

# Almac Voice

## QNMR – a modern alternative to HPLC



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Nuclear Magnetic Resonance (NMR) has long been the workhorse ID test used by the pharmaceutical industry for all stages of pharmaceutical development and manufacture, providing reliable structural data of active ingredients and impurities. More recently quantitative NMR (QNMR) has come to the forefront of the industry as an alternative to traditional HPLC assay analysis. Often perceived as inaccurate with sensitivity issues this article explores how QNMR is an attractive option for assay analysis of both active ingredients and impurities.

QNMR is an absolute analytical method, where an unrelated commercially available reference standard is used to quantify the compound of interest using quantitative <sup>1</sup>H NMR experiments. The United States Pharmacopoeia (USP), European Pharmacopoeia (EP), British Pharmacopoeia (BP) detail the use of NMR for quantification with the USP providing guidance on the validation of such methods for use in the quantification of drug product, drug substance and impurities.

Almac has invested in GMP compliant NMR instrumentation with cryoprobe technology allowing quicker NMR run times and increased sensitivity, which is often a critical parameter in impurity identification and quantification. This technology also allows the observation of multiple nuclei including <sup>13</sup>C, <sup>31</sup>P and <sup>19</sup>F that are commonly used nuclei for identification in addition to other more specialist nuclei as well as other complex NMR experiments. A white paper published on the use of NMR under GxP by Almac in conjunction with Bruker Biospin will give you some idea of the experiments, method development and applications that we have used NMR for at Almac 1

The combination of this technology and the limited material requirements for QNMR has been proven at Almac to be an advantage over traditional HPLC method development and validation.

Traditional HPLC analysis relies heavily on material related reference standards (or impurity markers), that can be costly to synthesize, isolate and recertify. These reference standards also add a further layer of complexity to projects, particularly to long term stability studies where the availability and shelf life of standards can put a project at risk. QNMR can be particularly useful for quantitative analysis of new compounds where no reference standard of the material is readily available. This is highly applicable in early-stage API development, where unknown impurities often arise. The use of commercially available reference standards for NMR removes this problem at any stage of the project.

Sample preparation for NMR requires only three components (sample, standard, solvent), there is no requirement to generate calibration curves and the QNMR experiments can take as little as a few minutes to perform. This gives NMR analysis further advantage over traditional HPLC analysis that tend to require complex sample preparation, generation of calibration curves and often have extended run times of 20mins plus leading to increased instrument and analyst time associated with this testing. HPLC method development and validation is a significant part of all development programs, with many variables to consider; as well as a more involved sample preparation, a considered approach must be taken with the screening of columns and eluent systems. NMR as an alternative can provide significant time saving as shown in the table below.

Typical time frame	HPLC	QNMR
Method development	2-4 weeks	5-8 days
Method Validation	4-6 weeks	5-10 days
GMP Release testing	1-2 weeks	2-5 days

*Example time frames for analytical work, these will vary depending on the complexity of the method and phase of clinical development*

Impurities that arise in an HPLC assay will require further interrogation prior to quantification. This will take the form of structural identification by other spectroscopic techniques and isolation or synthesis of these materials to allow genotoxicity to be assessed to ensure the safety of the drug is not affected by the presence of these impurities. Where the analysis is performed by QNMR the concentration of each element of the sample can be easily calculated. So, for example an early phase API with an impurity present will require an ID and an RRT to calculate the concentration using an HPLC assay. The same sample subjected to QNMR will provide a relative concentration of this impurity in the sample down to 0.1% accuracy via further interrogation of the generated data. In accordance with ICH Q3B the ID Identification threshold for novel drug products can be as low as 0.1% (dependent on the dose strength) so QNMR can provide a vital limit test for these impurities without the need for the costly investigational process to determine concentration via HPLC.

HPLC analysis is also limited by its use of detectors, there is no universal chromatographic detector across all HPLC methods, with different compounds requiring UV/Vis, RI or CAD detection. This often leads to problems when multiple compounds requiring different detectors are present in the same sample. QNMR avoids this pitfall with determination by <sup>1</sup>H atoms making the probe suitable for a wide range of compounds.

Each of these issues can require different analytical techniques to interrogate and it is not always clear which of these items has given rise to the issue. A lab operating a number of varied techniques can investigate a wider range of issues more efficiently and be able to close deviations and get the product. QNMR can often wrongly be perceived as inaccurate, however daily calibration of the instrument is performed as part of GMP compliance.



The use of NIST certified reference standards in the determination of results also gives reassurance in the results obtained in addition to the need for replicate agreement to be within 2%w/w and stability of the material determined as part of the method development. The use of cryoprobe technology gives enhanced sensitivity over standard room temperature probe configurations with Almac's instrument currently having the sensitivity specification limit set for  $^1\text{H}$  NMR at  $>1350:1$ . This sensitivity allows the quantification of low-level impurities in materials in the ppm range.

A single NMR analysis can provide a concentration value either as a percentage or ppm value of a target compound in a sample. This is particularly useful for crude mixtures from manufacturing streams, polymers and natural products.

QNMR is a powerful analytical tool which overcomes many of the issues with HPLC analysis. With excellent precision, accuracy and a wide range of applications assay by NMR may be the new gold standard release test in the future, making HPLC obsolete!

<https://www.europeanpharmaceuticalreview.com/whitepaper/70112/whitepaper-app-note-bruker-biospin/>

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