The Role of Benchmarking Critical Impurity Control in Therapeutic Oligonucleotide Phosphoramidites

The growing need for oligonucleotide therapeutics aimed at larger patient populations has driven the demand for high-quality nucleoside phosphoramidite which are building blocks for oligonucleotide therapeutics.

The global oligonucleotide therapeutics market is projected to grow by USD 13.44 billion between 2022 and 2027, at a compound annual growth rate (CAGR) of 19.87%, according to a report by Technavio. This growth, driven by the expanding RNA-based therapeutic modalities, has placed pressure on suppliers to provide consistent, high-purity phosphoramidites for large-scale manufacturing. The limited number of reliable suppliers further complicates this challenge, particularly as more companies scale up for clinical trials and commercial manufacturing.

To support the emergence of synthetic oligonucleotide therapeutics, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) have recently provided more clarity in regulatory expectations. In 2024, the FDA published a guidance that described the clinical pharmacology requirements while the EMA's guidance focused on manufacturing and control of synthetic oligonucleotides.

The recent EMA guidance reiterated the principles per International Conference on Harmonisation (ICH). This guidance reinforces the regulatory expectations of analytical rigor and process robustness needed for starting material by highlighting ICH Q9 Quality risk management and ICH Q11 Guideline on development and manufacture of drug substances. ICH Q11 is referenced in the context of starting materials selection, and Q9 is referenced to highlight how the benchmarking of certain impurities (referred to as "critical impurities" in the guidance) impacts process development. The guidance went on to list the specific analytical considerations, including impurity characterization, control of raw material quality, and batch-to-batch consistency.

This whitepaper aims to demonstrate how a deep understanding of benchmarking critical impurity profiles in nucleoside phosphoramidite products is essential to advancing oligonucleotide therapeutics. WuXi TIDES has developed precise, data-driven analytical methods that enable reliable impurity identification, control, and documentation, providing drug developers with the essential tools to navigate regulatory requirements and progress confidently from development to market. By thoroughly analyzing impurity sources and implementing rigorous quality assessments and manufacturing processes, we ensure that materials consistently meet the high standards required for both clinical and commercial production.

Supporting Development Pipelines through Data Transparency

For oligonucleotide therapeutics, critical impurities are key impurities that can impact the final drug substance (DS) by interfering with synthesis, leading to variations in mass and/or structure. The levels of starting material-derived impurities are determined by both critical impurity levels and the frequency with which product-related impurities are incorporated. While some critical impurities can be detected based on mass deviations from the DS molecular weight, others introduce structural impurities with identical molecular weights to the DS.

Per EMA's guidance, "particular attention should be paid to differences in levels of critical impurities compared to pre-clinical batches" in the regulatory filing. Without clearly defined impurity profiles, drug developers face increased scrutiny by health authorities as the clinical program advances. This is particularly important for regulatory starting materials, where data transparency is often lacking. The lack of consistent impurity profiling and data transparency complicates the assessment of product quality. These uncertainties in the lack of impurity data can lead to variability in the final oligonucleotide DS, increasing the complexity of managing process development and ensuring compliance with regulatory expectations.

Precision and Expertise: Addressing the Critical Need for Quality

Our extensive and in-depth analytical data set at WuXi TIDES allows us to develop robust manufacturing processes that ensure batch-to-batch consistency and scalability for clinical and commercial applications.

This rich data set also ensures that we can provide drug developers with the information needed to understand the downstream impact of the starting materials.

Our analytical methodologies include:

- Column selection, pH optimization and temperature screening to resolve impurities
- Advanced LC-UV methods and mass spectrometry (MS) for impurity identification and quantification
- Supplemental methods like LC-SFC to further resolve impurities when LC-UV-MS alone is not suitable for the impurity (i.e. water-sensitive impurities)

These extensive methods not only guarantee the quality and consistency of our products but also enable our partners to meet strict regulatory and quality benchmarks, reducing the risk of unforeseen issues during development and manufacturing.

Comparative Analysis: 2'-OMe Amidite Series

Taking the 2'-OMe amidite series as an example, an essential modification in all FDA-approved small interfering RNA (siRNA) drugs, we applied our analytical methods to benchmark impurity profiles from various commercially available products sourced from leading external suppliers in comparison to our own product.

Examining 2'-OMe C(Ac) amidite (CAS No. 199539-09-4), we identified and quantified critical impurities present and matched them to the corresponding impurities found in the synthesized oligonucleotide product. Table 1 outlines the critical impurities detected in the 2'-OMe C(Ac) amidite samples and their corresponding downstream impurity in the final oligonucleotide.

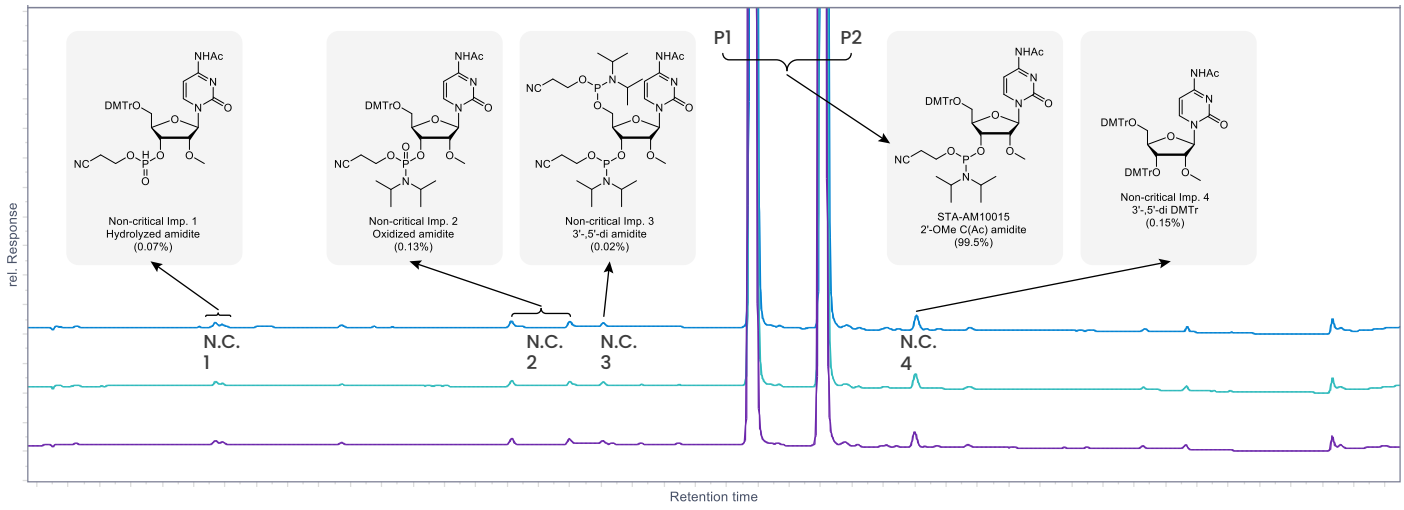
Table 1: Critical impurities in 2'-OMe C(Ac) amidite samples and their corresponding impurity in the final oligonucleotide

Compound	Vendor 1	Vendor 2	WuXi TIDES	Corresponding impurity on oligonucleotide
199593-09-4	99.1%	99.3%	99.5%	N/A
Impurity 1	0.03%	<LOD (0.02%)	<LOD (0.02%)	Isomer with same MS
Impurity 2	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Isomer with same MS
Impurity 3	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Isomer with same MS
Impurity 4	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Isomer with same MS
Impurity 5	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Isomer with same MS
Impurity 6	<LOD (0.02%)	0.04%	<LOD (0.02%)	M+14
Impurity 7	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	M+43
Impurity 8	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	n+1
Impurity 9	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Branch mer
Impurity 10	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Branch mer
Impurity 11	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Branch mer
Impurity 12	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Branch mer
Impurity 13	0.06%	0.07%	<LOD (0.02%)	Branch mer
Impurity 14	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Isobaric product
Impurity 15	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Isobaric product
Impurity 16	<LOD (0.02%)	<LOD (0.02%)	<LOD (0.02%)	Branch mer
Impurity 17	0.06%	0.07%	<LOD (0.02%)	Branch mer

The results from our analysis reveal that both external vendor products each respectively contain three critical impurities above our limit of detection, whereas the WuXi TIDES product is absent of impurities that transfer to the final oligonucleotide product above the limit of detection (less than 0.02%). Additionally, the purity of our product was the highest among the tested samples, achieving 99.5%. These results are consistent across multiple batches, as demonstrated in the chromatogram below, which presents three recent multi-kilogram batches, all reaching 99.5% purity. Each impurity detected above the limit of detection has been labeled with the structures identified to highlight our product's reproducibility, quality, and transparency.

Figure 1. Three reproducible batches of STA-AM10015 (2'-OMe C(Ac) amidite) at multi-kg scale with labeled non-critical impurities above detection limit

N.C.: Non-critical impurity



Batch 1: ET79467-11-P1; 20 kg
Batch 2: ET79467-12-P1; 15 kg
Batch 3: ET79467-13-P1; 20 kg

A Trusted Partner for Oligonucleotide Discovery, Development, and Manufacturing

As oligonucleotide therapeutics expand into broader clinical applications, maintaining stringent quality, impurity control, and regulatory compliance is essential. WuXi TIDES simplifies regulatory submissions for our customers by documenting select amidite products and PMO monomers in Drug Master Files (DMFs)—comprehensive FDA filings that provide detailed records of our manufacturing and quality control processes. In addition, our quality standards ensure that drug developers meet the regulatory requirements not only for US markets, but for successful therapeutic development across global markets.

Moreover, access to modifications beyond the traditional building blocks used in FDA-approved oligonucleotide therapeutics is paving the way for the discovery of new candidates targeting underrepresented indications. WuXi TIDES Catalog Products now offers over 600 amidite products, available for direct purchase online, with select amidites stocked globally in the US and Europe. By offering a broad portfolio of high-quality building blocks, WuXi TIDES Catalog Products enables drug developers a seamless progression from discovery research to commercial manufacturing. With WuXi TIDES, every step of the oligonucleotide drug development process is backed by the expertise and resources needed to bring innovative therapies to market efficiently and confidently.

About WuXi TIDES

WuXi TIDES, part of WuXi AppTec, is a unique Contract Research, Development and Manufacturing platform. WuXi TIDES offers our partners efficient, flexible, and high-quality solutions from discovery through the commercial supply of oligonucleotides, peptides and related synthetic conjugates ("TIDES" drugs).

WuXi TIDES has more than 1,000 scientists across 10 R&D and manufacturing sites, offering discovery synthesis, process development and manufacturing of novel monomers, linkers and ligands, oligonucleotides, peptides and complex synthetic conjugates at any scale. Beyond chemistry, we offer formulation development, manufacturing, packaging, labeling and distribution in a variety of oral & injectable dosage forms and filling formats. Our comprehensive analytical method development, validation and testing platform supports TIDES drug development from discovery through clinical to commercial for both drug substance and drug product. Moreover, our regulatory dossier preparation teams are experienced in global fillings of TIDES molecules with all major regulatory agencies.

Visit our website at **tides.wuxiapptec.com**

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