Internal Standard Reference Data for qNMR: Dimethyl Terephthalate [ISRD-04] 1st edition

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**Bureau International
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1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is now well-established as the pre-eminent method for the qualitative structural analysis of organic molecules. The potential for the application of quantitative nuclear magnetic resonance (qNMR) for organic analysis was also recognized soon after the technique became widely commercially available. [1] However it has only been more recently, as instrumental capabilities have achieved a level of accuracy and precision comparable to those attainable by chromatographic techniques, that this potential has begun to be generally realized. Quantitative methods based on NMR spectroscopy, particularly for the assignment of the purity of individual organic compounds, are now actively and extensively implemented. [2], [3], [4] As evidence, an editorial in the *Journal of Medicinal Chemistry* [5] highlighted and recommended the general utility of "absolute quantitative ^{1}H NMR spectroscopy to determine the purity of biologically tested research compounds". Purity assignment by qNMR spectroscopy also potentially meets the metrological requirements for a primary ratio measurement procedure. [6] Validated qNMR methods [7] are now being used, often in combination with data obtained by orthogonal chromatographic techniques, to assign the purity of organic materials intended for use as Primary Reference Materials [8] for individual organic analytes. [9], [10], [11] 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 through a calibration hierarchy to a specific pure material. [12]

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:

$$w_A = \frac{I_A}{I_S} * \frac{N_S}{N_A} * \frac{M_A}{M_S} * \frac{m_S}{m_A} * w_S \tag{1}$$

w_A is the mass fraction of A in the material subject to assignment, w_S the independently established mass fraction content of the internal standard S, I_A and I_S are the integrals of the quantified signals unique to A and S respectively, N_A and N_S the number of ^{1}H 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 individual aliquots of the analyte and internal standard material used to prepare the solution subject to the qNMR measurement.

In optimal cases and with the data processing carried out by experienced operators the standard uncertainty for purity mass fraction assignments for non-problematic systems has been reported to reach the level of 1 mg g^{-1} on an absolute basis, equivalent to 0.1 % relative. [13] However this level of uncertainty is difficult to achieve on a routine basis and in addition is limited on a case by case basis being contingent on the structural complexity and impurity profile of A. Factors including, *inter alia*, the lineshape and multiplicity of the signals integrated, the extent and nature of potential interferences from impurities present in the analyte, the nature of the internal standard and solvent used, the magnetic field strength, hardware settings and performance characteristics of the spectrometer and the approach taken to transform the time domain free induction decay (FID) signal generated by the NMR experiment and integrate the signals of the resulting frequency domain spectrum can all contribute to the overall uncertainty of the 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 ^{1}H NMR measurement using the internal standard approach to provide a measurement result traceable to the International System of Units (SI). [14] It should be noted that although these principles should be applicable in general to quantitative measurement involving any NMR-active nuclei the specific recommendations in this document are intended only for assignments by ^{1}H qNMR.

The second goal is to describe a set of internal standard reference materials (ISRMs) which the Bureau International des Poids et Mesures (BIPM) in collaboration with the National Metrology Institute of Japan (NMIJ) propose as a "universal" set of higher-order, SI-traceable internal standards for use in routine ^{1}H qNMR measurements. Different groups have proposed specific compounds or sets of compounds suitable for use as qNMR internal standards. [13], [15], [16], [17] Although there is some commonality between the internal standards recommended in this earlier literature and our proposal the focus of the earlier papers is the application of the materials for general use in purity assignments rather than, as is the case here, of their suitability as higher-order, SI-traceable primary measurement standards for qNMR. At least one ISRM compound should be suitable for use for the assignment of a given organic compound soluble in a specified NMR solvent. The compounds constituting the "universal" ISRM set together with an overview of their solubility and suitability for use in representative deuterated NMR solvents are described in Figure 1 below.

The third goal, and the focus of this document, is to provide guidance regarding the use and limitations of dimethyl terephthalate (DMTP) as an ISRM for qNMR analysis.

Ideally, a qNMR ISRM should consist of a stable crystalline solid which is:

- available as a high purity Certified Reference Material (CRM) whose value has been assigned by a National Metrology Institute (NMI) using methods independent of qNMR or which has been value assigned directly by qNMR using a high purity CRM as the internal standard;
- predominantly a single organic component ($w_S > 995$ mg g^{-1});
- value assigned with small standard uncertainty ($u(w_S) < 1$ mg g^{-1});
- providing unique NMR signals, preferably as singlet or simple multiplet resonances, having Lorentzian lineshape with a narrow signal width;
- free of impurities interfering with the areas to be integrated;
- inert in solution in suitable NMR solvent;
- soluble in the chosen NMR solvent 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 relative mass content contribution from the hydrogen atoms giving rise to the quantification signal below 5%.[1]

(1) When H-content exceeds 5% by mass, the aliquot size for the internal standard used for a typical analysis is small and the uncertainty associated with gravimetric operations becomes a limiting factor in the overall uncertainty of a qNMR assignment.

It is recognized that these characteristics constitute a "wishlist" rather than prescriptive requirements. However DMTP can potentially meet all the listed specifications, taking into account its instability in solution in CD$_3$OD (see discussion below).

The solubility estimates of the ISRMs in the individual solvents listed in Figure 1 are intended as also being indicative of those for solvents having similar capabilities. The four shown were selected as being the most readily available deuterated solvents. In practice the majority of

the reported applications of qNMR for purity assignment in solution have been undertaken using one of these solvents.

At least three ISRMs are applicable to each solvent class and provide quantification signals distributed across the standard ^{1}H chemical shift range.

DMTP is suitable for use as an internal standard for qNMR purity assignments of analytes soluble in CDCl3, DMSO-d_6 and related solvents such as CD$_3$CN and acetone-d_6. It can also be used in CD$_3$OD but only if using the aromatic proton signals for purposes of quantification. In this solvent *in situ* transesterification leads to the replacement of the methyl (CH$_3$) ester functional groups by deuteromethyl (CD$_3$), which precludes the use of the methyl ester signal for quantification studies.

The following section of this reference document and the attached annexes describes the specific properties and applications of DMTP for use as an ISRM for qNMR.

ISRM	KHP	BTFMBA	DMTP	MA	DMSO$_2$	BTMSB-d_4	DSS-d_6
Structure							
δ (ppm)	8.3-7.0 (4H)	8.4-8.5 (2H) 8.2-8.4 (1H)	8.1 (4H) 3.9 (6H)	6.3 (2H)	3.0 (6H)	0.2 (18H)	0.1 (9H)
Density (g.cm^{-3})	1.64 ± 0.17	1.72 ± 0.04	1.2 ± 0.24	1.53 ± 0.03	1.4 ± 0.03	1.0 ± 0.02	1.27 ± 0.03
H content (mg.g^{-1})	19.6	11.6	20.6 (4H) 30.9 (6H)	17.2	63.8	79.5	44.5
Solvent ⇩	Solubility (mg/mL)						
D$_2$O	> 10	< 1	< 1	> 5	> 10	< 1	> 5
d$_6$-DMSO	> 2	> 10	> 5	> 10	> 5	> 2	> 5
CD$_3$OD	> 2	> 10	> 2 *	*	> 5	> 2	> 5
CDCl$_3$	< 1	> 5	> 10	< 1	> 10	> 5	< 1

Figure 1 — qNMR ISRM Suite [18]

* soluble but only for quantifications based on the aromatic proton signal. Exchange of the methyl ester with CD$_3$OD precludes quantification based on the dimethyl ester.

* soluble but unsuitable for qNMR due to esterification reaction with CD$_3$OD

KHP — Potassium hydrogen phthalate

BTFMBA — 3,5-Bis-(trifluromethyl)benzoic acid

DMTP — Dimethyl terephthalate

MA — Maleic acid

DMSO$_2$ — Dimethyl sulfone

BTMSB — 1,4-*bis*-Trimethylsilylbenzene (R=H), BTMSB-d_4 (R = D), BTMSB-F$_4$ (R = F);

DSS-d_6 — 3-(Trimethylsilyl)-hexadeuteropropane-1-sulfonic acid [4,4-Dimethyl-4-silapentane-1-sulfonic acid-d_6]

D$_2$O — Deuterium oxide

DMSO-d$_6$ — Dimethyl sulfoxide-d_6 / Hexadeuterodimethyl sulfoxide

CD$_3$OD — Methanol-d_4 / Tetradeuteromethanol

CDCl$_3$ Chloroform-*d* /
Deuterochloroform

2. Properties of Dimethyl terephthalate

2.1. Physical Properties

Name	**Dimethyl terephthalate**
Structure	+
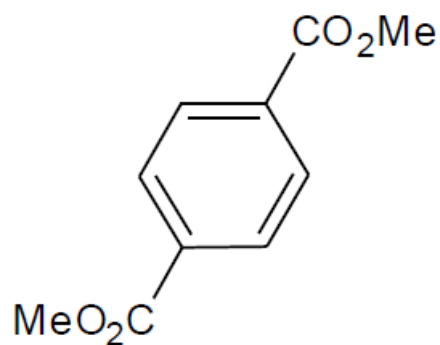
Synonym	Dimethyl 1,4-benzenedicarboxylate
CAS Registry Number	120-61-6
Molecular Formula	$C_{10}H_{10}O_4$
Molar Mass [19]	194.186 g/mol, $u = 0.009$ g/mol
Melting point [20]	141 °C
Density	1 075 kg/m^3 [20]
	1 200 kg/m^3 [21]
Appearance	White crystalline powder
^{1}H NMR [22]	δ8.1 (S, 4H) ; 3.94 (s, 6H) ppm
^{13}C NMR	δ166.2; 134.0; 129.6, 52.4 ppm
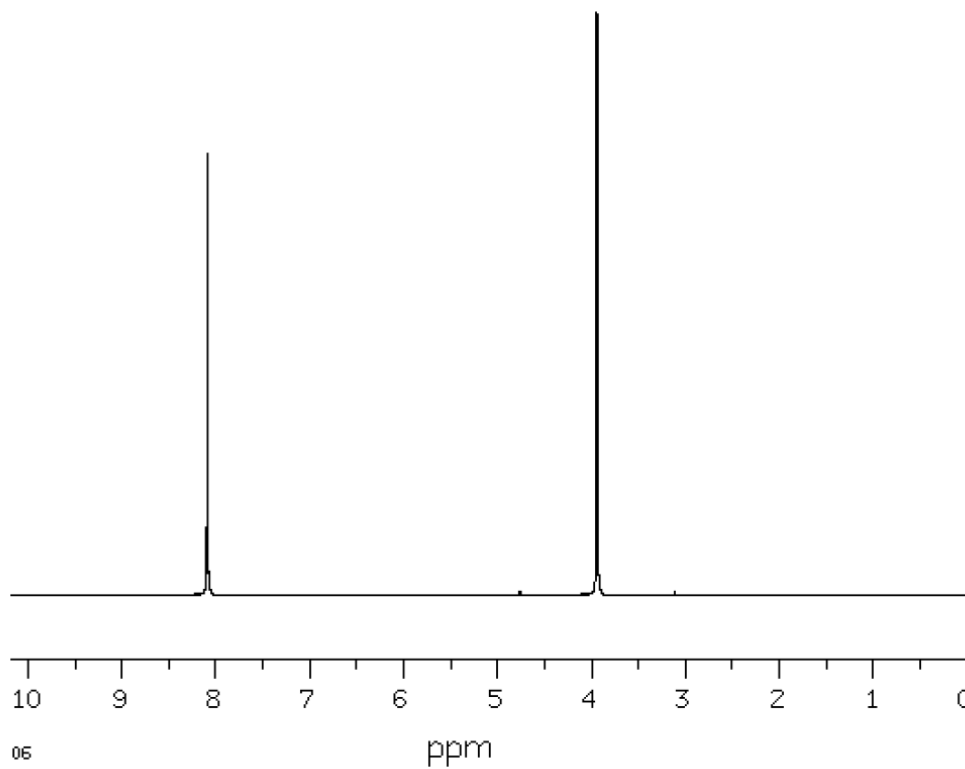
Figure 2 — ^{1}H NMR spectrum of DMTP in CDCl$_3$

2.2. NMR Solvent Compatibility

NMR solvents suitable for use with DMTP are CDCl$_3$ and DMSO-d_6. DMTP is soluble at levels in excess of $5\,\mathrm{mg\,mL^{-1}}$ in CDCl$_3$ and in excess of $2\,\mathrm{mg\,mL^{-1}}$ in DMSO-d_6. qNMR studies using DMTP as ISRM can also be undertaken in as solutions in acetone-d_6 or CD$_3$CN.

DMTP is moderately soluble in CD$_3$OD. The formation in situ of mono- and di-transesterification artefacts from exchange of CH$_3$O- by CD$_3$O- at the methoxycarbonyl substituents restricts its use for qNMR in this solvent to quantifications based on the aromatic proton signal. [23]

2.3. NMR quantification signals

The four magnetically equivalent aromatic and six equivalent methyl ester protons of DMTP are observed as singlets at a chemical shift of approximately 8 ppm and 4 ppm respectively. The position of the resonance is a function of factors including but not limited to the solvent, temperature and the concentration of DMTP and other analytes in the solution. For optimal quantification results the homogeneity of the spectrometer magnetic field should be optimized such that the full width at half maximum (FWHM) of the signal(s) used for quantification are less than 1 Hz while the base of each resonance retains a suitable Lorentzian peak shape.

2.4.   Impurities and artefact signals

Samples of DMTP analysed in our laboratory show some evidence of the presence of small levels of related structure impurities in the material. These are most likely either isomeric (dimethyl phthalate) or homologous (diethyl terephthalate) in structure.

As noted above, artefacts form if DMTP is taken up in solution in CD_3OD due to transesterification exchange. An NMR spectrum showing the appearance of free methanol in a solution of DMTP in CD_3OD subsequent to its preparation is shown in Appendix A1.3.

In practice the main interferences in a solution containing DMTP come from residual non-deuterated solvent. The chemical shifts of these signals are given in Table 1 below.

2.5.   Solvent recommendations & advisories

2.5.1. D$_2$O

DMTP is not sufficiently soluble in D_2O for use in qNMR applications.

2.5.2. DMSO-d_6 and related solvents

DMTP is sufficiently soluble in DMSO-d_6 for qNMR studies if less polar solvents are not suitable for the target analyte. CD_3CN and acetone-d_6 are solvents with similar solubilizing properties as DMSO-d_6 and are also suitable for use with DMTP

2.5.3. Methanol-d_4 and related solvents

As discussed in Section 2.2 and Section 2.4 above, despite its solubility it is advised to consider alternatives to the use of DMTP in CD_3OD for high accuracy qNMR assignments if possible. [23] However as the transesterification exchange with the solvent is slow and results in minimal change in the chemical shift of the aromatic proton signal, quantification based on this signal can be undertaken if desired.

2.5.4. CDCl$_3$ and related solvents

DMTP is soluble in $CDCl_3$ and it is a recommended choice as a solvent for use in qNMR measurements with this ISRM. It should also be suitable with non-polar deuterated solvents such as $C_2D_2Cl_4$, benzene-d_6 and pyridine-d_5.

Table 1.   Solvent Parameters for DMTP

Solvent	qNMR signal	Integration range (ppm) [(a)]	T_1 (s) [(a)]	Residual Solvent (ppm)	Comments:
	Singlet, 8.0 ppm (4H)				
	Singlet, 3.9 ppm (6H)				

D$_2$O		Not suitable			Insufficiently soluble
DMSO-d_6	8 (4H), 3.9 (6 H)	8.2 – 7.8, 4.1 – 3.7	3-4	2.5, 3.3	
CD$_3$OD	8 (4H) only	8.2 – 7.8	4-5	3.3, 4.8	Transesterification
CDCl$_3$	8 (4H), 3.9 (6 H)	8.2 – 7.8, 4.1 – 3.7	2.5-3.5	7.25	

(*a*) Indicative values only. The value in a specific qNMR solution will be a function of factors including concentration of DMTP 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), see p. 6). The mass fraction purity of the internal standard used for the measurement, the source of traceability to the SI for the value to be assigned to the analyte, is established by independent measurements undertaken prior to the qNMR experiment.

The gravimetric procedure used for the preparation of the NMR solution has to be fully validated and fit for its intended purpose, [24], [25] and the spectrometer performance, experimental parameters and the protocol for signal processing and integration must be optimized, [4], [7], [26] in order that the observed ratio of the integrals of the analyte and standard signals accurately reflects the molar ratio of the hydrogen nuclei giving rise to the signals. [27] When these conditions are met the assigned mass fraction purity of the analyte can be regarded as traceable to the SI. [9], [28], [29] Some general guidance for recommended practice for these critical steps is given in the following sections.

3.2. Internal standard

At least one of the internal standards listed in Figure 1 above should be suitable for use as the ISRM for a qNMR purity assignment. The material used should comply as far as possible with the criteria described in Chapter 1 above regarding composition, physical characteristics, inertness, solubility, impurity profile and relative hydrogen content by mass. In order to establish traceability of the result to the SI, the material should comply with the requirements of a reference measurement standard, and in particular of a Certified Reference Material, defined in the International Vocabulary of Metrology (VIM). [30]

For SI-traceability the internal standard should consist of one of the following:

1. Certified Reference Material (CRM) characterized for mass fraction purity and value assigned by a National Metrology Institute;

2. CRM provided as a high purity organic material by a Reference Material Producer accredited to ISO 17034:2016 [31] requirements;

3. High purity material subject to a validated measurement procedure for purity assignment by qNMR using as an internal standard a CRM of type 1) or 2).

3.3.   Gravimetry

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 described in a recent publication. [24] Achieving an overall relative standard measurement uncertainty for a qNMR assignment of 0.1 % will typically require the relative uncertainty associated with individual gravimetric operations be less than 0.03 %. If the combined standard uncertainty of a mass determination is $3\,\mu g$, a level achievable with modern electronic microanalytical balances, this corresponds to a minimum sample size of 10 mg. Care should be exercised to include an appropriate allowance for the uncertainty of each gravimetric operation within the overall uncertainty budget for a qNMR purity assay. It must take into account the contribution due to gravimetric uncertainty introduced as a consequence of the aliquot sample sizes and the performance characteristics of the balance used.

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 mass determinations the procedure for transfer of solids into the solution must be validated to address the assumption that the ratio of the 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 examples reported in the Appendix A1.2 below, gravimetric operations were undertaken using a balance associated with a measurement uncertainty estimate of $1.3\,\mu g$ for individual mass determinations. In this case a minimum sample size of 4 mg achieves a relative uncertainty in individual gravimetric operations below 0.03 %. In addition to the measurement uncertainty of the balance reading, for high accuracy measurements correction for sample buoyancy effects and the contribution to the overall measurement uncertainty associated with this correction should also be taken into consideration. [25]

As sample preparation for qNMR involves mass determinations in the milligram range using sensitive balances, the loss of even minute (almost 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. [32] It is recommended that mass determinations be performed in an area where the relative humidity is maintained in the range 30 % to 70 %.

The accumulation of surface electrostatic charges is a 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 deioniser is advisable prior to the mass determination. Where possible materials subject to qNMR analysis should be evaluated for their hygroscopicity, for example by measurement of the change in observed mass as a function of relative humidity using a dynamic sorption balance. 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 when assigning the purity of a compound by qNMR.

3.4. NMR spectrometer optimization for quantitative measurements

There is no specification of minimum NMR spectrometer field strength for purity measurements. Increasing field strength results provides enhanced signal separation and increases sensitivity, both of which should increase the accuracy and precision of qNMR measurements. Careful optimization of the lineshape (shimming) is mandatory and critical in order to achieve reliable qNMR results. [33] A general guidance is to choose the simplest signal in the sample, often the residual solvent peak, and to optimize the instrument shimming until this signal is symmetrical with a FWHM below at least 1 Hz. Experience has shown that these lineshape requirements are more easily achieved using an inverse probe than a direct type. For lower field magnets (< 300 MHz), this recommendation might not be attainable. If the lineshape is broader the level of measurement uncertainty associated with the assigned value will increase. In no case should a signal from a labile, exchangeable hydrogen or one subject to dynamic tautomeric exchange be used for quantitative measurements

Due to the relatively wide Lorentzian signal 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 the range one hundred times the FWHM either side of each signal to be integrated.

3.5. NMR acquisition parameters

The basic experiment to perform quantitative NMR experiments uses a simple 1D pulse sequence designed to minimise differences in the integrated signal intensities due to effects related to incomplete relaxation of the quantification resonances. For highest accuracy assignments use of broadband heteronuclear decoupling should be avoided if possible 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, this potential contribution to bias in a qNMR result is attenuated to a negligible level because of the low (1.1 %) natural abundance of the ^{13}C isotopomer.

The recommended 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 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 or 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. Ideally the pulse offset should be positioned as close as possible to the midpoint between the two signals to be quantified. This will not eliminate off-resonance effects but should result in them cancelling out in both signals.

Regarding the pulse length, 90° pulses are recommended for quantitative analyses. A 30° pulse experiment, providing a signal response approximately half that of a 90° pulse, has the potential advantage of needing a significantly shorter relaxation time to re-establish equilibrium magnetization compared with a 90° pulse while requiring only twice as many

transients to achieve an equivalent **signal** response. However this potential practical advantage is offset by the need for four times as many transients as a 90° pulse to achieve the same **signal to noise** ratio. The accuracy (trueness) of the results should not be impacted by the use of different pulse lengths but the acquisition times to achieve equivalent levels of signal precision (repeatability) will.

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 digital 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 digitisation 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 meaningful area measurements and suitable repeatability at typical sampling rates.

The relaxation delay between pulses in particular 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 DMTP in different solvents at the concentration and under the specific instrumental conditions used, 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 commonly used method to determine the T_1 constant is the inversion-recovery sequence generally available in the factory programmed pulse sequences installed with any NMR. The application of the inversion recovery experiment requires knowledge of the optimized 90° pulses for each quantified signal, which should also be determined for each mixture under investigation. The optimized 90° pulse values can be 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 RT is a multiple T_1. After a 90° pulse, if the available instrument time permits, 10 times T_1 of the signal with the longest relaxation time will lead to the recovery of > 99.995 % 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 will be sufficient for equivalent (even if incomplete) magnetization re-equilibration. At least 10 T_1 should be used as a minimum where highest accuracy results are sought.

Thus the pulse RT is given by:

$$RT = RD + AQ = n * T_1 \qquad (2)$$

$$(n = 10 - 15)$$

The number of transients (or scans) should be determined according to the concentration of the samples, 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 for can still lead to acceptable results, but the reported measurement uncertainties increase as the S/N ratio decreases.

Table 2.   Recommended NMR Parameters for quantitative measurements.

Parameter	Recommended Value	Explanation/Comments
Shimming	FWHM of lineshape signal (eg $CHCl_3$/acetone-d_6) $< 1\,Hz$	Optimization of field homogeneity is critical for uniform response over typical chemical shift range
Pulse Width	$90°$	Should not change the quality of the results, but the use of a 90° pulse with adequate recovery time leads to a smaller total acquisition time for a target S/N ratio.
Pulse Offset	Midpoint between signals	Theoretically makes off resonance effects equivalent for both signals.
Repetition Time	$10 - 15 \times T_1$	After 90° pulse, a delay of 10 T_1 of the signal with the longest relaxation time necessary for recovery of $> 99.995\,\%$ of magnetization for all quantified signals.
Number of Transients (scans)	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	$> 20\,ppm$	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 and 4 s has been found to be suitable for many applications.
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.

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 but it is important to be 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 independently weighed analyte/IS mixture (a minimum of two samples) should be measured at least three times in the NMR system. Independent measurements for a particular sample mixture should be non-continuous, where the tube is removed and the measurement process (tuning, locking, shimming) repeated each time for each sample.

3.6.   NMR signal integration

The integration range should extend on each side ideally at least seventy six times the FWHM of the signal being measured in order to integrate in excess of 99.9 % of the signal. The estimation of signal width should be done for the outer signals if a multiplet signal is subject to integration. A generally acceptable approximation is to use a range extending 30 Hz beyond the furthest ^{13}C satellites as the start and end points for the integration range, as this generally exceeds the above described width. In a complex spectrum where other signals are adjacent to one or both of the quantification signals and quantification over the full range is not possible apply a consistent approach to the integration of both signals.

It is important to apply a suitable algorithm for the baseline correction and check its validity by analysing standard samples. Practical experience has shown that currently manual baseline assignment is the most reliable general approach when high accuracy qNMR results are required. [31] A final data treatment parameter that can be applied is an adequate window function. For ^{1}H NMR, exponential multiplication by a factor not greater than 0.3 Hz should be used. The exponential multiplication factor in use at the BIPM with the JEOL-ECS 400 is typically no greater than 0.05 Hz -0.10 Hz and in some cases is not used at all.

3.7.   Measurement uncertainty

Evaluation of the measurement equation previously presented (Equation (1), see p. 6) identifies the factors influencing the input quantities for the measurement uncertainty as shown in the diagram in Figure 3.
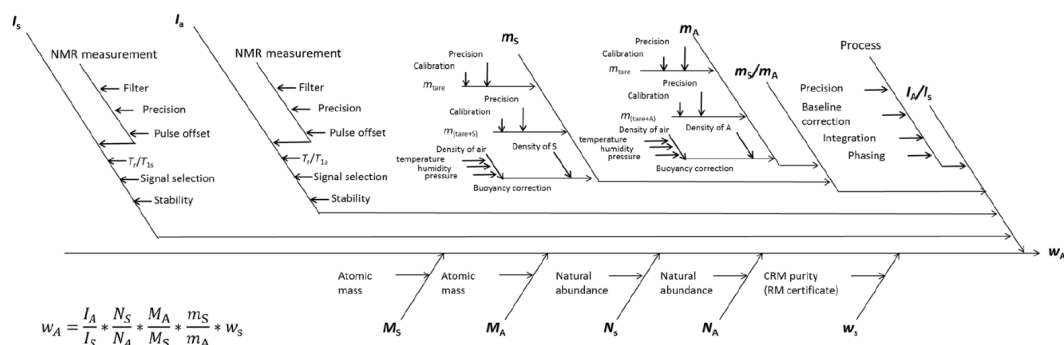
$$w_A = \frac{I_A}{I_S} * \frac{N_S}{N_A} * \frac{M_A}{M_S} * \frac{m_S}{m_A} * w_S$$

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. [24], [34] Since these measurements come from at least two independent solutions each containing different sample masses, the observed absolute 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 this 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 exist in addition to that due to the method repeatability. [9]

The final assigned value will be similar regardless of the approach used, although the contribution 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 uncertainties are estimated based on the "conventional" values for atomic weights given in Table 3 of the 2016 revision of the IUPAC Technical report of the Atomic weights of the elements, [19] the uncertainties of mass determinations are based on balance performance characteristics and are corrected for buoyancy effects [26] 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. [28], [35] Examples of "best case" measurement uncertainty budgets for qNMR analysis are provided in the examples given in Appendix A1.2.

4. Acknowledgements

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.
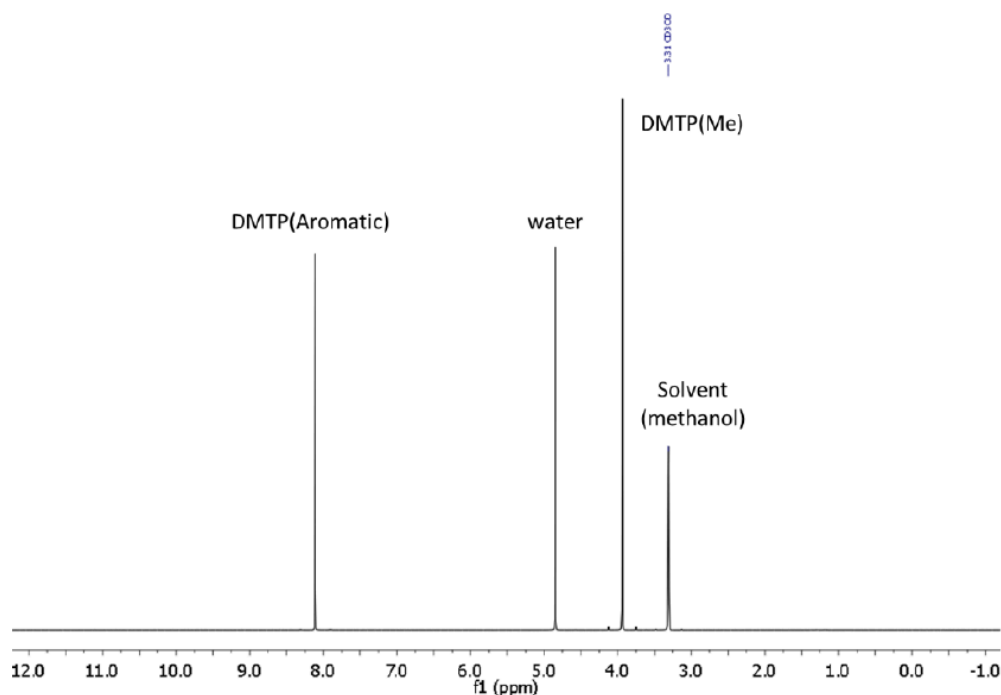
Appendix 1. Annexes

A1.1. Solution NMR Spectra of Dimethyl terephthalate

A1.1.1. DMTP in DMSO-d_6
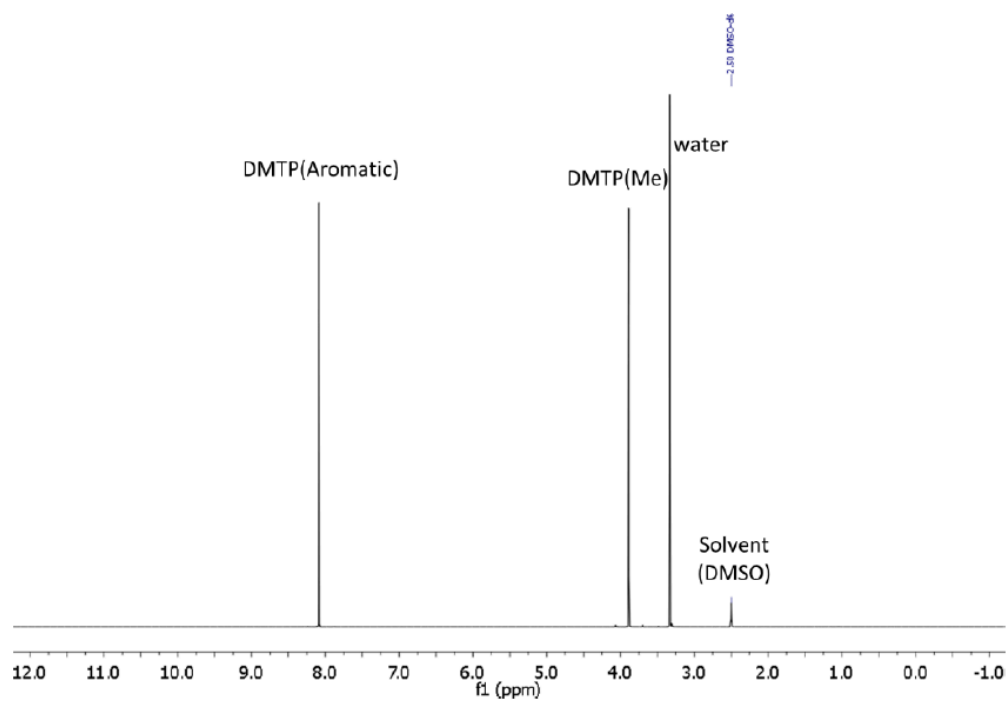
A1.1.2. DMTP in CD₃OD

A1.2. qNMR using DMTP as internal standard

Two examples are provided of the value assignment by qNMR of the mass fraction content of organic compounds using DMTP as the ISRM and the associated measurement uncertainty budgets. In the first DMTP was used in a solution in DMSO-d_6 with BTFMBA as analyte. In the second CD₃OD was the solvent with BTMSB-d_4 the analyte. [18]

These are intended as "best case" illustrations and should not be regarded as representative of the uncertainty budget achievable when quantifying more structurally complex compounds. The signals for quantification in these examples are clearly separated from each other, have narrow, well-resolved signal shape and there is no significant interference from impurities or solvent signals. As a result the uncertainty contribution due to the reproducibility of the signal integration is smaller (and the relative uncertainty contribution due to gravimetry and the purity of the internal standard correspondingly greater) than should be anticipated for more general applications.

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 DMTP was purchased from a commercial provider and was used without additional treatment. The purity of the DMTP was assigned in separate qNMR experiments in solution in CDCl₃ using high-purity CRMs as the internal standard. The mass fraction content of the DMTP assigned by our internal qNMR measurement was 999.3 ± 0.8 mg g^{-1}, consistent within its stated uncertainty with the purity value for the material reported in the documentation supplied by the material producer.

The BTFMBA was provided by NMIJ (NMIJ CRM 4601a) and the BTMSB-d_4 was donated by WAKO Chemicals (Product reference 024-17031). Both analytes were used without further

treatment or purification. Deuterated solvent was purchased from commercial suppliers and used as supplied. Borosilicate glass NMR tubes with 5 mm internal diameter rated for use in 500 MHz spectrometers purchased from a commercial supplier were used for all measurements.
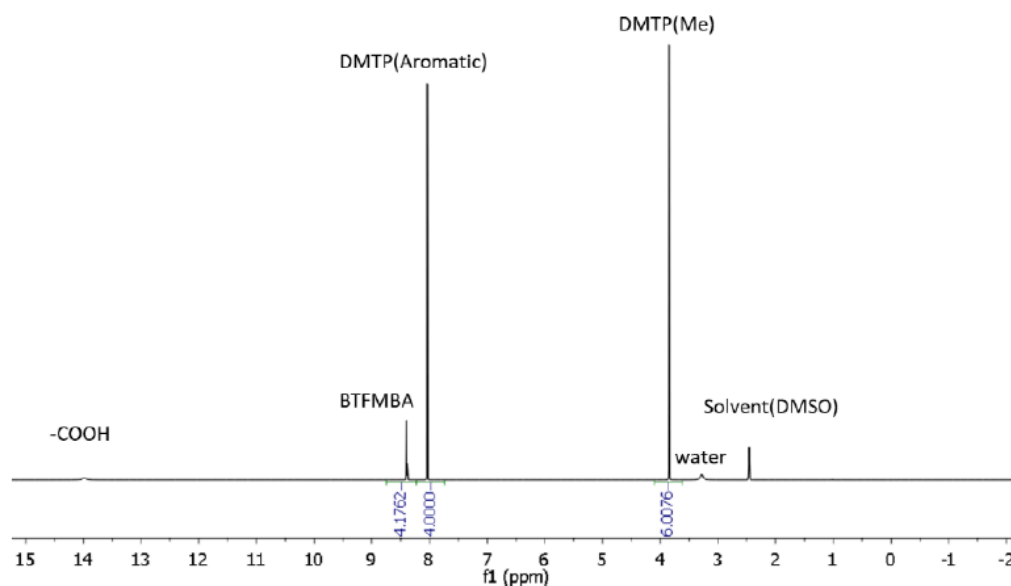
A1.2.1. DMTP (IS) & BTFMBA (Analyte) in DMSO-d_6

Figure 1.1 — NMR spectrum of DMTP + BTFMBA in DMSO-d_6

The optimized gravimetric and NMR parameters for the qNMR assignment using a JEOL ECS-400 spectrometer equipped with a Royal probe are given in Table 1.1. The sample was made up in solution in approximately 1 mL of CDCl$_3$ and 800 μL was transferred into the NMR tube for analysis.

Table 1.1. NMR parameters for BTFMBA purity assignment using DMTP in DMSO-d_6.

Parameter	Value
DMTP Sample size (mg)	3 – 6
BTFMBA Sample size (mg)	8 – 11
Number of Transients	32
Receiver gain	Automatic
Acquisition time (s)	4
Relaxation delay (s)	65
Pulse offset (ppm)	6.1

Spectral width (ppm)	400
Data points	639652
Temperature (K)	298
Spinning	Off
Integral ratio (BTFMBA:DMTP)	0.5 – 1.0 [DMTP –OMe signal] 0.7 – 1.4 [DMTP –ArH signal]

A baseline correction window extending one hundred times the FWHM of each integrated signal was applied. The integration range covered eighty times the corresponding FWHM. Four independent sample mixtures were prepared, each sample was measured four times and each quantification was made against the integrals of both the aromatic proton signal and the dimethyl ester signal of the DMTP. The measurement uncertainty budget for the samples quantified against the DMTP methyl ester signal is reproduced in Table 1.2. The integral ratio is a mean of the four replicate values obtained for each of the four samples, with the result normalized to take into account the different sample sizes. The standard uncertainty of the normalized ratio is the standard deviation of the mean. The other uncertainty components are Type B estimations. The relative contribution of each component to the uncertainty of the combined result for this sample is displayed in Figure 1.2. The mass fraction content of BTFMBA in the material assigned from the data obtained for this sample was $1\,000 + 0/- 1.2\ \text{mg g}^{-1}$.

Table 1.2. Uncertainty budget for BTFMBA purity by qNMR using DMTP in DMSO-d_6.

| Uncertainty sources	Value	Type	Standard Uncertainty	Sensitivity coefficient	Uncertainty Component
I_A/I_{IS} (repeatability)	0.8307	A	0.00014	1.203837613	1.83E-04
Analyte signal ^{1}H Nuclei	2.9994	B	0.0003	-0.333399302	1.00E-04
IS signal ^{1}H Nuclei	5.9988	B	0.0003	0.166698019	6.67E-05
Analyte Molar Mass	258.119	B	0.0058	0.003874168	2.25E-05
IS Molar Mass	194.186	B	0.0085	-0.005149691	4.37E-05
Analyte mass (mg)	7.7562	B	0.00124	-0.12892884	1.60E-04
IS mass (mg)	3.5147	B	0.00124	0.284518697	3.54E-04
IS purity (mg g^{-1})	999.3	B	0.40	1.000698354	4.00E-04
				Combined Uncertainty	0.00058
Purity of BTFMBA			$1\,000.0 + 0 / - 1.2\ \text{mg g}^{-1}$		
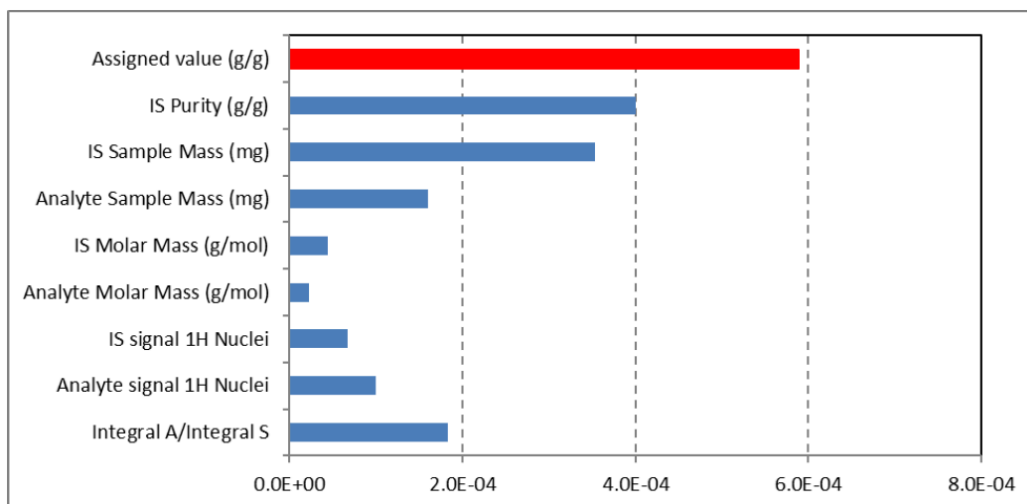
Figure 1.2 — Relative uncertainty components: BTFMBA assignment using DMTP in DMSO-d_6

The qNMR purity assignment of BTFMBA using DMTP as ISRM agreed within its associated uncertainty with the certified value of 999.6 ± 0.6 mg g^{-1} reported by NMIJ for the content of the material as assigned by mass balance and titrimetry.

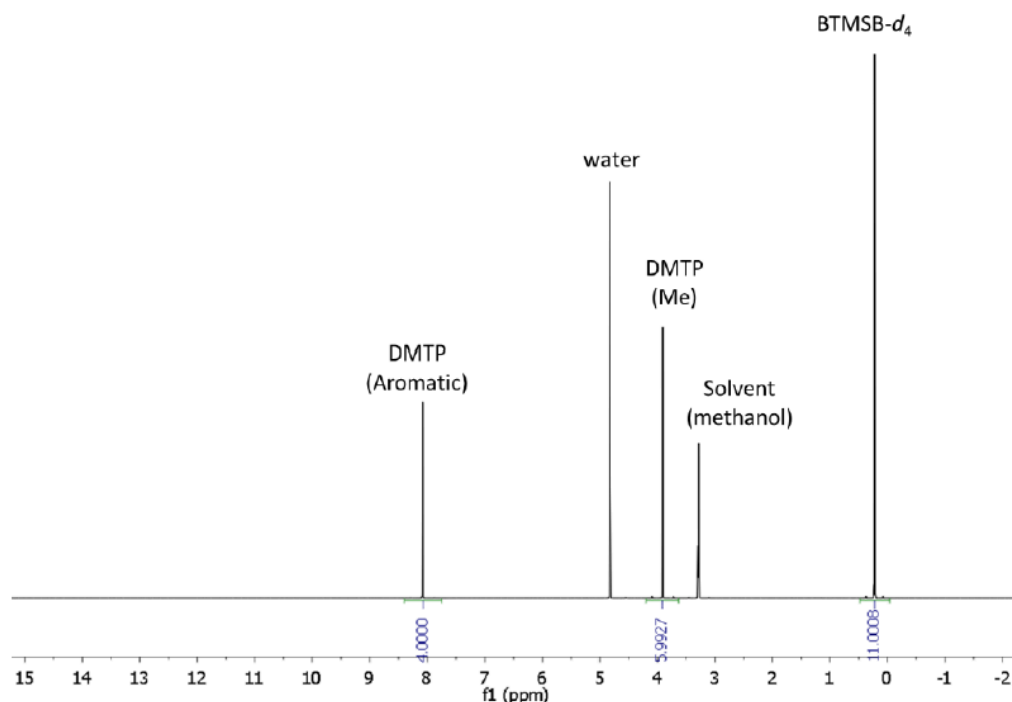### A1.2.2. DMTP (IS) & BTMSB-d_4 (Analyte) in CD$_3$OD

Figure 1.3 — ^{1}H NMR of DMTP and BTMSB-d_4 in CD$_3$OD.

The experimental NMR parameters used for the measurement are given in Table 1.3.

Table 1.3. NMR experiment parameters for BTMSB-d_4 assignment using DMTP in CD$_3$OD.

Parameter	Value
DMTP Sample size (mg)	2.4 – 2.8
BTMSB-d_4 Sample size (mg)	1.9 – 2.4
Number of Transients	32
Receiver gain	Automatic
Acquisition time (s)	4
Relaxation delay (s)	45
Pulse offset (ppm)	4.15
Spectral width (ppm)	400
Data points	39979
Temperature (K)	298
Spinning	Off
Integral ratio (BTMSB-d_4 : DMTP)	2.5 – 5.0 (DMTP ArH signal)

Results from four independent sample mixtures each measured four times were obtained. The measurement uncertainty budget for the combined results for the four sample determinations with the values assigned using the DMTP aromatic signal as reference is reproduced below in Table 1.4. The integral ratio is a mean of the four replicate values obtained for each of the four samples, with the result normalized to take into account the different sample sizes. The relative contribution of each component to the uncertainty for this sample is displayed in Figure 1.4. The mass fraction content of BTMSB-d_4 in the material assigned from the results for this sample was $999.0 + 1.0 / - 1.9 \, \text{mg g}^{-1}$.

Table 1.4. Uncertainty budget for BTMSB-d_4 purity by qNMR using DMTP in CD$_3$OD.

Uncertainty sources	Value	Type	Standard Uncertainty	Sensitivity coefficient	Uncertainty Component
I_A/I_S (repeatability)	3.1854	A	0.00037	0.313630465	1.35E-04

Uncertainty sources	Value	Type	Standard Uncertainty	Sensitivity coefficient	Uncertainty Component
Analyte signal ^{1}H Nuclei	17.9964	B	0.0003	-0.055513645	1.67E-05
IS signal ^{1}H Nuclei	3.9992	B	0.0003	0.249811515	7.49E-05
Analyte Molar Mass (g/mol)	226.502	B	0.013	0.004410751	5.53E-05
IS Molar Mass (g/mol)	194.186	B	0.0085	-0.005144788	4.37E-05
Analyte mass (mg)	1.9462	B	0.00124	-0.513331502	6.38E-04
IS mass (mg)	2.3565	B	0.00124	0.423953223	5.27E-04
IS purity (mg g^{-1})	0.9993	B	0.0004	0.999745591	4.00E-04

Combined Uncertainty $9.3*10^{-4}$

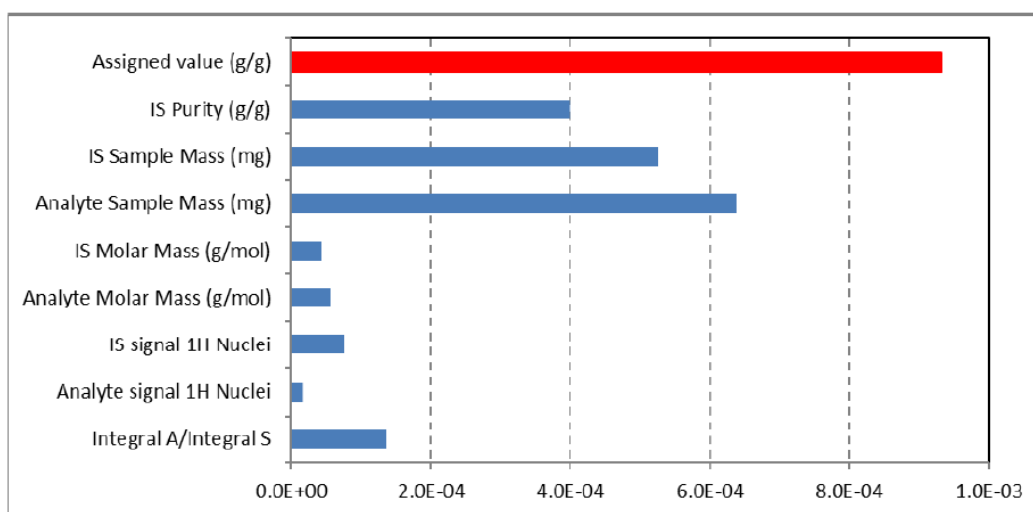Purity of BTMSB-d_4 $\quad$ 999.0 + 1.0 / - 1.9 mg g^{-1}

Figure 1.4 — Relative uncertainty components: BTMSB-d_4 assignment using DMTP in CD$_3$OD

The purity assignment of BTMSB-d_4 agreed within its associated uncertainty with the value reported by the material producer and with values assigned by qNMR analysis using other ISRM/solvent combinations.

A1.3. Time course for transesterification of DMTP in solution in CD$_3$OD

Figure 1.5 illustrates the formation over time of free methanol (singlet at 3.35 ppm) in the presence of CD$_2$HOD (from solvent) by transesterification after a sample of DMTP is taken up in solution in CD$_3$OD. [23]. The relative integration area of the released methanol was estimated as 4 % of the parent DMTP methyl ester signal after one week in solution.
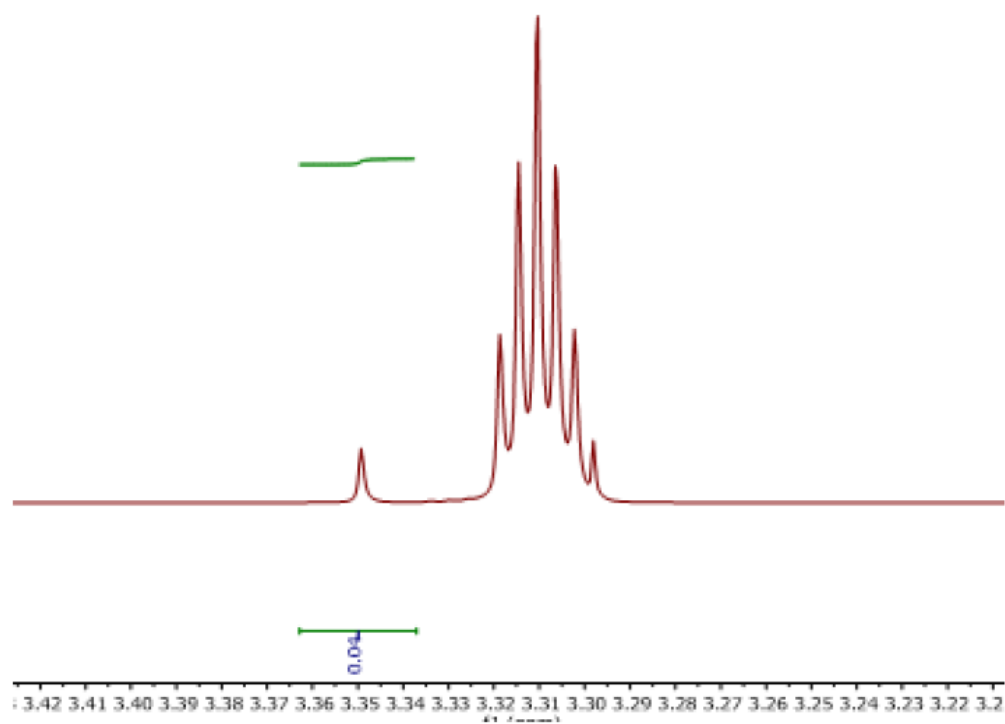
Figure 1.5 — ^{1}H NMR spectrum of solvent portion of DMTP in CD$_3$OD on storage.

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Document Control

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