APPLICATION NOTE

Purity Determination using the Residual Solvent Signal of Chloroform-*d* as an Internal Calibrant

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Introduction

Nuclear magnetic resonance (NMR) has been a hallmark of characterization techniques in organic chemistry, as it provides a plethora of information crucial for structural elucidation, such as: atom connectivity, chemical shift, multiplicity, and integration areas. Recently, NMR has steadily found its place in quantitative applications (qNMR) like purity determination, quantification of unknowns, etc. in sectors including pharmaceuticals, biotechnology, metabolomics, and many more.[1] While traditional high-field NMR spectrometers have been used for qNMR, some disadvantages that accompany the technique include significant upfront and recurring costs to purchase and maintain, the requirements for extensive training, and routine maintenance. With the increasing popularity of benchtop NMR, due in part to its ease of use, small footprint, and affordability, many industries are now able to incorporate NMR directly into their quality control or research workflows to obtain the same valuable information provided by high-field NMR.

NMR spectroscopy offers key advantages compared to traditional chromatography techniques (*e.g.*, gas/liquid chromatography), including simple sample preparation, non-destructive analysis, the lack of need for analytical standards specific to each target analyte, etc.[2,3] qNMR spectroscopy is a method that relies on the accurate direct integration ratios of signals in a spectrum, which is possible because the number of nuclei associated to a specific resonance is directly proportional to the area under the curve of that signal.[3] Thanks to NMR being an inherently quantitative technique, a calibration curve is not required, unlike for other techniques. Instead, the integration areas of an internal calibrant of known concentration are compared to the signals of interest to gather quantitative information (*e.g.*, purity). The internal calibrant is of utmost importance to obtain accurate results, therefore, one must select an appropriate internal calibrant that is completely soluble in the solvent of choice and also does not overlap nor interact with the analyte of interest.

Deuterated chloroform ($CDCl_3$, chloroform-*d*) is arguably the most popular solvent used to dissolve most organic compounds for NMR analysis because of its ability to solubilize a large variety of these and the ease of sample recovery of the analyte after analysis due to its volatility.[4] An often-overlooked peak in many spectra that use chloroform-*d* as the solvent is the singlet at 7.26 ppm, commonly only used to properly reference the chemical shifts in the spectrum. This singlet is produced by the non-deuterated residue of chloroform-*d* ($CHCl_3$) and, when used properly, can provide a generous amount of information. Under qNMR conditions, this peak can be treated as an internal calibrant, replacing the need for preparing stock solutions of expensive qNMR standards required for traditional qNMR.

The experiments presented in this application note are adapted from work done by Veliks *et al.* in *The Journal of Organic Chemistry* with slight modifications made to the concentration of the chloroform-*d* used.[4] Herein, we prepared an approximately 98% (w/w%) $CDCl_3$ solution and quantified it with the known qNMR standard, dimethyl terephthalate (DMT). This newly quantified 98% $CDCl_3$ solution was used to determine the purity of various laboratory chemicals (**Figure 1**), and the results were compared to those obtained using DMT as the internal calibrant. A 98% $CDCl_3$ solution is not strictly necessary, but we chose this concentration to optimize experiment times and sample amounts required to maintain a similar dynamic range between signals of interest.

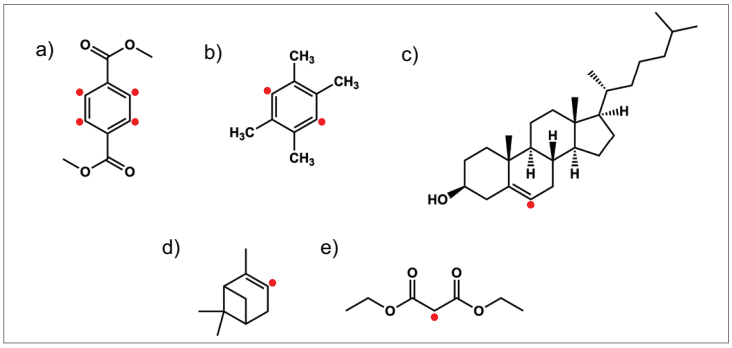

Figure 1. Structures used in this work: a) dimethyl terephthalate (DMT), b) 1,2,4,5-tetramethylbenzene, c) cholesterol, d) α-pinene, and e) dimethyl malonate. The red circles indicate the protons used for determining purity.

Experimental Procedure

Materials

Chloroform (99.9%), chloroform-*d* (99.85%), dimethyl terephthalate (TraceCERT®, 99.95%) 1,2,4,5-tetramethylbenzene (99.7%), cholesterol (99.7%), α-pinene (98.8%) and diethyl malonate (99.9%) were purchased from MilliporeSigma and used without further purification.

98% Chloroform-d Purity Determination

To make a 20 mL solution of 98% chloroform-*d*, chloroform-*d* (99.85%, 29.44 g, 244.59 mmol) and chloroform (99.9%, 55.29 mg, 4.63 mmol) were added to a re-sealable glass vial and thoroughly mixed. To determine the purity of the new chloroform-*d* solution, 0.6 mL (0.886 g, 7.36 mmol) and DMT (5.91 mg, 0.0304 mmol) were mixed in a vial and transferred to a 5 mm NMR tube. The T_1 values of the chloroform peak (7.26 ppm) and dimethyl terephthalate peak (8.10 ppm) were determined to be 7.5 and 3.5 seconds, respectively.

Purity Determination of Commercial Chemicals

The analyte and DMT were accurately weighed with a Mettler Toledo analytical balance (model: MS105DU) into a vial equipped with a screwing cap, with the goal of obtaining a millimolar amount as close as possible to the millimolar amount of residual chloroform in a 0.6 mL aliquot of 98% chloroform-*d* (~0.151 mmol) used to dissolve the sample. A screwing cap was used to minimize solvent evaporation of the 98% chloroform-*d* solution. The sample was vortexed until completely dissolved and transferred to a 5 mm NMR tube. The T_1 of the analyte signal of interest, chloroform and DMT were determined and the longest T_1 time was used to determine the optimal scan delay for accurate ^{1}H qNMR analysis.

To obtain accurate qNMR results, each spectrum was obtained in triplicates and using a scan delay of at least five times the longest T_1 value to ensure the full relaxation of spins between scans.[2] Additionally, the following parameters were used: spectral width, 40 ppm; spectral center, 10 ppm; number of points 16384; number of scans, 16; dummy scans, 2; interscan delay, time varies for each sample; pulse angle, 90°; receiver gain, auto. All spectra were manually corrected for phase and baseline distortions using the MestReNova software (v14.1.1).

Results and Discussion

The purity of the analyte was determined using the following equation:

$$P_x = \frac{I_x}{I_{IC}} * \frac{N_{IC}}{N_x} * \frac{M_x}{M_{IC}} * \frac{m_{IC}}{m_x} * 100\%$$

Note: The internal calibrant for this application note is either residual chloroform in chloroform-*d* or DMT. Therefore, the analyte purity, P_x, will be calculated via both $CHCl_3$ and DMT.

Where P_x is the analyte purity; I_x and I_{IC} are the integration areas of the analyte and internal calibrant peaks, respectively; N_{IC} and N_x are the number of protons used for quantification in the internal calibrant and analyte molecules, respectively; M_x and M_{IC} are the molar masses of the analyte and internal calibrant, respectively; and m_{IC} and m_x are the masses measured using the analytical balance for the internal calibrant and analyte, respectively.

Figure 2 shows one of the spectra used to determine the exact concentration of the 98% chloroform solution described in the procedure. The integration areas of DMT and the residual $CHCl_3$ peak were compared, and a purity of 97.1% chloroform-*d* (2.9% chloroform) was determined using Equation 1. The results of this analysis are summarized in Table 1.

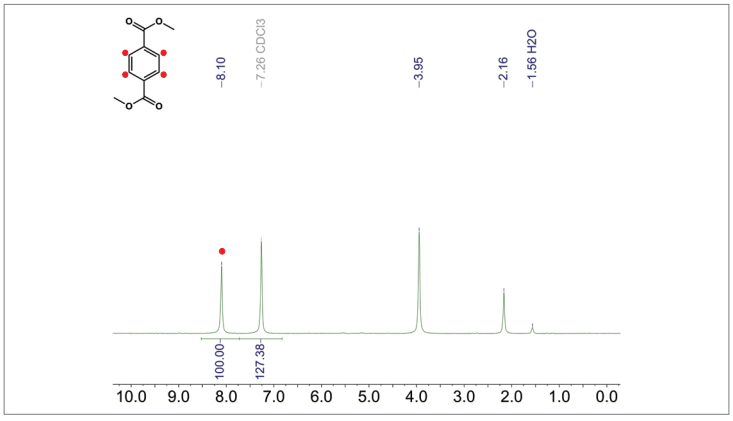

Figure 2. ^{1}H NMR (60 MHz) spectrum used for purity determination of chloroform-d using DMT as the internal calibrant. The red circle denotes the DMT signal used for quantification. The integration values were determined and tabulated into Table 1.

Table 1. Determination of chloroform-d solvent purity using DMT as an internal calibrant.

	$I_{chloroform}$	I_{DMT}	$N_{chloroform}$	N_{DMT}	$m_{chloroform}$ (mg)	m_{DMT} (mg)	$P_{chloroform}$
Run 1	127.38	100	1	4	871.22	7.37	97.9%
Run 2	127.21	100	1	4	875.13	7.84	97.9%
Run 3	128.29	100	1	4	869.24	7.63	97.9%

> "The Nanalysis benchtop NMR has allowed us to quickly analyze samples for time critical experiments, allowing samples to be prepared and analyzed without risk of precipitation or degradation. It has also helped with preliminary NMR method development for compounds which have non-typical relaxation properties."
>
> **— Lauren MacEachern**, Senior Scientist & Team Lead
> Solid State Pharma

Figure 3 shows one spectrum of the triplicates acquired for each commercial compound used for the purity determination via both the chloroform-*d* solvent and the DMT approaches. The results of these analyses are summarized in Table 2.

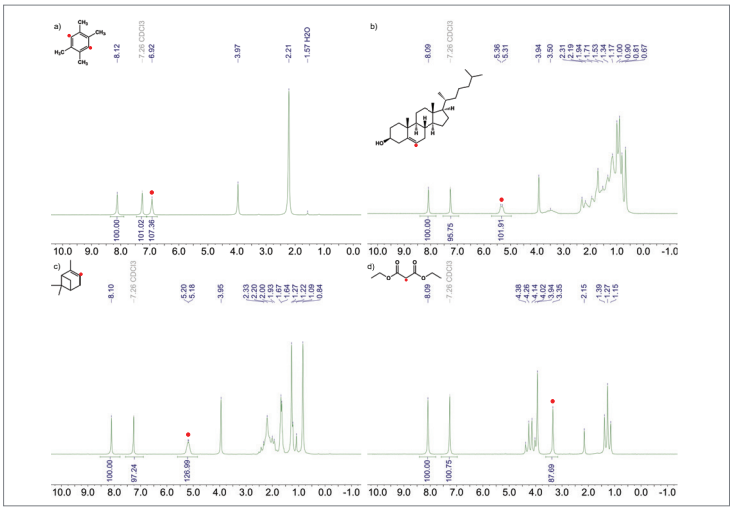

Figure 3. ^{1}H NMR (60 MHz) spectra used for purity determination of a) 1,2,4,5-tetramethylbenzene, b) cholesterol, c) pinene and d) diethyl malonate using the residual chloroform signal and DMT as internal calibrants. The red circles in the structures correspond to the proton(s) used for purity determination, as well as their corresponding peaks in the respective spectrum. The integration values were determined and tabulated into Table 2.

Table 2. Determination of commercial compound purity using the residual chloroform signal and DMT as internal calibrants.

Sample	I_x[#]	$I_{chloroform}$[*]	I_{DMT}[*]	N_x	$N_{chloroform}$	N_{DMT}	m_x (mg)	$m_{chloroform}$ (mg)	m_{DMT} (mg)	P_x via chloroform[*]	P_x via DMT[*]
1,2,4,5-Tetramethylbenzene	100	100.93	108.09	2	1	4	11.14	871.22	7.37	98.2 ± 0.7%	98.8 ± 0.5%
Cholesterol	100	94.92	100.73	1	1	4	62.97	875.13	7.84	99.6 ± 0.4%	99.8 ± 0.3%
α - Pinene	100	97.09	127.31	1	1	4	27.52	869.24	7.63	98.6 ± 0.3%	99.0 ± 0.5%
Diethyl Malonate	100	100.06	87.42	2	1	4	10.91	878.70	7.35	98.5 ± 0.5%	97.1 ± 0.2%

[#]Value was normalized to 100
[*]Value is the average of triplicate runs

As shown in Table 2, the purity determination of various commercially available compounds using the residual chloroform signal compares quite well with the certified qNMR standard, DMT. Care must be taken to ensure that the chloroform-*d* solution is stored cold to ensure minimal evaporation and water contamination, which would diminish the accuracy of the qNMR results.

Conclusion

The work presented in this application note demonstrates that the residual solvent peak of chloroform-*d* can be used to perform accurate qNMR using a benchtop NMR spectrometer. The results obtained compare favourably to those obtained using the more expensive certified qNMR standard, DMT. This agrees with previously published work demonstrating this on a traditional high-field NMR spectrometer.[4] With the growing field of benchtop NMR spectroscopy, small labs can easily incorporate the benchtop spectrometer into their workflow as it is inexpensive, easy to maintain, requires little sample preparation and is a non-destructive analytical technique. If you have any questions regarding this application or benchtop NMR in general, please do not hesitate to contact us!

References

[1] Holzgrabe, U.; Deubner, R.; Schollmayer, C.; Waibel, B. *J. Pharm. Biomed. Anal.* **2005**, *38*, 806-812.

[2] Rundlöf, T.; Mathiasson, M.; Bekiroglu, S.; Hakkarainen, B.; Bowden, T.; Arvidsson, T. *J. Pharm. Biomed. Anal.* **2010**, *52*, 645-651.

[3] Yang, Q.; Qiu, H.; Guo, W.; Wang, D.; Zhou, X.; Xue, D.; Zhang, J.; Wu, S.; Wang, Y. *Molecules.* **2015**, *20*, 12114-12124.

[4] Muhamadejev, R.; Melngaile, R.; Paegle, P.; Zibarte, I.; Petrova, M.; Jaudzems, K.; Veliks, *J. J. Org. Chem.* **2021**, *86*, 3890-3896.

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