VOL. 8 I AUG 2019

NMR VALIDATION

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Overview of the Newsletter

- ValidNMR Committee in Transformation
- October 2019: qNMR Events
- Certified Reference Materials for ^{19}F qNMR
- Proficiency Testing for qNMR
- Validation guidelines

BRUKER

Practical Applications of NMR in Industry Conference PANIC

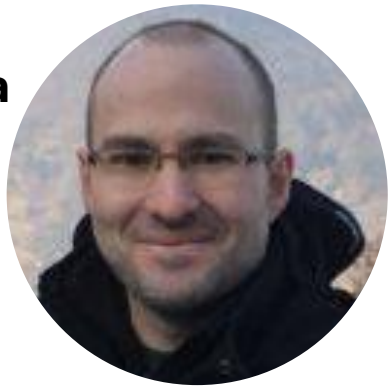
JEOL Solutions for Innovation

Wako

ValidNMR Committee in Transformation

The ValidNMR Committee wants to take the opportunity to thank **Jose G. Napolitano Farina** and **Claudia Boot** for their engagement in the ValidNMR Committee. Jose and Claudia have undertaken a number of tasks such as education and the organization of the NMR Wiki. For their professional and personal futures, we wish them all the best!

Jose G. Napolitano Farina



Claudia Boot

Our new Committee Members!

Klas Meyer

Klas is a researcher at Bundesanstalt für Materialforschung und -prüfung (BAM) in Berlin focusing on the fields of process spectroscopy and quantitative NMR spectroscopy. Klas worked at BAM during his PhD studies on high-pressure NMR spectroscopy in the gas-phase and process applications before joining benchtop NMR manufacturer Magritek in Aachen as an application scientist. At the end of last year he took the opportunity to join BAM again for a permanent position in the process analytical technology group.

Klas will support the Bureau International des Poids et Mesures (BIPM) close to Paris in the framework of international metrology working on the development of qNMR methods and reference materials.

Mike Bernstein

After graduating from the University of British Columbia with a PhD in NMR, Mike worked with NMR and small molecule drug discovery and development in Canada and the UK. His main interests have been structure elucidation and conformation, qNMR, and reaction monitoring. For the last 8 years he has worked for Mestrelab Research. As VP of R&D he is primarily involved with the development of software relating to qNMR and RM.

Bernie O'Hare

"I began my career with Bruker BioSpin providing NMR installation, service, and applications support for a diverse customer base. Soon after joining Bruker, I became involved with their ssNMR-DNP product. I was eventually responsible for all DNP installations and service in the America's and interacted directly with R&D as well as production to provide direct feedback to help improve the product. In 2017, I started a new chapter with GlaxoSmithKline as an NMR spectroscopist within both discovery and CMC spaces. I am currently focused on both small molecules as well as the characterization of higher order structure of biopharmaceutical macromolecules."

- Our October 2019 Events -

October 1, 2019

Bruker's Pharma World Tour, Rockville, MD

More information: page 5

October 2-3, 2019

Fifth International qNMR Summit, Rockville, MD

More information: page 6

October 4, 2019

ValidNMR Meeting, Gaithersburg, MD

More information: page 6

- Bruker's Pharma World Tour -

October 1, 2019

Bruker's Pharma World Tour will be coming to **Rockville**! Join us for a program to explore analytical solutions to current challenges in the design and analysis of biologic drugs. This joint program between **Bruker BioSpin**, and **Bruker Daltonics** and **Mestrelab Research** will provide the latest developments in mass spectrometry and NMR magnetic resonance tools as well as stimulate discussion and share valuable experience. Presentations will be made by both Bruker/Mestrelab as well as industry collaborators.
→ More information: **here**

Biologics

8.30 WELCOME

9.00 – 10.30 TALKS 1 – Chaired by Chen Peng (Mestrelab)

10 min - Corporate intro by VP applications – Clemens Anklin (Bruker)
10 min – Innovative Solutions for the Pharmaceutical Industry – Kate Holub (Bruker)
35 min – high order structure by NMR – Bruker / Mestre – Mike Bernstein (Mestrelab) & Clemens Anklin
25 min – real application of high order structure of biologics – customer TBA

10.30 – 11:00 BREAK

11-12.30 TALKS 2 – Chaired by Daltonics

25 min – MS talk – customer TBA
25 min – culture and mixture analysis– Bruker / Mestrelab – industrial leader TBC
25 min – MS talk – host cell analysis, intact mass, post-translational modification? – Bruker Daltonics

12.30 - 2.00 LUNCH

qNMR

2.30pm WELCOME and reception

3-4.30 pm SESSION 1 - Chaired by Amy Freund (Bruker)

10 min - Corporate intro – Kate Holub (Bruker)
25 min – qNMR intro: update on ISO, state-of the art and trend for the future – external speaker TBC
15 min – Automation solution for qNMR – Mike Bernstein (Mestrelab)
15 min – Compliance GxP – (introduce qNMR under GxP) – Christoph Freudenberger (Bruker)
25 min – qNMR Applications for Accurate Certification of Reference Materials – Markus Obkircher, Merck

4.30-5 pm BREAK – coffee and some pastries

5 -6.30 pm SESSION 2 - Chaired by Amy Freund (Bruker)

25 min - Kriste M. Adams, Steelyard Analytics Inc. – title TBC
25 min - Greg Walker, Pfizer – title TBC
25 min –potency determination of biotherapeutic drugs – external speaker TBC

6.30 pm DINNER

- Fifth International qNMR Summit -

The **United States Pharmacopeia (USP)** is organizing the **Fifth International qNMR Summit** on October 2-3, 2019 in **Rockville**, MD. The event will be conducted in partnership with the Center for Natural Products Technologies (CeNaPT) of the University of Illinois at Chicago. In the short time span since the inaugural Summit of 2016, three more summits – in Berlin, Tokyo and Würzburg – have taken place, along with the thematically linked events in Cologne, Bari and Tokyo. This underscores the great ongoing interest toward qNMR and the need to further spread the word about the technique's advantages within the broad analytical community.

→ More information: **here**

October 2-3, 2019

Topic A
qNMR Implementation and Practice in Regulated Environments (Industry and Regulatory Aspects)

Topic B
Purity and Impurity Profiling (LC vs. qNMR)

Topic C
qNMR Reference Materials / Method Validation Guidelines / Best Practices

Topic D
Practical Analysis of Chiral Compounds by qNMR

- ValidNMR Meeting -

October 4, 2019

Steelyard Analytics Inc. will welcome you at the next ValidNMR Meeting on October 4, 2019 in **Gaithersburg, MD**! Experts will discuss validation of instruments, software, processes and methods to answer the questions of how to guarantee that NMR results meet quality demands and comply with regulations. The meeting will be completed by a social event in the evening with good food and excellent discussion!

→ More information: here

STEELYARD ANALYTICS

- Article: Certified Reference Materials for ^{19}F Quantitative NMR Ensuring Traceability to "The International System of Units" (SI) -

(by Romana Rigger, Alexander Rück, Christine Hellriegel, Robert Sauermoser, Fabienne Morf, Kathrin Breitruck, Markus Obkircher markus.obkircher@merckgroup.com)

In recent years quantitative NMR (qNMR) spectroscopy has become one of the most important tools for content determination of organic substances and quantitative evaluation of impurities. The implementation of

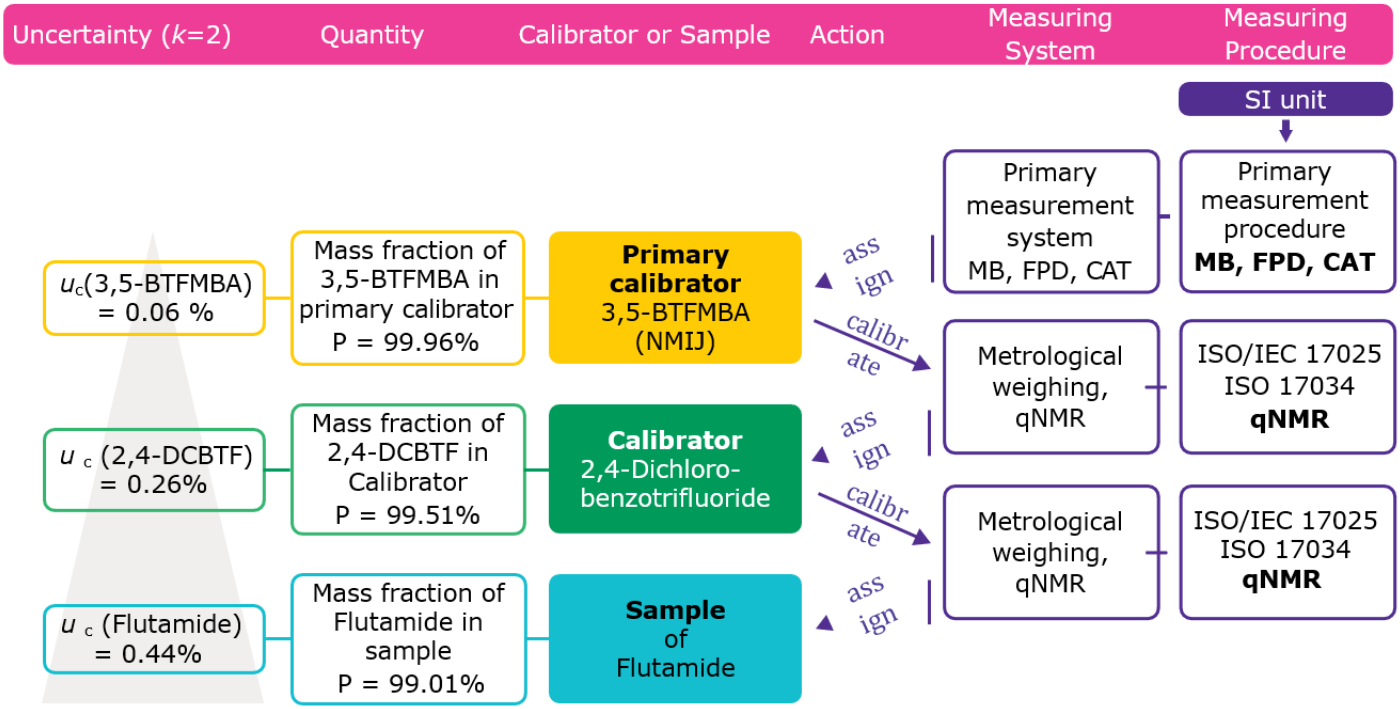
qNMR for new application fields, e.g., metabolomics, environmental analysis and physiological pathway studies, brings along more complex molecules and systems, thus making the use of ^{1}H-qNMR challenging. A smart workaround is possible through use of other NMR active nuclei, namely ^{31}P and ^{19}F.

At our manufacturing site in Buchs (Switzerland), we have been using qNMR since 2009 to produce certified reference materials (CRM) traceable to the SI unit, under ISO/IEC 17025 and ISO Guide 34 (since 2017: ISO 17034) accreditation (an example of a traceability chain is shown in **Figure 1**). The TraceCERT® product

range of organic CRMs suitable for HPLC or GC is certified using this technique and comprises over 200 products including pesticides, vitamins, amino acids, plasticizers, PAHs, antibiotics, FAMEs and many other product groups. In addition to this product range, we also provide a toolkit of qNMR standards traceable

to primary material from NIST (National Institute of Standards and Technology, USA) or NMIJ (National Metrology Institute of Japan), see SigmaAldrich.com/ qnmr. The expansion of this qNMR standard product line with new, interesting CRMs is ongoing and up-to- date 16 different ^{1}H qNMR CRMs with known purity values and small expanded measurement uncertainties have been developed. They cover the whole spectral and solubility range, enabling access to the qNMR certification of hundreds of organic products.

Figure 1. Traceability chain of Flutamide. Certification was done by comparison with 2,4-DCBTF (secondary calibrator) and 3,5-BTFMBA (primary calibrator) and finally to the SI unit. MB = mass balance, FPD = freezing point depression, CAT = coulometric acidimetric titration.



In certain cases, ^{1}H qNMR reaches its limits, especially regarding the certification of complex and larger molecules. However, new fields of application often also bring along the presence of heteroatoms, namely ^{31}P and ^{19}F. Thus we introduced 4 CRMs for ^{31}P qNMR with traceability to the SI.

In the following section, the development of CRM for the use in ^{19}F qNMR is described. This article is an excerpt from our AOAC paper published in 2017. Please refer to this reference for further information.[1]

3,5-Bis(trifluoromethyl)benzoic acid (3,5-BTFMBA, NMIJ CRM 4601-a) is a primary CRM for use in ^{1}H and ^{19}F qNMR certified by NMIJ. The NMR shift range of ^{19}F is very large but the window for linear excitation, which is necessary for ^{19}F qNMR, is quite small and depends on field strength and NMR parameter. Techniques to counter this dilemma were published earlier including the use of new NMR experiments. Therefore, we set out to develop qNMR CRMs with peaks in different shift regions which can further be chosen corresponding to the analytes' shift and employed in standard ^{19}F qNMR experiments. Two of the most common structure elements are CF_3 groups and fluorine atoms bound directly to substituted aromatic compounds. Shifts of ^{19}F in CF_3 groups arise around -55 to -90 ppm, while shifts of fluorine atoms bound to aromatics can be found between approximately -110 and -180 ppm.

Further structure elements show signals between -70 and -140 ppm (CF_2) or between -120 and -240 ppm (fluorine atoms in saturated and unsaturated aliphatic compounds). Recently three different ^{19}F qNMR CRMs were developed by us. They were selected based on various parameters including solubility, stability, homogeneity, purity and shift range. As a prerequisite to show traceability to the SI and the certification concept, we selected molecules that carry both ^{1}H and ^{19}F nuclei.

2,4-Dichlorobenzotrifluoride (2,4-DCBTF, cat. no. 53396) is liquid and the CF_3 group shows a singlet at -61.2 ppm in the ^{19}F spectrum, depending on the solvent (DMSO-d_6). The three aromatic protons show analyzable signals between 7.5 and 8.5 ppm in the ^{1}H spectrum 2-Chloro-4-fluorotoluene (2Cl4FT, cat. no. 80730) is also liquid and the fluorine atom bound to the aromatic ring shows a multiplet at -115.3 ppm (DMSO-d_6) in the ^{19}F spectrum. In the ^{1}H spectrum, again three aromatic protons show peaks between

7.0 and 8.0 ppm and an additional peak can be found for the methyl group at around 2.3 ppm (DMSO-d_6). 4,4′-Difluorobenzophenone (4,4′-DFBP, cat.no. 07563) is solid and the two symmetrical fluorine atoms show a multiplet at around -106.5 ppm (DMSO-d_6) in the ^{19}F spectrum. Eight aromatic protons give signals between 7.0 and 8.0 ppm (DMSO-d_6). All three compounds are soluble in common organic NMR solvents. Molecular weights are 215 g/mol (2,4-DCBTF), 144.57 g/mol (2Cl4FT) and 218.2 g/mol (4,4′-DFBP). Purity values, expanded measurement uncertainties, NMR solvent specific shifts and relaxation times (T1) can be found in **Table 1**.

Technical aspects of ^{19}F qNMR

A characteristic of ^{19}F NMR is given by ^{13}C and ^{12}C satellites that are present in the NMR spectrum. The interaction of ^{19}F with ^{12}C and ^{13}C leads to an isotopic effect and thereby to unsymmetrical satellites on the one hand and to multiple satellites around the main peak in non-decoupled spectra on the other hand.

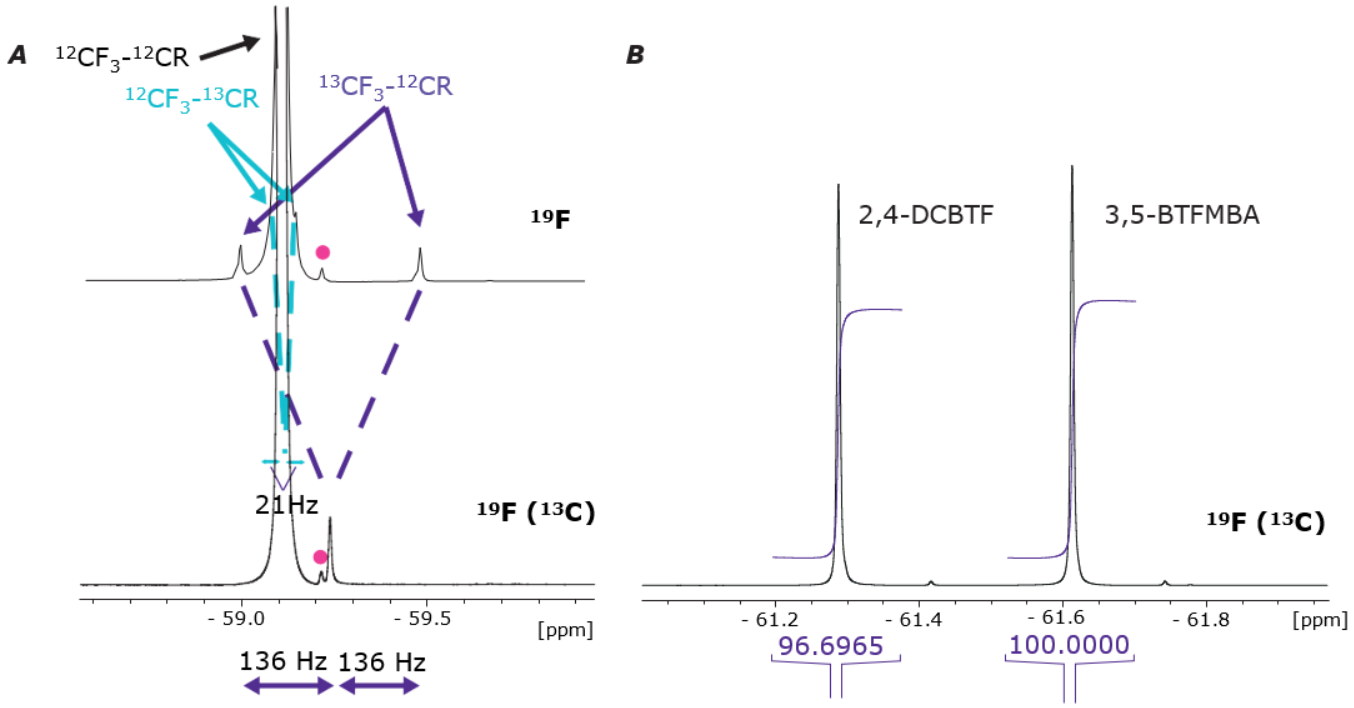
Additionally, peak shapes are different depending on the structure element. In general, CF_3 peaks show singlet signal pattern and aromatic bound ^{19}F atoms multiplet signal pattern.

As with ^{31}P qNMR, inverse gated decoupling was used during ^{19}F qNMR data acquisition. Using this method instead of an e.g., power-gated decoupler minimizes NOE (Nuclear Overhauser Effect) build-up. With this experiment, decoupling is applied only during data acquisition and thus allows the spin system to reach equilibrium between decoupling steps. By applying inverse gated decoupling, only one satellite appears that is on only one side of the main peak (**Figure 2**). When performing pretests, a set of decoupled and coupled spectra was recorded to distinguish between satellites and impurities. Integration of decoupled spectra (**Figure 2**) was done either including both satellites, only the ^{12}C satellite, or if possible no satellite. No matter which possibility was chosen, integration was performed in the same way for the internal standard and the sample compound with regard to the line width. Similar to ^{13}C, the ^{19}F nucleus has a wide chemical shift range. To perform quantitative measurements, broadband excitation over the full spectral width is required. Due to insufficient available radiofrequency power for pulsed excitation, signal intensities and thus signal integration can be error-prone. The effect leads to relatively narrow ranges of frequencies (15 - 30 kHz, 600 MHz NMR, 90 ° pulse) where an accurate quantification can be guaranteed.

Table 1: Summarized data of ^{19}F qNMR CRMs. Solubility tests were done at room temperature using commercially available NMR solvents. T1 relaxation times were recorded for the CRM only (c = 10 to 20 mg/mL at 25 °C). u_c(CRM) is the combined measurement uncertainty of the CRM and d(ppm) is the chemical shift in the ^{19}F spectrum (k=2).

					$CDCl_3$		DMSO-d_6		CD_3OD		CD_3CN	
Substance	Cat. No.	Package Size	Purity (%)	u_c (CRM) (%)	δ (ppm)	T1 (s)	δ (ppm)	T1 (s)	δ (ppm)	T1 (s)	δ (ppm)	T1 (s)
4,4′-Difluoro-benzophenone	**07563**	1g	99.82	0.30	-105.8	2.4	-106.5	1.4	-108.1	2.8	-108.3	2.3
2,4-Dichloro-benzotrifluoride	**53396**	1g	99.51	0.26	-62.5	2.3	-61.2	1.2	-65.4	3.3	-63.0	2.9
2-Chloro-4-fluorotoluene	**80730**	1g	99.57	0.24	-115.8	4.4	-115.3	3.3	-117.7	4.8	-117.3	4.7

Figure 2. A: ^{19}F NMR Signal of Flutamide in coupled (^{19}F) and decoupled ($^{19}F(^{13}C)$) spectra. The pink point is an impurity. Satellites are assymetrically arranged around the main peak in coupled spectra. B: Example for the integration of 2,4-DCBTF (analyte) and 3,5-BTFMBA (internal standard). Both signals were integrated without the outer satellite.



This requires sound pretesting, followed by accurate adjusting of spectral width and transmitter frequency offsets. Furthermore it is important to set the acquisition time as short as possible to avoid NOE build up, but long enough to avoid loss of spectral quality by truncation of the Free Induction Decay (FID). That requires an additional analysis of the FID prior to quantitative measurements. All ^{19}F NMR experiments were performed on a Bruker Avance III 600 MHz NMR instrument equipped with a Prodigy TCI probe head.

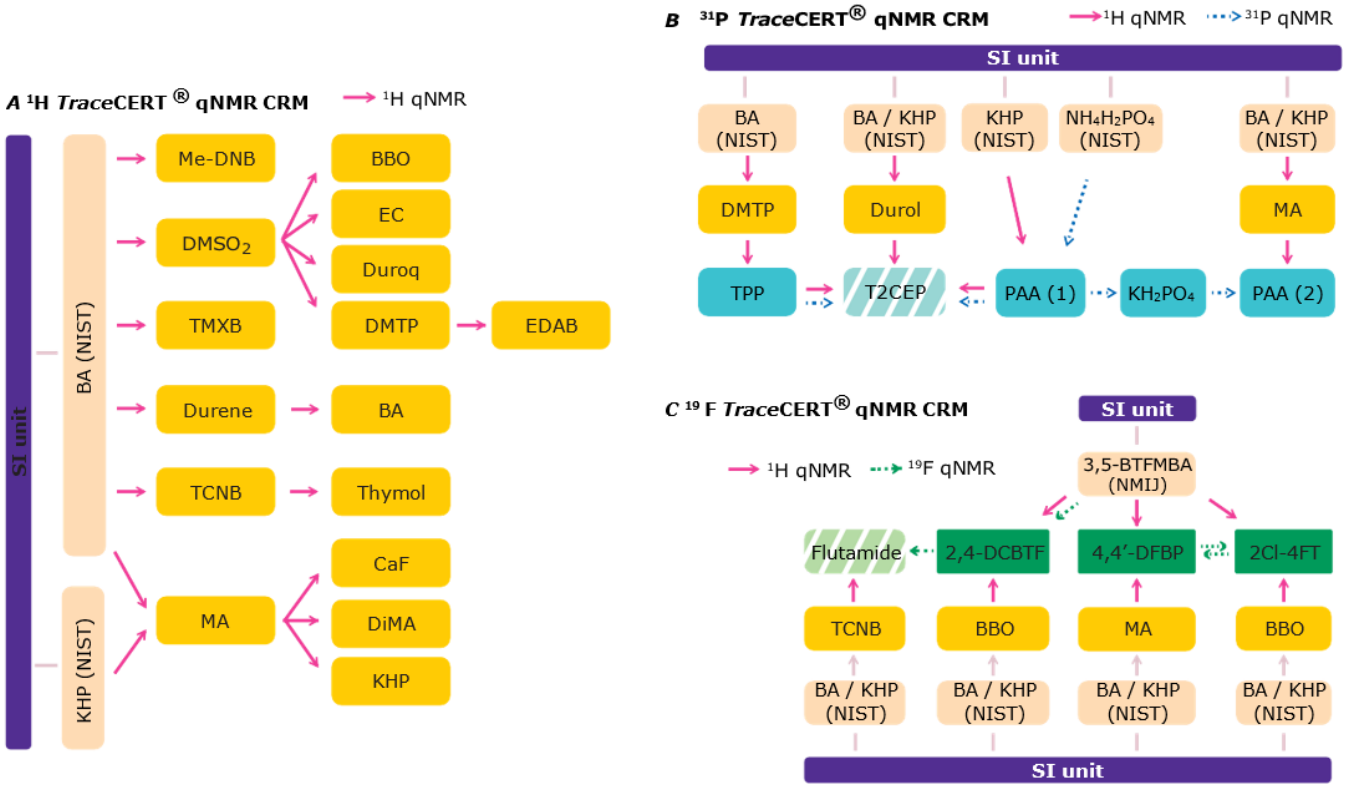
Even though a standard probe (instead of a dedicated ^{19}F probe) was used, a good spectral quality could be ensured. Background distortions by probe head and sample tube materials, pulse breakthrough and ringing artifacts influence the spectral quality, especially the baseline (rolling baseline), which is typical for ^{19}F, ^{11}B and ^{29}Si and increases when measuring over large spectral width. This can be counteracted by either applying additional processing steps (FID repair by cutting the first data points before data transformation) or by increasing the pre-scan delay. For ^{19}F qNMR experiments during the development of our CRMs, an increased pre-scan delay was used and no FID cutting was done.

T1 times were determined by inversion recovery experiments. Typical T1 times for ^{19}F qNMR CRMs are between 1.2 and 4.8 s depending on the concentration, of the mixture and solvent. Multiplying T1 times by a factor of 7-10 gives D1 times between 20 and 35s.

CRM for ^{19}F qNMR - traceability to the SI through primary CRM

Similar to the study published for 31P, a traceability scheme for ^{19}F qNMR CRMs was elaborated to guarantee the traceability to the SI unit and show the comparability of ^{1}H and ^{19}F qNMR experiments and thus the independency of the result of the measured nucleus (**Figure 3**, C). As primary reference material, 3,5-BTFMBA of the National Metrology Institute of Japan was selected. This reference is highly pure (99.96 %), has a very small expanded measurement uncertainty (0.06 %, k=2) and the two symmetrical CF3 groups show a sharp ^{19}F signal at -61.3 ppm (in DMSO-d_6). The three aromatic protons give signals around 8.2 - 8.6 ppm (DMSO-d_6), depending on the solvent. 3,5-BTFMBA is soluble in all common organic solvents and is specified by NMIJ for ^{1}H and ^{19}F qNMR.

Figure 3. Traceability chains for ^{1}H (A), ^{31}P (B) and ^{19}F (C) qNMR CRMs. Pink arrows symbolize ^{1}H qNMR measurements, blue arrows ^{31}P measurements and green arrows ^{19}F qNMR measurements. Light grey boxes indicate primary reference material, dark grey boxes ^{1}H *Trace*CERT® qNMR CRM, dark blue and dark green boxes ^{31}P and ^{19}F *Trace*CERT® qNMR CRM and light blue and light green boxes testing substances (chromatography *Trace*CERT® CRM).



The purity value of 2,4-DCBTF was certified by ^{19}F and ^{1}H qNMR using 3,5-BTFMBA. In a second way, certification was done with ^{1}H qNMR using 1,2,4,5-Tetrachloro-3-nitrobenzene (TCNB, cat. no. 40384) with traceability to the primary CRM BA (NIST SRM® 350b). The three purity values and their expanded measurement uncertainties are in perfect accordance (SD = 0.015, **Figure 4**). Values for uc(CRM) (k=2) are also comparable between the different experiments (0.25 – 0.29 %). Due to different signal shapes and spectral regions of peaks, 2Cl4FT and 4,4'-DFBP were certified by another route. Traceability to the SI for 2Cl4FT was achieved by determining a mass fraction via ^{1}H qNMR using 3,5-BTFMBA. In a second way, Benzyl benzoate (BBO) was used as internal standard. A third value

is assigned by ^{19}F qNMR using 4,4'-DFBP as internal standard. The purity values from the three different measurements are overlapping within their expanded measurement uncertainties and again show good accordance (SD = 0.053, **Figure 4**). The uncertainty values uc(CRM) (k=2) are similar to that of 2,4-DCBTF (0.24 – 0.41 %).

The purity value of 4,4'-DFBP was certified via ^{1}H qNMR using 3,5-BTFMBA, and in a second way Maleic acid (MA, cat.no. 92816), as internal standard. ^{19}F qNMR certification was performed using 2Cl4FT, showing again the independency of the result of the observed nucleus. All three values are comparable and the SD of the three results is small (SD = 0.055, **Figure 4**). The values of uc(CRM) are slightly higher compared with the other two ^{19}F qNMR CRMs (0.30 to 0.37 %). The increased uncertainties (e.g., 0.41 %, 2Cl4F2 and 0.37 % 4,4'-DFBP) do not result from the measurement procedure but are caused by a higher uncertainty contribution by the internal standard (4,4'- DFBP, MA) and homogeneity of the material. In all other ^{19}F certifications the overall repeatability of the measurement represents the most significant uncertainty contribution.

A last experiment was done to assign a purity value to the TraceCERT® Flutamide CRM. It was possible to show, that via ^{19}F qNMR and using 2,4-DCBTF as internal standard, comparable results were achieved as by the common route via ^{1}H qNMR using an established CRM (BBO).

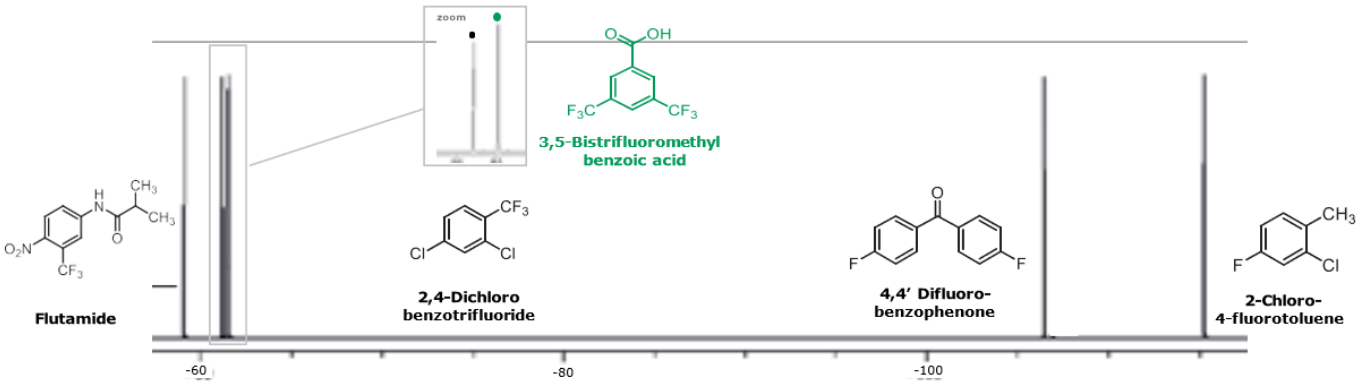
Again, overall repeatability of the measurement represents the most significant uncertainty contribution, which is in the same order for certification via ^{1}H and ^{19}F. The purity values are overlapping within their expanded measurement uncertainties (**Figure 4**), which is again a clear indicator that ^{19}F qNMR can be used routinely

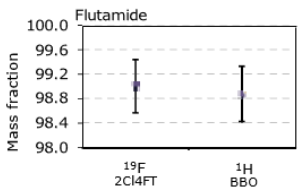
as a stand-alone method to assign the purity of fluoroorganic substances.

Conclusion

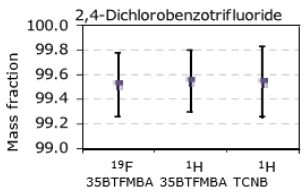
In summary, qNMR using ^{1}H, ^{31}P, or ^{19}F TraceCERT® CRMs is a very valuable method. We outlined sensitive aspects that are important for an accurate qNMR certification and need particular awareness by the operator. The presented set of ^{1}H, ^{31}P, and ^{19}F qNMR CRMs is produced fulfilling the requirements for a reference material producer under ISO 17034 accreditation, covering additional data such as homogeneity of the material and short-term and long-term stability.

Figure 4. ^{19}F spectrum showing shifts of different secondary CRMs and the primary CRM 3,5-BTFMBA. Depending on the structure element (CF_3 or aromatic bound F) peaks are shifted to different regions. Results for the purity determination of secondary CRMs via ^{19}F and ^{1}H qNMR and using different internal standards are shown. Different values for purity of an analyte (P_{CRM}) are within their expanded measurement uncertainties u_{CRM}. The certified values for 2,4-DCBTF, 4,4'-DFBP and 2Cl4FT are shown in bold, and CRM 4601-a was selected as the primary CRM for the three. Due to chemical shifts in the NMR spectrum, direct comparison on the basis of ^{19}F was only possible in the case of 2,4-DCBTF. In the cases of 4,4'-DFBP and 2Cl4FT, ^{1}H qNMR had to be used, but also referencing to CRM 4601-a. Flutamide could be measured in both ways.

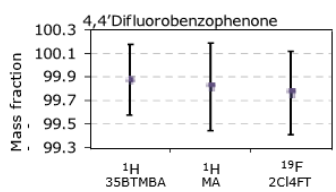




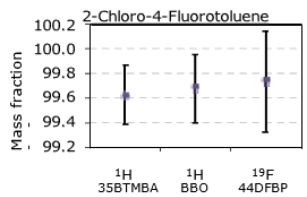
P_{CRM} (%)	99.01	**98.87**
u_{CRM} (%)	0.44	**0.46**



P_{CRM} (%)	**99.51**	99.54	99.53
u_{CRM} (%)	**0.26**	0.25	0.29



P_{CRM} (%)	**99.82**	99.76	99.71
u_{CRM} (%)	**0.30**	0.37	0.35



P_{CRM} (%)	**99.57**	99.62	99.68
u_{CRM} (%)	**0.24**	0.28	0.41

Reference

1. Rigger R, Rück A, Hellriegel C, Sauermoser R, Morf F, Breitruck K, Obkircher M (2017) Journal of AOAC International, Vol. 100, No. 5, 1365-1375.

For an overview on our qNMR products visit us at **SigmaAldrich.com/qnmr**

The full portfolio of organic TraceCERT® certified reference materials (CRMs) can be found at **SigmaAldrich.com/organiccrm**

Featured Products

Description	Qty.	Cat.No.
TraceCERT®, certified reference material for ^{19}F-qNMR		
4,4′-Difluorobenzophenone	500 mg,1 g	**07563**
2,4-Dichlorobenzotrifluoride	500 mg	**53396**
2-Chloro-4-fluorotoluene	500 mg	**80730**

Related Products

Description	Qty.	Cat.No.
TraceCERT®, certified reference material for ^{31}P-qNMR		
Triphenyl phosphate	1 g	**05498**
Potassium phosphate monobasic	1 g	**92214**
Phosphonoacetic acid	1 g	**96708**
Triethyl phosphate	1 g	**90999**

Description	Atom %	Cat.No.
Deuterated solvents		
Chloroform-d6	99.96	**151858**
Dimethyl sulfoxide-d6	99.96	**156914**
Methanol-d4	99.96	**444758**
Acetonitrile-d3	99.96	**233323**

- New Post -

ValidNMR Blog

News, Information and Exchange

3M Science. Applied to Life.™

Implementation of a Benchtop NMR in a Manufacturing Environment

Author: Travis Gregar, Anthony Busche, Terry Downey, 3M Center, St Paul, MN 55144

Read it now!

- The Impact of Weighing Accuracy and Data Integrity for qNMR Applications -

Abstract

One of the major sources for measurement uncertainty in quantitative NMR applications is weighing of reference and analyte. Weighing has a strong bearing on the final qNMR results. A balance must be consistently accurate, which is achieved by calibrating the device periodically and by determining the minimum weight and the safe weighing range. Weighing sample sizes in the safe weighing range reduces the measurement uncertainty of the weighing process below a predefined threshold. Further to accurate weighing, data integrity of plays a fundamental role in regulated environments. With automated transfer of weighing data and associated metadata the traceability of the weighing process is established and operator errors can be avoided.

The significance of measurement uncertainty and minimum weight

Weighing is a critical step for qNMR analysis. It strongly and directly influences the accuracy of the final result because the weight of the net sample and of the reference standard have a direct correlation on the determination of sample purity or content. To ensure that weighings are accurate, laboratory managers often rely on quality management systems to define a weighing process. This includes proper recording criteria, calibration of the instrument and determination of measurement uncertainty.

To better understand minimum weight, it is important to recognize that the stand out prerequisite for traceable and accurate weighing is the effective calibration of weighing instruments, which must include an estimation of measurement uncertainty.

Historically, many laboratories have set up their own calibration procedures due to the lack of nationally or globally recognized calibration guidelines. Based on international cooperation from subject matter experts in the field of metrology, efforts have been made to globally harmonize the methodology of calibration of weighing instruments1.

The benefit of these harmonization activities is that the state-of-the-art calibration concepts not only stipulate how to estimate measurement uncertainty at the time of calibration, but provide guidance for estimation of uncertainty during the day-to-day usage of the instrument. This concept leads to the calculation of the minimum sample weight, often referred to as the minimum weight. This is the smallest amount of net substance that must be weighed in order to achieve a specified degree of accuracy.

All weighing instruments act in a similar manner across the weighing range - as the sample size decreases, the relative measurement uncertainty increases. Eventually, with a small enough mass, the relative weighing uncertainty can become high enough that the weighing result is no longer accurate. The measurement uncertainty then becomes larger than the specified threshold. This accuracy limit is the minimum weight (Figure 1).

Based on the risk associated with the weighing process, it is also recommended to apply a safety factor to this value. This factor increases the minimum amount that should be weighed on a particular balance and defines the starting point of the so-called safe weighing range. The safety factor accounts for performance fluctuations caused by environmental factors (air drafts, temperature, vibrations, and different user techniques) that can affect the balance during normal use between calibrations.

With the benefit of measurement uncertainty and the resulting minimum weight defined, it is important to realize that typical calibration certificates only contain measurement uncertainty values. An Accuracy Calibration Certificate (ACC) contains both components, the measurement uncertainty and the minimum weight for the required weighing tolerance. Therefore, it links the performance of the weighing instrument to the weighing process tolerances required by the user for their specific application.

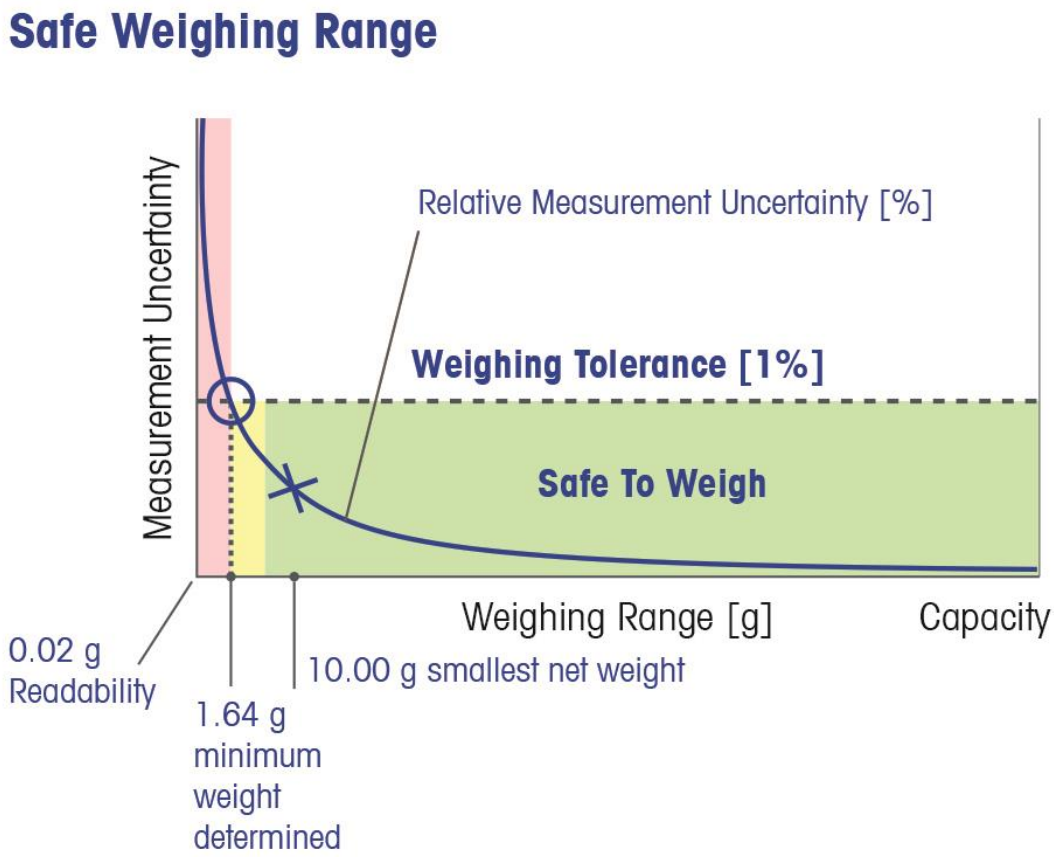


Figure 1: *Typical behavior of measurement uncertainty across the weighing range of a balance*

The minimum weight is an extremely important characteristic when performing quantitative NMR analysis because small sample sizes are often used for the purpose of minimizing costs or limited valuable amount of samples. The associated weighings of the samples and standards have a direct impact on the analysis results. Therefore, weighing above the minimum weight under consideration of an appropriate safety factor, i.e. weighing in the safe weighing range of the instrument, is extremely critical.

Based on the defined safety factor, the ACC allows the safe weighing range to be determined for each particular balance. This level of detail from a calibration enables balance users to improve the quality of their weighing, increase confidence in the weighing results and avoid weighing errors.

Ultimately, understanding and implementing a quality system that adheres to weighing sufficiently more substance than the minimum weight and thus working in the safe weighing range of the balance, ensures instrument accuracy and minimizes the risk of errors that could affect the correctness of analysis results.

With respect to measuring instruments, many regulations and guidelines now require **complete data derived from all tests**...[2]. This includes the raw data generated through the course of an analysis and the associated metadata. Metadata is the contextual information required to understand data[3].

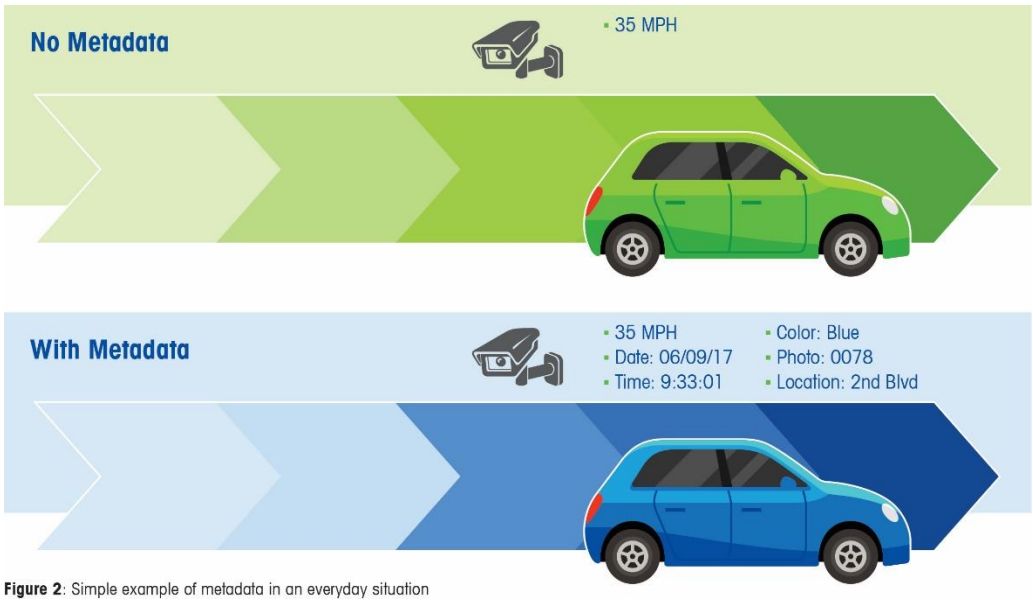


Figure 2: Simple example of metadata in an everyday situation

Figure 2: *Simple example of metadata in an everyday situation*

Avoiding incomplete data and achieving compliance

To help comply and meet the requirements on data integrity, especially in the regulated environment like pharmaceutical laboratories, it is also important to understand the benefits of incorporating the components of the weighing process in an integrated data management system. In recent years, an increasing number of assessments and FDA warning letters have revealed incomplete data, the lack of audit trails, and falsification of results. The problems with data integrity could be eliminated by first focusing on the sample file generated from the sample during the course of analysis. Many labs have turned toward LIMS systems with the idea of replacing the manual workflow. These systems are designed primarily to aggregate result data from an array of analytical tests, rather than to automate and document bench top workflows or bind instrument metadata to the measurement.

An example of the use of metadata in an everyday situation is shown in Figure 2. If a car speeds through a traffic enforcement camera and the only information captured is the image, the speed of the automobile,

and the associated unit of measure, there isn't enough information to link the car to the speed. However, if the date, time, color of the car, unique picture identifier, and location is included, the necessary contextual information is then available to link the car with the speed.

When the same principle is applied to the regulated laboratories , every critical weight measurement that is recorded should not only include the weight and unit of measure, but the additional metadata necessary to be considered "complete data" (Figure 3).

Figure 3: *Examples of metadata available from laboratory instrumentation*

Automated data transfer and standardization of weighing workflows

Many labs have discovered that transferring metadata from bench top analytical instruments is much more complex than only the transfer of a few parameters, such as sample weight and unit of measure. Leveraging the potential of appropriate software technology, such as LabX, enables users to transfer weighing results with all the associated metadata directly to their LIMS systems - thereby ensuring the data is complete and traceable.

Additionally, the weighing workflow can be automated and standardized to the specifications of the unit or lab (Figure 4). This guarantees and proves that the same weighing process is used for each sample, regardless of who performs the steps – ensuring consistency in every analysis. For example, the administrator can elect to have the balances locked down every morning until an analyst logs in and performs an adjustment of the balance by means of the built-in weights. Only once that has been completed can the balance user proceed to a guided weighing process on the terminal of the balance.

Another example of a benefit the software provides is the ability to capture not only the net weight of the substance, but the weight of the tare vessel used in each weighing event. This allows the analyst to provide documentation during trial, confirming the tare vessel weight was not included in the net weight of the substance in question.

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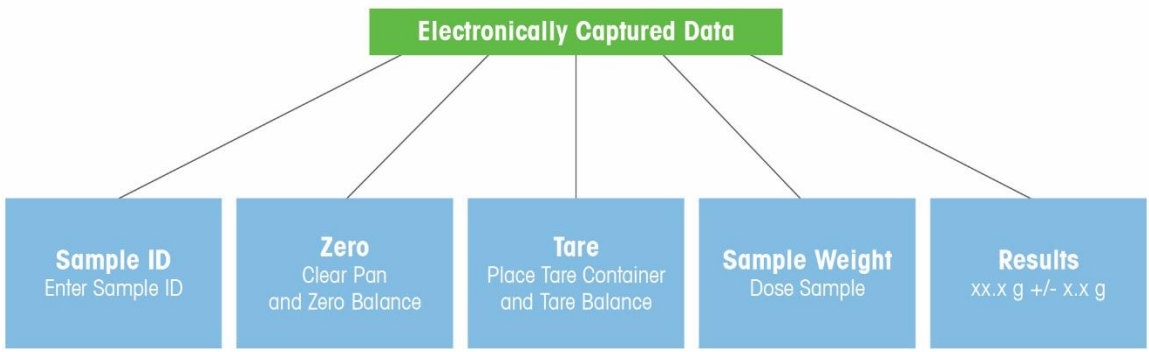

Figure 4: *Example of a standardized weighing method*

Conclusion

To increase accuracy of qNMR analysis, it is crucial to minimize weighing and sample preparation uncertainty. Error elimination, process simplification and data traceability are the keys to succeed in qNMR application which can be supported by the following.

- Establish a harmonized approach to the calibration of balances
- Ensure all weighing is performed in the safe weighing range, well above the minimum weight
- Automate data capture and transfer of weighing data to ensure traceable data and to reduce operator error

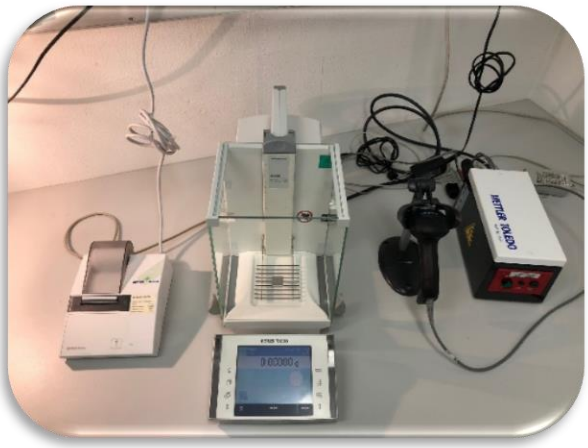

Figure 5: *XP205 Semi Micro Balance*

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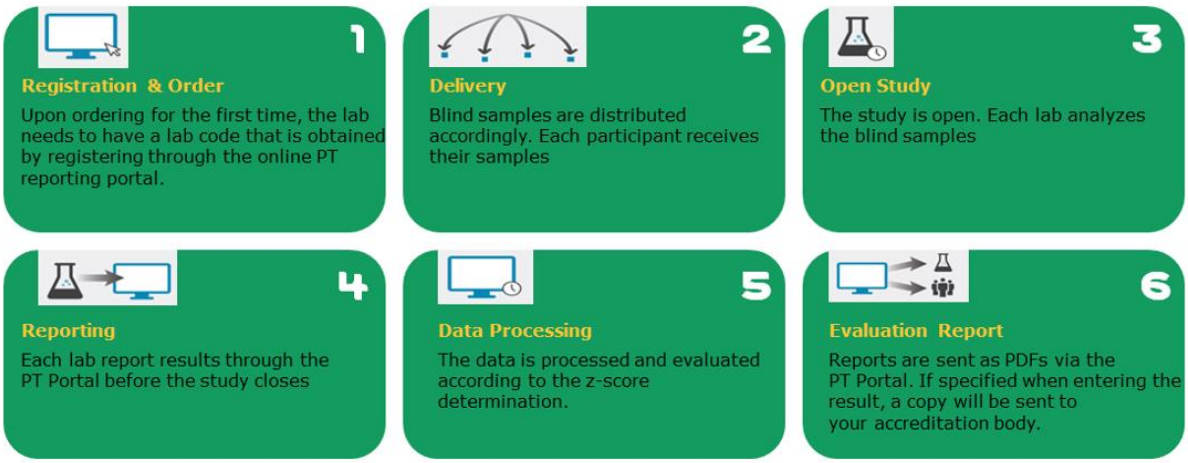
The most common use for proficiency testing is to demonstrate to a regulatory or an accreditation body, that a laboratory is capable and competent to perform a specific analytical test or technology. A second and sometimes overlooked benefit of proficiency testing is its use as critical tool for quality assurance and continuous improvement since it can provide a true picture of a laboratory's testing quality over time.



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- Future validation guidance from USP and ICH -

By ValidNMR Committee,

Lead author: Dan Sorensen

Both the USP and the ICH have initiated activities to update their guidance documents to incorporate the application of scientific knowledge and quality risk assessment to the development and validation of analytical procedures. These principles, that can be summarized and described as **Quality by Design (QbD),** are fully embraced and endorsed by the ValidNMR group. We are very excited about these initiatives and encourage the wider NMR community to pay close attention to the developments in this area.

The manufacture of pharmaceuticals is highly regulated and managed by the continuously improving **Good Manufacturing Practices** (GMP). The evolution of GMP can be traced back to the beginning of the 20th century, when the **quality of drug products** mainly relied on the ethical integrity and craftmanship of individual pharmacists. With increasing industrialization, the need to manage the quality of products evolved into the system that we know today as **cGMP (current GMP)**. However, many fundamental concepts and principles of GMP still rely on traditional procedures and technologies and many benefits of modern industrialization and technology remain unrealized. The 2004 publication from the **FDA** entitled **"Pharmaceutical cGMPs for the 21st Century – a Risk-based Approach"** was a clear indication that a fundamental shift towards the use of modern quality management systems was [and still is] required.

From the technical and scientific perspective of NMR spectroscopists, it remains problematic that prescriptive guidance **documents for validation of analytical procedures (e.g., ICH Q2(R1), USP <1225>)** are based on chromatography and its traditional practices. **Many of the analytical performance characteristics and parameters, that regulatory compliance officers expect to be able to verify, are not always applicable to NMR or commensurate with scientific rationale and quality risk management for the intended purpose.** Yet, the question of "validation" often becomes a **case-by-case topic** of debate between the officer and the NMR scientist – a debate where the officer has the ultimate authority and will almost always enforce adherence to guidance documents.

The USP established a Validation and Verification Expert Panel to develop **new guidance for development, validation and lifecycle management of analytical procedures** and the outcomes and proceedings of this expert panel have been published in a series of stimuli articles in the Pharmacopeial Forum [www.uspnf.com/pharmacopeial-forum] and culminated with the proposal to create a new **USP General Chapter <1220> "The Analytical Procedure Lifecycle"**.

This enhanced approach to development and validation of analytical procedures starts with specification of an **Analytical Target Profile (ATP)** and a **Target Measurement Uncertainty (TMU)** and then progresses to a QbD approach to design of the analytical procedure. The procedure is then qualified to demonstrate that the performance is acceptable for the intended purpose and the state of validation is continually monitored and assessed throughout the use and lifecycle of the procedure. Based on the firm scientific basis and comprehensive body of knowledge established for qNMR, this analytical technique is particularly well-aligned with this proposed new framework, where the process of validation is **rational, objective and technically agnostic**. With **SI-traceable certified reference materials (CRMs)** now readily available, qNMR can, as just one example, be used for measurements of mass fractions of drug substances and provide accurate assay results without the need for a reference standard of the substance itself.

In parallel with the USP, the ICH acknowledged that the current **Q2(R1) guidelines for validation of analytical procedures** do not incorporate the scientific principles of spectroscopy (e.g., NMR) and spectrometry and that that the scientific knowledge gathered during design and development of the analytical procedure was practically "lost in translation" – not subject to regulatory assessments. **Thus, an Expert Working Group (EWG) was established to prepare revised Q2(R2) guidelines and prepare a guideline Q14 for analytical procedure development** [https://www.ich.org/products/guidelines/quality/quality-single/article/validation-of-analytical-procedures-text-and-methodology.html]. It is notable that **Dr. David Keire** from the FDA is actively involved in the ICH Q2(R2)/Q14 EWG as the regulatory chair and his subject matter expertise in NMR will hopefully be reflected in the new guideline documents. The EWG is on track with their work and has prepared internal drafts and held meetings and we are looking forward to the public drafts and consultations that are **planned for 2020**.

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