Roundtable Session 1 – Table 3 – MS of RNA and Oligonucleotides: Characterization and Quantitation of CQAs

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Abstract:

In this roundtable, we will review state-of-the-art technologies and methodologies in the characterization of RNA and oligonucleotides using mass spectrometry. Focus will be on the identification of critical quality attributes (CQAs), and quantitative measurements of such CQAs by mass spectrometric methods when appropriate.

Discussion Questions:

1. What are common MS characterization methods for RNA and oligonucleotide characterization?

2. Are there software available for accurate and efficient data processing?

3. Is it common that quantitative information is derived from mass spectrometric analyses?

4. Are there standards or controls that can be used for method performance monitoring and control?

5. Are there examples of successful method validation? QC implementation?

6. What are the resolutions and sensitivities for impurities or minor variants? Are methods sufficiently stability-indicating?

7. Please bring your questions to the discussion!

Notes:

General question about the usefulness of intact mRNA sequencing via LCMS.

- What value does this bring over other sequencing techniques such as Sanger or NGS?
    - General discussion about mRNA can be capped well and have the PolyA tail, but there is still lot to lot variability when measuring potency. Are there modifications we still don't fully understand? Potentially MS sequencing can see these impurities where sequencing cannot?

- General discussion about enzymes for digestion for sequence coverage notably: RNAse4, RNAse T1, and Rapizyme MC1. Also cited a long separation gradient spanning 4 hours to achieve sequence coverage.

Is the structure of siRNA important?

- Do regulatory agencies require characterization of the phosphorothioate backbone?
- It is not possible to measure, but there may be bioactivity differences. Does Chirality affect potency? We cannot chromatographically separate.

What is the pain point for LCMS separations?

- Issue with sourcing high quality reagents such as ion pair reagents. Most are still quite salty and low purity.
- Option to use HILIC. Not as straightforward as IPRPLC, but in the right hands can work very reproducibly.
- IPRP MS does enhance sensitivity. HFIP can drastically improve sensitivity. HILIC can be as much as 50x worse sensitivity.

General discussion regarding size of polyA tail and a need to understand how subtle changes in tail length or composition can affect potency. If 100 polyA is 100% potent is 120 PolyA 50% potent?

General discussion around chemical stability and potential degradation pathways. Right now, in purity assays for mRNA, peaks before the main peak at called shortmer impurities and longer are called longmers, with no standardized way to investigate those impurities further. Is oxidation present? Clipping may be the major degradation pathway. Is the secondary structure of mRNA important to potency? Does the secondary structure of pDNA affect transcription efficiency? For sgRNA hairpins are important. How do these impurities affect the expression of the target protein? There is still quite a bit we need to study.