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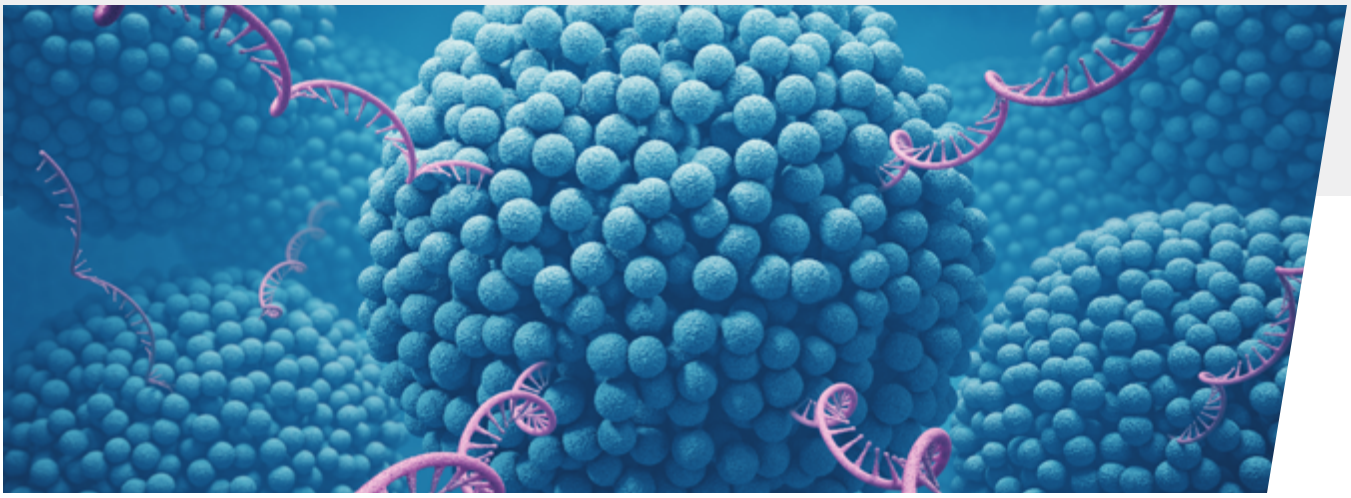
Driving the Field of Oligonucleotide Therapeutics

State-of-the-Art Solutions for Oligonucleotide Characterization, Purification, and Manufacturing

LabX

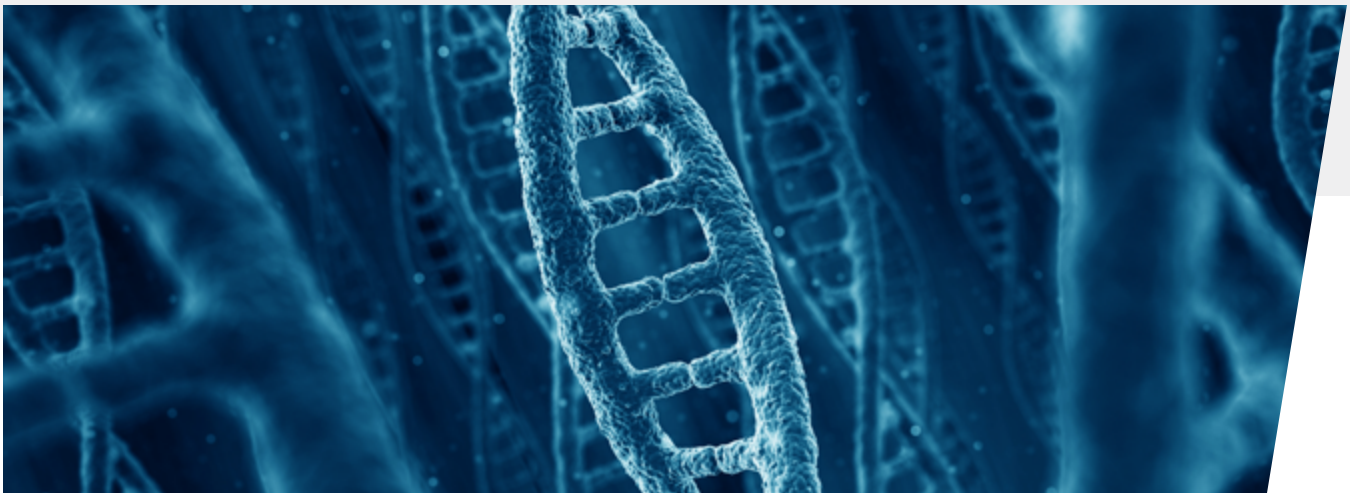


Introduction

The research and development of oligonucleotides represents a revolutionary approach to discovering new and important therapeutic agents. There are challenges in the synthesis, purification, and large-scale production of oligonucleotides. These challenges must be overcome to realize their true therapeutic potential.

Analytical testing solutions are essential in driving research to confidently identify and validate new therapeutic candidates. These analytical solutions include raw/starting material analysis for oligonucleotide synthesis, purity analysis, sequence confirmation, and purification techniques to support validation and quality control for downstream applications. Preparative solutions are necessary to enable oligonucleotide production and scale-up to manufacturing.

This eBook highlights the associated complexities of oligonucleotide synthesis and the range of technologies designed to streamline and advance oligonucleotide characterization, purification, and scale-up to production.



Chapter 1: Introduction to Oligonucleotide Therapeutics

Oligonucleotides or oligos are short single- or double-stranded nucleic acid polymers produced from natural or synthetic sources. Bioactive oligos can elicit responses by interaction with complementary sequences on target RNA or DNA, which can take the form of gene silencing, gene activation, splice modulation, or other activities. Some bioactive oligos known as aptamers (Figure 1 far right) can affect protein targets through secondary structural interactions[1].

Therapeutic oligos can be used to modulate expression via a range of processes and have potential therapeutic applications for many indications. Highly specific lead compounds can be rationally designed based on the known sequence of the therapeutic target. Furthermore, unique genetic elements such as single-nucleotide polymorphisms (SNPs) and expanded repeats can be targeted without silencing wild-type RNA or DNA[1]. Thus, therapeutic oligos have the potential to treat rare diseases in a highly precise and patient-specific manner[2].

How many oligonucleotide therapeutics have been developed?

To date, a total of 18 synthetic oligo therapeutics have been approved by the FDA, with one currently going through pre-registration[3]. There are over 130 programs currently in human clinical trials involving 80 oligonucleotides. In total, these oligo therapeutics span over 100 different indications, covering 14 therapeutic areas and targeting 66 different genes.

What are oligonucleotide therapeutics disease indications?

Approved therapeutics leverage antisense (ASO), splice-switching (SSO), small interfering RNAs (siRNAs), microRNAs (miRNAs), and aptamer approaches. Thus far, diseases targeted by approved oligo therapeutics include homozygous familial hypercholesterolemia, spinal muscular atrophy, Duchenne muscular dystrophy, hereditary transthyretin-mediated amyloidosis, familial chylomicronemia syndrome, acute hepatic porphyria, and primary hyperoxaluria.

Novel oligo therapeutics are being developed for a wide range of disease indications—including cancers, neurological, metabolic, musculoskeletal, sensory, and cardiovascular diseases—with a broad pipeline currently in late clinical development[4].

Chapter 1: Introduction to Oligonucleotide Therapeutics

Figure 1. Double stranded, single stranded, and aptamer oligonucleotide structures

What are the most common oligonucleotide mechanisms of action?

Antisense oligos account for the highest number of approved therapeutics and can be divided into two groups according to the mode of action—gene-expression inhibitors and splicing modulators[4] (Figure 2.). Gene-expression inhibitors work by targeting RNA and can be further divided into steric blocking and enzyme recruitment (Figure 2. left and right side, respectively). In steric blocking, an oligo drug binds to complementary mRNA and prevents that segment of mRNA from being processed. In the enzyme recruitment approach, the oligo binds to mRNA and recruits enzyme RNAse-H to degrade mRNA—or binds to the RNA-induced silencing complex (RISC) and guides it to degrade target mRNA.

What are the manufacturing challenges associated with synthetic oligonucleotides?

Most small oligos (such as ASO, siRNA, miRNA, etc.) are synthesized via solid-phase phosphoramidite chemistry using a four-step cyclic process of the growing oligomer. The manufacturing batch consists of both the target and closely failed related sequences. These failure sequences include:

- *Shortmers* **– oligos missing one or more nucleotides**
- *Longmers* **– oligos with more than the intended number of nucleotides**
- *Missing protecting groups* **– the lack of groups intended to decrease reactivity and increase stability**

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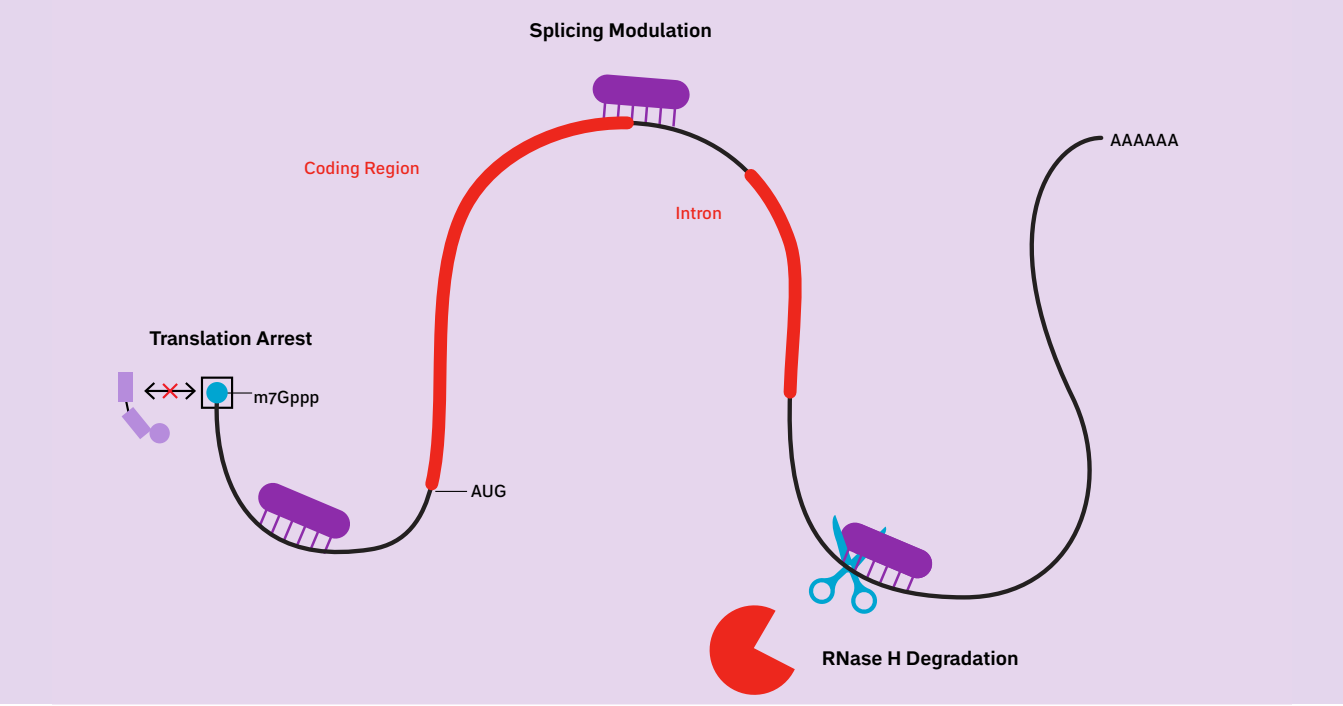

Figure 2. Mechanism of action for three types of bioactive oligonucleotides. Steric blocking at the site of translation initiation or splice modulation can result in misreading or loss of gene expression. Enzyme recruitment or binding to the RNA-induced silencing complex can lead to mRNA transcript degradation and loss of gene expression.

Other examples of product-related species are phosphodiester analogs, de-purinated sequences, partially deprotected sequences, and aggregated sequences.

Why is oligonucleotide characterization important?

Production of high-fidelity oligo preparations, free from impurities, is essential for downstream chemical modification to support stability and favorable pharmacokinetic and -dynamic properties[1]. Phosphorothioates and other modifications can directly affect affinity, nuclease resistance, chemical and mechanical stability, as well as modulations of hydration and protein binding[1].

One of the main limitations of ASOs, and oligo drugs in general, is their poor cell penetration and the lack of tissue specificity. To this end, several technologies are under development to improve cellular uptake and tissue-specific targeting. Post-synthesis modification with sugar moieties, such as GalNAc conjugation (Figure 3 right), has shown success in targeting the liver, while lipid nanoparticles (LNPs) (Figure 3 left) have demonstrated strong capabilities as delivery vehicles[3]. Agilent has created systems and columns to detect LNP and help advance this technology[5].

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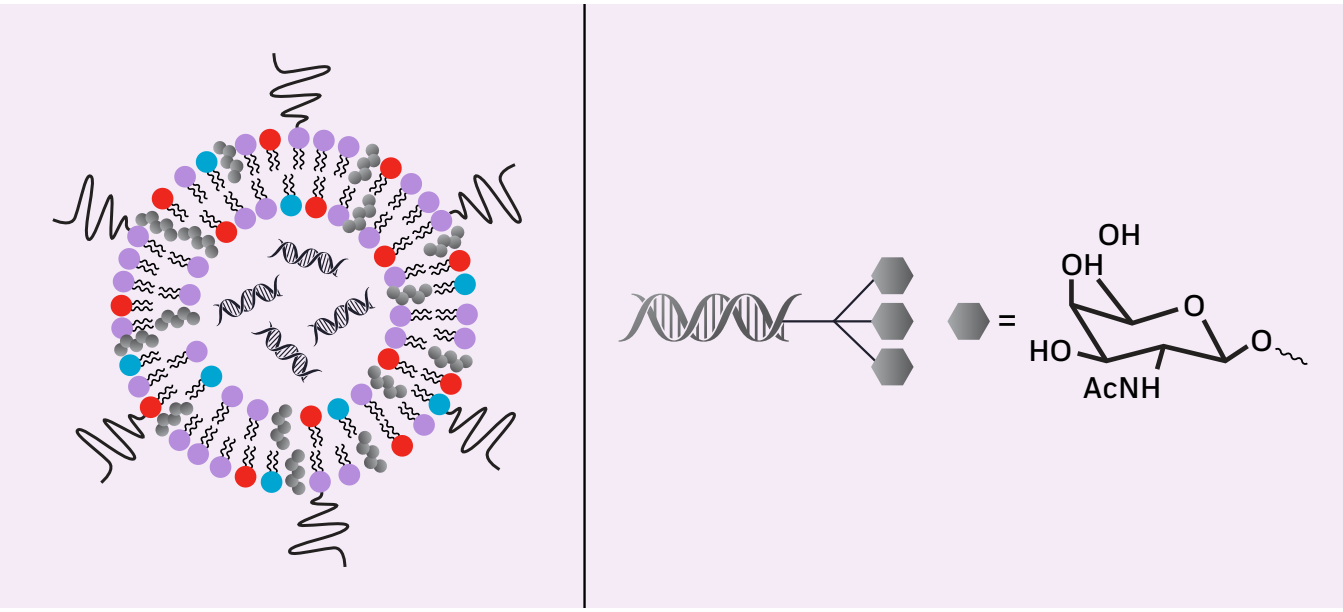


Image 3. Two approaches towards increasing target specificity and efficacy of oligonucleotide therapeutics. Lipid nanoparticles (LNPs) loaded with therapeutic bioactive oligonucleotides (left) facilitate tissue and cell targeting. GalNAc sugar moieties directly conjugated to therapeutic oligonucleotides (right) enhance target cell penetration.

High-performance synthesis and purification techniques are pivotal to producing high-quality oligos suitable for appropriate modification, delivery, and testing. Furthermore, considerations must be made when scaling up from analytical to preparative processes to ensure high yields of functional oligos are maintained.

What are the regulatory considerations for synthetic oligonucleotides?

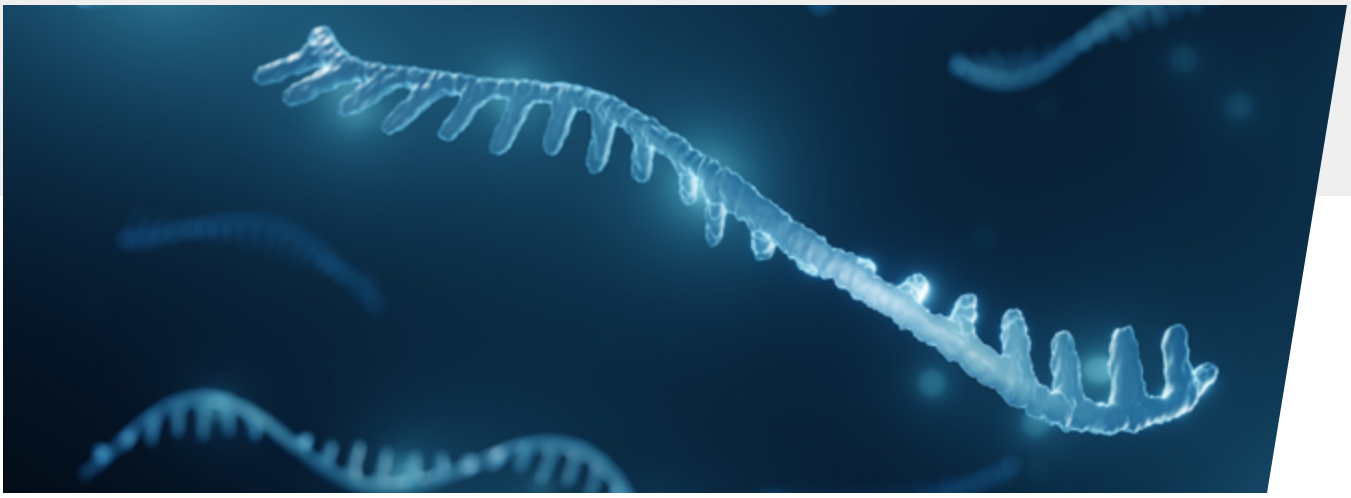
There are currently no ICH or FDA regulatory guidelines that specifically address the quality standards for oligo drugs. Despite their large size, synthetic oligo drugs are considered more alike to small molecule (SM) drugs than biologics, as they are chemically synthesized and manufactured.

Furthermore, there is no consensus about impurity identification and qualification thresholds. Most exist as mixtures of closely related components; some impurities are largely intact parent oligos cross-linked to another molecule of the parent oligo.

What are some solutions to these regulatory challenges?

The Oligo Safety Working Group has developed a framework for the safety assessment and control of impurities, reporting, identification, and qualification thresholds that have not been set by the FDA or the European Medicines Agency (EMA) and instead require a case-by-case approach.

ICH Q3C(R6)[6] and ICH Q3D(R1)[7] guidelines that cover residual solvents and elemental impurities, respectively, are applicable to oligonucleotide products.



Chapter 2: Challenges for Oligonucleotide Synthesis and Production

Developed in the 1980s and later enhanced with solid supports and automation, **Oligo Synthesis using the Phosphoramidite Method** is the preferred method for DNA synthesis and manufacturing. The synthetic process involves four main steps[8,9,10].

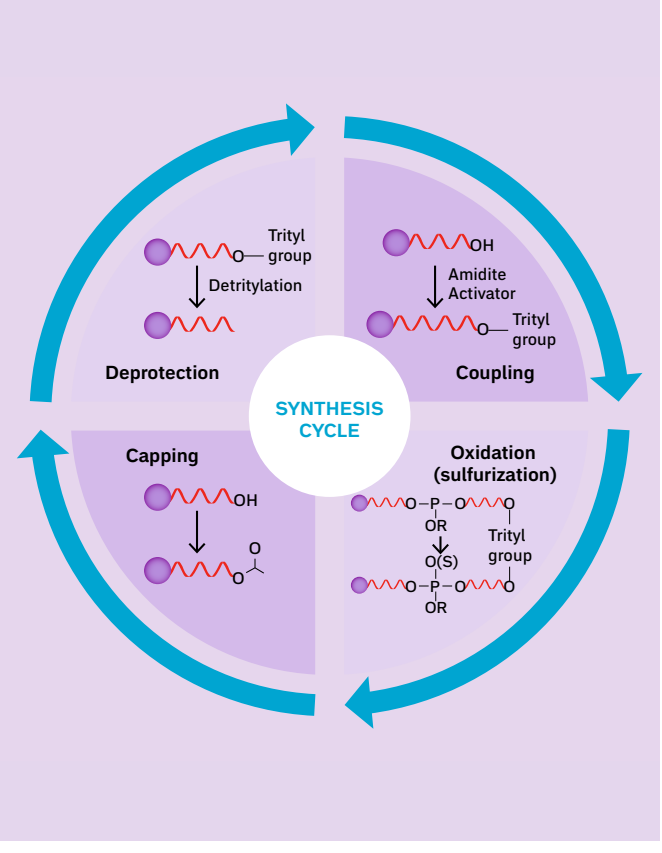
1. **In the first step—deprotection and detritylation—the 5'-DMT (4,4'-dimethoxytrityl) protecting group of the solid-support linked nucleoside is removed.**

2. **The second step involves coupling the next successive nucleoside through reaction with the 5'-OH group of the solid-support nucleoside.**

3. **In the third step, an oxidation reaction converts the unstable phosphite triester linkage between the two nucleosides into a more stable phosphorus species.**

4. **Unreacted solid-support nucleosides are capped during the final step to ensure these don't react during further cycles.**

The process repeats with the addition of each nucleoside until the desired sequence of oligo is obtained. At the end of synthesis, the oligo is cleaved from the solid support, leaving a free 3'-OH group. Following cleavage, the oligo is heated in concentrated aqueous ammonia to remove protecting groups from the bases and phosphates.



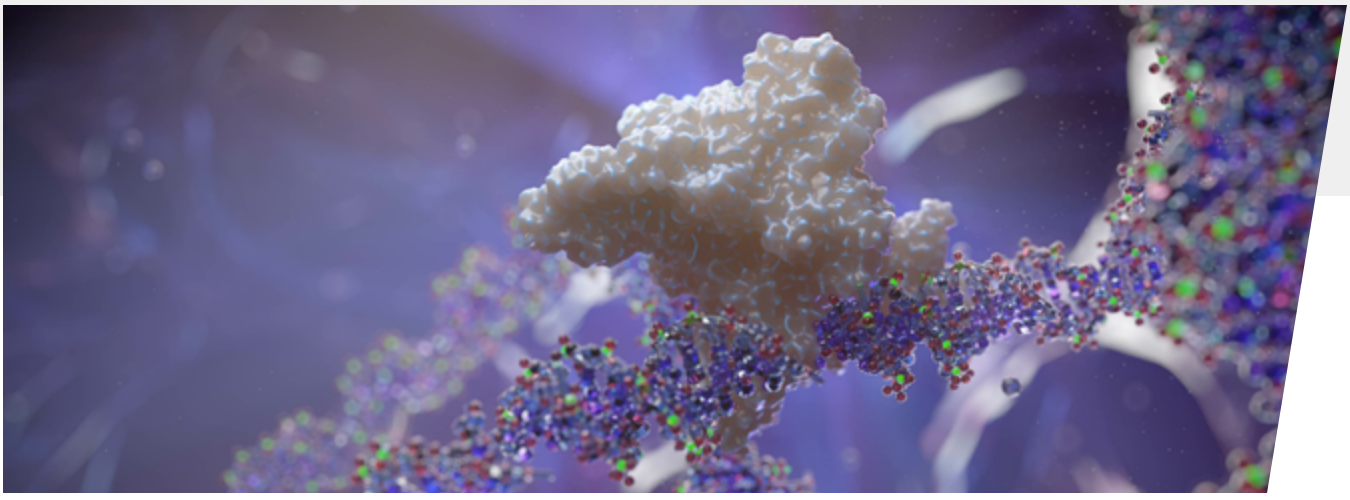
Chapter 2: Challenges for Oligonucleotide Synthesis and Production

The yield of oligonucleotide sythesis is affected by several steps of the process, most notably the coupling efficiency. Deprotection, cleavage, and purification also impact yield to varying degrees. At 95% coupling efficiency, the overall reaction yield decreases with each successive cycle, arriving at <36% after 20 cycles (*i.e.,* 20-mer oligo). It is essential to optimize the synthesis steps and reaction yield to maximize the efficiency of production.

Throughout the oligo synthesis, testing, and production pipeline, there are many stages that present challenges and require solutions.

- **Raw material (or starting material) analysis is needed to identify contaminants and ensure chemical stocks are of sufficient purity to prevent the related impurity carryover.**
- **Synthesis must be optimized to minimize side reaction impurities and maximize yield.**
- **Oligo purity analysis must be performed to confirm the purity of the intended oligo, the related product yield, and determine the presence of potential impurities arising from synthesis.**
- **Verification is needed to ensure the oligo product sequence, molecular weight, and other defining characteristics.**
- **Structural characterization is required to ensure proper functional confirmation.**
- **Considerations must be given to transitioning oligo production from analytical to preparative scale.**

Precise and sensitive analytical solutions are required to verify the intended products and resolve (and remove) impurities.



Chapter 3: Solutions for Oligonucleotide Analysis and Purification

End-to-end solutions for oligo analytical characterization and preparative separation are available to address these challenges. These solutions leverage spectroscopy, chromatography, and mass spectrometry-based tools, consumables, and software tools to ensure confident oligo analysis.

For example, purity analysis during oligonucleotide synthesis can be challenging and must be addressed to maximize efficacy and yield of the desired oligo. Impurities—including truncations, incomplete thiolation, and base loss—must be detected and identified during synthesis optimization, and methods must be in place to effectively remove these from the final samples. Purity analysis is essential to maximizing biological activity and clinical efficacy as well as ensure the downstream safety for intended use.

- **Raw Material Identification using Raman, FTIR, and HPLC/UHPLC**
- **Purity Analysis using LC and LC/MS**
- **Target Plus Impurities Analysis using LC/MS**
- **Sequence Confirmation using LC/MS/MS**
- **ID Confirmation using UV-Vis**
- **Trace Elemental Impurities Analysis using ICP/MS**
- **Residual Solvent Analysis using GC and GC/MS**

Chapter 3: Solutions for Oligonucleotide Analysis and Purification

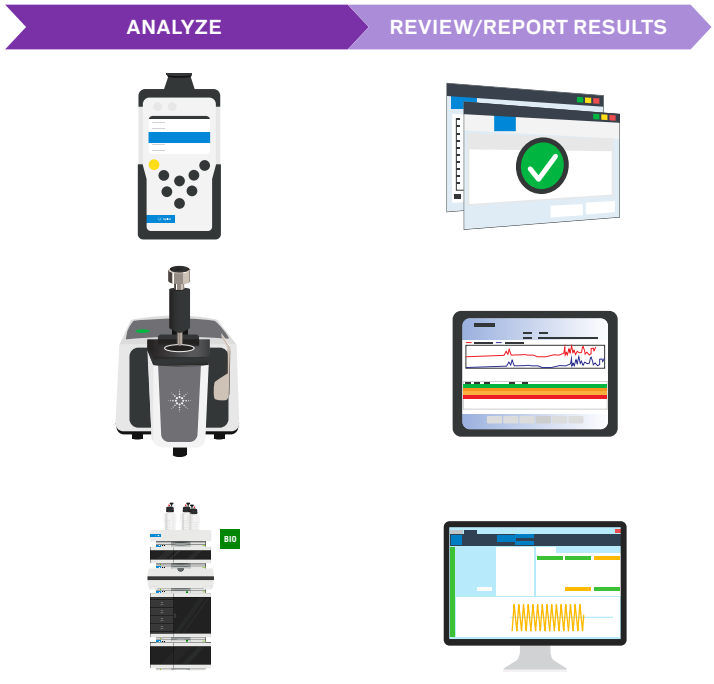
Raw Material Analysis

Optimizing oligo synthesis or large-scale production starts with the use of quality raw (or starting) materials. Materials can be tested for quality, including the presence of impurities, using multiple analytical platforms depending on the type, scale, and location of the test analytes. These may include:

- **Raman Spectroscopy**
- **FTIR Spectroscopy**
- **Liquid Chromatography (HPLC/UHPLC)**

Raman spectroscopy is a non-destructive technique that can be used to inspect raw materials in the warehouse. This includes direct analysis through opaque and colored packaging and containers, without the need for sample removal and workup. The approach protects contents, reduces costs, and enables detection to be run on-site in warehouses or receiving sites in addition to lab environments. It also preserves sterility, prevents cross-contamination, and helps to maximize the shelf life of air-sensitive materials while creating a safety measure by avoiding user exposure to high-potency reagents and APIs.

Workflow Solutions for Raw Material Identification and Assessment Prior to Oligo Synthesis



Chapter 3: Solutions for Oligonucleotide Analysis and Purification

Vaya Handheld Raman Spectrometer

Performs raw materials identification through transparent and colored packaging and containers, including white or colored tubes, FIBCs, paper bags, and amber bottles. The system is easy to use and includes:

- **A dedicated raw materials identification workflow**
- **Clear pass/fail analysis**
- **Intuitive method development wizard**
- **Minimal training requirements**

Learn More

Cary 630 Benchtop FTIR Spectrometer

Performs raw materials identification in a laboratory environment. It is a quick method ideal for small sample amounts, and the modularity offers sample flexibility for analysis of solids, liquids, powders, and gases. The Cary 630 FTIR spectrometer's intuitive and easy-to-use design is matched by the Agilent MicroLab software suite, providing stepwise processing steps:

- **Attach the required sampling module**
- **Follow the picture-driven software guidance and load the sample**
- **Instantly receive color-coded, actionable results**

Learn More

1290 Infinity II Bio UHPLC System

Utilizes biocompatible materials, solvents, and sample flow paths to ensure the integrity of biomolecules is preserved through minimal surface interactions.

- **The systems are resistant to high salt and extreme pH conditions**
- **Provides the highest resolution and lowest dispersion at pressure up to 1300 bar**
- **A variety of biocompatible flow cells are available for sensitive UV detection**

Learn More

AdvanceBio Oligonucleotide Column

Offers high-quality, high-resolution ion-pair reversed-phase (IP-RP) separations. The columns enable analytical characterization through purification with seamless method scale-up.

- **High-efficiency particles for increased resolution of target product from closely related impurities (n+/1) (2.7 and 4.0 µm Poroshell)**
- **Robust column chemistry stable at elevated pH and temperature for better separation performance under denaturing conditions**
- **Preparative columns for improved yield and purity of target product (4 µm Poroshell)**

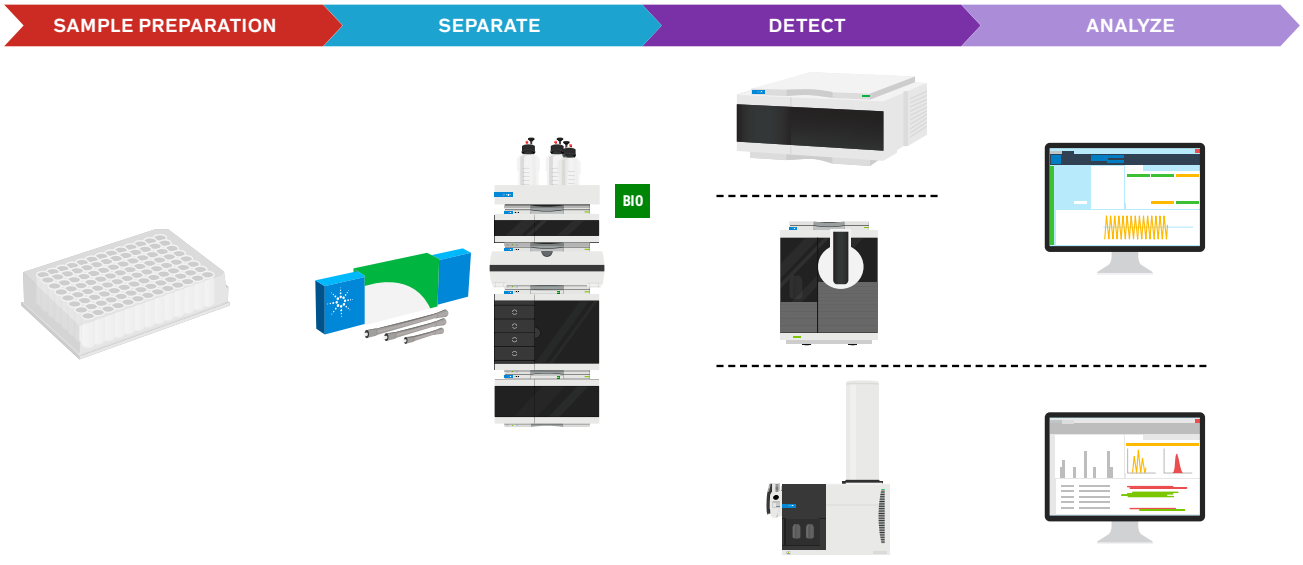
Learn More

Chapter 3: Solutions for Oligonucleotide Analysis and Purification

Oligonucleotide Purity Analysis

Purity analysis of synthetic oligos can be performed by a single technique or multiple techniques in tandem. Sample preparation columns allow the removal of acids and salts ahead of chromatographic separations. HPLC or UHPLC systems using high-performance columns designed for oligo separations can be coupled with UV detection and/or mass spectrometry for high-sensitivity purity assessments. Deconvolution software can identify the presence of impurities as well as the relative concentration of full-length oligo products.

Workflow Solutions for Assessment of Oligo Purity



- **Oligo purity analysis begins by enrichment using strong anion exchange (SAX) or ion-pair reversed-phase (IP-RP) chromatography.**
- **Samples are then analyzed for oligo purity using either LC/UV or LC/MS methods.**
- **The 1290 Infinity II Bio LC/UV or SQ MS system coupled with OpenLab CDS software is used for initial oligo purity analysis.**
- **The 6230B TOF LC/MS system coupled with the 1290 Infinity II Bio LC front end is integrated with MassHunter BioConfirm 12.0 software for data deconvolution and in-depth purity analysis.**

Orthogonal Purity Analysis

Orthogonal tools can be leveraged in assessing oligos (up to 60mer), confirming their identity, and detecting potential impurities. The use of orthogonal approaches expands the search space and helps uncover contaminating species that may have been masked using single techniques alone.

The **Agilent Oligo Pro II system** utilizes UV detection along with parallel capillary electrophoresis to provide single nucleotide resolution and direct assessment of oligonucleotide purity.

Chapter 3: Solutions for Oligonucleotide Analysis and Purification

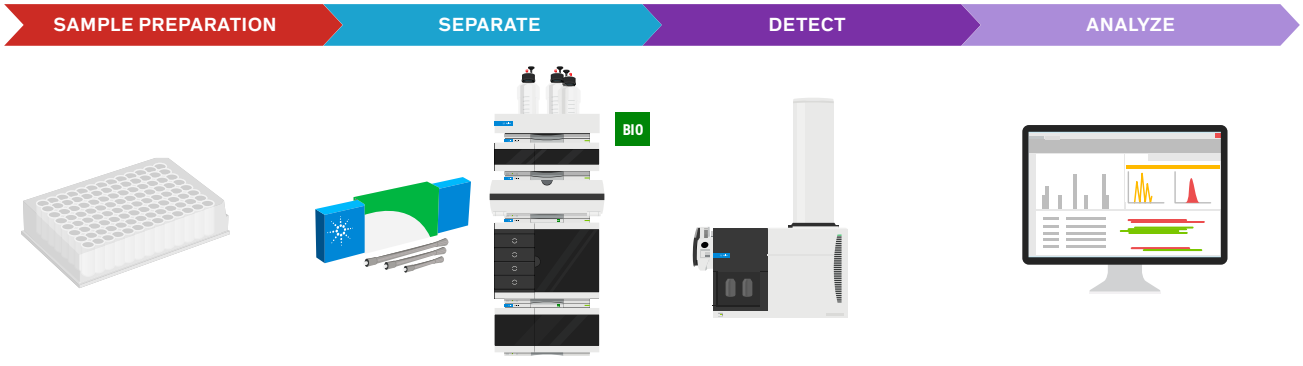
- **High-resolution separations allow direct detection of both ssDNA and ssRNA**
- **Quickly adjusts to demands with either a 12-capillary, 24-capillary or 96-capillary arrays**
- **Software automates capillary conditioning, sample injection, electrophoresis separation, and data processing**

Product-Related Impurities Analysis

Product-related impurities can be generated during oligo synthesis reactions. These can include:

- **Shortmers (N-1) – oligos missing one or more nucleotides**
- **Longmers (N+1) – oligos that include more than the intended number of nucleotides**
- **Lack of protecting groups (derivatives of existing functional groups that decrease reactivity and increase stability)**
- **Other product-related impurities, including Phosphodiester analogs, depurinated sequences, partially deprotected sequences, and aggregated sequences**

Workflow Solutions for Product-Related Oligonucleotide Impurities Characterization



- **The product-related impurities are analyzed using the 1290 Infinity II Bio LC coupled with the 6230B TOF LC/MS system.**
- **MassHunter BioConfirm 12.0 data analysis software enables quick setup of impurity analysis and sequence confirmation workflows using preferred definitions for building blocks, linkers, and modifications.**
- **The 6545XT AdvanceBio LC/Q-TOF can alternatively be used for high-resolution analysis across multiple workflows.**

Process-Related Impurities Analysis

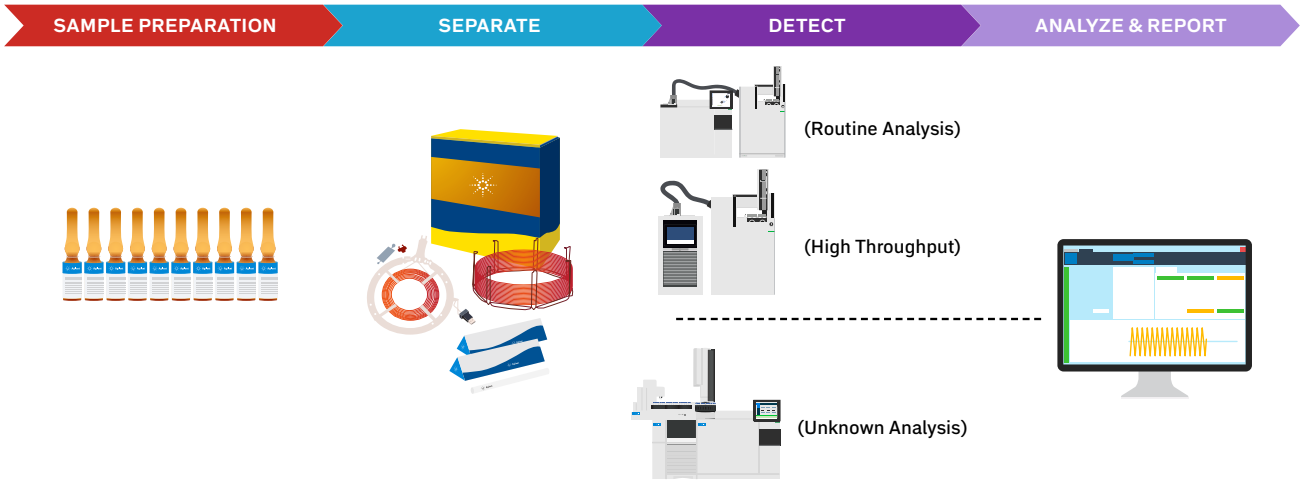
Process-related impurities such as residual solvents are common concerns arising from oligo production and pharmaceutical preparation in general. Exchange of process-related solvents for those with higher volatility is often desired during chemical synthesis. However, this may not be possible in many processes due to negative effects on solubility and yield. Moreover, the solvent may in fact be a critical parameter in the synthetic process.

Chapter 3: Solutions for Oligonucleotide Analysis and Purification

Determination and quantification of residual solvents is part of the release testing requirement process, and ICH, USP, and EP guidelines serve to dictate safety and minimal allowable levels in pharmaceutical preparations[4].

Gas chromatography (GC), alone or coupled with mass spectrometry (GC/MS), is used for residual solvent analysis. GC/MS is ideal for identifying known and unknown solvents depending on the nature of the materials and the synthesis protocol. High-quality calibrants are used for quantitation.

Workflow Solutions for Residual Solvent Analysis by GC and GC/MS



- **The residual solvent analysis workflow begins with the use of compatible GC calibration standards and GC columns.**
- **Depending on GC throughput needs, the 8890 GC with 8697 headspace sampler is used for routine GC analysis. The Intuvo 9000 GC with 8697-XL Tray is best for high-throughput sample analysis needs.**
- **For GC/MS applications, the 8697HS/8890 GC coupled with 5977 GC/MSD is designed to excel at unknown residual solvent analysis.**
- **OpenLab CDS software is used for data deconvolution and analysis.**
- **All instruments are capable of delivering USP/ICH compliance requirements.**

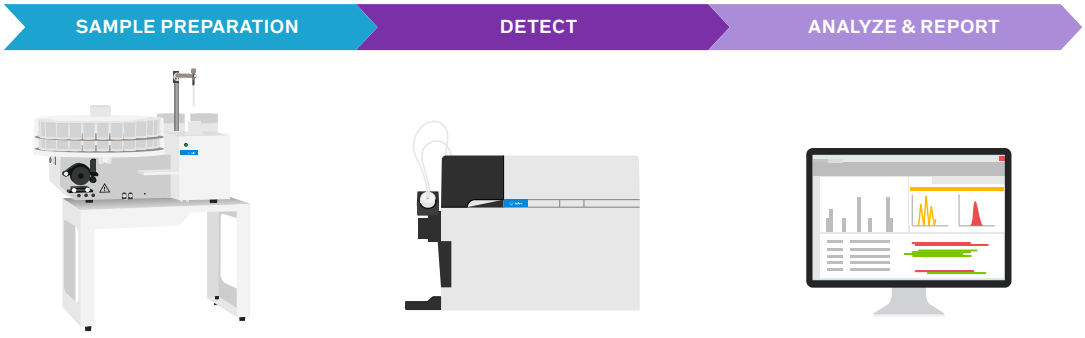
Oligonucleotide process-related trace elemental impurity analysis

Elemental impurities in oligo synthesis arise from several potential sources. They may appear from catalysts used during synthesis or originate from interactions with reaction vessels and containers used throughout the synthesis process[6]. These elemental impurities can impact activity and can lead to a shorter shelf-life or unexpected side effects. Some contaminants, such as heavy metals, are inherently harmful. Therefore, they should be monitored to ensure the intended oligo activity in downstream processes.

Chapter 3: Solutions for Oligonucleotide Analysis and Purification

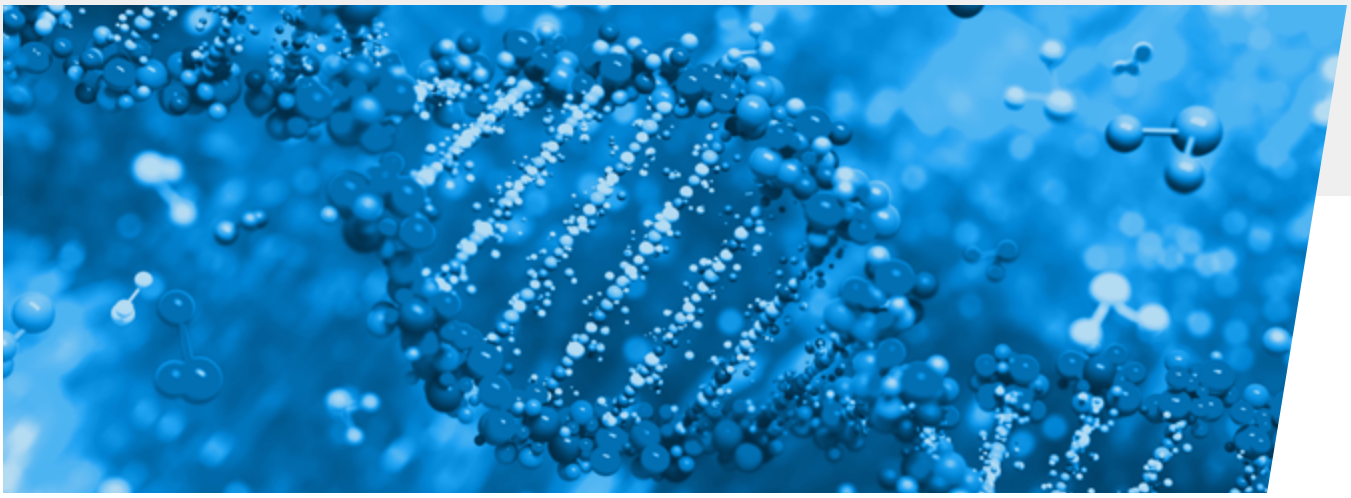
ICP-MS is the preferred method for quantitative analysis of elemental impurities, even in trace levels. Testing for 24 elemental impurities—including cadmium, lead, arsenic, mercury, and other elements defined in USP/ICH—is an essential part of release testing requirements during manufacturing.

Workflow Solutions for ICP-MS Trace Elemental Impurity Analysis



The Agilent 7850 and 7900 ICP-MS instruments each meet requirements for USP/ICH Elemental Impurities.

- **The Agilent 7850 supports fast setup and streamlines analysis for great results in typical applications. The system is cost-effective with very robust operation, including matrix tolerance, stability, and control of typical interferences.**
- **The Agilent 7900 exhibits high-performance flexibility to handle demanding applications while being ideal for advanced, single-particle/single-cell applications.**

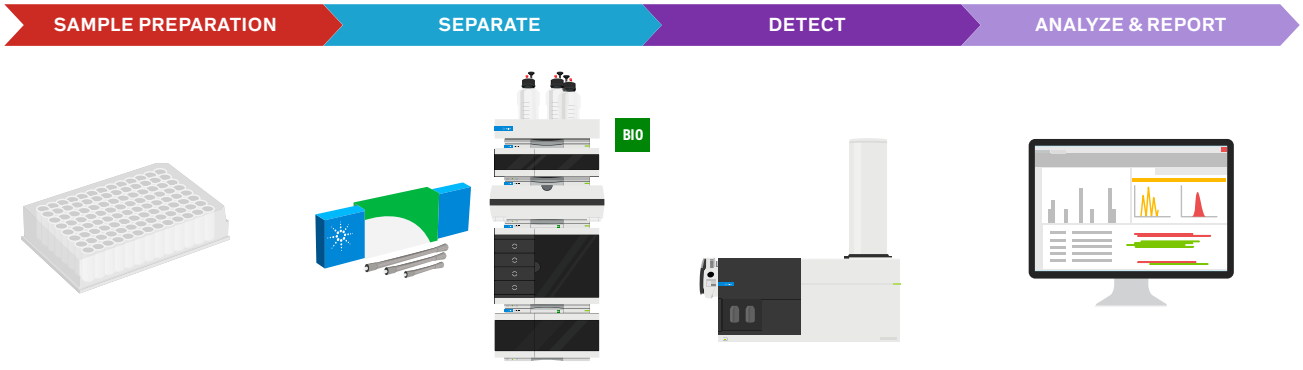


Chapter 4: Solutions for Oligonucleotide Sequence and ID Confirmation

Sequence verification is an essential part of the oligonucleotide synthesis process. Characterizing the structural identity and the location of specific chemical groups is critical for downstream modifications and associated activities. Multiple analytical solutions are available for sequence verification and variants identification, including size exclusion analysis, melting point analysis, and mass spectrometry-based molecular weight determination.

Workflow Solutions for Synthetic Oligo Identity: Sequence and Variants Determination

The challenge of sequence determination includes identifying small differences in masses of related but non-identical species. Isotopic resolution is sometimes required to discern a desired oligo sequence from a highly related impurity.

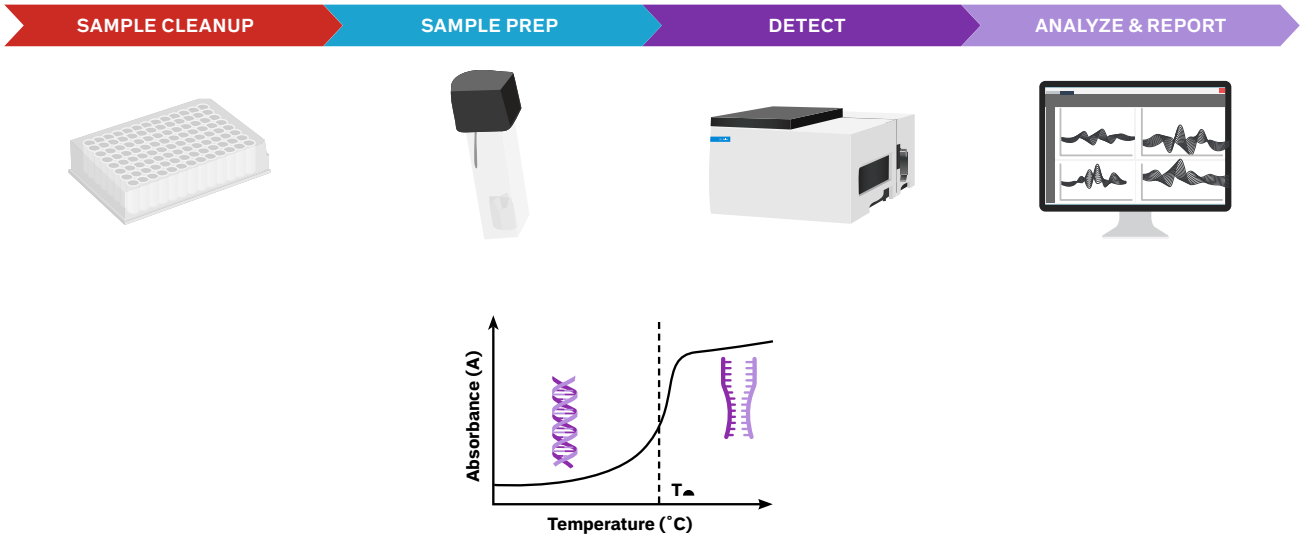


High-performance LC and LC/Q-TOF systems—such as the **Agilent 1290 Infinity II Bio-LC** and **Agilent 6545XT AdvanceBio LC/Q-TOF**—can be leveraged to assess full length products and impurities with high accuracy and precision. The **MassHunter BioConfirm software** is an essential part of the Oligo Target Plus Impurity Workflow.

Chapter 4: Solutions for Oligonucleotide Sequence and ID Confirmation

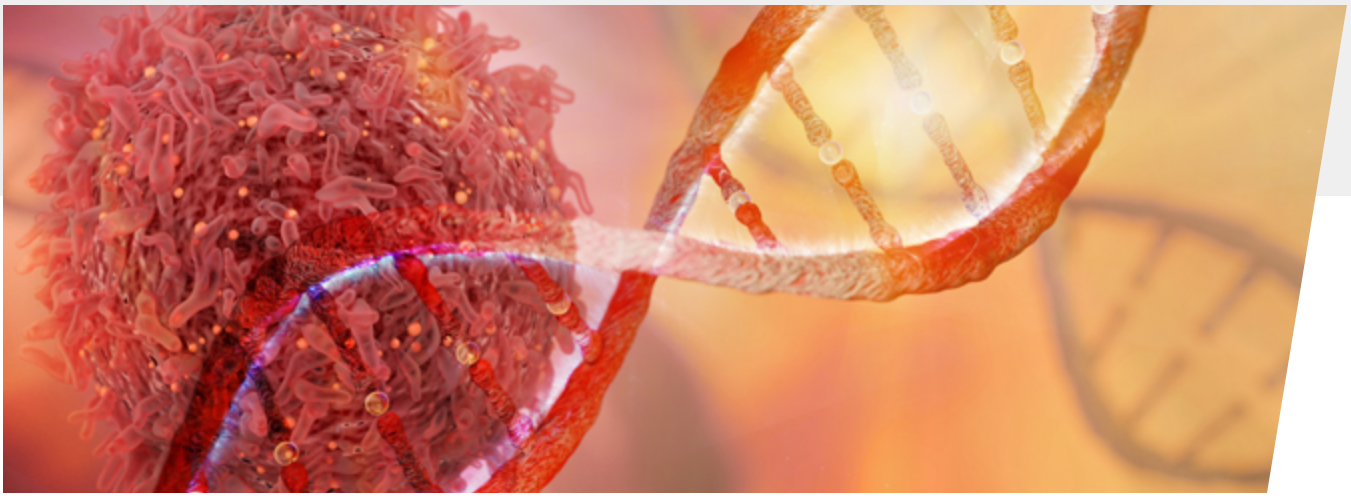
Workflow Solutions for Synthetic Oligo Identification Confirmation: Melting Point Analysis

Melting point analysis is a method to reveal nucleotide content and secondary structure implicit in oligonucleotides. Shifts in the melting point can disclose whether discrete sequence changes are present, thereby affecting high-order structural interactions within the nucleotide sample. These changes could indicate homogeneity in the preparation or alteration of the stability of synthesized oligonucleotides. A common modification in oligo purification is cross-linking between products.



The **Cary 3500 UV/Vis Spectrophotometer** is an easy-to-use system with an integrated air-cooled Peltier-driven temperature control ideal for melting point analysis.

- **Permanent optical alignment and no moving parts together serve to reduce maintenance requirements**
- **Fast and accurate temperature measurements are possible between 0 to 100°C at 30°C increments**



Chapter 5: Transitioning from Analysis to Purification

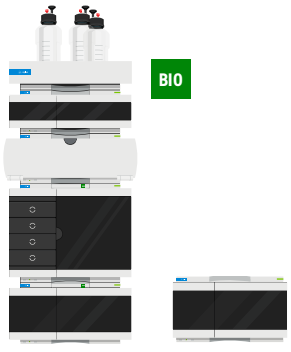
One of the broader objectives of therapeutic oligonucleotide development is the ability to purify the intended oligo. Once the oligo is identified and characterized, the workflow can transition to the preparative scale for purification. Key to this transition is achieving performance and yield benchmarks while increasing scale and throughput.

Shifting from analytical to preparative scale oligo purification comes with challenges. During the preparative workflow, there are critical stages that require analytical sampling to be sure quality purification standards are met. These include target confirmation, purity analysis, and purified sample screening, among others. Important steps include:

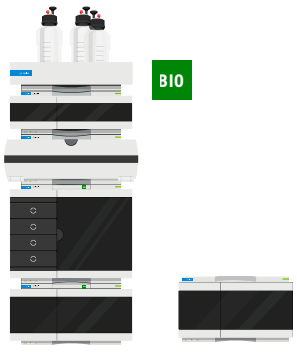
- **Verifying the sample formulation with respect to solvent, concentration, and additives**
- **Confirming LC method compatibility given the type of oligo**
- **Validating optimal LC column phases, separation, resolution, and sample loading maximum**
- **Determining the prep column size and loading capacity**
    - **To facilitate this, the Preparative LC Scaling Calculator is an effective tool that can be used for scaling up analytical methods while reducing the amount of manual computation that is required**
- **Scaling analytical conditions to the prep column and confirming compatibility with instrumentation**
- **Purifying the target compound and ensuring objectives of purity and yield are met**

Versatile Oligonucleotide Purification Solutions include LC Systems that range from analytical to full-preparative for scaling up purification and production.

Chapter 5: Transitioning from Analysis to Purification



1260 Infinity II Bio-Inert Analytical-Scale LC with fraction collector

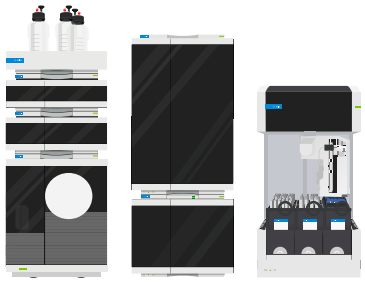


1290 Infinity II Bio LC System (preparative)

AdvanceBio Oligonucleotide column (21.2x150 mm, 4 µm, 120 Å)

Column option:
PLRP-S prep column
(25 x 150 mm, 6 pm, 100 Å)

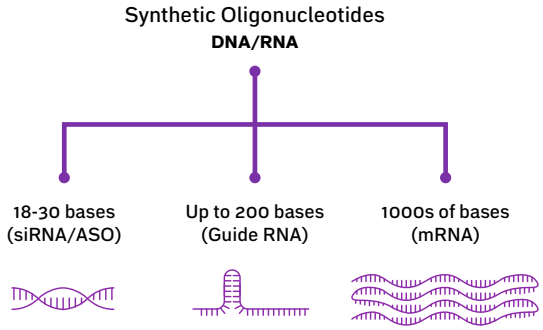
or



1290 InfinityLab LC/MSD XT System (preparative)

OpenLab ChemStation

Oligo Columns use chemistry and pore size that is optimized and independent of target oligo size or purification scale. These columns provide maximum separations performance and a range of functionalities and scales.



✓ Reversed-phase ✓ Anion exchange	Small synthetic oligos (siRNA/ASO/aptamers/ miRNA)	Guide RNA (100-200 bp)	mRNA
AdvanceBio Oligonucleotide	✓	✓	
PLRP-S	✓	✓	✓
Bio-SAX	✓	✓	
PL-SAX	✓	✓	

Chapter 5: Transitioning from Analysis to Purification

Considerations for ion pair-reversed phase (IP-RP) vs. anion exchange:

Oligonucleotide Size

- **Longer oligos are hard to elute from anion exchange media without destructive conditions—which can lead to poor recovery**
- **IP-RP is better suited for oligos >200 nucleotides**

Detection method

- **Buffers and high salt concentrations typically used for anion exchange are not MS-friendly**
- **IP-RP is better suited for MS detection**

Cost and Environmental Health and Safety

- **Ion pairing reagents and organic solvent can become cost prohibitive and may favor anion exchange**
- **High organic solvent consumption at the prep scale**
- **Volatile ion pairing reagents are less environmentally friendly**

The AdvanceBio Oligonucleotide columns offer a high efficiency, high resolution superficially porous particle chemistry.

- **Available in 2.7 and 4 µm particle sizes with 120 Å pore size**
- **Fully scalable column chemistry platform with dimensional offerings from analytical through 21.2 mm ID prep**

The Agilent PLRP-S ion-pair reversed-phase columns use organic solvents and volatile ion-pairing agents and support separations compatible with UV and LC/MS.

- **Ion pair reversed-phase chemistry**
- **Four pore sizes: 100, 300, 1000, and 4000 Å**
- **Six particle sizes: 3, 5, 8, 10, 20, and 30 µm**
- **Ideal for larger oligos, such as mRNA, and large prep scale purification**

The Agilent PL-SAX polymeric anion exchange columns leverage strong anion exchange functionality and covalently link to a fully porous chemically stable polymer, extending both the operating pH and temperature.

- **Best-in-class polymeric anion exchange media**
- **Two pore sizes: 1000 and 4000 Å**

Chapter 5: Transitioning from Analysis to Purification

- **Four particle sizes: 5, 8, 10, and 30 µm**
- **For purification of small synthetic oligos to guide RNA**
- **Ideal when mass spectrometry detection is not needed**
- **Scalable and available in preparative dimensions**

The Bio SAX columns are comprised of a nonporous particle structure, which promotes better mass transfer than fully porous particles.

- **Best used when high-resolution separations at analytical scale are needed**
- **Ideal for UV analysis**
- **Four particle sizes: 1.7, 3, 5, and 10 µm**
- **PEEK hardware available in select dimensions in 5 and 10 µm particle sizes**

Chapter 6: Oligonucleotide API Manufacturing

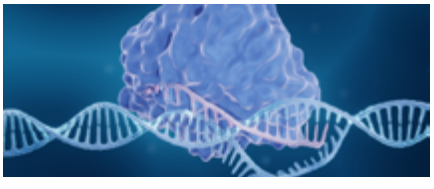
Agilent offers oligo manufacturing for late stage and commercial applications as well as comprehensive testing to support early-state development. These oligo manufacturing operations employ current good manufacturing practices (cGMP) as defined by ICH for providing active pharmaceutical ingredients (APIs). Two manufacturing facilities and a broad range of synthesis and purification equipment provide the capability and capacity to serve toxicology and pre-clinical uses – as well as late-stage clinical trials and commercial launches.

Current product offerings include:

Early-Stage Clinical Development
Agilent can supply you with material throughout your clinical trial development program.

Analytical and Chemical Development
Agilent experts transfer, develop, qualify, and validate analytical methods from investigational new drug (IND) to new drug application (NDA) and biologics license application (BLA). Agilent finds to positively identify impurities and uses quantitative mass spectrometry for critical impurities.

Late Stage & Commercial Development
Agilent can offer assistance in the development and manufacturing of late-stage and commercial APIs.



ClinGuide CRISPR sgRNA for Human Therapeutics
Agilent ClinGuide CRISPR sgRNA provides the high quality you need to meet the rigorous requirements of human clinical trials. For robust, scalable, efficient, and safe sgRNA manufacturing, Agilent adheres to strict cGMP practices such as documentation, traceability, and quality standards.

Chapter 7: Solutions to Support the Oligonucleotide Development Pipeline

Agilent offers oligo workflow-based bundles that provide instruments and consumables targeting specific stages in the oligo production pipeline. These include Purity Analysis, Impurity Detection, Identity and Sequence Confirmation, and Analytical & Preparative Separations.

Sample Preparation, Instrumentation, Consumables, Software, Service, and Financing Options

Agilent provides labs everything that is needed to setup and run current methods

- **Sample preparation expertise and collaboration with leading vendors**
- **High-performance, easy-to-use instruments and software**
- **Complete services for installation, commissioning, and qualification**
- **Preset methods define settings and reporting tools to quickly get a lab operational**
- **Elemental impurities SOP/start-up documentation to guide a lab through method setup**
- **Spares and solutions, including CRM stocks for preparation of USP/ICH calibration standards**
- **A range of compliance software options suitable for any size and type of laboratory**
- **A worldwide team of industry and technical experts to provide training and support**

Agilent offers instrument finance solutions to help maximize return on investment and lower total cost of ownership.

- **Flexible payment plans to help acquire the latest technology while allowing you to pay for instruments as you use them without a large capital expenditure.**

Chapter 7: Solutions to Support the Oligonucleotide Development Pipeline

- **Maintain performance through regular and ongoing instrument updates and improvements**
- **Preserve shrinking capital budgets for other critical business needs**
- **Use payment plans for immediate projects or constrained budgets**
- **Available bundling options to combine hardware, software, services, and consumables in a single monthly payment**

Agilent CrossLab is ready to help you design a custom lab instrument financing solution that matches your analytical and business needs.

Learn More

Agilent offers Certified Pre-Owned Instruments that are factory refurbished and rigorously tested to deliver rock-solid reliability.

- **Certified used chromatography and spectroscopy systems and modules have options for installation and training to expedite productivity.**
- **Pre-owned instruments come with a one-year factory warranty (the same warranty as new instruments) and pass a comprehensive operational qualification and performance verification to ensure risk-free performance in your lab.**

Certified Pre-Owned Chromatography and Mass Spectrometry Instruments
Used Agilent GC, GC/MS, LC, and LC/MS instrumentation—expertly refurbished with like-new performance.

Certified Pre-Owned Spectroscopy and Life Science Instruments
Innovative Technologies with Certified Spectroscopy and Life Science Instruments

Agilent provides a lab instrument trade-in and buyback program, which allows you to refresh technology by trading in outdated instruments.

- **Trade in or sell your older and unused chromatography, mass spectrometry, cell analysis, and electrophoresis instrumentation.**
- **Transfer value from older instruments to new and unlock the value in your lab.**
- **Achieve environmentally responsible disposal and sustainability goals.**

Learn More

Summary

The field of therapeutic oligonucleotides is growing rapidly, with many unique candidates in the development pipeline. The identification of novel oligos and their characterization and synthesis present many challenges that must be overcome to clarify purification and maintain activity for downstream applications. Scaling up oligo purification and production presents additional considerations to ensure clinical efficacy and manufacturing compliance.

Agilent offers a broad portfolio of solutions—from instruments and consumables to services and support—aimed to address the challenges of oligo synthesis and to drive the development of oligonucleotide therapeutics.

Chapter 7: Solutions to Support the Oligonucleotide Development Pipeline

References

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Key Application Notes for Oligonucleotide Analysis

Purity & Impurity Analysis

- Best Practice for Nucleic Acid Thermal Stability Measurements Using the Cary 3500 UV-Vis Spectrophotometer
- Identification of Commercially Available Oligonucleotide Starting Materials Directly Through Containers
- Analyzing Raw Material for Oligonucleotide Synthesis
- An Integrated Workflow for the Analysis of Oligonucleotides and Their Impurities by Agilent High-Resolution
- Composition Analysis of Lipid Nanoparticle Components with Agilent 1290 Infinity II ELSD

Identity & Structure Characterization

- Comprehensive and Integrated Workflow for Oligonucleotide Sequence Confirmation by Agilent High-Resolution LC/Q-TOF
- Fast and High-Resolution Reversed-Phase Separation of Synthetic Oligonucleotides
- High-throughput Mass Spectrometry Analysis of Synthetic Oligonucleotides
- Comparability Studies for the Analysis of Nucleotides on Four Different LC Systems
- Evaluation of Different Ion-Pairing Reagents for LC/UV and LC/MS Analysis of Oligonucleotides
- Mass Spectrometric Characterization of Antibody-siRNA Conjugates using the Agilent 6545XT AdvanceBio LC/Q-TOF

Chapter 7: Solutions to Support the Oligonucleotide Development Pipeline

- Rapid Analysis of mRNA 5' Capping with High Resolution LC/MS
- Analysis of mRNA Poly-A Sequence Variants by High-Resolution LC/MS
- Analysis of Oligonucleotides by Capillary Gel Electrophoresis with the Agilent 7100 Capillary Electrophoresis System
- Fluorescence Measurement of Hybridization Between Quencher (DABCYL)-Labeled PNA Probes and a Fluorescein-Labeled DNA Using the Fluorescence BioMelt Package
- High-throughput, Ion-Pairing-Free HILIC Analysis of Oligonucleotides Using Agilent RapidFire Coupled to Quadrupole Time-of-Flight Mass Spectrometry
- Oligonucleotide Analysis with Ion-Pair Reversed-Phase Chromatography and Agilent 1260 Infinity II Prime LC
- Analysis of Oligonucleotides with Ion Exchange Chromatography and Agilent Infinity II UHPLC
- Fast Determination of Thermal Melt Temperature of Double-Stranded Nucleic Acids by UV-Vis Spectroscopy

Purification

- Purification of Single-Stranded RNA Oligonucleotides Using High-Performance Liquid Chromatography
- Direct Analysis of In-Process Oligonucleotides Without Manual Purification
- Fast and Selective Purification of Oligonucleotides Using Preparative HPLC/MS and Software Support

Resource pages for additional Application Notes, Brochures, Column Selection Tools, and On-Demand Webinars:

- https://www.agilent.com/about/newsroom/media-room/oligonucleotides.html
- https://www.agilent.com/en/solutions/biopharma-pharma/synthetic-oligonucleotide-therapeutics
- https://www.agilent.com/en/product/biopharma-hplc-analysis/oligonucleotide-analysis/oligonucleotide

For more information about the products in this eBook visit

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