

Table 4: MS of RNA and Oligonucleotides: Characterization and Quantitation of PQAs

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Table Scope:

The size range for the category of “RNA and oligonucleotides” continues to expand with the development of ever newer and larger types of mRNA. On the low end of size, oligonucleotides are still anchored with aptamers as small as a few thousand daltons. On the upper end, self-amplifying RNAs (saRNAs) can reach masses measured in the multiple megadaltons. Additionally, while these family members are based on the same basic monomer units, they grow in their chemical diversity with increasing types of modifications. As we learn more about these molecules, new types of biophysical characteristics are being added to the list of relevant product quality attributes (PQAs) or critical quality attributes (CQAs).

While LC-MS and other hyphenated MS approaches are not the only analytical tools, they have evolved to become critical platforms for oligonucleotide and RNA characterization and quantitation in both GMP and non-GMP contexts. In addition to the more established MS-based assays such as quantitative bioanalysis, 5'-capping confirmation and quantitation, PolyA tail characterization, sequence confirmation, intact identity/integrity analysis and sequence confirmation, newer types of assays such as mRNA adduct analysis or lipid/LNP analysis are also becoming more widely used.

Please join us for a discussion of how we are discovering new types of quality attributes for RNA and oligonucleotides that can be addressed with mass spectrometry and how we can best implement assays for these PQAs/CQAs from discovery through product release.

Questions for Discussion:

- Question 1: What MS-based analyses for quality attributes are used for QC/Release of RNA and oligonucleotides? Is the net trend for more or less MS-based QC/release assays?
- Question 2: What MS-based analyses for quality attributes are used in analytical development or earlier?
- Question 3: How have new developments (sample preparation, hardware, software) in RNA mapping increased its use for measuring/monitoring multiple quality attributes?
- Question 4: How will disruptive MS technologies (ion mobility-cyclic or trapped, asymmetric track mass analyzer, electron dissociation, charge detection) change how we measure quality attributes?
- Question 5: Are there disruptive, new technologies that we expect to displace mass spectrometry for some RNA and oligonucleotide analysis?
- Question 6: What unique challenges do we face in transitioning a quality attribute assay from qualitative to quantitative?

Discussion Notes:

- Question 1: What MS-based analyses for quality attributes are used for QC/Release of RNA and oligonucleotides? Is the net trend for more or less MS-based QC/release assays?

Intact MS analysis is utilized in QC/release for smaller oligonucleotide constructs, e.g., siRNA. It's highly possible to see requirements from regulatory agencies for sequence confirmation by LC-MS for RNA as a release test in the next few years. Currently, NGS is the standard method for RNA sequencing, however it does not provide information on the nucleotide linkage or modification. There is a potential to implement MS as a release assay for mRNA even though detailed sequencing is challenging.

- Question 2: What MS-based analyses for quality attributes are used in analytical development or earlier?

The mRNA 5' capping efficiency and Poly(A) tail polydispersity are routinely monitored by MS. Typically, MS instrument calibration is performed on the day of analysis to mitigate mass accuracy issue. If dedicated instrument is used for IPRP-LC-MS analysis, occasionally switching to positive mode running mobile phases with formic acid additive may help address metal adducts issue.

If capping reagent is good enough to achieve consistently high capping efficiency and the process is robust enough, 5' capping assay and Poly(A) dispersity assay may be taken out of QC panel. Run the assay once or twice during clinical development to demonstrate the good understanding of the process. The assays can be run as characterization instead of release testing.

The intact LC-MS analysis to assess mRNA integrity is very challenging but still feasible and the feasibility is dependent on the size of the mRNA. At CASS MS 2022, Moderna showed intact MS data for mRNA up to ~2000nt length with mass accuracy less than 50ppm. SEC-MALS, mass photometry, and AEX HPLC are good orthogonal techniques to assess RNA integrity.

- Question 3: How have new developments (sample preparation, hardware, software) in RNA mapping increased its use for measuring/monitoring multiple quality attributes?

Andrew Dawdy from Pfizer talks about "Oligonucleotide Mapping via LC/MS/MS to Enable Comprehensive Primary Structure Characterization of an mRNA Vaccine Against SARS-CoV-2" in "Session V: New Technologies, Approaches and Methods" on 9/8/2023.

The group also discussed the potential for mRNA oligo mapping fingerprint without MS detection to be used as a second identity test, mirroring the peptide map identity test for therapeutic protein.

Restriction enzymes, DNazyme technology, limited RNase digestion are worth evaluation to develop top-down or middle-down approaches for impurity analysis.

- Question 4: How will disruptive MS technologies (ion mobility-cyclic or trapped, asymmetric track mass analyzer, electron dissociation, charge detection) change how we measure quality attributes?
- Charge detection mass spectrometry - Megadalton published paper on characterizing DNA ejected from AAV capsid.
 - Charge reduction - move to higher m/z range to increase resolution. McLuckey group is working on gas phase ion reaction for negative mode charge reduction.
 - Ion mobility – look at the higher order structure of oligonucleotide, which may impact mRNA potency.

- Potential to develop MS as the platform potency assay.

Key recommendations from discussion group:

1. There is a need for mRNA analytical reference standard material. This would be analogous to NISTmAb for protein therapeutics. The first stage would be a globally available lot of mRNA drug substance (naked mRNA). An organization like NIST would be an ideal steward for this material. Just as was done for NISTmAb, extensive characterization across multiple organizations and platforms could be pursued with subsequent publications published to describe the characterization. This would require some sort of consortium to agree upon the specifics of what that individual mRNA would be (i.e., length, base composition, ORF, UTRs, tail, cap, etc.). The second stage would be an LNP-mRNA drug product. A similar approach would be recommended.
2. There is a need for intact MS analysis of mRNA.
3. There should be a focused discussion on MS for characterization of RNA drug product PQAs as well. As RNA drug substances and products are advancing so quickly, the team felt it would be appropriate to devote a significant portion of the 2024 CASSS MS agenda to RNA characterization.