Designing a specific characterization or quality control strategy for oligonucleotides presents many challenges driven by the complexity of structure and absence of dedicated regulatory guidance.

Our oligonucleotide expertise

Intertek, on behalf of our clients, have supported characterisation and quality control programs for oligonucleotide based therapeutics since the inception of commercialization of this product class.

Our oligonucleotide team develop the strategy and analytical approach to support comprehensive characterisation, stability and long term quality control programs. Incorporating a range of analytical approaches, these programs are delivered from our Good Manufacturing Practice (cGMP) laboratories to the latest regulatory guidance.

Expertise and experience in the field is essential to ensure that the optimal analytical approach is applied, a consequence of the complexity of oligonucleotide chemistry and structure. For examples, oligonucleotides are known to be sensitive to alkali metal adduct formation, which directly impact the sensitivity of mass spectrometry-based analysis. To address this challenge, Intertek has commissioned dedicated chromatographic and accurate mass spectrometry capabilities which minimise the risk of alkali metal adduct formation and minimise baseline noise.

In addition to considering the purity and impurity profile, as well structural and physiochemical integrity the molecule, biological potency of oligonucleotide products is often critical to product registration and quality control process.

- Method Development & Validation
- Identity and Assay
- GMP Release and Stability Testing
- Physico-chemical Characterisation
- Structure, Sequence, Chain Length
- Internucleoside Linkages Analysis
- Higher-order Structure Determination
- Cell Based Assay for Potency
- Purity Determination & Impurities Analysis
- ICH Stability Studies
- Extractables / Leachables

Structure, sequence, chain length

Mass spectrometry is a critical tool in supporting oligonucleotide analytics. Intertek utilise high-resolution mass spectrometry to determine accurate mass and monitor molecule heterogeneity, applying LC-MSMS for sequence confirmation.

An extensive and diverse selection of additional instrumentation is required to provide comprehensive assessment with emphasis often placed on the application of orthogonal approaches. For example, when considering assessment of chain length distribution, capillary gel electrophoresis (CGE) and ion exchange high-performance liquid chromatography (IEX-HPLC) are often applied in combination.

Nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR) are utilised to assess a number of key quality attributes, such as monitoring the distribution and type of internucleoside linkage (phosphodiester P=O, phosphorothioate P=S, methyl phosphonate, phosphonate or any other modified phosphate).

In addition, ^{31}P NMR can also be used to monitor general composition of the molecular backbone.

A detailed characterisation package would include:

- Accurate mass and sequence confirmation by mass spectrometry
- Nucleobase composition by High performance liquid chromatography (HPLC)
- Internucleoside linkages modifications (ion exchange HPLC and ^{31}P NMR)
- Chain length using gel electrophoresis (CGE, PAGE) or ion exchange HPLC
- Molecular backbone composition (PS/PO ratio by ^{31}P NMR)
- Spectroscopic profile (FTIR, NMR)

Physicochemical characterisation

- Optical rotation
- pH, pKa and moisture content
- Intact molecular weight by Electrospray ionization (ESI-MS or High resolution ESI-MS
- Melting temperature (Tm) by NMR, or Circular Dichroism (CD)
- UV spectra (λ_{max}, λ_{min}) and determination of Extinction coefficient

Purity and impurities analysis

Determination of purity and levels of product and process-related impurities presents a fundamental attribute for continued quality control of oligonucleotides products. Our scientists apply a combination of chromatographic and spectroscopic methods to determine product related impurities including:

- Addition sequences (n+1, n+2, etc.)
- Deletion sequences (n-1, n+2, etc.)
- Phosphodiester analogs
- Depurinated sequences
- Partially deprotected sequences
- Aggregated sequences

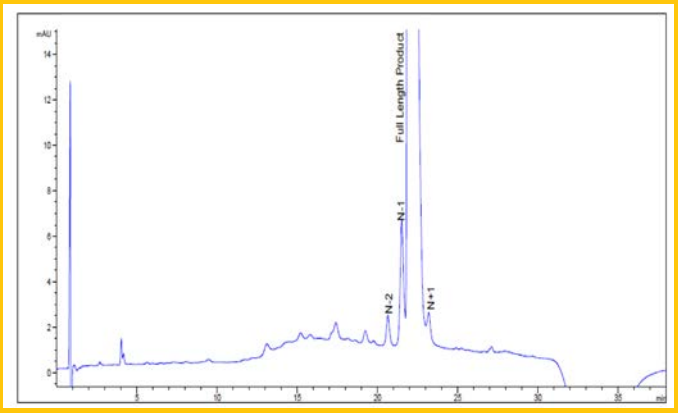
Prior to implementation of any purity and impurity quality control strategy, our team perform comprehensive product characterisation to ensure the final remit of analytics incorporates all potential product or process impurities. This would typically include stressing of material and identification of potential degradation pathways as well as inherent impurity identification using mass spectrometry and or NMR. Once degradation is understood, our team develop and validate methods for assessing all degradation products including those formed via depurination, depyrimidation or aggregation. Application of orthogonal approaches is critical for ensuring complete coverage and includes methods such as gel electrophoresis (SDS-PAGE), size exclusion chromatography (SEC) and dynamic light scattering (DLS).

Successful quality control strategy is not based solely on analysis of the final product, but includes testing of amidite starting materials and assessment of batch to batch variation in raw material quality which has significant impact on the purity of the final product.

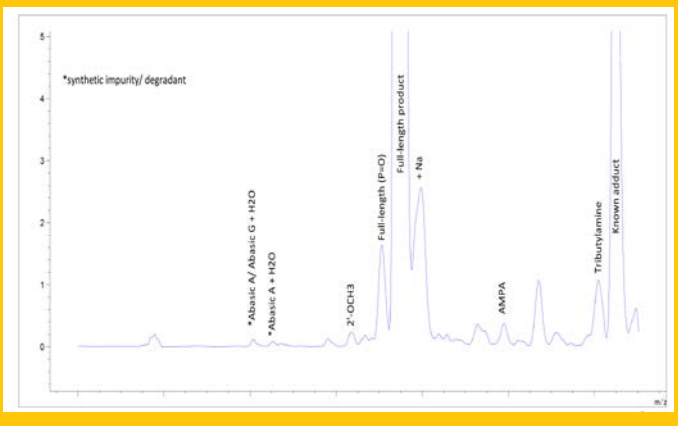
CASE STUDY: Purity Analysis and Impurities Determination by RP-LC-UV-MS

The purity of the an oligonucleotide drug product can be characterised with reverse phase LC-UV-MS which allows highly efficient separation of synthetic oligonucleotides for example to meet the needs of a stability program time point test.

This approach presents a rapid method of determining the % purity, impurity profile and identity of the oligonucleotide product by comparing the main peak retention time with that of a reference material, the % purity can be calculated. Individual impurities and total impurities can also be determined.



a) Drug Product UV Chromatogram showing the oligonucleotide main peak and other peaks indicating presence of n+1, n-1 and n-2 sequences



b) Drug Product MS spectrum under main peak

Process residual impurities including organic volatile impurities (OVI) or residual solvents, are typically quantified by gas chromatography (GC) with flame ionization detection (FID) or mass spectrometry (MS).

Our specialists quantify inorganic molecules, metals, inorganic salts, catalysts, cleavage reagents, and counterions, quantified by coupled plasma (ICP), MS or OES. Alkali metal, adducts (in particular sodium) are determined using HPLC methods.

Stability programs

We conduct integrated stability programs for oligonucleotides incorporating ICH storage and testing to cGMP. We also provide method development (and validation) of stability indicating tests.

- Appearance, water content, pH
- Assay by HPLC
- Sulphur content
- Sodium content by ICP-MS or Sodium adduct by HPLC
- Mass Spectrometry (including accurate ms)
- Impurities or s –truncation determination by LC-UV-MS, IEX
- Aggregation by SEC
- Higher Order Structure by CD
- Biological activity
- Melting temperature (Tm) by NMR
- Microbiology

Total Quality Assurance

Our teams have over 15 years of experience in the strategic application of orthogonal analytical techniques for oligonucleotides therapeutics with a heritage of supporting GMP manufacturing and regulatory submission requirements. Our Total Quality Assurance expertise can support your development helping you to progress from initial characterisation and CMC through to longer-term long term quality control. We deliver innovative and bespoke Assurance, Testing, Inspection and Certification solutions for our customers' operations and supply chains.

Characterization and Impurity Analysis of Oligonucleotide Therapeutics

PHARMACEUTICAL TECHNOLOGY 41 (1) 2017

A. Wake, Biologics Director, Intertek

UK +44 116 296 1620

oligos@intertek.com

intertek.com/oligonucleotides

intertek
Total Quality. Assured.