



Quantitative NMR of Challenging Materials

Case study

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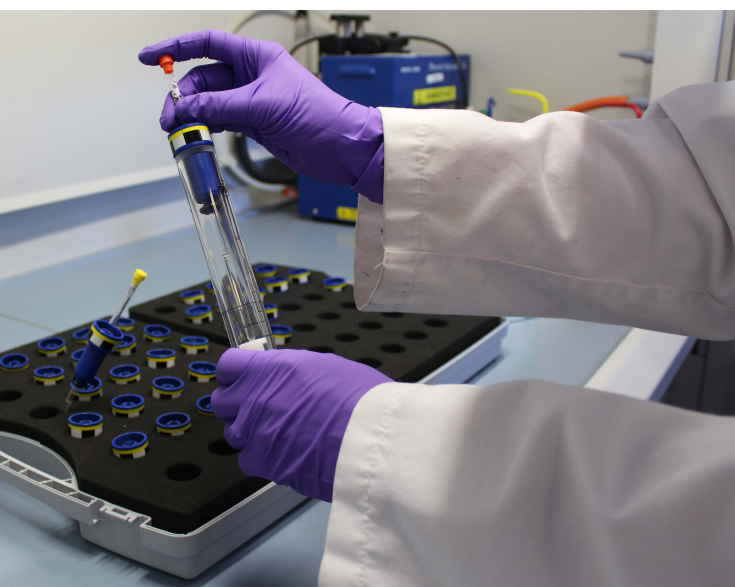
Quantitative analysis plays a key role in certification of primary and secondary reference standard materials. This is achieved using a direct measurement technique such as a chromatographic assay method against a certified material, or indirectly via mass balance after accounting for all impurities. In later phase studies it is prudent to include an orthogonal technique such as Assay by NMR, as part of the release specification to verify the certified purity value.

In instances where the material exhibits sub-optimal behaviour under typical testing conditions - such as reaction with the internal standard - generating a suitable Assay by NMR method may prove challenging. For the scenario described in this case study, the material was best measurable using ^{31}P NMR in Deuterium Oxide (D_2O). The properties of the molecule resulted in signals of interest exhibiting unacceptable signal drift and occlusion when using commercially available ^{31}P Certified Reference Materials as an internal standard. The traditional solution is to use an external standard as described in USP <761>, either via the use of coaxial NMR tubes to measure the solutions together without interaction or by use of ERETIC or similar techniques to measure the test solutions separately.

The drawback to using external quantification is that as the measured species no longer exist in a common solution, volumetric accuracy is introduced as a potential source of error. The volumetric reproducibility of NMR tubes must also be considered as the measured response is a function of both the solution concentration and the effective volume of solution observed by the RF coil. The volumetric reproducibility of commonly used 5mm NMR tubes was verified as part of the study, with an 1.2% RSD observed when analysing the same standard solution.

The primary limitation of ERETIC as executed in Topspin 4.0.2 is that the quantification is performed off a reference generated via single standard acquisition. Variation in instrument performance throughout a run, results in variation of the measured response, which in turn can lead to inaccuracy in the determined purity of the analyte. As such, the study included a check on the repeatability of acquisitions. An RSD of <1.0% for 5 acquisitions of the same replicate was confirmed.

Based on the findings of the study it was confirmed that steps to minimise the impact of instrument performance, particularly shimming, must be taken to ensure that the method is adequately accurate and repeatable. The impact of tube volume variation and instrument performance of the calibration standards was reduced by preparing 5 aliquots in separate NMR tubes. The response for each was determined and averaged for use as the quantitative reference. The sample sequence consisted of three independently prepared replicates of the sample, each using an average of two acquisitions. The validity of the calibration and instrument response was verified by use of bracketing check standards acquired before and after the analyte in duplicate. Acquisition of two separate datasets allowed for the averaging of responses for each tube, mitigating the impact of instrumentation performance on measured response. Following the combination of these measures, the method was successfully tested and issued for release testing.



Release testing using the established method resulted in an RSD of 0.3% across 5 replicates of standard, standard concordance of $\pm 1.0\%$ relative to the certified value for the standard. The agreement of the two check standard replicates was 1.0% and precision of 0.3% RSD for the sample replicates (n=3) was obtained. The mean assay result was in good agreement with the certified purity of the material.

At Almac we have the necessary experience and instrumentation to overcome challenging samples and are able to establish robust Assay by NMR testing methods in accordance with current regulatory guidance using our state-of-the-art GMP qualified Bruker Avance NEO 500MHz NMR spectrometers fitted with Prodigy BBO Cryoprobes.

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