ANALYTICAL

UNDERGRADUATE EXPERIMENT

Using qNMR to Determine the Purity of Commercial Chemicals

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Introduction

NMR spectroscopy is one of the most powerful analytical techniques available to chemists due to the wealth of information that can be obtained from a spectrum. Historically, it has been primarily used as a tool for structural elucidation as the combination of chemical shift, multiplicity, and integration of the signals provide a detailed picture of how atoms are connected within a molecule. More recently, the technique has been applied to quantitative applications (qNMR), particularly in natural products and medicinal chemistry.[1,2]

qNMR spectroscopy is reliant on the fact that the integral of each signal is directly proportional to the number of nuclei responsible for that individual resonance, and that response is linear.[3] Therefore, unlike other analytical methods, a calibration curve correlating the known concentration of the analyte and its signal response is not needed. The concentration of the analyte is then obtained by directly comparing the integral of the chosen analyte signal with the integral of the signal(s) from the internal calibrant of known concentration. As such, selection of an appropriate internal calibrant is vital for a qNMR experiment. The calibrant must be inert and its resonance must be distinct from the analyte. To ensure the highest precision, the calibrant should be of high purity and materials that are volatile and/or hygroscopic avoided.[3]

The precision of the measurement is also dependent on accurate integrations of the signals of the analyte and the calibrant.[4] To meet this criterion, a long interscan delay is used to ensure that every nucleus in the sample has fully relaxed before the next pulse is applied. The appropriate interscan delay will depend on the T_1 relaxation times of the analyte and the calibrant. Typically, the interscan delay is set to 5–7 times the longest T_1.

In this application note the purity of commercial chemicals will be determined with the NMReady-60 via an internal calibrant and compared with 400 MHz NMR data and the manufacturer's label.

Procedure

Preparing samples for analysis

Using a 5 decimal place analytical balance, the analyte and internal calibrant, maleic acid (≥99% purity) or 1,2,4,5-tetrachloro-3-nitrobenzene (qNMR standard), were weighed into the same vial and the masses recorded. The mass (~20-50 mg depending on molecular weight) was such that approximately 0.3 M solutions were achieved. The solids were then dissolved in 0.6 mL of DMSO-d_6 or $CDCl_3$ and mixed thoroughly, making sure the compounds were fully dissolved. The resulting solution was transferred into an NMR tube.

Recording the NMR spectra

The NMR samples were placed into the NMReady-60 and left to equilibrate for 5 minutes. The ^{1}H NMR spectra were then recorded in triplicates using the following parameters:

spectral width: 40 ppm	interscan delay: 14 or 50 sec*
spectral center: 10 ppm	number of points: 16384
number of scans: 16	dummy scans: 0
receiver gain: auto	pulse angle: 90°

**14 sec (if maleic acid is the internal calibrant) or 50 sec (if 1,2,4,5-tetrachloro-3-nitrobenzene is used)*

Processing the NMR spectra and calculating analyte purity

For each NMR spectra, the phase and baseline were manually corrected. The signals of the calibrant and analyte were then integrated and the purity of the analyte was calculated using the following equation:

$$P\,[\%] = \frac{n_{IC} \cdot Int_A \cdot MW_A \cdot m_{IC}}{n_A \cdot Int_{IC} \cdot MW_{IC} \cdot m_A} \cdot P_{IC} \quad \textbf{(1)}$$

where *n* = number of protons;
Int = integral area;
MW = molecular weight;
m = mass;
P = purity;
IC = internal calibrat;
A = analyte.

Results & Discussion

Prior to starting the qNMR experiments, the T_1 relaxation times of each analyte and internal calibrant were measured on the NMReady-60. It was found that in all cases the signals from the internal calibrants had the longest T_1 relaxation time and the interscan delay was set appropriately. As well, the purity of maleic acid was established in a separate experiment while the purity of 1,2,4,5-tetrachloro-3-nitrobenzene was obtained from the certificate of analysis supplied by the manufacturer.

An example of a ^{1}H NMR spectrum recorded on the NMReady-60 to determine the purity of 4′-hydroxypropiophenone is displayed in Figure 1.

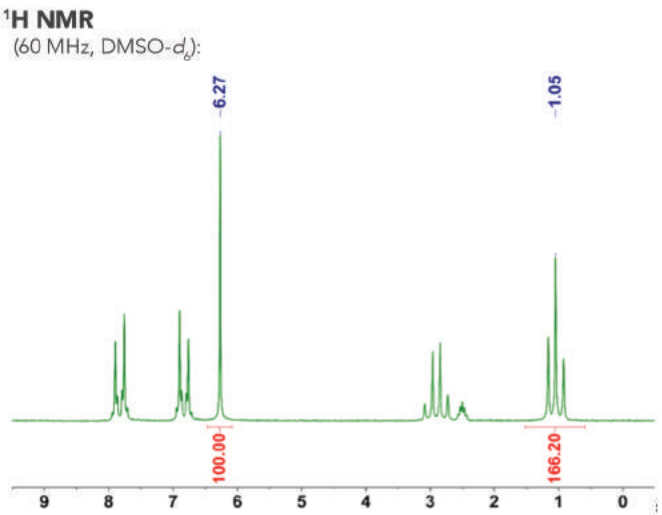

Fig 1. ^{1}H NMR spectrum of 4′-hydroxypropiophenone and maleic acid in DMSO-d_6.

In this example, the signal of the internal calibrant (maleic acid) at 6.27 ppm was integrated while the triplet at 1.05 ppm was chosen as the signal to integrate for 4′-hydroxypropiophenone. The purity of 4′-hydroxypropiophenone was then calculated with equation 1 and the results, along with the other compounds evaluated in this study, are listed in Table 1.

Table 1. Percent purity of the compounds tested using the NMReady-60 and a 400 MHz NMR spectrometer as well as the manufacturer's specifications.

Compound	60 MHz Experimental[a]	400 MHz Experimental[a]	Label
4′-hydroxypropiophenone[b]	98.76	98.45	98
ibuprofen[b]	100.03[d]	99.92	≥98
indomethacin[b]	99.40	99.68	≥99
fluorene[b]	98.58	98.64	98
1,4-dinitrobenzene[b]	98.44	98.58	98
1,2,4,5-tetramethylbenzene[c]	98.64	98.62	98
dimethyl terephthalate[c]	99.76	99.81	≥99

[a]Experimental values are an average of 3 separate acquisitions.
[b]Maleic acid used as the internal calibrant.
[c]1,2,4,5-tetrachlorobenzene-3-nitrobenzene used as the internal calibrant.
[d]The +0.03% difference between the determined qHNMR purity (100.03%) and the theoretical maximum of 100% is well within the accuracy of typical laboratry settings (NMR method and balance validation).

Excellent correlation was observed between the purity determined experimentally and what was stated from the manufacturer. As well, the results obtained with the NMReady-60 matches very well with the results obtained with a 400 MHz NMR spectrometer.

Conclusions

In this experiment the purity of several commercial chemicals was determined with qNMR experiments on the NMReady-60. The values obtained matched well with the manufacturer's specifications. Furthermore, there was excellent agreement between the values found from the NMReady-60 and a high field NMR spectrometer. The experiment is simple to perform in undergraduate laboratories and highlights an important application of NMR spectroscopy as a quantitative analytical technique.

References

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Data Accessibility

The data can be processed directly on the NMReady-60 and printed and/or exported directly to a USB or networked file where it can be worked up using third party NMR processing software.

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