Table 8: MS of RNA and Oligonucleotides - Minimizing Adducts and Quantitation Best Practices

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Scope:

The favorable regulatory environment, adoption, and breadth of applications as well as a less complex bioprocess propel the rapid growth of RNA-based therapeutics in recent years. The pipeline of short oligonucleotides, such as ASO and siRNA, continues to expand. The seminal work on product-related impurities completed by Capaldi in 2017 continues to guide those working in chemistry, manufacturing and control. Due to the low levels required for impurity identification required for therapeutic release, LC-MS is increasingly adopted. LC-MS benefits from high selectivity, a broad dynamic range and multiplexity enabling low-level quantification. In addition to product-related impurities, there is also the concern of process-related impurities. Oligonucleotides are known to form adducts with alkali metals that they encounter. Adduct formation increases MS spectral complexity, reduces parent ion intensity, directly impacts the sensitivity and accuracy of MS-based quantification. In this round table discussion, we will focus on oligonucleotides and concerns surrounding adduct formation and quantitation.

Questions for Discussion:

1. What are the main sources of metal adducts: samples, solvents, reagents, LC? How to monitor adduct level? What is the acceptable level of adducts?
2. What type of MS are used for oligonucleotide quantification, Triple Quad or HRMS? How to perform data analysis on each type of MS platform?
3. Do you anticipate that the learnings and methods of shorter oligonucleotide can be applied to longer oligonucleotide like gRNA and mRNA? What additional analytical needs do you anticipate in this area?

Discussion Notes:

The session was agile as we switched to focus on mRNA (longer nucleic acids) with some discussion around lipid nanoparticles to better suit the needs of the attendees.

1. Metal adducts – inconsistent between different instrument vendors. Some MS systems like a Qtof with the same sample produce more adducts than others. Inert column chemistry is said to help. Developing a good cleaning method and setting a regular cleaning routine provides more

consistent results; results of cleaning or contamination were checked by running in positive mode. One user also mentioned that they are dedicating an LC-MS system to nucleic acids. In this thread of thoughts we also discussed instrument induced changes of the compounds causing conformational changes (temperature induced changes could be one example).

2. MS mentioned were HRMS. The benefits of MS and understanding not just how much but how much of the correct amount was emphasized. MS/MS is required to confirm sequence and modifications. Fragmentation optimization and alternative fragmentations are needed to improve accuracy. For mRNA, MS compliments the next generation sequencing data and provides an added benefit of requiring small amounts of sample. The higher resolution instruments are used in identification and quantification of modifications like methylation. All attendees mentioned all vendors platforms as well as third party software, PMI. Software solution is needed to simplify data processing.

3. The bulk of the time was spent focused on mRNA. We discussed that mRNA is more stable than RNAi or siRNA. As long as RNAseA, found on so many surfaces including our skin, is kept away from the mRNA it can even remain intact and room temperature for a period of time. Due to its large size most of the conversation was around oligo mapping, starting with the enzyme considerations. RNaseA is the favored enzyme at this time but we anticipate to see more specific cuts like those presented in the Moderna talk. We also discussed the benefit of having RNaseA conjugated to a magnetic bead so that enzymatic cleavage could be better controlled. When considering the CQAs for these nucleic acids they are consistent with their shorter counterparts: ID, sequence, impurities, modifications. For the modifications it is still under investigation whether they are just product quality attributes or a CQA.

In addition to the aforementioned topics we did spend time on lipids and what might be in the future to encapsulation studies. Although FLR tends to be the method of choice a more sensitive technique that looks at individual populations and not just the bulk solution would be interesting. For example, are some LNP holding more mRNA than others and is mRNA sometime sitting on the exterior of the LNP? The final technique we talked about was the benefit of the CAD for LNP and the wish for a 2D analysis with sample siphoned to the multiple detectors.