Table 7: Best Practices in NMR Data Acquisition and Analysis

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Scope:

The use of NMR for HOS characterization of biotherapeutics has become well established in the literature and is seeing increased adoption for industry application. However, as interest in NMR for HOS characterization grows, we have seen a proliferation of new data acquisition strategies as well as data processing and analysis methods reported. It is therefore incumbent to establish fit-for-purpose applications of these various NMR methods and to develop standard operating procedures for data collection and analysis to ensure analytical rigor and to meet regulatory requirements. This roundtable discussion will focus on recent advances in NMR for HOS characterization of biotherapeutics and other complex biologics and how they may best be adopted and deployed in a regulated industrial environment.

Questions for Discussion

1. NMR has an established role in discovery and development, what are the prospects of NMR in regulatory filings and/or QC labs? What issues need to be addressed to move NMR into these realms?
2. Considering the relative performance characteristics and fit-for-purpose of 1D proton versus 2D heteronuclear correlation NMR methods, should NMR be viewed as a single comprehensive HOS characterization platform, or should the 1D and 2D modes be treated as distinct analytical platforms? How about other NMR methods such as TD-NMR?
3. Currently, NMR data are generally handled using a "tier-3"-like visual inspection by spectral overlay. However, chemometric methods have demonstrated better sensitivity for the detection of spectral differences and provide quantification of spectral similarity. Are these chemometric methods needed (or do they "see too much")? If so, how do we best evaluate performance characteristics and integrate into analysis workflows?
4. In terms of data acquisition, what is the primary concern, sensitivity (*i.e.*, experimental time) or spectral integrity/resolution?
5. What about a question of automation? How much does NMR need to turn into a 'black box' for the non-expert operator?

Discussion Notes:

1. NMR has an established role in discovery and development, what are the prospects of NMR in regulatory filings and/or QC labs? What issues need to be addressed to move NMR into these realms?

To satisfy regulatory requirements R&D has to demonstrate structure integrity principally using 13C methyl fingerprint spectra for comparability of innovator/biosimilar in the QC lab. A question was posed if the QC spectrometer needs to be GxP to help ensure compliance but there was disagreement that this would be necessary. Another point of disagreement was whether or not fragments of innovators and their biosimilars would suffice for the comparison and potentially produce better metrics than intact biologics, e.g. mAb. One point of view is that the intact molecule would be required, and another viewpoint was that if 2D methyl fingerprint spectra of fragment and intact biologic were superimposable, comparison of fragments would suffice. Regarding the establishment of universal criteria using for example the NIST mAb, it was generally agreed that universal criteria would not encompass the modality specific, platform specific, different spectral quality samples which will have different benchmarks established using criteria developed in-house. It was agreed that these internal qualification criteria must be verified.

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1D and 2D fit for purpose spectral characteristics are distinct analytical platforms, not a single platform. The 1D approach requires orthogonal techniques because of its exquisite sensitivity to many factors other than HOS. For an R&D filing, secondary and tertiary structure must be retained. Comparability and similarity is faster with 1D spectra but it was generally agreed that 2D spectra inform more on structural integrity relative to 1D spectra. A point was made about making sure that the temperature of long 2D measurements is kept well below (~10C) the Tm1 of the mAb to avoid/minimize temperature-induced structural changes.

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Historically the visual inspection by spectral overlay was what was used and recently is used digitally measuring CCSD with peak lists, but it was agreed that chemometrics produces better statistics. It was stated though that, like 1D, it can be too sensitive, i.e. not necessarily changes involving HOS. Still the preference is for analysis by ECHOS & PCA, especially since PCA moves away from peak lists and thus is less dependent upon processing thereby avoiding the introduction of spectroscopist bias in peak picking.

4. In terms of data acquisition, what is the primary concern, sensitivity (i.e., experimental time) or spectral integrity/resolution? performance characteristics and how to integrate into workflows and software packages

A primary concern is the establishment of a validated pipeline from acquisition-->processing-->analysis. Universally agreed that MATLAB scripts would never be deemed compliant.

5. What about a question of automation? How much does NMR need to turn into a 'black box' for the non-expert operator?

Unanimous approval of using automation because automation: 1) eliminates/lessens knowledge requirement of NMR, 2) affords high-throughput, 3) reduces errors/omissions, 4) minimizes operator subjectivities and, 5) standardizes the measurement for acquisition and processing. It was noted that Fragment Based Drug Discovery by NMR measurements are exclusively done using automation, serving as a precedent. Also noted was that a stable, optimized instrument directly effects the quality of data from automation making that part of the method's verification process. It was greed that automation would ensure compliance of the methodology.