**Introduction:** As a growing number of therapeutic oligonucleotide compounds continue to be introduced into the clinical pipeline, and advancing into larger, late phase clinical trials, an increasingly stringent demand is placed upon the phosphoramidite supply chain.

With global raw material suppliers scaling up production to meet market demands, heightened concern surrounds the increased potential for generating novel, as well as previously identified, impurities. The impurities found within the material supply chain can directly impact the quality of phosphoramidite synthesis, and thus potentially affect the quality of a therapeutic oligo.

Therefore, an increased focus has been placed upon controlling incoming raw materials, understanding the impact to phosphoramidite chemistry, including impurity profile, and subsequent effects to oligo purity. Thermo Fisher Scientific Milwaukee continues to undertake a comprehensive approach to supply chain management through partnerships with raw material suppliers, as well as customers, to define raw material specifications, including control of impurity levels to satisfy the dynamic quality requirements.

Recently, the Process Development team performed a deep investigation into the quality of an integral raw material, 4'-4'-Dimethoxytrityl Chloride (I, DMTr-Cl). The team capably identified the role and potential deleterious impact of two potential impurities, 4'-Acetoxy-4'-methoxytrityl Chloride (ii, AMTr-Cl) and 4'-Hydroxy-4'-methoxytrityl Chloride (iii, HMTr-Cl), in the synthesized phosphoramidite (illustrated in Synthetic Schemes, below).

---

**Discussion:** AMTr-Chloride in DMTr-Chloride generates non-critical impurity B. HMTr-Chloride generates the critical impurities C, D, E and F. Impurities A, B, C, D and F have been synthesized and further characterized by 31P NMR, HPLC and LC-MS.

All the critical impurities will produce deletion sequences during oligo synthesis. The number and percentage of total impurities in an oligonucleotide due to these critical impurities in phosphoramidites will depend on the sequence length. For example; a 0.1% critical impurity in the phosphoramidite, may generate up to 2.0% of impurities in a 20mer oligo.

**Synthetic Schemes:** 5'-DMT-N4-Bz-dC-Phosphoramidite (A); 5'-AMT-N4-Bz-dC-Phosphoramidite (B); 5'-HMT-N4-Bz-dC-di Phosphoramidite (C); HMT Phosphoramidite (D); HMT-di Phosphoramidite (E); and N4-Bz-dC-3',5'-di Phosphoramidite (F).

---

**Conclusions:** Thermo Fisher Scientific collaborates with our suppliers and therapeutic oligo manufacturing and developmental partners to offer phosphoramidites that reflect the high standards for which our TheraPure Phosphoramidites have been known since 2002.

Minimizing and controlling upstream single critical impurities as demonstrated in DMTr-Cl, can help reduce stringency of oligo purification and increase overall yields.

Our continuous commitment to the pursuit of deeper control, analytical refinement and quantitation of the phosphoramidite supply chain will maintain Thermo Fisher Scientific as an industry leader and a sustainable partner for the continued growth and safety of oligo therapeutic medicines.

---

**Acknowledgement:** We thank Amal Audi, Syed Raza, Rick Matusik, Roger Knight, Amy Pasley, and Michael Singer for their support.