

qNMR on samples from the production plant

Quantify multiple analytes without sample preparation and in the presence of protonated solvents with a single measurement.



In a previous application note we described the advantages and prerequisites for performing qNMR with an internal standard for the purity determination of a final product. However, this method is not easy to implement on liquid samples taken directly from the production line. In this case, the main complication comes from the fact that the type of sample preparation required to add an internal standard is not always possible in the production environment. Moreover, as samples need to be measured in the presence of protonated solvents, solvent suppression methods are necessary to attenuate the large solvent signals to remove overlapping with the signals of the compounds of interest. Depending on the solvent suppression method, the amplitude of some of the signals in the spectrum may get affected requiring a different calibration strategy. While for conventional experiments a single reference substance is enough, for the general case we propose the use of a reference sample for each compound to be quantified in the sample. By using as external calibrant a sample with a known concentration of the same substance, the effect of the solvent suppression sequence can be eliminated. In this way it is possible to quantify several components in parallel with a single measurement in a precise and robust way. There are several other cases in which qNMR with internal standard is not applicable, for example in conversion control in large reactors or composition control of screening experiments. Here, one can use qNMR with external standard. In this application note we demonstrate this method exemplary for different mixtures of histidine, sucrose and polysorbate. Different combinations of these three substances are used in many formulations in drug development. Histidine is used as a buffer, polysorbates as surfactant and sucrose as stabilizer.

For the successful implementation of qNMR with external standards, a set of reference samples with known concentration is needed. For this application note we prepared three reference samples with only histidine, sucrose, and polysorbate 20 dissolved in H₂O, respectively. Three mixtures with varying compositions of these three substances in H₂O were prepared to demonstrate the high accuracy that can be achieved when the method is implemented on our Spinsolve benchtop NMR spectrometer. To ensure the linearity between the reference samples and the mixtures, all spectra must be recorded with identical parameters (saturation power and frequency, flip angle, repetition time, etc.). All spectra shown in this application note were recorded on a Spinsolve 80 MHz Carbon Ultra spectrometer with 10 s repetition time, 2 dummy scans, 32 scans, 90° flip angle, solvent suppression active on the water peak and a total experimental time of 6 min. Figure 1 shows a stack plot with the proton spectra of the three reference samples and one mixture sample. For the quantification of histidine, the aromatic peaks between 7 and 8 ppm were used, for sucrose the peak at 5.36 ppm and for polysorbate 20 the peak group around 1 ppm.

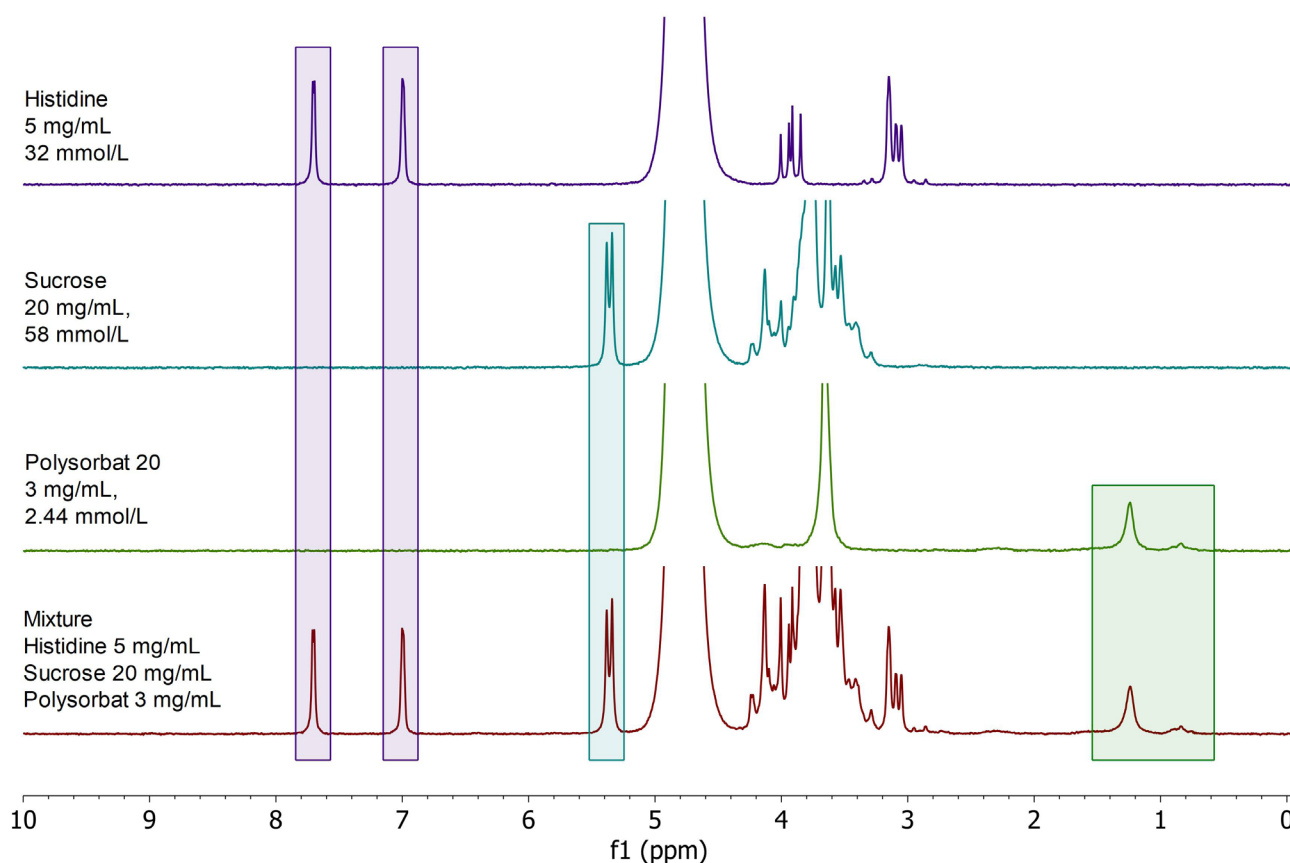


Figure 1: Stack plot of the three reference spectra and one spectrum of one mixture measured on a Spinsolve 80 MHz Carbon Ultra spectrometer.

The three mixture samples contain different amounts of histidine, sucrose and polysorbate 20 as shown in Figure 2. By measuring the samples with the three single substances we obtained three calibration values in form of integral/(mg/mL). With these values we were able to determine the concentrations of the different components in the mixtures. Each mixture sample was measured five times. For histidine there are two peaks that can be used. Figure 3 shows the concentrations determined for both peaks. The mean of the two concentrations are the results for histidine which are shown in Figure 3.

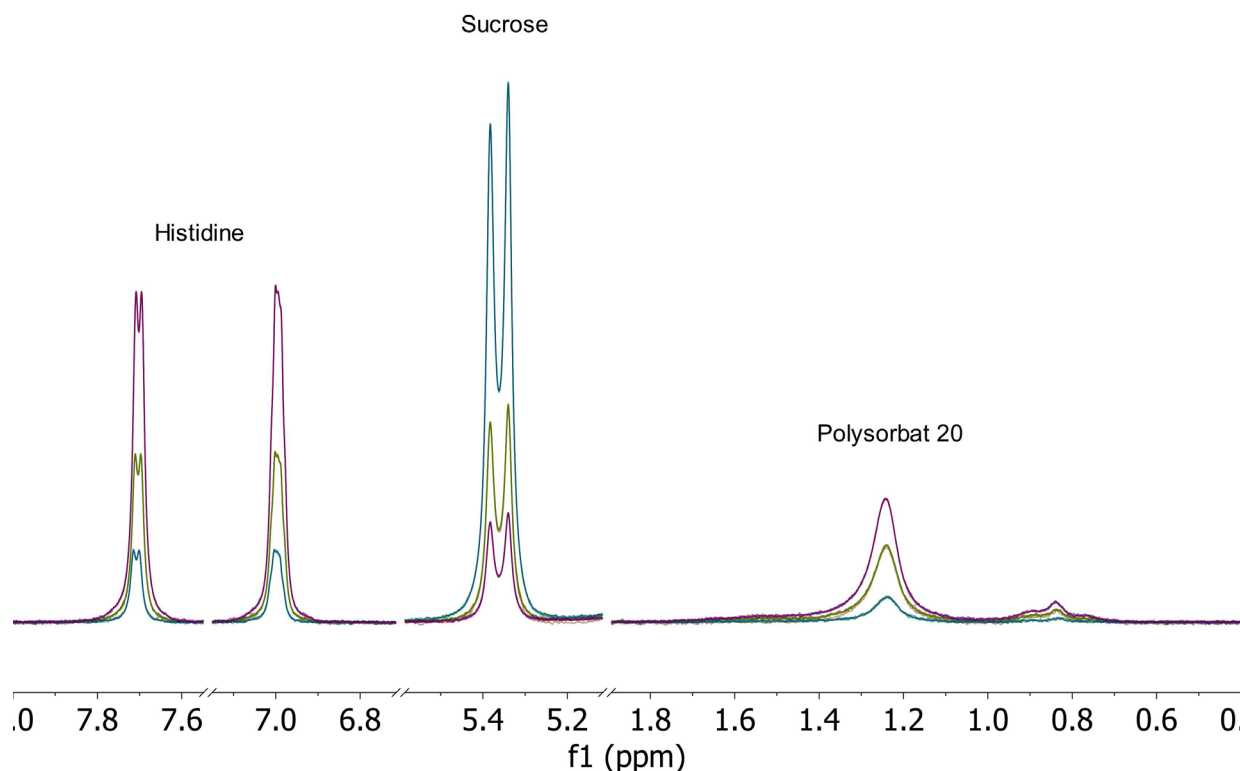
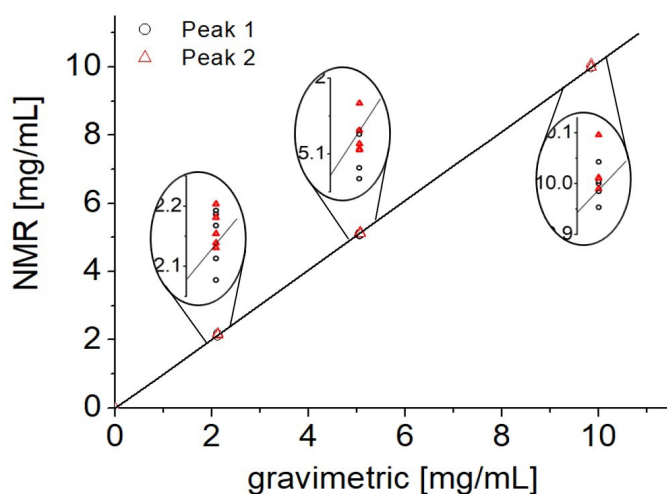


Figure 2: Overlay of three mixture samples showing the different concentrations of histidine, sucrose and polysorbate 20. For each sample the overlay of 5 repeat measurements is displayed in the graph proving the excellent reproducibility of the measurements.

The excellent reproducibility of the recorded spectra is demonstrated by the low standard deviation of the five measurements. Although these low concentrated samples contain more than 95 % of protonated solvent and weighing errors can not be excluded the results show a relative deviation of < 2 % compared to the expected gravimetric results.



| | Mix 1 | Mix2 | Mix3 |
|---------------------|-------|------|-------|
| NMR [mg/mL] | 5.11 | 2.15 | 10.02 |
| std [mg/mL] | 0.03 | 0.05 | 0.04 |
| gravimetric [mg/mL] | 5.06 | 2.11 | 9.84 |
| Deviation % | 0.96 | 1.78 | 1.75 |

Figure 3: Results of the concentrations determined by qNMR of the two peaks of histidine compared to the gravimetric values.

The results for sucrose are shown in Fig. 4 and have the same quality as the results for histidine.

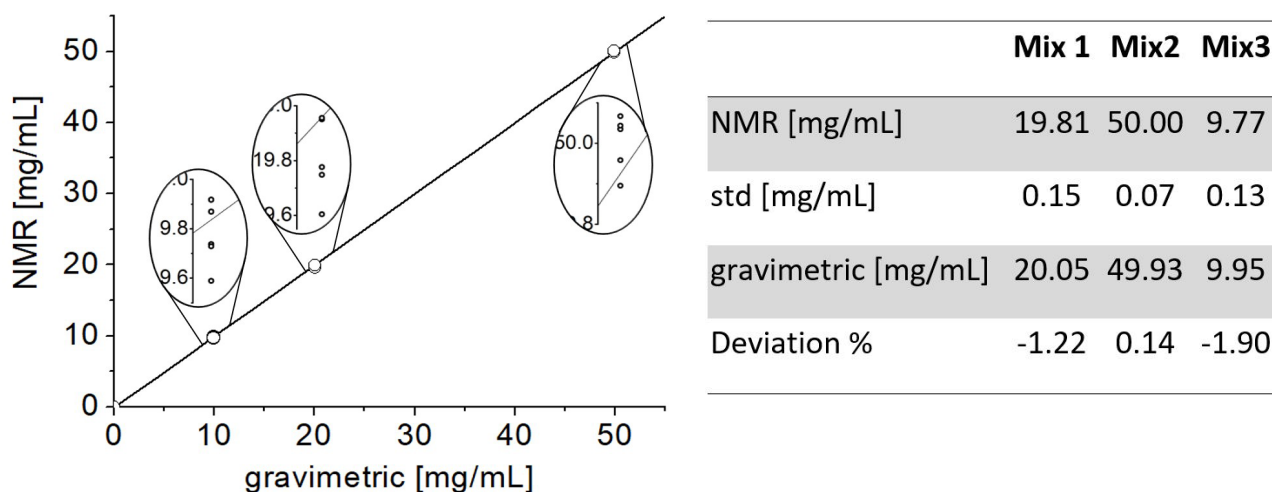


Figure 4: Results of the concentrations determined by qNMR of the sucrose peak compared to the gravimetric values.

Finally, the results for polysorbate 20 are shown in Figure 5. Mixture 1 and 3 show relative deviations similar to the ones discussed for the other components. While Sample 2 shows a higher relative deviation, which is expected as the sample concentration is much lower, the absolute deviation achieved is similar.

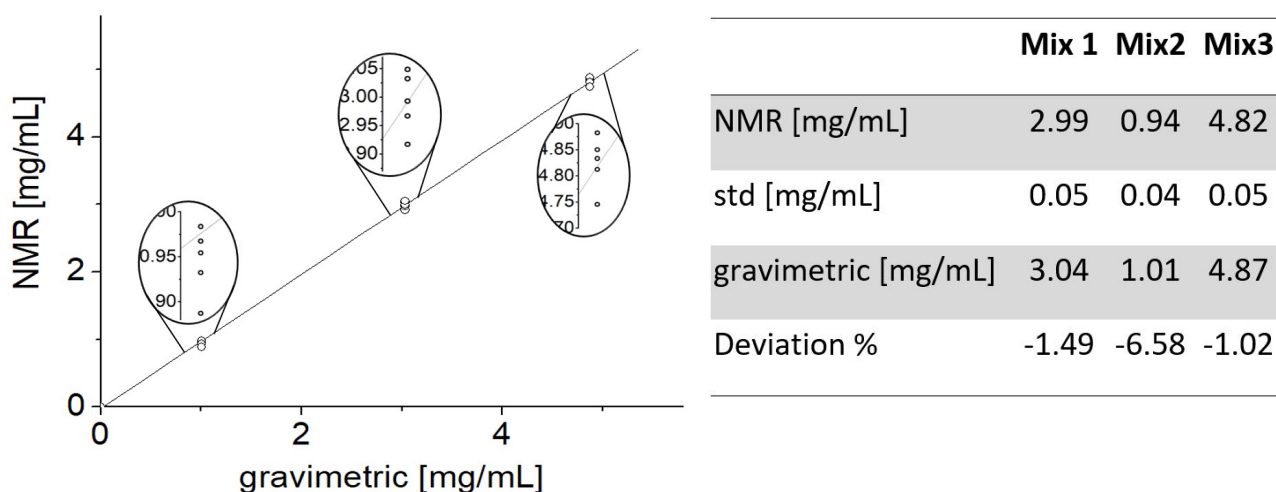


Figure 5: Results of the concentrations determined by qNMR of the polysorbate 20 peak compared to the gravimetric values.

In this application note we demonstrated the excellent reproducibility and precision one can achieve with our Spinsolve spectrometer using quantitative NMR with external standard. This method is advantageous where fast and reliable quantification of components in liquid products is required. The samples can be directly transferred to the NMR spectrometer without any need of sample preparation resulting in fast, accurate and robust methods eliminating weighing or pipetting errors.

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