ALMAC

Partnering to Advance Human Health

Case study

Impurity level confirmation by Nuclear Magnetic Resonance (NMR) Spectroscopy

ALMAC
Partnering to Advance Human Health

Case study

Impurity level confirmation by Nuclear Magnetic Resonance (NMR) Spectroscopy

This case study discusses two approaches taken when different clients had a requirement for determination of the level of known process impurities in multiple batches of Active Pharmaceutical Ingredient (API). In both cases the detection of the process impurity presented technical challenges due to the inability of most common specific techniques (Liquid Chromatography Mass Spectrometry, Gas Chromatography Mass Spectrometry or Inductively Coupled Plasma Mass Spectrometry) to adequately detect the impurity.

Approach 1:

In the first example, the API material and impurity were assessed and found to have suitable solubility characteristics to allow the use of a simple solvent/internal standard matrix, typically used for Assay by NMR analysis. It was therefore possible to directly observe the impurity alongside the API and quantify against a National Institute of Standards and Technology (NIST) traceable certified internal standard. The solvent afforded good resolution between impurity signals and those of the API material, allowing robust phasing and baseline correction to be performed. The impurity was introduced by spiking, and solution stability and relaxation delay were determined prior to performing a full spike study to test the recovery of the method.

Spiking studies were performed equivalent to 50, 25 and 10 ppm impurity content relative to the sample weight under quantitative conditions, and spike recovery at each level was measured. An unspiked sample was analysed alongside the series and was used to correct for possible intrinsic content for measuring spike recoveries. The recoveries at 50 and 25 ppm were determined to be between 100-105%, and at 10 ppm was found to be 127%. A signal was observed in the same region as the impurity, and whilst it was not possible to definitively confirm that this was the target impurity, the size of the peak equated to approximately~7 ppm impurity as a worst case.

Sample	Theoretical Spike (ppm)	Impurity (ppm)	Recovery (%)	Signal:Noise
Unspiked	N/A	7	N/A	2.0
50ppm Spike	51	59	101.9	13.0
25ppm Spike	26	34	104.8	6.2
10ppm Spike	10	20	127.0	4.0

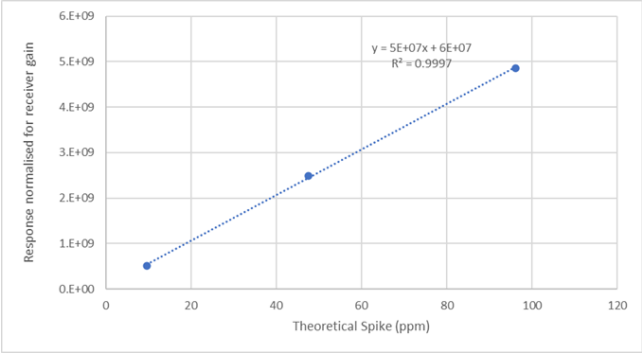
From the impurity spike recovery results, including possible intrinsic impurity content, it was determined that the detection limit for the method was 20 ppm, and the quantification limit was 34 ppm with an experiment time of ~1.25 hours per replicate. Based on the parameters used there is limited scope to further increase the detection limit and quantification limit using this approach.

Approach 2:

In the second example, during initial screening of the second API it proved possible to dissolve both components in a mixture of solvents, however even with optimisation, the resolution of the impurity signals against other matrix components proved to be poor due to the complexity of the API structure. It was possible to visually resolve one of the impurity signals down to 100ppm, however due to poor resolution it was not possible to get good spike recovery, and under quantitative conditions the experiments were prohibitively long at ~2 hours per replicate.

Resolution and signal accumulation were determined to be the biggest barriers to further reducing the detection limit for the method. Based on this assessment, impurity observation after extraction coupled with a limit test approach was determined to be the optimal way forward. Resolution was improved by employing a liquid/liquid extraction to extract the impurity from the API. The API was first fully dissolved in aqueous media to ensure that no encapsulated impurity was excluded from the analysis. The impurity was then extracted into an immiscible deutero-organic phase in which the API was not soluble, allowing for the phase to be collected and analysed with no further work-up. The spectra exhibited significantly improved spectral resolution allowing more accurate peak identification and processing. The detection limit was further improved by reducing the relaxation delay for the experiment and increasing scans to allow greater accumulation of signal.

The method was executed by preparing and analysing a series of spiked samples alongside the API batches equivalent to ~100, 50 and 10 ppm impurity content relative to the sample weight being analysed. The suitability of the approach was checked during execution in accordance with validation guidance for limit tests confirming response, specificity and sensitivity, with all parameters yielding positive results.
The absolute integrals of the impurity spiked API samples were normalised for receiver gain and plotted against the theoretical impurity spike level, resulting in a fit with an R^2 of >0.999, confirming the consistency of the extraction and spectrometer response across the range of interest for the study.



The final method conditions yielded a detection limit of 10 ppm with a mean signal to noise of >6:1 and an acquisition time of <20 minutes per sample, allowing for expedient bulk screening of API batches for the known impurity. This approach affords overhead to further reduce the detection limit through a combination of pulse sequence optimisation and scan increase and gives the opportunity to convert the method to a fully quantitative technique by validation and inclusion of check standards.

At Almac Sciences we offer established “off the shelf” methodology, as well as bespoke method development tailored to fit any use case required by the client. The dedicated NMR laboratory, located in Almac Sciences’

Craigavon Headquarters is outfitted with two state-of-the-art Bruker Avance NEO 500MHz NMR spectrometers equipped with high sensitivity Prodigy BBO Cryoprobes. The laboratory and instrumentation are fully qualified in accordance with cGMP requirements and are maintained and operated by a dedicated team of subject matter experts with decades of experience within the Spectroscopy Services group. Almac Sciences specialises in supporting projects of all sizes from early clinical phase all the way to process validation and commercial manufacture in compliance with all current market specific regulatory requirements.

NMR analytical services available at Almac Sciences include:

- Structural elucidation employing a suite of experiments to confirm the structure of known materials ranging from small building blocks to multi amino acid peptides.
- Structural elucidation for unknown impurities in conjunction with orthogonal techniques.
- Standalone purity determination and impurity quantification employing individually optimised methodology to determine the assay of a compound of interest using commercially available NIST traceable standards. The capabilities of the Prodigy BBO Cryoprobes allow the determination of various impurities to levels consistent with industry staples such as Gas Chromatography with significantly reduced turnaround times for results.
- Phase appropriate method development for recurring sample qualification for Identity/Purity/Impurity quantification in accordance with ICHQ14 and current Pharmacopeial guidance.
- Phase appropriate method validation in accordance with ICHQ2 and current Pharmacopeial guidance.

The above services are available as a standalone offering, or as part of a manufacturing development and support package. Additional services include continuous support of manufacturing projects to provide raw material certification, isolated intermediate certification and in process control checks.

Author: Alexander Kornev, Analytical Technical Leader

almacanalytical@almacgroup.com

Contact us:

Global HQ
+44 (0) 28 3833 2200

US HQ
+1 (215) 660 8500

Ireland
+353 90 646 0200