

NMR under GxP in Drug Development and Manufacturing

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Abstract

This paper summarises multiple examples of where NMR is used in regulated environments (i.e. GLP and GMP: known as GxP) and describes how the technique adds value during various stages in the progression of compounds from research, via development and then into commercial manufacturing.

A series of examples are described in detail, with the target species being classical small molecule drugs or biopharmaceuticals.

The NMR experiments used in these analyses range from the ubiquitous 1D characterisation (^1H or ^{13}C) through to more complex experiments including 2D e.g. ^1H - ^1H COSY, ^1H - ^{13}C -HSQC and ^1H - ^{13}C HMBC.

Overall, it is clear that NMR is used widely under GxP and is an important technique that supports drug research, development and manufacturing.”.

* Almac has a dedicated NMR team operating two Bruker NMR spectrometers to meet the requirements of both external client’s pharmaceutical manufacturing needs and its own

manufacturing and development processes. In addition to these instruments a further Bruker NMR system is dedicated to its drug discovery pipeline. Analysis performed using NMR encompasses all aspects of the drug lifecycle from early phase research and development through to commercial drug products. Below we will detail several key requirements of NMR systems when used in a GMP environment and discuss the use of NMR in the discovery, development processes and at all stages of the manufacturing process.

** Bruker BioSpin has a dedicated Pharmaceutical business team that are actively developing multiple different applications of magnetic resonance as applied to Pharmaceuticals and BioPharmaceuticals. This includes lead discovery and optimisation, medicinal chemistry support, metabolite identification and elucidation, impurity identification and elucidation, drug potency determination, phase purity ... to the very end of the drug cycle with for example contactless vials and syringes fill check. The further development of the portfolio of Bruker BioSpin’s products that support operations in compliant environments is a strong theme within the dedicated team.

Introduction

From the development phase through to commercial manufacturing, all analytical methods employed by the pharmaceutical and biopharmaceutical industries must be designed carefully, validated appropriately and operated according to relevant SOPs by trained and competent personnel.

In addition, all methods must pass multiple evaluations and assessments (by both internal auditors and also by external regulators, for example the Food and Drug Administration (FDA) and the Medicine and Healthcare products Regulatory Agency (MHRA)). Initial assessments are triggered by the new-product filing process, but also take place periodically during subsequent manufacturing operations.

It is relatively uncommon for the details of analytical methods used under GxP to be reported in the open literature. In part, this is because they may contain commercially confidential details, but most likely it is due to the fact that for very widely used analytical techniques (for example those based upon the separation sciences), the implementation details of a specific method do not contain much, if any, information that is generically useful to others i.e. they do not “teach” anything important about the analytical technique itself.

However in the case of NMR, outside of a relatively small group of technique specialists, it would appear that the technique is not widely known for being used in GxP environments¹. This is a surprising perception since the technique has been widely used in GLP and GMP environments for decades and the assays performed form the basis of numerous important technical functions in the pharmaceutical and biopharmaceutical industries, including product release to patients.

This white paper is designed to redress this generally held, but inaccurate perception by describing multiple methods that are in current use within this sector. Clear examples are disclosed, many of which are drawn from the work of the company Almac, who are part of the pharmaceutical industry². The text also contains multiple literature references including a series of methods defined in the United States Pharmacopoeia (Appendix 1).

The Drug Discovery Phase

NMR use in drug discovery does not require the same stringent controls that are required in the highly regulated drug manufacturing phase: drug discovery instruments are generally not required to be GMP compliant.

A brief description of early phase discovery is provided here, mainly for the sake of context, but also to preface the various changes that are required for operation under GxP during later stages. It is also worth highlighting that there is a strong current trend to expand good practices such as adherence to the principles of data integrity into discovery environments.

It is noted that the use of NMR during the drug discovery phase is extensive and the techniques is an indispensable aid to chemists employed in drug discovery. However, the discovery stage of is not a focus for this paper and only a couple of examples will be given.

There is an ever growing availability of fragment based libraries and NMR has become a key to the development of new drug targets³. Within Almac a dedicated 500 MHz Bruker AVANCE NMR with Prodigy TCI CryoProbe is used for fragment based screening activities in addition to performing routine analysis to support medicinal chemistry. Fragment based screening identifies targets for their drug discovery pipeline providing key information to the research and development chemist.

Overview of Pharmaceutical Development

Figure 1 shows the phases of drug development that follow on from the initial discovery process, right through to commercial manufacture of Active Pharmaceutical Ingredients (APIs) and final products. All phases of this development sequence make extensive use of NMR, and it is also noted that many of the types of analysis performed during manufacturing are derived directly from the development and validation of NMR assays that take place during the earlier phases.

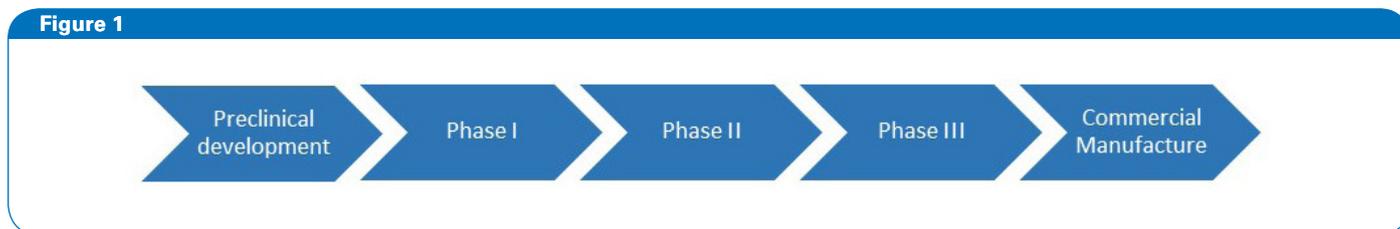


Figure 1: Phases of drug development

¹Kellenbach, E. and Dani, P., “NMR in Pharmaceutical Manufacturing”, eMagRes, 4(2), 2015. DOI: 10.1002/9780470034590.emrstm1397

²This paper is partially based on a webcast delivered by Dr Kerry Hughes on 28th Jan 2020 that can be accessed through the following link: <https://www.bruker.com/events/webinars/the-application-of-nmr-for-drug-development-and-manufacturing-in-a-good-manufacturing-practice-setting.html>, accessed 30Mar20

³<https://www.theresonance.com/fragment-screening-by-ligand-observed-nmr>, accessed 30Mar20

The “Transition” to GxP

Moving from the discovery phase through to the process development and manufacturing phases requires the principles of GxP to be adhered to: the regulatory requirements escalate in line with the phase in which the material is to be used.

To allow the use of NMR in these later stages, the requirements of working under GxP must be met, and a number of steps must be taken before the instrument can be brought into use. The regulatory authorities have issued guidelines for the steps required to reach GxP compliance⁴: the process of getting an NMR instrument into a GxP ready state (as with any instrument in a GxP environment) is known as instrument qualification.

Instrument Qualification

Instrument qualification must be thorough and can take several months to complete - Figure 2 shows the steps required to qualify a GMP instrument of any type. This process was recently followed by Almac for the qualification of a 500 MHz AVANCE NEO NMR spectrometer equipped with a Prodigy CryoProbe. Qualification begins before the instrument is even purchased with the NMR spectrometer first being selected based on its capabilities compared to the technical requirements of the analytical methods for which it is to be used. This selection is undertaken through the drafting of a User Requirements Specification (URS) which systematically evaluates the purpose for which the instrument is to be used and ensures that the specifications will meet multiple regulatory requirements such as compliance with the 21 CFR Part 11 guidelines. As the purchase of an NMR is also a significant financial investment, consideration also needs to be given to the needs of the business in the future which may of course evolve and change. In collaboration with Bruker Almac’s URS was then matched to the instrument specifications that can be supplied from a range of components (i.e. magnets, probes, consoles, accessories and servicing). Once it was established that the requirements were met then it was possible to proceed with the purchase.

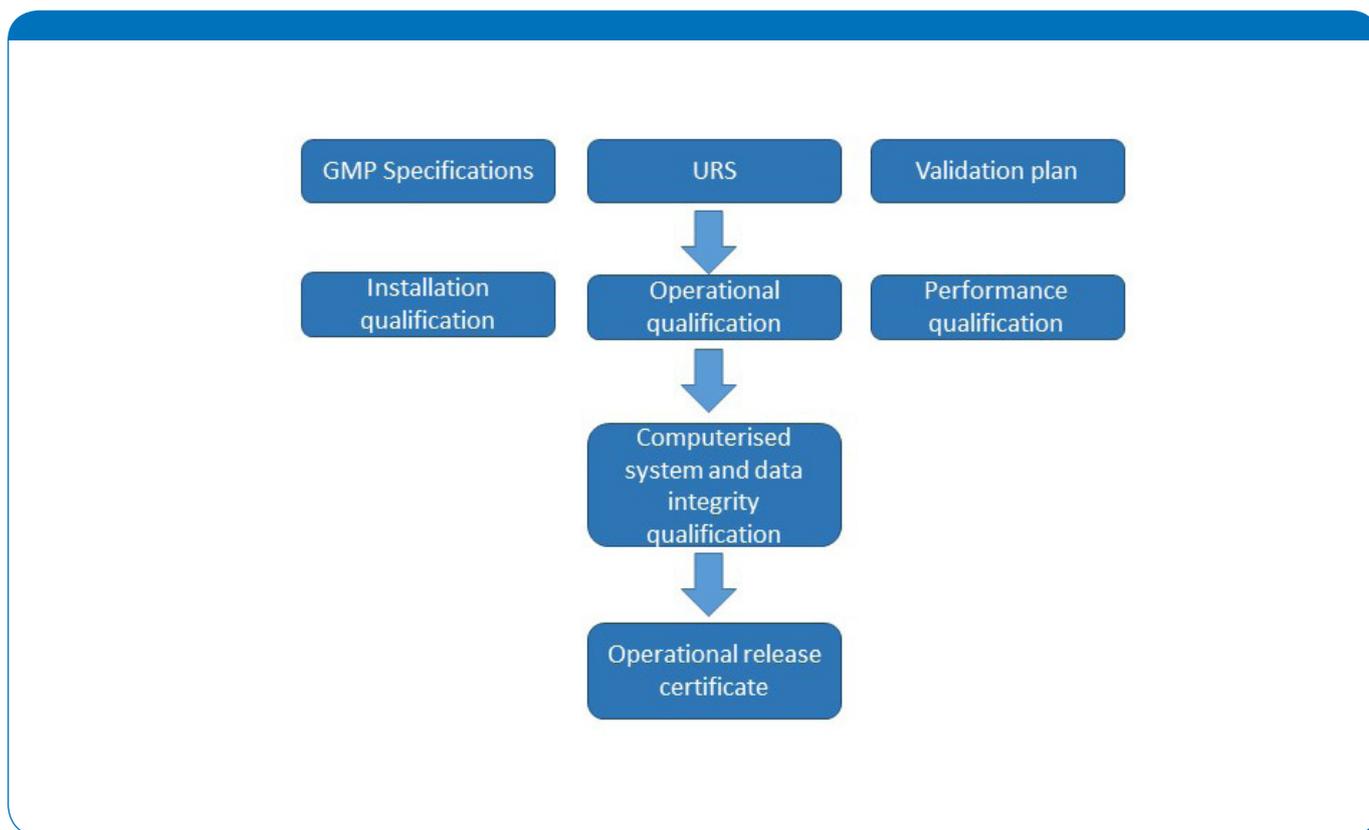


Figure 2: Instrument qualification flowchart

⁴<https://www.fda.gov/media/92841/download> accessed 09 Dec 2019 (on the date of access, this page indicated that it has last been updated on Jul2015)

On-site instrument commissioning includes an extensive and rigorous installation and operational qualification phase. This is undertaken by GxP trained Bruker engineers and includes testing of the operation of the system so as to establish that it meets Bruker's factory specifications and also to test the computer system to ensure the requirements of 21 CFR Part 11. Part of Bruker's qualification is then incorporated into Almac's own instrument performance qualification to ensure that the instrument is operating to the required specifications, throughout its lifetime.

Detailed impact and data integrity assessments are also completed in order to ensure full instrument compliance. Part of this testing also involves assessing comparability with previously generated methods to ensure that the same results can be obtained on the new instrument. In Almac's case this required running a number of methods that had previously been performed on an AVANCE I 500 MHz NMR and an AVANCE 1 400 MHz NMR to ensure that the results obtained on the new AVANCE NEO 500 MHz NMR were comparable. See figure 3 for an example of an assay by ¹H NMR for a commercial material performed on the AVANCE II 400 MHz with QNP and AVANCE NEO 500 MHz NMR with a Prodigy CryoProbe, as performed during instrument qualification.

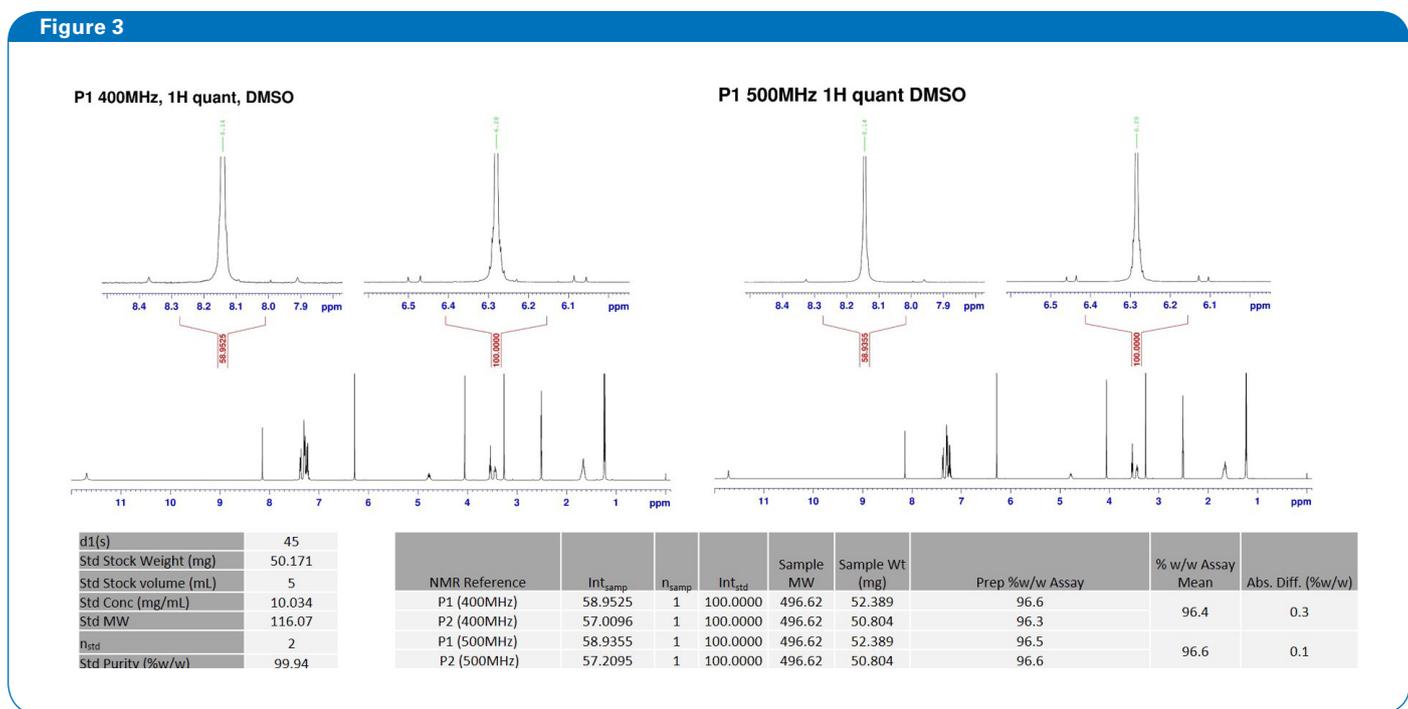
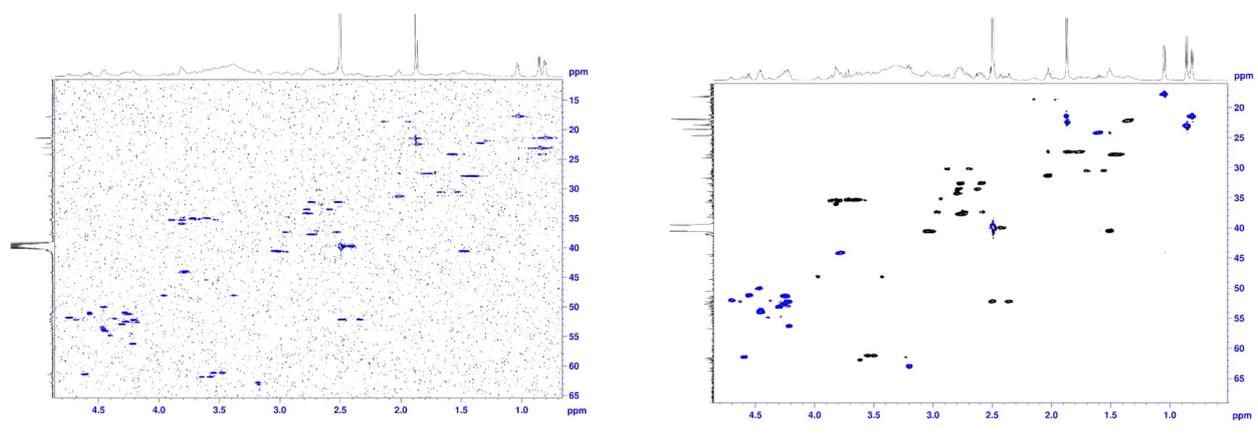


Figure 3: Assay by NMR comparison during instrument qualification

As a further example of this comparability work, Figure 4 shows the comparison of 2D NMR spectrum acquired on the AVANCE III 400 MHz NMR with a QNP probe and the AVANCE NEO 500 MHz NMR with Prodigy CryoProbe. As can be seen, there is no impact on the results of the qualitative assay although the spectra acquired by the up-to-date NEO with a Prodigy probe clearly have much better signal to noise and were acquired much more quickly (<10% of the time required on the older system).

Upon completion of a successful data integrity assessment and performance qualification, the instrument is then formally approved for use and an operational release certificate is issued.

It is noted that formal release of the NMR instrument for GMP use is not the end of the performance testing for the instrument. For a system to be used in a GxP environment it must have continued scheduled maintenance and performance qualification (PQ) completed at set intervals to ensure performance is maintained within specifications. These maintenance activities and PQ tests takes the form of daily and weekly performance checks of line shape and sensitivity, in addition to quarterly, biannual and annual maintenance and calibration to ensure initial qualification specifications continue to be achieved. Additionally, a record must also be kept of any unscheduled maintenance and repairs and this includes the trending of breakdowns or repairs to ensure that issues do not escalate since these could subsequently affect instrument performance, and ultimately of course have an impact on patient safety.

Figure 4

^1H - ^{13}C HSQC, QNP, ns=8 run time = 4.5 hours.
Scaled in order to see all peaks of interest, high background noise clearly visible.

^1H - ^{13}C HSQC, Prodigy, ns=2 run time = 20 mins.
Scaled in order to see all peaks of interest, no background noise visible

Figure 4: Identification by NMR comparison during instrument qualification

A high level of qualification activities are performed on each instrument in order to bring it into GMP use and should any component part of the instrument need to be replaced then it must be on a 'like for like' replacement. Where this is not possible then additional change control⁵ and performance verification must be implemented to ensure that the instrument maintains its GMP compliance.

Reference Standards

Before material is released for commercial use there is a requirement for a reference standard to be available to ensure subsequent production meets the standards of the material that was originally released. There is also the need for impurity reference standards to be made available and it is common for a manufactured API to have multiple impurity reference standards to be in place. Reference standards require full characterisation and NMR is commonly used as an identification test with quantitative NMR⁶ being used in conjunction with other quantification techniques such as GC and water content to provide purity data. These reference standards must also have an expiry date and will require re-certification at set intervals to ensure that the material continues to meet pre-defined specifications. Identification by NMR often forms a critical test at re-certification with the results obtained over a number of years being assessed to detect any trends that may be present. Impurity reference standards are also certified to the same exacting requirements as API reference standards as they can form a critical part of impurity identification in future

manufacturing campaigns (as either qualitative or quantitative standards). It is noted that the amount and type of testing that is required for these materials is dependent on their end use: quantitative reference standards may only require identification testing while quantitative standards will require identification and quantitative analytical testing such as purity assessment.

Stability Testing

APIs and formulated drug products are both required to go through an appropriate amount of stability testing prior to their regulatory approval for commercial use. The material must be tested at the conditions it is expected to be stored at prior to use, in addition to numerous other conditions that are designed to stress-test the material. These stressing conditions take the form of varying temperature and humidity storage conditions over a set period of time. The temperature and humidity storage conditions are set out in the ICH guidelines Q1A (R2) to Q1E⁷. This testing is typically performed over several years to ensure adequate data has been compiled to satisfy regulatory standards. Figure 5 shows an example of a stability testing protocol for an API or drug product. Identification by NMR testing detailed in this example protocol involves comparison of the spectrum of a stressed sample to that of a spectrum of the reference material to confirm that structure has not changed. Again, although NMR is used routinely in stability testing as an identification test, it may also be used to confirm the identity of degradation products and, and quantify them in the parent material.

⁵https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/MagneticResonance/NMR/brochures/T173714_pharma-change-control.pdf, accessed 30Mar20

⁶Goda, Y., "Introduction of qNMR to the Japanese Pharmacopoeia (JP) for specification of marker compounds used for standardization of herbal medicines", *Planta Med*, 79 - SL79, 2013. DOI: 10.1055/s-0033-1351904 .

<https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0033-1351904>, accessed 30Mar20

⁷ICH Topic Q 1 A (R2) Stability Testing of new Drug Substances and Products https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-1-r2-stability-testing-new-drug-substances-products-step-5_en.pdf (retrieved Apr2020)

Figure 5

- Storage conditions:
 - 25°C ± 2°C/60% ± 5% relative humidity (RH) over a period of 36 months
 - 40°C ± 2°C/75% ± 5% RH over a period of 6 months.
 - With samples at 30°C ± 2°C/65% ± 5% RH over a period of 6 months and samples at 5°C ± 3°C over a period of 36 months. As backup in case of failure at the above conditions.
- Testing requirements:
 - Appearance
 - Identity by ¹H NMR
 - Purity and Impurities by HPLC
 - Water Content by KF titrator
 - Melting Point (DSC)
 - Particle Size Distribution
- Time points:
 - T=0, 1, 2, 3, 6, 9, 12, 18, 24, 36 months

Figure 5: An example stability testing protocol for an API / drug product⁸

Process Development

Process development is an essential step in the transition between drug discovery and drug manufacturing and covers many of the studies that need to be undertaken as part of pre-clinical and clinical trials. Often, such studies are run alongside the production of small scale demonstration batches prior to a full manufacturing campaign. This allows the development of many of the parameters that are to be used for future synthesis, manufacturing campaigns and stability testing activities. The data generated from this phase often forms part of the filing process with the regulatory bodies such as the FDA or MHRA. NMR is used extensively in the characterisation of the intermediates, final products and impurities that are identified during this phase. This may also involve the validation of meth-

ods for identification by NMR. Purity of materials and quantification of impurities are also determined during this stage and again such activities may lead to the development of validated quantification methods for use in subsequent manufacturing campaigns. The requirements for these validations follow the International conference on harmonisation (ICH) guidelines for pharmaceuticals for human use. These guidelines bring together the regulatory authority requirements and pharmaceutical industry best practices worldwide.

Detailed in Figure 6 below are the various phases of the drug manufacturing process where NMR is employed. Manufacturing can vary in scale from a small number of kilograms per batch up to multiple tonnes per batch. Many of the methods employed in the identification or quantification of the materials described later in this document will have first been developed at an earlier phase of the drug development process based on small gram scale chemist synthesis up to multiple 100 g demo batch syntheses.

Figure 6

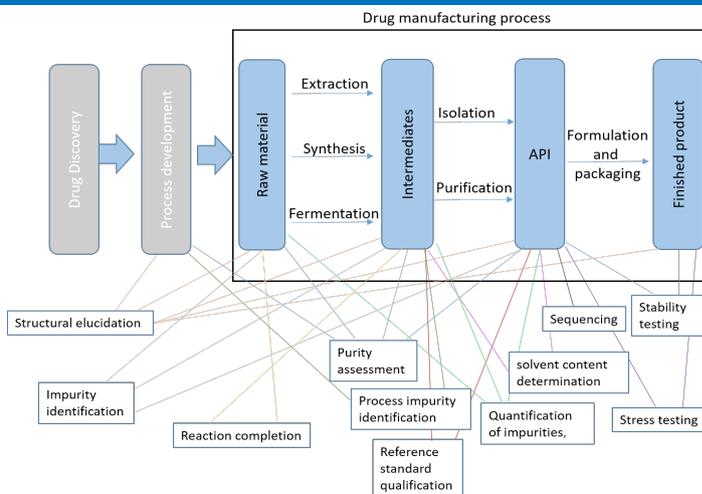


Figure 6: NMR in the drug manufacturing process

⁸Details are from a stability protocol in use at Almac

⁹ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf (retrieved Apr 2020)

Drug Manufacturing

Shown in Figure 6 is the drug manufacturing process which also details numerous examples of how NMR is employed in this phase. The use of NMR for each of these materials is discussed below in more detail.

Raw Materials

Testing of raw materials involves the characterisation of the raw material to ensure that it is as expected and for example enables assessment of the purity of raw material to ensure adequate charging of reactors. NMR provides a fast and efficient way to assess these raw materials across multiple batches. In many cases this is performed using a simple 1D proton NMR experiment, with acquisition taking less than one minute, followed by comparison with a previously generated reference spectrum. Purity is often assessed with a quantitative NMR experiment performed against an external standard. Once these tests have been performed and purity and suitability have been established, the material is then

released into the manufacturing process. This testing is necessary to ensure consistent supply and conformity of these raw materials, which are often required in large quantities for a manufacturing campaign. Testing of multiple batches from each potential supplier is also completed before primary and secondary suppliers are chosen. This is a particularly important step for materials required in the manufacture that are described as 'critical raw materials.' Shown in Figure 7 is an example of a supplier qualification test using on NMR based analysis of a critical raw material required for a manufacturing campaign. As can be seen from the ^1H NMR data there are differences in the material from the two suppliers, but clearly there is greater batch to batch variability in the spectra from one supplier that are shown on the right hand side of the figure (particularly in the 1-2ppm region). This analysis provided information on the impurity profile of the material from a number of suppliers and enabled an informed purchase decision of material suitable for the campaign at kilogram quantities. Testing of raw materials also provides critical information that allows production chemists and chemical engineers to better manage the manufacturing process and the quality of the final API produced.

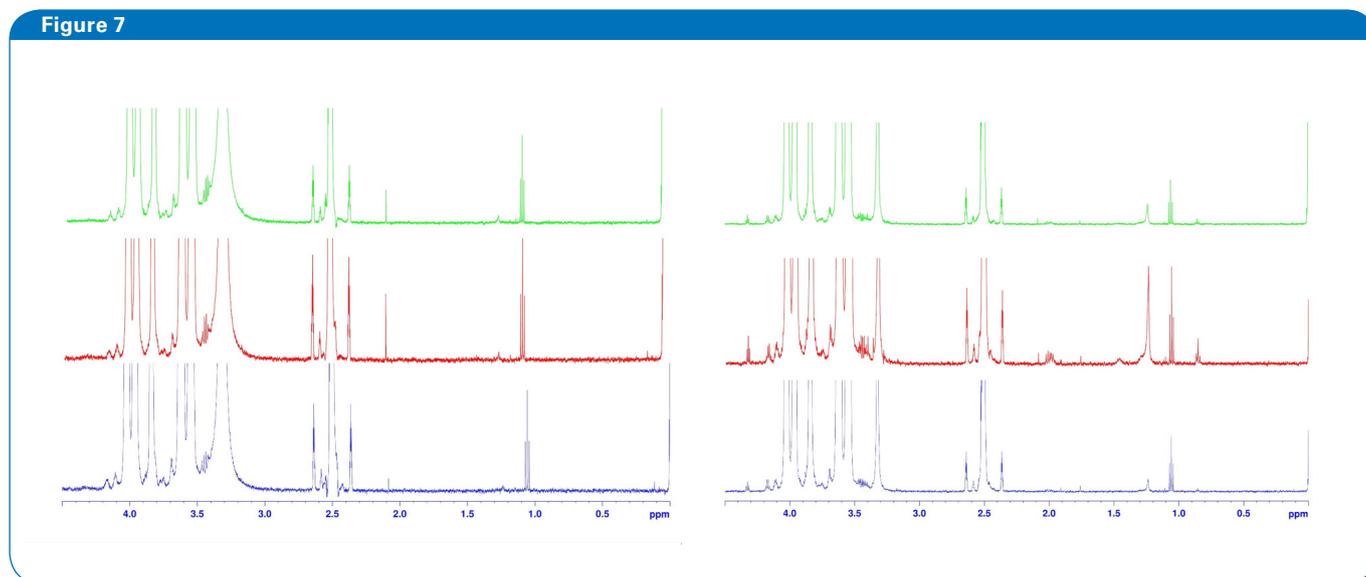


Figure 7: Overlaid spectra of different batches of a critical raw material from two suppliers.

In-Process Testing

Testing during the drug manufacturing process can be very important but can vary greatly depending on the step in the process, the criticality of the step and the type of material produced in the subsequent manufacturing steps. Often these tests are key to ensuring a smooth manufacturing run and they are also often time critical with manufacturing running 24/7. Many tests will be performed by plant chemists using manufacturing directives or shift Quality Control (QC) analysts working with previously developed and vali-

dated analytical methods. Analysis may take the form of a simple chemist checking on the process to ensure formation of the desired product or reaction completion as part of an in-process control (IPC) test. In many instances, there is also testing performed on isolated intermediates (IIRs) to ensure formation of the correct material prior to sign off for continued manufacturing. Chemist checks, IPCs or IIRs for identification often take the form of a simple ^1H NMR qualitative analysis performed with comparison to a previously generated reference spectrum. Purity may also be required for the intermediate and will determine the charging details for subsequent steps.

This testing is performed by the NMR analysis of a sample of material against a quantitative reference standard of known purity using pre-determined quantitative experimental parameters. The assays are performed in duplicate or triplicate to ensure replicate agreement and that precision criteria of <2% absolute are met. Shown in Figure 8 is an example of the quantitative analysis of an IIR. Details of commonly used sample quantities and the experimental parameters are detailed. The assay results obtained as a % w/w are given for two replicates in addition to the results of the stability testing performed on the samples. All results shown meet the required precision criteria. Results will also have to meet the pre-defined specification limits for that material. In some cases this will be a simple reported result and the value is then used for charging calculations. It may for example have a limit such as greater than 95% w/w for the specification limit if this purity value has been pre-determined as being required in order for subsequent steps are to perform as required.

obtained from this will then determine if further material processing (e.g. additional drying) is required prior to its use. Many solvent content methods are developed based on relative quantification by NMR. Here the integral of a sample peak is compared to the integral of the solvent peak to determine the concentration within the material. NMR provides an easy means of assessment with quick sample preparation for both the identification and quantification. With relatively fast turnaround times for analysis and processing and basically no method development, using NMR methods means minimal delays in manufacturing. A number of these methods are in place and in use across numerous manufacturing campaigns at Almac that assess isolated intermediates for release. Shown in Figure 9 is an example of methanol content determination performed on an API. Steps similar to this are taken for the development of a solvent content method for an IPC or IIR with spiking performed over a designated range to ensure that the method gives repeatable results over the range for which it is to be used.

In some instances there is also a requirement to carry out a solvent content determination as levels of solvent present can often impact on subsequent processes. The result

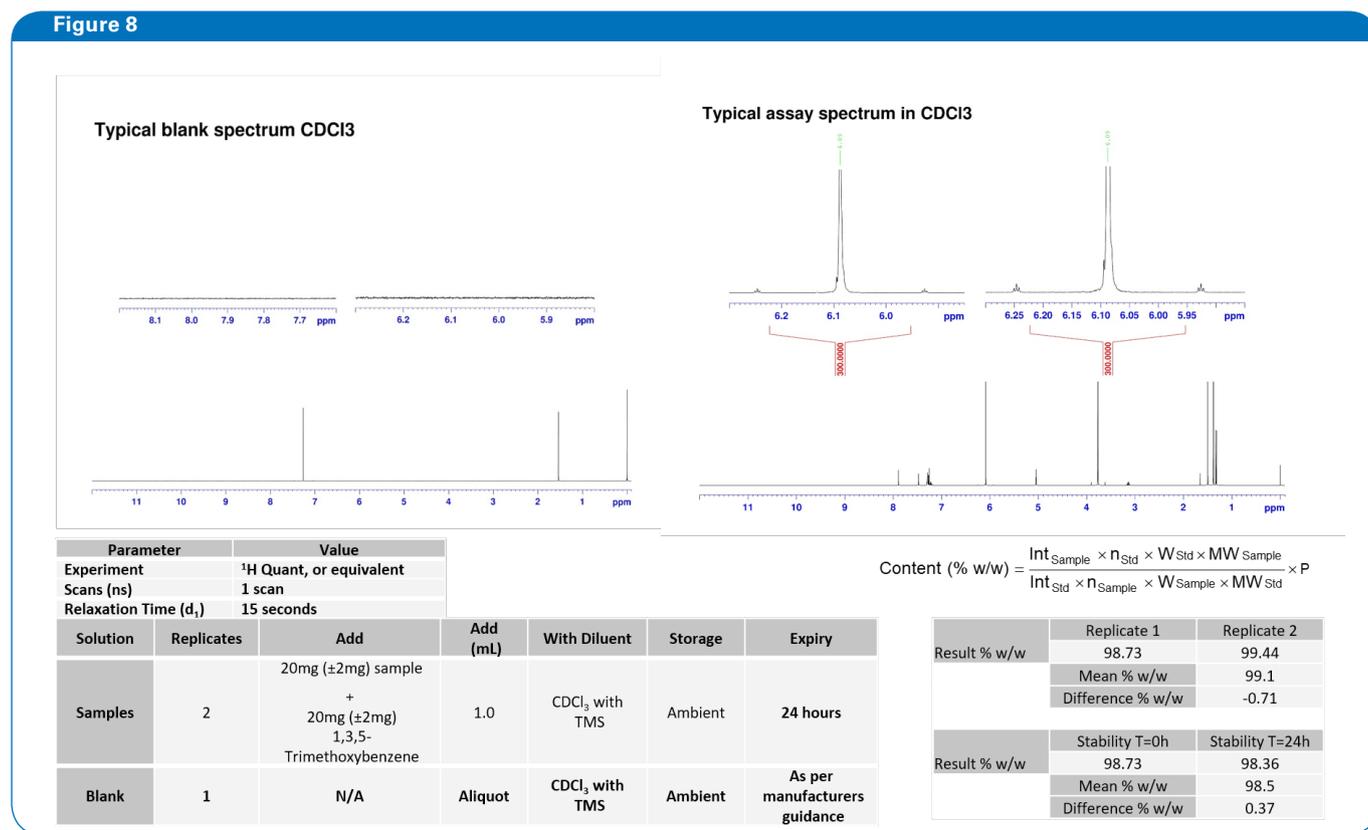
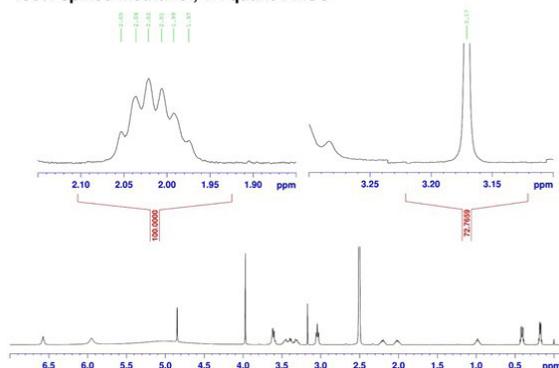


Figure 8: Example of quantitative ¹H NMR method for an IIR

Figure 9

150% spiked Methanol, 1H quant DMSO



Sample	Signal :Noise	Mean Signal :Noise
LOD-1	11.2	11.7
LOD-2	10.6	
LOD-3	13.4	

Sample	Spiked Recovery (%)	Mean spiked recovery (%)	RSD (%)	Overall RSD (%)
50% P1	104.8	102.8	1.8	7.8
50% P2	103.0			
50% P3	100.4			
100% P1	88.2	89.9	2.4	
100% P2	88.1			
100% P3	93.2			
150% P1	93.5	104.3	6.7	
150% P2	104.9			
150% P3	111.8			
150% P4	107.0			

LOD determination 1H quant, DMSO

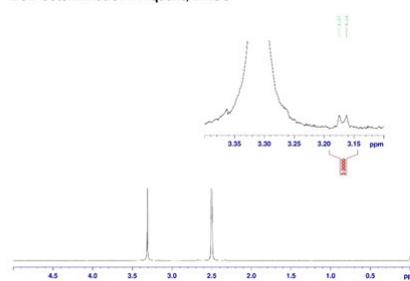


Figure 9: Validation of methanol content in an API

Final products: active pharmaceutical ingredients

Analysis of final APIs is governed by the requirements of the regulatory authorities and the market into which the final drug product is to be released. This testing usually requires a wide range of analytical techniques to cover multiple variables and for which individual specification limits have been set: and all of which must be met to allow release. Much of this testing relies on the use of validated methods and specification limits that are in line with those set by the international council for harmonisation (ICH) in their guidelines. Validation of NMR methods takes the same form as the validation of other analytical methods. For example identification testing require validation for specificity whilst quantitative methods require validation of accuracy, precision, specificity, linearity, stability and range. There is also the requirement for all impurities formed in the manufacture of APIs (at > 0.1%)¹⁰ to be identified and quantities of these impurities in the API batch to be assessed. Solvent content of final APIs must also be assessed. This is often done in conjunction with GC and for NMR may take the form of a solvent quantification against an external standard or a limit test. This again relies on validation of methods in accordance with the internationally recognised ICH guidelines. Shown in Figure 9 is an example of a methanol content method validation performed on a material in Almac. As per the ICH guide-

lines determination of the levels of impurities is performed as a limit test and must be validated for the following parameters: specificity and limit of detection. The example shows the results of spiking of the sample at 50-150% of the nominal concentration of methanol with recovery assessed and %RSD calculated. The %RSD achieved is in line with that achieved for a solvent content method performed by GC. The use of validated limit tests by NMR offers an alternative to GC with much less development time required.

APIs also require full characterisation as part of their release and a number of identification and analytical characterisation techniques may be employed. NMR is very commonly used and a raft of NMR experiments may be employed to ensure full and unambiguous assignment of the chemical structure of the material being studied. The most basic list of tests that are performed include ¹H, ¹³C, ¹³C-DEPT, ¹H-¹H COSY, ¹H-¹³C-HSQC and ¹H-¹³C HMBC. This experiment list is typically performed to aid the structural elucidation of small molecules by ¹H and ¹³C NMR.

For batches that are regularly manufactured on a production plant there will frequently be a reference reference spectrum available to allow quick confirmation of structure between batches. Shown in Figure 10 is the ¹H-¹³C correlation experiment (HSQC) assignment of a small peptide molecule performed with the aid of the NMR experiments listed above. Manufacture of APIs however does not always take the form

¹⁰ICH Q3A (R2) Impurities in new drug substances <https://www.ema.europa.eu/en/ich-q3a-r2-impurities-new-drug-substances> (retrieved April2020)

Figure 10

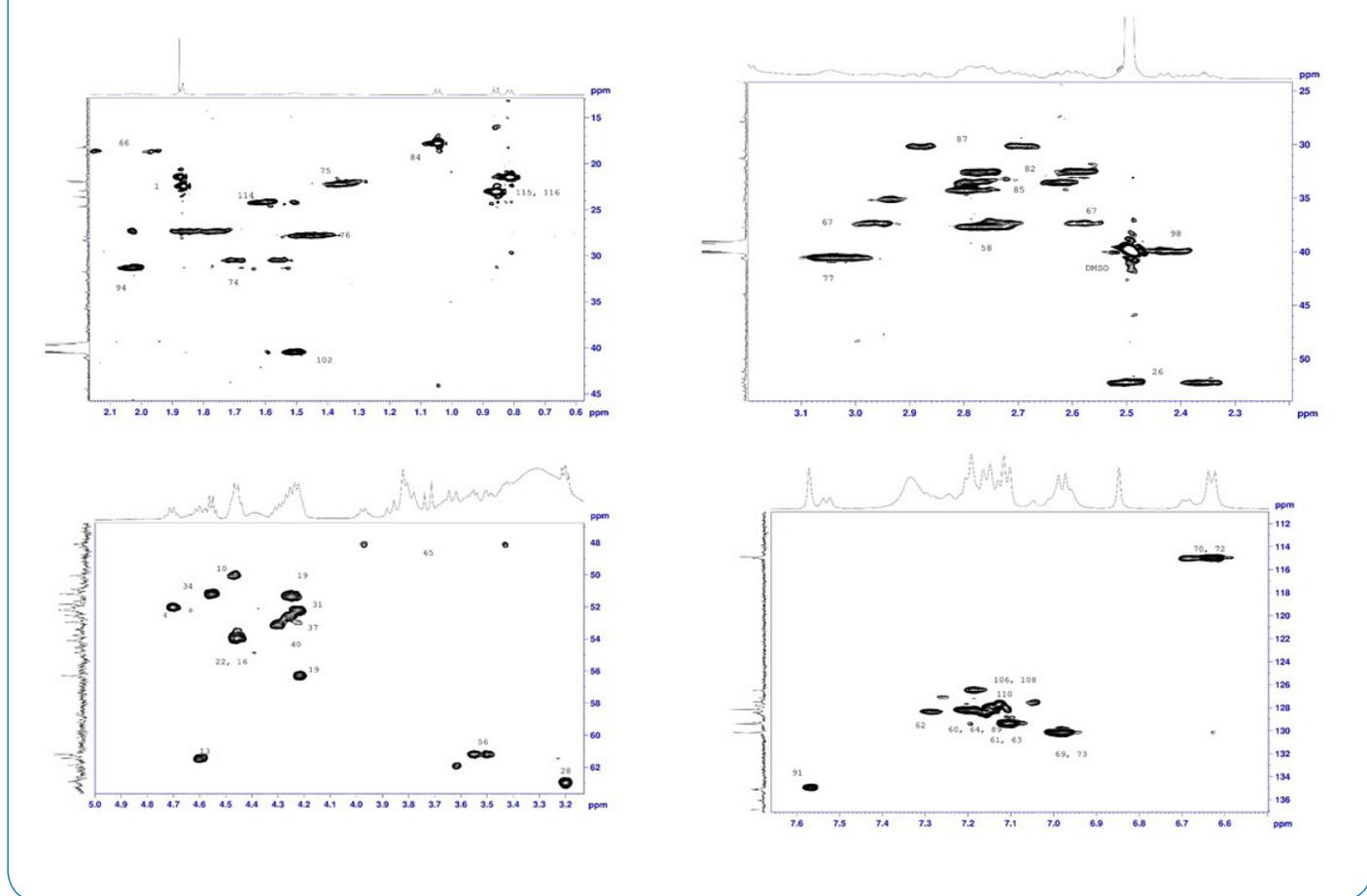


Figure 10: Annotated HSQC assignment of a small peptide

of small molecules, there can also be larger molecules that are synthesised. There is, in fact an increasing number of large peptide molecules coming through the drug discovery pipeline to manufacturing for early phase clinical trials. These again require full characterisation, of course in addition to peptide sequencing. The various advances in NMR technology over the last decade or so have allowed the characterisation of these larger peptides to be performed with sufficient speed, sensitivity and resolution. The use of a Prodigy cooled CryoProbe has allowed Almac to undertake the characterisation and sequencing of peptides, consisting of 10 to 40 amino acids. Prior to the purchase of this new probe, characterisation of these peptides was difficult and in some cases of limited use, however this service is now routinely offered by Almac. To carry out the full characterisation of these peptides and peptide sequencing the acquisition of the above experiments in addition to ^1H - ^1H TOCSY, ^1H - ^{15}N HSQC, ^1H - ^1H ROESY and ^1H - ^1H NOESY are required. Figure 11 shows the peptide sequencing performed on a 13 amino acid peptide performed using the NMR experiments detailed above.

Final products: vaccines

A collection of NMR methods applied to carbohydrate conjugate vaccines have been described in several conference proceedings and a book chapter¹¹. The book chapter contains the following phrase:

“High field NMR spectroscopy has been established as an extremely useful and robust method for tracking the industrial manufacturing process of these vaccines from polysaccharide bulk antigen through to the final formulation.”

A recent insightful paper from the GSK’s vaccine unit in Siena, Italy that has recently been published¹² entitled:

“NMR Assays for Estimating the O-Acetyl Content of Meningococcal Polysaccharide Serogroup A in Quadrivalent Conjugate Vaccine Formulation”

¹¹Berti, F. and Ravenscroft, N., “Characterisation of Carbohydrate Vaccines”. In: Lepenies, B. (ed) “Carbohydrate-Based Vaccines”. Methods in Molecular Biology, 2015, Vol 1331. Humana Press, New York, NY.

¹²Berti, F. et al “NMR Assays for Estimating the O-Acetyl Content of Meningococcal Polysaccharide Serogroup A in Quadrivalent Conjugate Vaccine Formulation”, ACS Omega, 2019, 12, 12827-12832.

Figure 11

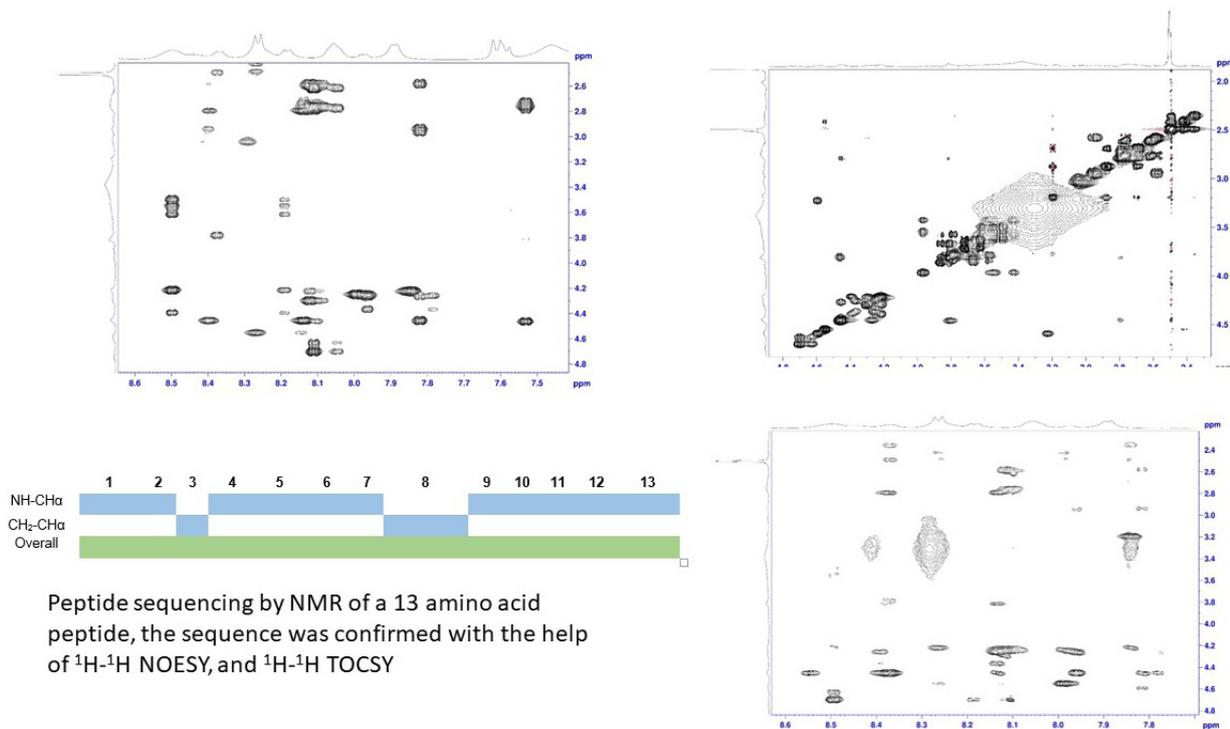


Figure 11: Peptide sequencing by NMR

Analysis Following Material Release

Analysis of API materials does not end once the manufacturing process is complete. There are a number of additional steps required to ensure continued quality of future manufacturing campaigns and quality once a product has been placed on the market. The testing undertaken at this stage can be continuous and be required by the regulatory bodies to ensure material quality and integrity is maintained throughout the shelf life. In some instances the country in which the drug product is to be used will also have additional testing requirements to ensure the material meets certain quality standards. This testing may need to be performed and shown as a customs check prior to the entry of the drug into the country. Testing of this nature may be required by the regulatory authority of that country on all batches of the material or on a randomised selection of batches.

One prominent example is the testing of heparin following a contamination crisis. The quality issues with this important product (12-15 KD glycosaminoglycan used for several indications) that date from approximately 2006 are well known in

the technical literature. What is perhaps less well known is the important role that has been played by NMR in the determination of the cause of those quality issues. An extract from a prominent publication¹³ of the time is reproduced below:

“Recently, several analytical tests have detected differences in suspect versus control lots (5). Screening of heparin lots by a combination of optical rotation, capillary electrophoresis, and one dimensional NMR indicates a defined pattern that can be used to distinguish suspect from control lots. In the case of capillary electrophoresis, suspect lots contain an additional, leading edge peak in addition to the broad peak associated with heparin. Proton NMR analysis indicates distinctive differences between suspect and control lots, most prominently in the acetyl region of the spectrum (2-2.2 ppm). Given the nature of these analyses, and the differences observed upon comparison of suspect versus control lots, the source of the major contaminant was surmised to be a highly sulfated “heparin-like” contaminant (5).

To understand further the structure or structures of the contaminant(s) present within specific lots of heparin, we sought to identify the major contaminant. This exercise required the use of multiple orthogonal techniques, including multidimensional NMR, to overcome the challenges inherent

¹³Guerrini, M et al, “Oversulfated Chondroitin Sulfate is a major contaminant in Heparin associated with Adverse Clinical Events”, Nat Biotechnol. 2008, 26(6), 669–675.

in the analysis of complex polysaccharides, including heparin, which in and of itself comprises a complex mixture of glycosaminoglycan chains. In doing so, we were able to determine definitively the structure of the contaminant(s), isolate it, and confirm the structural identity by comparison to a chemically synthesized standard."

The contamination of heparin led to the requirement that every batch destined for the US market to be tested for contamination and for the results obtained to be included with its shipping paperwork and checked at customs¹⁴. It was determined that ¹H NMR was a suitable means to ensure that the levels of contaminant were below the required level. Almac works with a number of pharmaceutical companies performing this type of compliance testing and this includes the use of NMR to ensure drug products meet regulatory requirements and pass customs checks.

A further example is filgrastim, a 18.8 kDa biomolecule used to treat low blood neutrophils. The US Food and Drug Administration have published their work on the assessment of the Higher Order Structure (HOS) of filgrastim using NMR, online, with the title 'CDER (Centre for Drug Evaluation and Research) Scientists Use Modern Measurement Tools for Quality Assurance and Comparability of Complex Drugs'¹⁵. A direct extract from that page is reproduced below and it is noted that the page contains a useful reference paper¹⁶:

"To test the variability and reliability of NMR methods, OTR scientists have collaborated with scientists in the United States and abroad in a series of experiments to study the structure of distinct filgrastim biosimilar products at four independent NMR laboratories. The group of scientists found that by calibrating their NMR instruments properly, they could generate uniform results across the four independent laboratory settings, even though the samples were handled by different individual investigators from different countries, using NMR instruments of varying magnetic power. The NMR data were so precise and reproducible that the structural information can be securely used for years to come, providing a cost-effective basis for the development of additional filgrastim biosimilar products in the future".

Concluding Remarks

On initial inspection, the number of formally reported NMR methods appears to be relatively small. However, the examples shown in this paper cover a variety of molecules over a range of sizes and describe a number of different objectives. Overall, this is clear evidence that NMR is firmly established as a method that is used in pre-clinical, late stage pharmaceutical development and manufacturing i.e. in GLP and GMP environments.

In this white paper, multiple approaches employed by Almac during drug development and manufacturing are described, several standard methods from the USP are briefly disclosed, together with more recent descriptions of methods for much larger molecules (up to 18.8 kDa).

The underlying NMR methods are based on 1D or 2D approaches, typically using proton and carbon as the observe nuclei. The NMR methods described are designed for the analysis of raw materials, excipients, isolated intermediates and also for APIs and final products. The detailed objectives of such measurements include confirmation of identity of the main components, identity and quantification of impurities and the determination of potency. Work at Almac has shown the identification and peptide sequencing of larger molecules of up to 40 amino acids to date. In addition the literature shows an example of NMR used for the determination of higher order structure. It is clear that NMR is extensively used in GxP environments and in recent years the diversity of its application has increased greatly. NMR offers an analytical technique that is rapid, cost effective and convenient: it can be applied to multiple manufacturing steps and campaigns.

¹⁴Szajek, A.Y et al; "The US regulatory and pharmacopeia response to the global heparin contamination crisis" Nat Biotechnol. 2016, 34(6), 625–630. doi: 10.1038/nbt.3606"

¹⁵<https://www.fda.gov/Drugs/NewsEvents/ucm603877.htm> accessed 24th October 2018 (on the date of access, this page indicated that it has last been updated on 06th April 2018)

¹⁶Ghasriani, H., Hodgson, D.J., Brinson, R.G., McEwen, I., Buhse, L.F., Kozlowski, S., Marino, J.P., Aubin, Y., Keire, D., Nat Biotechnol., "Precision and robustness of 2D-NMR for structure assessment of filgrastim biosimilars", 2016, 34(2), 139-4.

¹⁷ <http://www.usppf.com> - accessed January 2020 (N.B. New users must register for an account)

Appendix #1: USP Methods

As a measure of the maturity of NMR in terms of its use in GxP environments, it is noted that a number of methods are described in the United States Pharmacopoeia (USP)¹⁷. This interesting source of methods includes examples where the objectives include the confirmation of the identity of a substance (which may be an API or an excipient) and the determination of potency of an API. It is also noted that the methods described are based on 1D experiments using ¹H or ¹³C as the observed nucleus (see Table 1 below).

Substance	Function	Method Objective	NMR method Details
Gadoversetamide Injection	MRI Contrast Agent	Determine Performance	Relaxometry
Poloxamer	Excipient	Composition	¹ H method
Hydroxypropyl Betadex	Excipient		¹ H method
Orphenadrine Citrate	API	Impurity	¹ H method
Enoxaparin Sodium	API	Identification	¹ H method
Dalteparin Sodium	API	Identification	¹ H method
Goserelin Acetate	API	Identification	¹³ C
Polyoxyl 10 Oleyl Ether	Excipient	Polymer Length	¹ H method
Polyoxyl 20 Cetostearyl Ether	Excipient	Polymer Length	¹ H method
Goserelin Acetate	API	Amino Acid Content	¹³ C method (Quantitative)
Oxytocin	API	Identification	¹ H method
Chitosan	Excipient	Composition	¹ H method
Heparin Sodium	API	Identification	¹ H method
Hydroxypropyl Pea Starch	Excipient	Assay	¹ H method
Hydroxypropyl Potato Starch	Excipient	Assay	¹ H method

About Almac

The Almac Group is an established contract development and manufacturing organisation providing an extensive range of integrated services across the drug development lifecycle to the pharmaceutical and biotech sectors globally. Its Sciences Business Unit offers services that include API development and manufacture, radio labeling, biocatalysis solutions, peptides and protein technology, pre-formulation, solid state and analytical services.

The international company is a privately owned organisation which has grown organically over the past five decades, now employing over 5,600 highly skilled personnel across 18 facilities including Europe, the US and Asia.

About Bruker

Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, pre-clinical imaging, clinical phenomics and proteomics research and clinical microbiology.

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