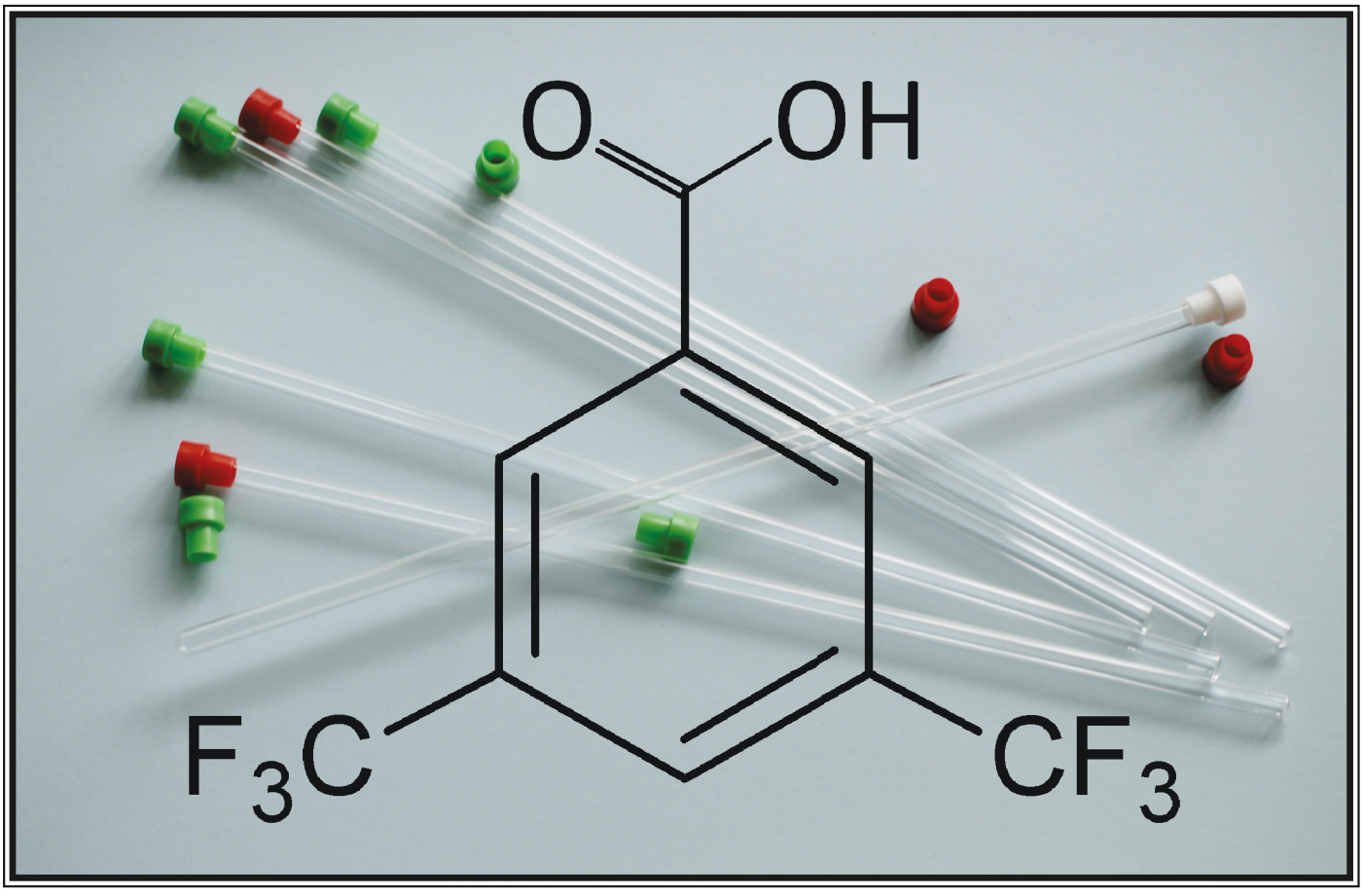
Bureau International des Poids et Mesures

qNMR Internal Standard Reference Data (ISRD)

Internal Standard Reference Data for ^{19}F qNMR: 3,5-Bis(trifluoromethyl)Benzoic Acid [ISRD-09]



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BIPM ISRD-09: ^{19}F qNMR using 3,5-Bis(trifluoromethyl)benzoic Acid as Internal Standard

Table of Contents

1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is well-established as the pre-eminent method for the qualitative structural analysis of organic molecules. The potential for its application for quantitative organic analysis was recognized soon after NMR instruments became commercially available.[1] However it has only been recently, as spectrometer capabilities have achieved a level of accuracy and precision comparable to those attainable by chromatographic techniques, that this potential has been widely realized in practice. As a result, quantitative NMR (qNMR) methods, particularly for the assignment of the purity of individual organic compounds, are now actively and extensively employed.[2–5]

Purity assignment by qNMR spectroscopy potentially also meets the metrological requirements for a primary ratio measurement procedure.[6] Validated qNMR methods[7–9] are now being used, generally in combination with data obtained by orthogonal chromatographic techniques, to assign the purity of organic materials intended for use as Primary Reference Materials[10] for individual organic analytes.[11,12] The availability of properly characterized Primary Reference Materials is in turn an essential initial step in establishing the metrological traceability for measurement results for an organic analyte linked in a calibration hierarchy to a specific pure material.[13]

Fluorinated compounds today play an increasing role in various fields that range from pharmaceuticals (for example skin disease, antifungal and antitumoral drugs, drug analysis),[14-16] agrochemicals (for example pesticides),[17–19] cosmetics (for example perfluorinated carboxylic acids),[20] biomolecule analysis (for example fluorinated amino acid probes)[21] or functionalized materials.[22] The magnetic properties (spin number, natural abundance, gyromagnetic ratio, Larmor frequency) and the required operating frequency to acquire ^{19}F NMR spectra are very similar to those for ^{1}H NMR. The 100 % isotopic abundance makes ^{19}F NMR about as sensitive as ^{1}H NMR while the chemical shift dispersion and the sensitivity of ^{19}F nuclei to the local environment are much higher due to the nine-electron cloud around the nucleus.[23] These features combined with the usually lower number of fluorinated sites in the organic molecules result in well-separated NMR signals in most cases.

The assignment of the mass fraction purity of an organic analyte A by qNMR in solution using an internal standard S is based on measurement equation 1 below.

$$w_{\mathrm{A}} = \frac{I_{\mathrm{A}}}{I_{\mathrm{S}}} * \frac{N_{\mathrm{S}}}{N_{\mathrm{A}}} * \frac{M_{\mathrm{A}}}{M_{\mathrm{S}}} * \frac{m_{\mathrm{S}}}{m_{\mathrm{A}}} * w_{\mathrm{S}} \qquad \text{Equation 1}$$

w_A is the mass fraction of the analyte in the material subject to assignment, w_S the independently established mass fraction content of the internal standard, I_A and I_S are the integrals of the quantified signals, N_A and N_S the number of ^{19}F nuclei contributing to each

quantified signal, M_A and M_S the molar masses of the analyte and internal standard and m_A and m_S the masses of the samples of the analyte and internal standard used in preparation of the solution, subject to the qNMR measurement.

The uniform excitation of the large chemical shift spread of ^{19}F signals is hampered by the limited available radiofrequency power. Off-resonance effects lead to a symmetrical distortion of signal intensities around the excitation offset. Adjusting the transmitter frequency offset midway between the two evaluated resonance signals is the most common approach to minimize quantification bias, although other methods to improve the excitation bandwidth exist[24]. In optimal cases where the data processing is carried out by experienced operators, the relative standard uncertainty for purity mass fraction assignments can reach levels ≤ 0.5 %.[25–27] Factors including lineshape and multiplicity of the signals integrated, the extent of overlap with the main peak of interfering signals from impurities present, the nature of the internal standard and solvent used, the magnetic field strength, baseline distortions induced by NMR probe background signals, the hardware settings and performance characteristics of the spectrometer, as well as the approach taken to process and transform the free induction decay (FID) signal generated by the NMR experiment and to integrate the signals of the resulting frequency domain spectrum, all contribute to the overall uncertainty of the final assigned value. Evidently, regardless of the precision of a qNMR measurement, the overall (relative) measurement uncertainty of a qNMR assignment can never be smaller than that associated with the purity of the internal standard used to obtain the result.

The first goal of this document is to furnish general recommendations for the design of a qNMR experiment and for the undertaking of a quantitative ^{19}F NMR measurement using the internal standard approach to provide a measurement result traceable to the International System of Units (SI).[28] It should be noted that although these principles should apply generally to quantitative measurement involving any NMR-active nuclei, the recommendations in this document are only intended for assignments by ^{19}F qNMR.

There are a number of literature reports on the use of specific compounds as ^{19}F qNMR internal standards.[25,29–31] The focus of these papers are generally a specific application of ^{19}F qNMR rather the general application of an individual compound as a higher-order, SI-traceable primary measurement standard for qNMR. The second goal of this report is to establish the desirable properties for individual compounds to serve as versatile SI-traceable internal standards for purity assignment by ^{19}F qNMR. The longer-term aim will be to identify a “suite” of organofluorine ISRMs able to cover the broad ^{19}F NMR chemical shift range and that are compatible with a range of solvents. At least one ISRM compound should be suitable for the purity assignment by ^{19}F qNMR of a given organofluorine compound soluble in a specified NMR solvent.

Ideally a ^{19}F qNMR ISRM should consist of a stable crystalline solid which is:

- a Certified Reference Material (CRM)[32] produced and characterized by a National Metrology Institute (NMI) using methods other than qNMR **or** has been assigned by qNMR using an NMI CRM as the internal standard;
- predominantly one organic component (w_S > 995 mg.g^{-1});
- value assigned with small associated standard uncertainty ($u(w_s)$ < 2 mg.g^{-1});
- providing unique NMR signals, either as singlets or simple multiplet resonances, having Lorentzian lineshape and narrow signal width;
- free of significant impurities interfering with areas to be integrated;
- inert in solution and soluble at a level in excess of 2 mg.mL^{-1};
- readily handled for accurate mass determinations:
    - non-hygroscopic
    - non-volatile
    - not subject to electrostatic effects
- having a ratio of quantifiable fluorine atoms to the molar mass of the ISRM suitable to allow for convenient gravimetric operations.

It is recognized that these characteristics constitute a “wishlist” rather than prescriptive requirements. It is also recognized that pragmatically, not all materials within a suite of ISRMs for ^{19}F qNMR will be able to meet all these specifications.

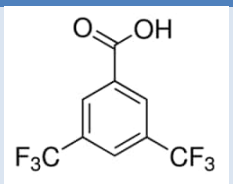
The third goal, and the focus of this specific document, is to provide guidance regarding the scope, use and limitations of 3,5-bis(trifluoromethyl)benzoic acid (BTFMBA) as an ISRM for ^{19}F qNMR analysis.

BTFMBA is soluble in a range of deuterated solvents with intermediate to low polarity, as listed in Table 1. It is suitable for use as an internal standard for qNMR purity assignments of analytes soluble in methanol-d_4, DMSO-d_6 and solvents with related solubilizing properties. It is recommended that for a purity assignment using BTFMBA as ISRM the ^{19}F resonance to be quantified should have a chemical shift within a range ± 26 kHz of the BTFMBA resonance at -60 ppm – i.e from -130 ppm to +10 ppm at 376.17 MHz for ^{19}F.

The attached annexes present ^{19}F NMR spectra and example applications of BTFMBA.

This proposal was developed by the Chemistry Department, Bureau International des Poids et Mesures (BIPM) working in collaboration with scientists from the National Metrology Institutes of Japan (NMIJ), Brazil (INMETRO), Germany (BAM) and Argentina (INTI).

Table 1. Key properties of BTFMBA

ISRM	BTFMBA
Structure	
^{19}F δ (ppm)*	-64 to -61 (6F)
Solvent	**Solubility (mg/mL)**
D_2O	< 1**
DMSO-d_6	≥ 20
Methanol-d_4	≥ 20
Chloroform-d	≥ 5
Acetonitrile-d_3	≥ 20
Acetone-d_6	≥ 20

*Chemical shift ranges for different solvents.

**10 mg/mL in 0.1 M NaOD/D_2O.

KEY

BTFMBA: 3,5-Bis(trifluoromethyl)benzoic acid

D_2O: Deuterium oxide

DMSO-d_6: Dimethyl sulfoxide-d_6 / Hexadeuterodimethyl sulfoxide

Methanol-d_4: Tetradeuteromethanol

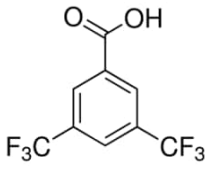
Chloroform-d: Deuterochloroform

Acetonitrile-d_3: Trideuteroacetonitrile

Acetone-d_6: Hexadeuteroacetone

2. Properties of 3,5-Bis(trifluoromethyl)benzoic Acid

2.1 Physical Properties

Name:	**3,5-bis(trifluoromethyl)benzoic acid**
Structure:	
CAS registry number:	725-89-3
Molecular formula:	$C_9H_4F_6O_2$
Molar mass:[33,34]	258.117 g/mol, u = 0.006 g/mol
Melting point:[35]	142 °C
Appearance:	white crystalline powder
δ ^{1}H NMR:	8.1 ppm to 8.6 ppm (2H)
	8.0 ppm to 8.4 ppm (1H)
δ ^{13}C NMR:	164.8 ppm to 168.4 ppm (1C)
	131.5 ppm to 135.2 ppm (1C)
	130.9 ppm to 133.4 ppm (2C)
	129.6 ppm to 131.1 ppm (2C)
	126.5 ppm to 127.5 ppm (1C)
	123.0 ppm to 124.7 ppm (2C)
δ ^{19}F NMR (Figure 1):	-64.4 ppm to -61.4 ppm (6F)

(Indicative chemical shift ranges for different solvents; ^{13}C data for ^{1}H and ^{19}F decoupled spectrum.)

^{1}H NMR spectra of BTFMBA in different solvents are available from the Spectral Database for Organic Compounds (SDBS).[36]

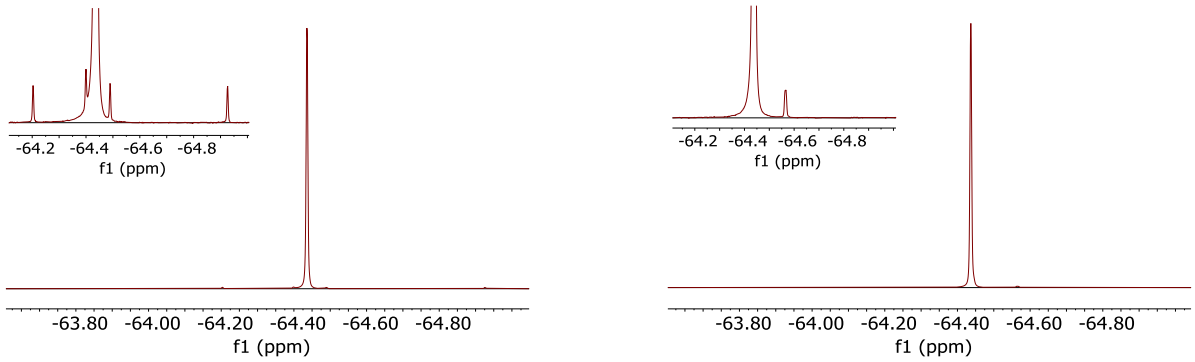


Figure 1. ^{19}F NMR spectra of BTFMBA in methanol-d_4 acquired on JEOL ECS-400 spectrometer with Royal probe: ^{19}F on the left; ^{19}F{^{13}C} on the right.

Spectra of BTFMBA in other NMR solvents are reproduced in Annex 5.1.

2.2 Solvent Compatibility

NMR solvents suitable for use with BTFMBA include methanol-d_4, DMSO-d_6, acetonitrile-d_3, acetone-d_6, and chloroform-d. BTFMBA is not sufficiently soluble in aqueous solvents at neutral pH or below, but at pH > 9 it is reported to be soluble at the level of 10 mg/mL.

2.3 Quantification Signal

The six magnetically equivalent fluorine atoms of BTFMBA give rise to a single absorption at a chemical shift in the range of -64.4 ppm to -61.4 ppm on the δ scale. The position of the resonance is a function of factors including, but not limited to, the solvent, temperature, pH, instrument referencing, and the concentration of BTFMBA and analyte in the solution. The homogeneity of the spectrometer magnetic field should be optimized such that the peak is not distorted, and the base of the resonance retains a suitable Lorentzian peak shape.

BTFMBA fluorine nucleus presents long-range couplings to hydrogen, hence its peak in

the ^{19}F NMR spectrum is not a narrow singlet. For optimal quantification results, the homogeneity of the spectrometer magnetic field should be optimized, and this can be verified in the ^{1}H NMR spectrum of the sample, where the full width at half maximum (FWHM) of a singlet signal (from residual solvent or other analyte) should be less than 1 Hz and the peak should retain a suitable Lorentzian peak shape.

2.4 Impurities and Artefact Signals

Samples of BTFMBA analyzed in our laboratory have not presented evidence of significant levels (> 0.1 %) of related structure impurities in the material.

2.5 Solvent Recommendations and Advisories

Among factors such as solvent, sample concentration and temperature, the solvent has the largest influence on the ^{19}F chemical shifts.[37] Therefore testing a different solvent can be useful when the integrated region is partially overlapped with impurities or other peaks from the spectrum. Solvent information for BTFMBA is presented below and in Table 2.

2.5.1 **D_2O and related solvents**

BTFMBA is not sufficiently soluble in neutral or acidic D_2O to use it directly in qNMR applications. If the pH of the solution is raised (by addition of NaOD), its solubility increases significantly, and it can be used for qNMR assignments of materials that are also stable at high pH in aqueous solution.

2.5.2 **DMSO-*d*$_6$**

BTFMBA is soluble in DMSO-d_6. It is recommended for use for qNMR studies where the target analyte is also soluble in this solvent.

2.5.3 **Methanol-*d*$_4$**

Although in principle the use of methanol-d_4 as solvent could result in BTFMBA esterification, this was not observed during laboratory studies for the preparation of this document, even after more than one week in solution at room temperature. Therefore, use of methanol-d_4 as solvent is a suitable option for BTFMBA.

2.5.4 **Chloroform-*d* and related solvents**

Chloroform-*d* is not the first choice for BTFMBA due to its lower solubility, however it can be an option if the target analyte is not soluble in other solvents.

2.5.5 **Acetonitrile-d_3**

BTFMBA is soluble in acetonitrile-d_3. It is suitable for use qNMR studies where the target analyte is also soluble in this solvent.

2.5.6 **Acetone-d_6**

BTFMBA is soluble in acetone-d_6. It is suitable for use for qNMR studies where the target analyte is also soluble in this solvent.

Table 2. Solvent parameters for BTFMBA

Solvent	qNMR signal multiplet*, 6F (ppm)	Integration range (ppm)**	T_1 (s)**	Comments
D_2O	-	-	-	Poor solubility
DMSO-d_6	-61.4	-60.8 to -61.9	1.4	
Methanol-d_4	-64.4	-63.9 to -65.0	1.6	
Chloroform-*d*	-62.9	-62.4 to -63.5	1.6	Moderate solubility
Acetonitrile-d_3	-63.4	-62.9 to -64.0	2.0	
Acetone-d_6	-63.4	-62.9 to -64.0	2.0	

* Depending on the magnetic field of the instrument, the BTFMBA peak resembles a wide singlet due to poor resolution for the resonance lines from the coupling to ^{1}H.

**Indicative values only. The observed value in a specific qNMR solution will be a function of factors including concentration of BTFMBA and analyte, solution temperature, instrument, etc.

3. Good Practice Guidance for SI-Traceable qNMR Measurement Results

3.1 Introduction

The first step in any purity assignment by qNMR should be the confirmation by qualitative NMR or other techniques of the identity of the analyte subject to purity assessment. In addition to confirming that the molar mass (M) and the number of nuclei (N) contributing to each signal subject to integration are appropriate, obtaining qualitative NMR spectra also provides a check for the occurrence and extent of any interfering signals in the sections of the NMR spectrum subject to integration.

Once the qualitative identity of the analyte has been appropriately established the

input quantities that influence qNMR measurement results must be evaluated. These are identified from the measurement equation (Equation 1, Section 1). The purity of the internal standard used for the measurement, and the source of traceability to the SI for the value assigned to the analyte should be established independently prior to the qNMR experiment.

The gravimetric procedure used for the preparation of the NMR solution has to be fully validated and fit for purpose,[27,38] and the spectrometer performance, experimental parameters and the protocol for signal processing and integration must be optimized,[7,31,39] in order to produce a result for the ratio of the integral of the analyte and standard signals that accurately reflects the amount of substance fraction of the fluorine nuclei giving rise to the signals. Only when these conditions are met can the assigned mass fraction purity of the analyte also be regarded as properly traceable to the SI.[40] Some general guidance for recommended practice for these critical steps is given in the following sections.

3.2 Internal Standard

The internal standard used in qNMR should comply as far as possible with the criteria described in the Introduction regarding composition, physical characteristics, inertness, solubility, impurity profile and suitability for accurate gravimetry. In addition, in order to establish traceability of the result of the qNMR assignment to the SI, the material should comply with the requirements of a reference measurement standard, and in particular a reference material, as defined in the International Vocabulary of Metrology (VIM).[41]

To maintain SI-traceability the sources of the internal standard should be either a:

a. CRM characterized for mass fraction purity and value assigned by an NMI;
b. CRM produced by a Reference Material Provider accredited to ISO 17034:2016[42] requirements;
c. High-purity material subject to a validated measurement procedure for purity assignment by qNMR using as an internal standard a CRM of type (a) or (b).

3.3 Gravimetry and Sample Size

The realization of accurate and precise qNMR measurements relies on the application of a properly implemented gravimetric procedure for the mass determinations of the internal standard and analyte. Recommended practice in this area in the specific context of qNMR sample preparation has been published.[27] Achieving an overall relative standard measurement uncertainty for the result of a ^{19}F qNMR assignment of 0.5 % requires the relative uncertainty associated with individual gravimetric operations typically to be less than 0.15 %. If the combined standard uncertainty of a single mass determination is 3 µg, a level achievable with modern electronic microanalytical balances, this corresponds to a minimum sample size of 2 mg. When the overall standard uncertainty is significantly smaller than 0.5 %, a higher sample size can be used to reduce the contribution to the measurement uncertainty from gravimetric operations. In all cases, the minimum sample amount described in the internal standard certificate shall be pursued and a higher sample size for the analyte should be considered in cases of more heterogeneous materials.

In addition to suitable control for each mass determination, if the receptacle used for the final solution preparation is not the same as that used for both mass determinations, the procedure for transfer of solids into the solution must address the assumption that the ratio of the gravimetric readings from the balance operations is equivalent to the ratio of the masses of each compound in the solution subject to the qNMR analysis.

For the example reported in the Annex 5.2 below, gravimetric operations were undertaken using a balance associated with a measurement uncertainty estimate of 1.3 µg for individual mass determinations. In this case a minimum sample size of 1 mg achieves a relative uncertainty in individual gravimetric operations below 0.15 %. In such cases, the amount of sample to be weighed is usually defined by the minimum sample size to ensure a representative measurement. In addition to the measurement uncertainty of the gravimetric operations, high accuracy measurements require additional correction for sample buoyancy effects.[38] As opposed to ^{1}H qNMR, correction of quantification signals for isotope composition is not needed for ^{19}F qNMR due to the 100 % isotopic abundance of ^{19}F. The fluorine content of BTFMBA (442 mg/g) lies conveniently in the range of most fluorinated compounds, which rarely extends beyond 600 mg/g (insecticides such as sulfluramid or LPOS are rare exceptions). This allows for the weighing of suitable amounts of BTFMBA and the target analyte compound, which helps achieving relative uncertainties of the gravimetric operation below 0.15 % and NMR signals of the same order of magnitude.

As sample preparation for qNMR involves mass determinations in the milligram range using sensitive balances, the loss of minute (effectively invisible) quantities of powder during the gravimetric procedure will have a measurable influence on the balance reading and hence

on the input quantities for the qNMR assignment. Environmental conditions for gravimetry and qNMR sample preparation should be controlled throughout the process, subject to minimum change and kept within the operating range recommended by the manufacturer.[43,44] It is recommended that mass determinations be performed in an area where the humidity is maintained at a relatively stable level in the relative humidity range 30 % to 70 %.

The accumulation of surface electrostatic charges is another potential source of bias for mass determinations, particularly for high-polarity, hygroscopic compounds. In these cases, pre-treatment of the sample with an electrostatic charge remover or deionizer is advisable prior to the mass determination. Materials subject to qNMR analysis should be evaluated for their hygroscopicity, for example by measurement of the potential for change in the observed mass of a sample as a function of relative humidity using a dynamic sorption balance.[45] This allows for assessment of the likely impact of variation in the relative humidity in the local environment on the results of gravimetric operations for a given compound. A minimum of two independent gravimetric sample preparations should be undertaken.

3.4 NMR Spectrometer Optimization

There is no specification of minimum NMR spectrometer field strength for purity measurements. Increasing the field strength enhances signal separation and sensitivity, both of which should increase the accuracy and precision of qNMR measurements. Careful optimization of the lineshape (shimming) is critical in order to achieve reliable qNMR results.[39]

Due to the relatively wide Lorentzian shape of NMR resonances the separation of the signals to be quantified from each other and from the remainder of the NMR signals in the spectrum should be considered carefully. Ideally there should be no interfering signals within a range one hundred times the FWHM on each side of each signal to be integrated.

3.5 NMR Acquisition Parameters

The basic experiment to perform quantitative ^{19}F NMR experiments uses a simple 1D pulse sequence designed to minimize differences in the integrated signal intensities due to differential rates of relaxation. For highest accuracy assignments, use of broadband heteronuclear decoupling should in general be avoided as it can lead to undesired nuclear Overhauser effects introducing a bias in the intensities of individual measured signals. However, in the common case of ^{13}C-decoupling to remove satellite signals, the potential for bias is attenuated because of the low (1.1 %) natural abundance of the ^{13}C isotopologue even though the decoupling efficiency for individual ^{13}C satellite signals is variable. The potential

for the introduction of additional bias due to ^{13}C-decoupling is negligibly small in most cases.

The basic sequence for a qNMR measurement consists of a “delay-pulse-acquire” experiment. There are critical parameters associated with each phase of the sequence in order to achieve a reliable, unbiased and quantitative signal response. Assuming the experiment starts from an equilibrium magnetization state, the first phase in the experiment is the pulse, which itself is preceded by a delay.

In the pulse phase, the two critical parameters for good qNMR measurement results are the pulse offset and pulse length (also called pulse width and related to the tip angle). When a single “hard” pulse is applied to the bulk magnetization of each compound, off-resonance effects can occur if the frequency offset of the initial pulse is relatively far from that of the signals of interest. This is particularly important for ^{19}F qNMR because ^{19}F signals can span a wide range of the spectrum (near 700 ppm for general compounds, or 200 ppm for organic molecules) and NMR probes present power handling limitations to excite uniformly a large bandwidth using ordinary square hard pulses. Therefore, the **pulse offset should be carefully positioned at the midpoint between the two signals to be quantified**.[31] This will not eliminate off-resonance effects but should result in cancelling out in both signals.

Regarding the pulse length, radiofrequency pulses have an effective excitation bandwidth that depends inversely on the duration of the pulse.[46] In other words, smaller tip angles such as 30° and 10° deliver an overall broader excitation profile, which is desirable for ^{19}F NMR. However, our studies showed that signal losses for peaks equidistant upfield and downfield to the pulse offset were less symmetric when smaller tip angles were used, resulting in experiments with less potential to cancel out off-resonance effects for analyte and internal standard.[47] Correspondingly, the use of a digital filter for data acquisition resulted in more uniform response over the same frequency range but was detrimental to the symmetry of signal loss. For those reasons, **90° pulses are recommended** for quantitative analyses using ordinary square pulses, even resulting in bigger (but more symmetrical) signal losses due to off-resonance effects. In addition, the effect of **digital filters should be evaluated before qNMR analyses** as their configurations vary depending on the instrumentation. When using 90° pulses without digital filtering, our results presented symmetrical signal losses in a range of 70 ppm (26 kHz) at 376.17 MHz for ^{19}F, with qNMR precision and accuracy similar to ^{1}H qNMR. This showed the quantitative potential of ^{19}F NMR when the distance between the analyte and the internal standard is < 26 kHz. An experiment to study the uniformity of response (related to both irradiation profile and receiver linearity) can be carried out by acquiring a series of spectra setting the transmitter offset at the peak of interest and at frequency intervals upfield and downfield. A plot of the peak integral versus the difference between the transmitter offset and the peak chemical shift (Δδ) is useful to evaluate the extent of off-resonance effects (Figure 2).

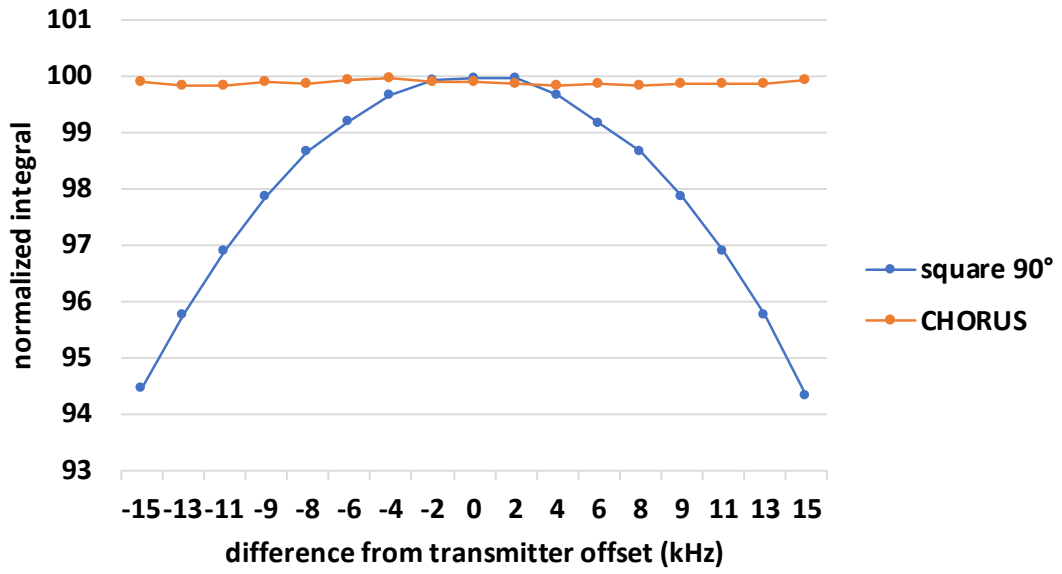


Figure 2. ^{19}F NMR excitation profile obtained at BIPM (JEOL-ECS 400) for square 90° pulse and CHORUS sequence with shifted laminar pulses; pulse width approximately 9.2 µs (calibrated before the experiment), pulse attenuation 3.5 dB.

Alternatively, sequences of shaped pulses designed for uniform broadband excitation have the potential to be less influenced by off-resonance effects. The application of this type of sequences for qNMR should be evaluated in the laboratory instrumentation before their use in qNMR measurements and should consider the limitations of their relatively long duration of pulse application (for example a few milliseconds instead of a few microseconds for single hard pulses). The potential drawbacks include relaxation before acquisition (which could affect differently internal standard and analyte when their relaxation times or lineshapes are not similar) and evolution of homonuclear coupling for polyfluorinated compounds, yielding distorted peaks.[24] The CHORUS sequence was tested in our laboratory and, after implementation of shifted laminar pulses to avoid problems of receiver linearity, it presented a uniform excitation profile in a range of 244 kHz (650 ppm for 376.17 MHz for ^{19}F), with qNMR trueness and precision similar to those obtained with square 90° pulses.[24,47] This can be particularly useful either for very distant analyte and internal standard peaks (over 26 kHz) or when more than two peaks must be quantified in the same spectrum and acquiring multiple spectra for different pairs of IS and analyte is not possible.

Additional parameters requiring optimization in the acquisition phase are the spectral window width, the acquisition time, the digital resolution, and the relaxation delay time

between acquisitions. The spectral window chosen will depend on the design and performance of the instrument used. The theoretical justification for the use of a large spectral window is that oversampling the FID will produce noise filtering. However, the efficiency of filters varies by instrument and the appropriate spectral window should be evaluated on a case-by-case basis.

The acquisition time should be at least 2.5 s to avoid truncation of the signals and to allow good digitization of the spectrum. The ideal acquisition time is the smallest time for which no truncation is observed. Use of longer acquisition times than necessary primarily results in addition of noise to the spectrum. The digital resolution should not exceed 0.4 Hz/pt in order to have accurately defined signals that will give accurate area measurements and suitable precision at typical sampling rates.

The relaxation delay between pulses has to be carefully established for each sample mixture. To determine the optimum repetition time for a given qNMR measurement it is critical to determine the longest T_1 time constant of the signals to be quantified. This document presents some observed values measured for BTFMBA in different solvents at the concentration and under the specific instrumental conditions used (Table 2), but these should be regarded as indicative only, and in any event they are not the determining factor in cases where the T_1 of the analyte quantification signal is longer.

As the T_1 constant arises from a process of spin-lattice relaxation, its values are strongly dependent on the composition of the solution being measured and it should be determined for each signal to be quantified in each mixture on a case-by-case basis. The most used method to determine the T_1 constant is the inversion-recovery sequence, which is generally available in the factory programmed pulse sequences installed with any NMR instrument. The application of the inversion recovery experiment requires knowledge of the optimized 90° pulse, which should also be determined for each mixture under investigation. The 90° pulse is used for both the T_1 determination and the quantitative measurements.

The repetition time between pulses should correspond to the full loop time in the pulse sequence and not simply the relaxation delay. Since most of the time intervals involved in NMR measurement are negligible relatively to the T_1 values, the repetition time (RT) can be estimated as the sum of acquisition time (AQ) and relaxation delay (RD), where the RD is a multiple of T_1. After a 90° pulse, if available instrument time permits, a repetition time equivalent to 10 times T_1 of the signal with the longest relaxation time will lead to the recovery of > 99.99 % of the magnetization for all quantified signals. In cases where the T_1 of the quantified signals are similar in magnitude, a shorter relaxation delay may be sufficient for equivalent (even if incomplete) magnetization re-equilibration.

Thus the recommended pulse RT for high accuracy quantification is given by:

$$RT = RD + AQ = n * T_1$$ Equation 2

(n = 10 to 15)

The number of transients (scans) should be determined according to the concentration of the sample, the nature of the signals and the available instrument time. To achieve small uncertainty, a signal to noise (S/N) ratio of at least 1000 should be achieved for each signal subject to quantification. Smaller S/N values can still lead to acceptable results, but the reported measurement uncertainties increase as the S/N ratio decreases.

Recommended parameters for qNMR are presented in Table 3.

Table 3. Recommended NMR parameters for quantitative measurements

Parameter	Recommended value	Explanation/Comments
Shimming	FWHM of lineshape signal in ^{1}H-NMR ideally < 1 Hz	Optimization of field homogeneity is critical for uniform response over typical chemical shift range. Due to ^{19}F-^{1}H *J* coupling, ^{19}F signals are usually multiplets or they may appear as "wide singlets" when instrument resolution is not enough to resolve the peaks. Therefore, the FHWM measurement for shimming evaluation should be performed in the ^{1}H spectrum, using a singlet peak from internal standard, analyte or residual solvent.
Pulse width	Calibrated for 90° square hard pulses	The use of 90° tip angle provided the most symmetrical results in relation to the excitation profile of the experiment. Hence 90° pulses presented better performance to cancel out off-resonance effects from internal standard and analyte provided that both peaks are equidistant pulse offset. The use of sequences of shaped pulses is discussed in item 3.5.
Pulse offset	Midpoint between signals	This is a very critical parameter for ^{19}F thus it should be carefully set considering the centre of ^{19}F main peaks of analyte and internal standard to compensate for off-resonance effects.
Repetition time	10 to 15 × T_1	After a 90° pulse, a delay of 10 × T_1 of the signal with the longest relaxation time is necessary for recovery of ≥ 99.995 % of magnetization for all quantified signals.
Number of transients	As needed for adequate signal to noise ratio	Evaluate on a case-by-case basis. Minimum requirement is S/N > 1000 for each signal quantified.
Spectral window	> 300 ppm	The use of a wide spectral window for data recording (oversampling) has been reported to yield better results in some instruments because of the noise filtering it produces in the quadrature detection scheme. This is instrument dependent and should be evaluated.
Acquisition time	> 2.5 s	The correct acquisition time is essential to give the best digital resolution for good quantitative results. If too short, lower digital resolution and truncated signals result. If too long, excessive noise is introduced. A minimum of 2.5 s is a useful starting point.
Digital resolution	< 0.4 Hz/pt	The digital resolution is the reciprocal of the acquisition time. Suitable signal shape sensitivity requires not less than 0.4 Hz/pt.
Signal integral ratio	1:1	The preference are sample sizes such that the integral ratio for the quantification signals is close to equivalent. However, in practice this ratio can vary within the range 10:1 to 1:10 provided the S/N ratio of the lower intensity peak is > 1000.

Good practice for performing quantitative experiments is to prepare, in addition to the

sample mixtures, one sample consisting of a solvent blank, one with the analyte only and one with the internal standard only in the same solvent. These additional NMR spectra should be acquired prior to the preparation of sample mixtures to check the suitability of the proposed mixture in terms of the absence of interferences from one compound (or impurities present in it) in the other. Other NMR techniques such as 2D HSQC or COSY may be applied to demonstrate the uniqueness of the signals used for quantification and the absence of overlapping contributions from impurities while aware that the sensitivity of such techniques is low, and the absence of observable interferences does not guarantee a signal free of such interferences.

Each analyte/ internal standard mixture should be measured at least three times in the NMR system. To correct for potential instrument drift, independent measurements for a particular sample mixture should be non-continuous. The sample tube should be ejected from the spectrometer probe and the measurement process (tuning, locking, shimming) repeated for each replicate for each sample. To avoid potential unwanted contributions due to spinning sidebands, it is recommended to undertake the measurement using sample spinning disabled. This presumes a high degree of field homogeneity has been achieved.

3.6 NMR Signal Integration

Integration approaches conventionally used for ^{1}H NMR cannot be directly applied to ^{19}F NMR for two reasons: (a) the main ^{19}F peak is not centered between ^{13}C satellites due to isotope effect (^{19}F chemical shifts depend on whether a ^{12}C or a ^{13}C is bonded to the ^{19}F nucleus); and (b) the ^{13}C-^{19}F coupling constants are usually stronger than equivalent ^{13}C-^{1}H. The approach based on 30 Hz beyond furthest satellites, commonly used for ^{1}H qNMR,[48] is not enough to cover the entire visible tail of the main peak downfield (to the left side). Similarly, approaches based on the FWHM, such as 76 × FWHM[48] or 80 × FWHM[31] extending on each side from the centre of the main peak can fail to include the furthest ^{13}C satellites (resulting from $^{1}J_{CF}$). From our experience, an integration range extending 120 Hz downfield (to the left side) and 30 Hz upfield (to the right side) beyond the outermost ^{13}C satellites is recommended to integrate in excess of 99.9 % of each quantified signal. A consistent approach should be employed for all signals subject to integration.

It is also important to apply a suitable procedure for the baseline correction and check its validity by analyzing standard samples. Practical experience has shown that manual baseline correction currently works best when very high accuracy qNMR results are required.[39] This method proved to give good results even for the ^{19}F baseline, which presents a rolling aspect.[26] A window function can be applied as a final data treatment parameter to enhance the S/N ratio.[8] To avoid line broadening effects, an exponential multiplication factor

not greater than 0.3 Hz should be used.

3.7 Measurement Uncertainty

Evaluation of the measurement equation previously presented (Equation 1) allows for the identification of individual factors potentially influencing the input quantities for the measurement uncertainty as shown in the diagram in Figure 3.

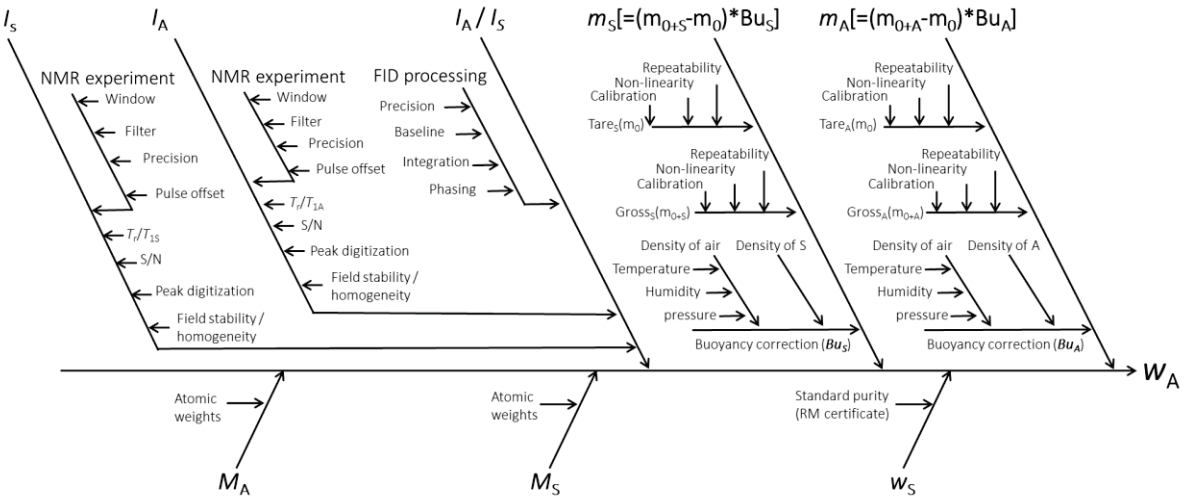


Figure 3. Ishikawa diagram for input quantities considered for the measurement uncertainty estimation by qNMR.

The observed repeatability of the integral area ratios, which incorporates contributions from the input factors for excitation, population, detection efficiency and data processing, is amenable to a type A statistical evaluation.[9,49,50] Since these measurements should come from at least two independent solutions each containing different sample masses, the area ratios will vary on a sample-by-sample basis.

The measurement uncertainty of the value obtained for each preparation can be evaluated separately and the individual purity results for each sample combined statistically. Another approach is to pool the purity values from the replicate results for the separate samples. Analysis of these combined data by ANOVA produces an assigned value and provides an estimate of the intermediate precision of the overall process. It also identifies if additional variance contributions from sample preparation and signal processing contribute significantly to the observed precision in the value assignment in addition to that arising from the method repeatability.

The final assigned value will be similar regardless of the approach used, although the contributions of the factors to the measurement uncertainty of the result may differ.

The standard uncertainties for the other major input quantities are type B estimates and are straightforward to evaluate. Molar masses and their associated uncertainties were calculated based on the values for atomic weights in the 2021 revision of the IUPAC Technical report of the Atomic weights of the elements,[34] the uncertainties of individual gravimetric operations are based on balance performance characteristics corrected for buoyancy effects[38] and the uncertainty of the purity of the internal standard is assigned by the material provider.

Other approaches to the evaluation of measurement uncertainty for qNMR and the combination of results from qNMR with orthogonal techniques for purity evaluation have also been reported[7,51,52] including a Bayesian approach using a Monte Carlo calculation of the results of replicate sample analysis – proposed to ^{1}H NMR but can be useful for ^{19}F NMR as well.[53] An example of a measurement uncertainty budget for qNMR analysis using BTFMBA as the ISRM is provided in Annex 5.2.

4. Acknowledgements

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All NMR studies were carried out by the authors of this document in the course of secondments at the BIPM and joint collaborations. The support of the parent institution of each scientist in making them available for secondment to the BIPM is gratefully acknowledged.

DISCLAIMER: Commercial NMR instruments, software and materials are identified in this document in order to describe some procedures. This does not imply a recommendation or endorsement by the BIPM nor does it imply than any of the instruments, equipment and materials identified are necessarily the best available for the purpose.

5. Annexes

5.1 Solution NMR Spectra of BTFMBA

5.1.1 **BTFMBA in DMSO-d_6**

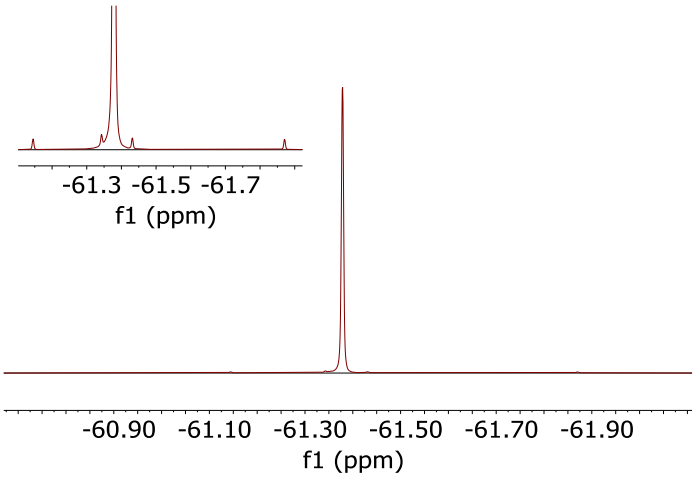

Figure 4. ^{19}F NMR spectra of BTFMBA in DMSO-d_6 acquired on JEOL ECS-400 spectrometer with Royal probe.

5.1.2 **BTFMBA in acetonitrile-d_3**

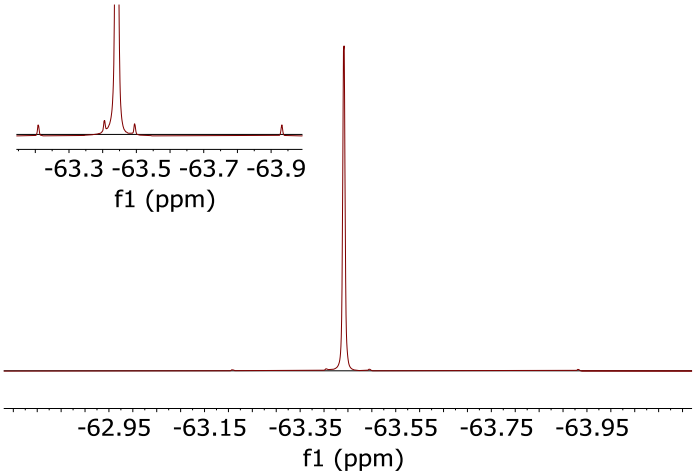

Figure 5. ^{19}F NMR spectra of BTFMBA in acetonitrile-d_3 acquired on JEOL ECS-400 spectrometer with Royal probe.

5.2 qNMR using BTFMBA as Internal Standard

An example is provided of the value assignment by qNMR of the mass fraction content of DFB using BTFMBA as the ISRM. The measurement uncertainty estimation is also presented.

This is intended as a “best case” illustration and should not be regarded as representative of the uncertainty budget achievable when quantifying more structurally complex compounds. The signals for quantification in this example are clearly separated from each other, have well-resolved signal shape and there is no significant interference from impurities (Figure 6).

A thorough shimming procedure was used to maximize the homogeneity of the instrument field. Gravimetric determinations were carried out using a microbalance with a readability of 0.1 µg and a measurement uncertainty for individual mass determinations of less than 100 mg net of 1.3 µg.

The BTFMBA was provided by NMIJ as a high-purity CRM (NMIJ CRM 4601-b). The mass fraction content of BTFMBA in the material certified by NMIJ was (0.9996 ± 0.0003) $kg.kg^{-1}$ (k=2). DFB and deuterated solvents were purchased from commercial suppliers and were used without further treatment or purification. Borosilicate glass NMR tubes with 5 mm internal diameter rated for use in 800 MHz spectrometers and purchased from a commercial supplier were used for all measurements.

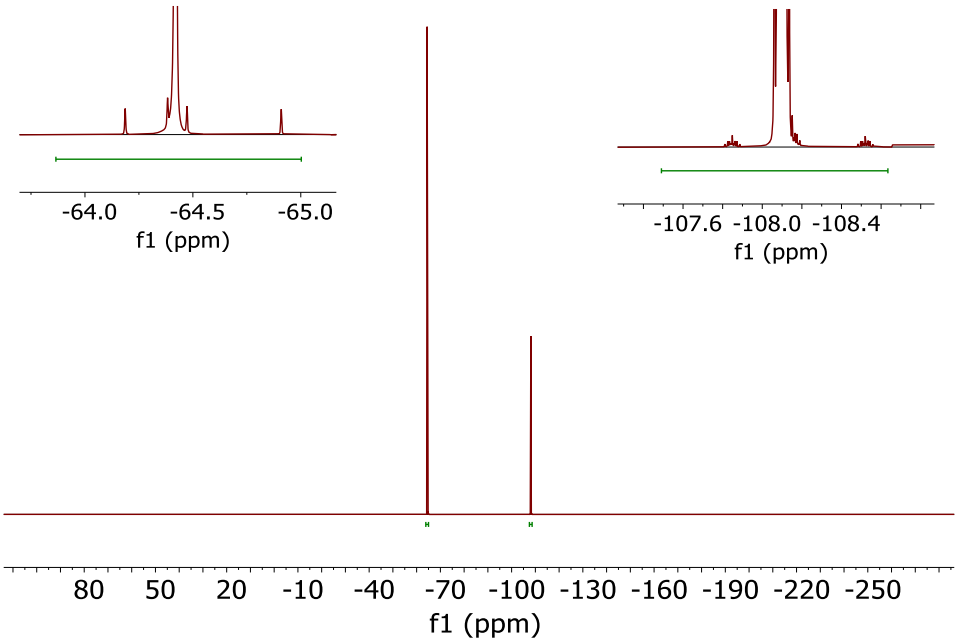

Figure 6. **^{19}F NMR spectrum of BTFMBA (δ -64.4 ppm) and DFB (δ -108.1 ppm) in methanol-d_4; sequence: relaxation delay – square 90° pulse – acquisition.**

The optimized gravimetric and NMR parameters for the qNMR assignment using a Jeol ECS-400 spectrometer with 5 mm Royal probe are given in Table 4. Analyte and internal standard were dissolved in 1 mL of methanol-d_4. After vortex homogenization, the solution was transferred into the NMR tube for analysis.

Table 4. NMR parameters for DFB purity assignment using BTFMBA in methanol-d_4.

Parameter	Value
BTFMBA sample size (mg)	5
DFB sample size (mg)	11 to 13
Number of transients	32
Receiver gain	38
Acquisition time (s)	3.5
Relaxation delay (s)	57.6
Pulse offset (ppm)	-86.26
Spectral width (ppm)	400
Data points	524288
Temperature (K)	298.15
Spinning	off
Integral ratio (DFB:BTFMBA)	1 (approximately)

The integration range covered 120 Hz downfield and 30 Hz upfield beyond the outermost ^{13}C satellites. Multipoint baseline correction was used with points placed about 20 Hz beyond integrated ranges for each signal. Four independent sample mixtures were prepared, and each sample was measured four times. The measurement uncertainty budget is reproduced in Table 5. The repeatability combined mass fraction variations between different samples and between acquisitions from each sample. Its value was expressed as the standard deviation of the mean relative to 1. The other uncertainty components were type B estimations. The relative contribution of each component to the uncertainty of the combined result for this sample is displayed in Figure 7. The mass fraction content of DFB assigned for this sample was 997.3 ± 1.3 $mg.g^{-1}$.

Table 5. Uncertainty budget for DFB purity by ^{19}F qNMR using BTFMBA in methanol-d_4

Uncertainty source	Value	Type	Standard uncertainty	Sensitivity coefficient	Uncertainty component (mg.g^{-1})
Repeatability	1	A	0.00056	997.315	0.555
IS mass (mg)	5.08	B	0.00129	196.258	0.253
Analyte mass (mg)	11.09	B	0.00165	-90.008	0.149
Analyte molar mass (g/mol)	218.20	B	0.00900	4.573	0.041
IS molar mass (g/mol)	258.12	B	0.00600	-3.866	0.023
IS purity (mg/g)	999.6	B	0.15000	0.998	0.150
				Combined uncertainty	0.648
Purity of DFB	**997.3**	**±**	**1.3**	**mg.g^{-1} (*k*=2)**	

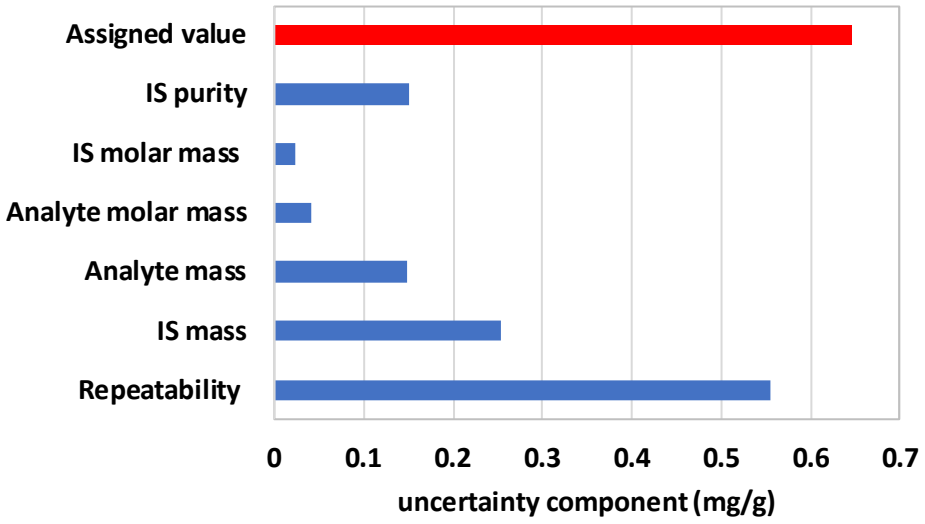

Figure 7. Uncertainty components for DFB purity assignment using BTFMBA in methanol-*d*4.

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