



NMR in Pharma: Performance Qualification (qPQ) for Quantitative NMR Applications (qNMR)

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qNMR PQ Test with Dedicated Certified Reference Material in a Standard Work Flow

For pharmaceutical development and production facilities, the requirement to perform process qualification is well known.¹ Vendors of sub-systems and instruments that are incorporated within (or otherwise support), pharmaceutical development and manufacturing typically supply tailored qualification documents (IQ/OQ/PQ/CSV). This is exactly the case for Bruker BioSpin, where the overall approach to qualification of high resolution NMR instruments has recently been described in a white paper.²

In that publication from Bruker BioSpin, the PQ step is classified as being slightly different in the sense that end users often perform PQ using a test sample that they “know” very well, as it is regarded as providing a particularly relevant test for the samples that are typically analysed. This is actually part of Bruker BioSpin’s standard approach to supply PQ supportive software (Assure SST – System Suitability Test) that can be configured to use samples which are generated and controlled by the end user, in addition to standard NMR tests like lineshape or ¹H sensitivity based on certified reference samples supplied by Bruker BioSpin.

This note focuses on qNMR and more specifically on potency determination by qNMR i.e. the case where NMR is used to determine the amount of active materials in samples: such samples may be subsequently used as reference materials to support other analytical techniques or for the purposes of batch release. It is noted that NMR has a long history of being used for quantitative applications and it does appear to be an ever more popular approach, as evidenced by some recent publications in this field.³

There are clear advantages in using a performance qualification reference material that has been manufactured by a recognised high-quality manufacturer of such materials. This is partly because of convenience and speed. The ability to generate, test and certify high quality reference materials for the purposes described in this note is not normally available in a typical NMR laboratory. Additionally, separation of the process of generation of the reference standard from that of testing provides a clear degree of independence.

A collaboration between Bruker BioSpin and Merck KGaA, Darmstadt, Germany [*] has led to the development of a certified reference material (CRM) tailored to qNMR. This

¹ Annexe 15 of the EU GMP Guidelines on Qualification and Validation is to be found via this hyperlink: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2015-10_annex15.pdf. Retrieved February 2019

² https://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/MagneticResonance/NMR/brochures/pharma-instrument-qualification_apps.indd.pdf

³ Webster, G.K., and Kumar, S., *Anal. Chem.*, 2014, 86, 11474-11480, dx.doi.org/10.1021/ac502871w; and Pauli, G.F. et al, *J. Med Chem.*, 2014, 57, 9220-9231, dx.doi.org/jm500734a

mixture is specially designed for PQ of qNMR and potency determination with the internal standard method. The new CRM consists of a binary mixture of two well-characterized constituents: 1,2,4,5-Tetrachloro-3-nitrobenzene (TCNB) and 1,3,5-Trimethoxybenzene (TMXB) in a DMSO-*d*₆ solution. The CRM is delivered as a set of 5 ampules, accompanied by a certificate.

These samples are manufactured using current best practices in the Merck KGaA, Darmstadt, Germany [*] manufacturing facility located in Switzerland which works in accordance with the highest industry standards applicable to this sector i.e. of ISO/IEC 17025 and ISO 17034 (see figure 1 below for a typical front page of the certificate supplied with each sample set). The certified mass fraction values result from a combination of gravimetry and qNMR on a Bruker 600 MHz instrument.

Figure 1

SIGMA-ALDRICH® **TraceCERT®**
Traceable Certified Reference Materials

Certificate of Analysis – Certified Reference Material

Bruker quantitative PQ (qPQ) Certified Reference Material

Product no.: 42350
Lot no.: BCBZ9945
Description of CRM: 1,3,5-Trimethoxybenzene (TMXB) and 1,2,4,5-Tetrachloro-3-nitrobenzene (TCNB) in DMSO-*d*₆ (solution)
Expiry date: DEC 2020
Storage: 20-25°C; storage under Argon
Chemical formula: C₉H₁₂O₃ (TMXB); C₆HCl₄NO₂ (TCNB)
Molecular mass: 168.19 g/mol (TMXB); 260.89 g/mol (TCNB)

Produced in double accredited
 laboratory fulfilling
 ISO/IEC 17025 and
 ISO 17034

Constituents	Certified value	Expanded uncertainty, $U=k \cdot u$ ($k=2$) <small>[1][2]</small>
1,2,4,5-Tetrachloro-3-nitrobenzene	23.51 mg/g	0.18 mg/g
1,3,5-Trimethoxybenzene	5.00 mg/g	0.04 mg/g

Excerpt from a typical sample certificate showing critical information on the front page

The production process for this CRM has been carefully designed and thoroughly tested during the development of multiple standards for ¹H and ³¹P qNMR. This includes homogeneity assessments and stability monitoring also at elevated temperature.⁴ The combined standard uncertainty is calculated by combination of the standard uncertainties of the input

estimates according to Eurachem/CITAC Guide “Quantifying Uncertainty in Analytical Measurement”⁵ and ISO17034.⁶

The uncertainty contributions are illustrated by the cause-effect diagram on the top of the following page:

⁴ Weber M, Hellriegel C, Rueck A, Wuethrich J, Jenks P, JPBA 93 (2014) 102-110

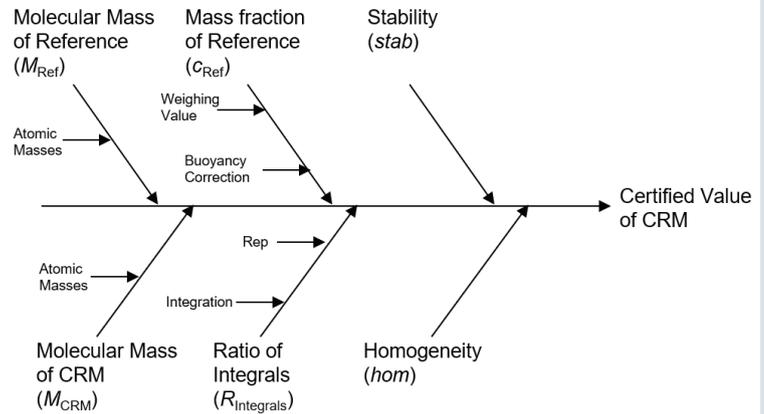
⁵ Eurachem/CITAC Guide, 3rd Ed. (2012), “Quantifying uncertainty in analytical measurement”

⁶ ISO 17034:2016, “General requirements for the competence of reference material producers”

Figure 2

Typical relative contributions are:

$U(C_{Ref})$	<0.20 %
$U(M_{Ref})$	<0.001 %
$U(C_{CRM})$	<0.001 %
$U(R_{Integrals})$	<0.01 %
U_{hom}	<0.01 %
U_{stab}	<0.40 %



Example Ishikawa diagram illustrating the potential sources of uncertainty in the reference value

With the availability of this certified reference material, it is important to acquire and process the NMR data following a convenient, robust, standardized and validated workflow. Additionally, the software must be designed in a way that helps to minimize the possibility of operator errors. For that reason, the qPQ described in this note runs in full automation (which is orchestrated by the package IconNMR™) with the frequency

and timing of the PQ test being defined by the lab manager. As the new qPQ test is incorporated within the well-established AssureSST software, it can be easily combined with other system suitability tests such as lineshape or sensitivity in order to perform a tailored performance qualification of the entire NMR system. As a result the user will get a combined pass/fail report for all tests (see below for an example).

Figure 3

¹H qNMR Performance Qualification Test

Perform qPQ Test

Sample Position

8

Shim Program

topshim

Lot. No.

BCBZ9945

1,2,4,5-Tetrachloro-3-nitrobenzene

23.51 mg/g

Uncertainty

0.18 mg/g

1,3,5-Trimethoxybenzene

5.00 mg/g

0.04 mg/g

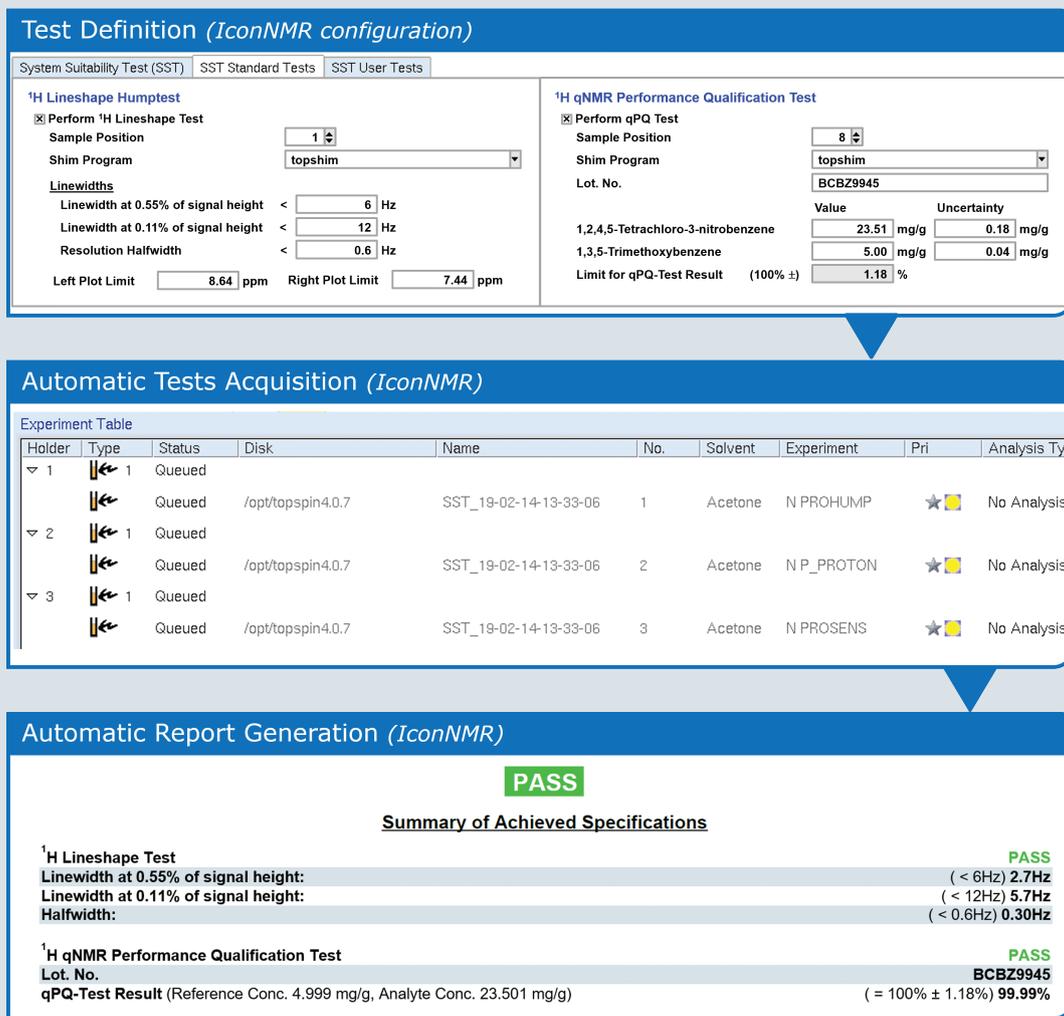
Limit for qPQ-Test Result

(100% ±)

1.18 %

In this dialog box, the operator enters the data on mass fraction values and uncertainties from the certificate and the software calculates the tolerable overall uncertainty value in % (in the box, which cannot be edited)

Figure 4



This figure is a composite screen shot illustrating the overall work flow:

Upper part - This screen shows the IconNMR configuration where the performance qualification tests are selected and parameters defined. This will now include the qPQ test

Central part - This screen shows IconNMR experiment tables, with several performance qualification tests queued, waiting for the acquisition to start

Lower part - performance qualification (also called system suitability) report generated after the tests have been run

[*]The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.