

Spinsolve

Quantification of single components in complex mixtures by ¹³C NMR



¹³C spectrum of a mixture of 13 hydrocarbon molecules acquired on a Spinsolve 80 MHz.

NMR is a powerful analytical method widely used to quantify the concentration of chemical compounds present in a sample. Compared to other techniques, NMR offers a number of advantages. The integral of the signals in the NMR spectrum is directly proportional to the concentration of the particular molecule in the full concentration range. Moreover, the signal integrals are independent of the matrix where the analytes are dissolved. Hence, if the concentration of a calibrant (internal or external) is known, the concentration of all components in the mixture can be determined by using simple linear relations. Although this property holds for all NMR active nuclei, quantitative NMR methods are typically based on the detection of ¹H signals, as they provide the highest sensitivity. However, when looking at complex mixtures, signals in the ¹H spectra may suffer from severe overlapping due to the small chemical shift spreading of this nucleus. In such cases, ¹³C can be used to improve the signal separation, as this nucleus provides a much larger chemical shift dispersion. The big disadvantage of ¹³C NMR is its low sensitivity due to the small gyromagnetic ratio and the low natural abundance of this nucleus. To mitigate this limitation, NMR methods transfer polarization from ¹H to ¹³C before the ¹³C signal is measured. Polarization transfer increases the sensitivity of ¹³C in an important factor, but not all ¹³C nuclei are enhanced by the same factor. Hence, the amplitude of the signals within one spectrum cannot be directly compared, as it is typically done in the ¹H spectra. Although signal amplitudes cannot be converted to concentrations by using the same reference substance, the linear relationship between signal amplitude and concentration is preserved for each peak. In this way, the analytes of interest can be accurately quantified if their signals are calibrated against external calibration standard containing known concentrations of the same analytes.

In this application note we describe a procedure developed to quantify the individual components present in a hydrocarbon mixture by using ¹³C NMR. This procedure has been implemented on a Spinsolve 80 MHz benchtop spectrometer. In the petrochemical field, samples are complex mixtures of hydrocarbon molecules that define the physical properties of these liquids. As a proof of concept, we present here an external calibration procedure able to quantify the concentration of 10 model molecules. The performance of the method has been determined by using a set of mixtures prepared with known concentrations.

Considered molecules and NMR spectra

To demonstrate the performance of this method, a set of samples was prepared mixing 10 commercially available liquid compounds covering different families of molecules typically present in petrochemical samples, like alkane, alkene, cycloalkane, and aromatics. 2-propanol was included as an example of a fuel additive. One of the compounds consists of a mixture of xylene isomers (including ethylbenzene), defining a total of 13 molecules in the samples. It should be noticed that xylene isomers are easily distinguishable by ¹³C NMR, giving the opportunity to quantify them individually but, like in our case, the proportion of each xylene isomer was not known so we consider it as a single compound and named it xylenes. The chemical structures of the compounds mixed in the samples are presented in Figure 1.



Figure 1: Molecules used in this study to test the accuracy of ¹³C NMR on a Spinsolve 80 MHz benchtop spectrometer.

A first synthetic mixture (called mix10) was prepared by mixing the 10 compounds with the same volumetric proportion of compounds (10% each). Figure 2 shows a very quick (1 min) ¹H spectrum measured on a Spinsolve 80.



Figure 2: 1D ¹H NMR spectrum (80 MHz) of mix10 (10% of each considered compound). The spectrum was collected with 4 scans, a flip angle of 90°, and a repetition time of 15 s. The total measurement time was 1 min.

While some signals of the components can be well resolved, it can be directly noticed that many others overlap, especially in the aliphatic (0 - 1 ppm) and the aromatic (6 - 7 ppm) regions. This overlapping is expected to be even more pronounced for real hydrocarbon samples, limiting the ability to quantify the individual components from the ¹H spectra. Therefore, ¹³C NMR appears as an alternative to explore. Figure 3 shows the ¹³C spectrum recorded for the same mixture. As expected, ¹³C NMR provides a much better peak separation than ¹H. The assignment of the ¹³C peaks was performed by comparison against the ¹³C NMR spectra of each individual component. A stack plot of the ¹³C spectra of the mix10 and the 10 single compounds is shown in Figure 3. When two (or more) peaks were too close to be assigned in this way, a spiking experiment was carried out.



Figure 3: Stack plot of the ¹³C spectra of the mixture (bottom) and the single compounds. Spectra were manually aligned. These data were collected averaging 4096 scans acquired with a pulse angle of 45° and a repetition time of 4 s.

A total of 43 NMR peaks could be well identified. From these peaks, 23 are well resolved, 17 show acceptable overlapping, and 3 overlap so much that they cannot be used for quantification. As explained above, ¹³C NMR spectra used for this quantification procedure are not acquired under the typical quantitative NMR conditions. The strategy here is to get a good sensitivity-over-time compromise, by combining a short repetition time and a small flip angle. This induces a peak-dependent but constant systematic deviation that can be simply overcome by using the same compound as external calibration standard.

	Mix A	Mix B	Mix C	Mix D	Mix E
cyclooctane	20	0	15	10	5
n-pentane	5	10	20	15	0
n-hexane	15	5	10	0	20
cyclohexane	0	20	15	5	10
isooctane	5	20	0	10	15
2-propanol	0	15	10	20	5
1-hexene	10	0	20	5	15
toluene	10	15	5	0	20
xylenes	20	5	0	15	10
benzene	15	10	5	20	0

Table 1: Concentrations used in the calibration mixtures

The method was calibrated by means of 5 samples prepared with different concentrations of the ten components. In each mixture, two compounds were dissolved at 20% (vol.), two at 15% (vol.), two at 10% (vol.), two at 5% (vol.), and the last two were absent. Within the 5 mixtures, each compound is present one time with each concentration in the range from 0 to 20% to produce a calibration curve. The individual concentrations were chosen in a random fashion and are presented in Table 1. The calibration samples were measured three times each and each spectrum was recorded in 4.5 h (4096 scans). The ¹³C spectra of these mixtures are presented in Figure 4. The resulting calibration curves are presented in Figure 5.



Figure 4: ¹³C NMR (1H-decoupled) spectra of the calibration mixtures acquired using 4096 scans, a pulse angle of 45°, and a repetition time of 4 s. As examples, the amounts of iso-octane (peak at 52.5 ppm), 2-propanol (peak at 62.8 ppm) and 1-hexene (peak at 113.4 ppm) are labelled in the picture.



Figure 5: Calibration curves with linear fits of the ¹³C NMR signals

The accuracy of the linear fits demonstrated by the corresponding R^2 values, show that the procedure is reliable enough for quantification. One can see that each peak, even from the same molecule, presented its own slope. For example, the six different carbons of the 1-hexene had different slopes due to the different relaxation and NOE characteristics of each carbon nucleus. One of the carbons has a significantly lower slope than the others (the vinylic carbon), it is likely that this carbon had a significantly longer T_1 and suffered more than the others from the partial saturation effect. In any case, when several signals can be used for the same molecule, the results can be combined to improve the quantification accuracy.

In this work only the 23 peaks that appeared well separated from the rest were used for quantification. Finally, to evaluate the performance of this procedure we used the calibration curves to quantify the different molecules present in the mix10 sample. Figure 6 and Table 2 show the results obtained for the different components.



Figure 6: Quantification of the different components present in sample mix10 performed by using the calibration curves obtained from the mixtures A to E. The error bars represent the standard deviation over 20 measurements acquired with 1024 scans and a repetition time of 4 seconds.

It can be seen so far, that for all components, except for n-hexane, average volume concentrations between 9.79 and 10.28 were determined, with a standard deviation that varied from sample to sample but was always smaller than 0.5%. The n-hexane shows a larger deviation which is most likely due to a pipetting error during the sample preparation. From these results, we can conclude that for such type of mixtures an absolute error smaller than 0.5% vol% can be achieved in a measurement time of about one hour. This proves that ¹³C NMR is a valid alternative for quantifying multi component mixtures in cases where ¹H NMR does not provide sufficient line separation. Depending on the accuracy required for different applications the measurement time can be adjusted and a further decrease of measurement times might be possible exploiting the use of relaxation agents.

	v/v (% Vol)	std (% Vol)
n-pentane	10.26	0.41
n-hexane	10.85	0.14
1-hexene	10.13	0.26
cyclohexane	10.09	0.15
cyclooctane	10.21	0.12
isooctane	9.79	0.27
2-propanol	9.65	0.18
benzene	10.28	0.43
toluene	9.84	0.32
xylenes	10.06	0.49

Table 2: Volume concentration and standard deviations determined for the different components in mix10.

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