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Quality control of small organic molecules using quantitative NMR and automated structure verification based on one-dimensional proton NMR spectroscopy

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INTRODUCTION

The quality control of small organic molecules includes structure verification, quantification and determination of the purity. These three topics can be addressed by nuclear magnetic resonance (NMR). The human interpretation of the NMR data is the bottleneck. The work presented here helps opening this bottleneck.

Figure 1: NMR equipment for automation and high throughput acquisition

CONCEPT

NMR data of more than 100 samples of non-trivial organic compounds was acquired and analysed including manual quantification.

- The NMR quantification accuracy and precision was studied. In this context also the quality of results from Bruker CMC-q auto-quantification package was verified.
- For an automatic NMR spectra assignment algorithm rules were formulated and defined to mimic the human interpretation of 1D ^{1}H NMR spectra.
- A method for purity estimation solely based on the information derived from 1D ^{1}H NMR spectra was defined.

RESULTS

Quantification

The precision of quantitative NMR was investigated with five individually prepared samples of quinine with a targeted concentration of 20 mM. A relative standard deviation of 2.5 % was found including errors from sample preparation (see Figure 2a). The comparison of the concentration determined in automation with the manually determined concentration is shown in Figure 2b. Almost every concentration value of the 74 samples was within a reasonable confidence interval of ±20% of the expert interpretation.

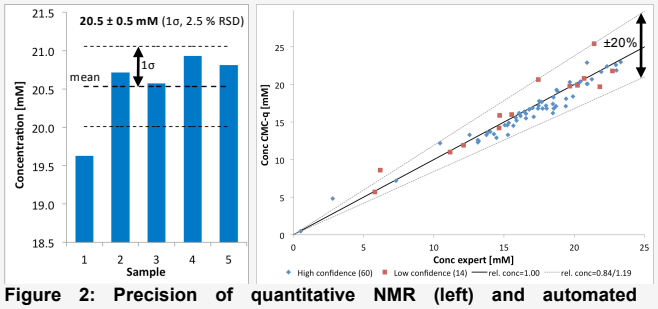

Figure 2: Precision of quantitative NMR (left) and automated concentration determination (Bruker CMC-q) in comparison with the manually determined concentration (right).

Structure Verification

Several interpretation rules (dependencies) were defined. As an example, the allowed differences in chemical shift between the signals observed in the proton NMR spectrum of the propyl group next to an oxygen atom is shown in Figure 3. A range for the difference in chemical shift between two signals was defined by investigating several spectra of compounds containing this substructure. A structure verification process using such rules is less dependent on an accurate chemical shift prediction.

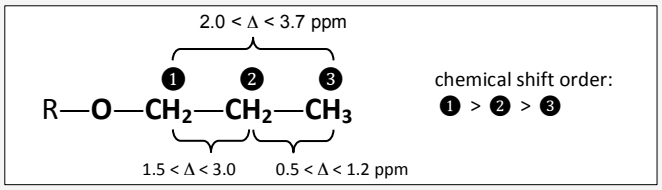

Figure 3: Example for chemical shift dependencies: propyl group next to an oxygen atom

Purity

A proton NMR spectrum with different identified impurities is shown in Figure 4.

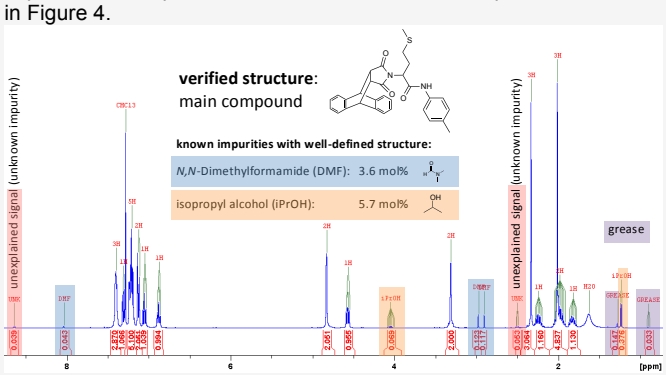

Figure 4: Proton NMR spectrum of a sample containing known (DMF, iPrOH and grease) and unknown impurities.

Several possible definitions for a single NMR purity statement have been analysed and evaluated (see Table 1). The proposed "single number purity estimation" only takes unknown impurities into account but not residual solvents (P_4). Well-defined impurities can be reported independently as molar percentage.

	impurity	NMR purity
known impurities: DMF and iPrOH	9.3 mol%	P_1 89.7 %
known impurities: DMF and iPrOH	2.5 area%	P_2 96.6 %
known impurities: grease	0.6 area%	P_3 96.5 %
unknown impurities	0.3 area%	**P_4 99.7 %**

Table 1: Impurity values and calculated NMR purity (different methods)

CONCLUSION

NMR quantification is very accurate and precise. The automatic analysis software almost always agrees with the manual concentration determination.
It could be shown that the usage of dependency rules supports the verification task and integration in an automated process is possible.
A purity estimation recipe was proposed which estimates the overall purity in form of a single number for any 1D NMR spectrum based on the percentage between signal area assigned to the molecular in question and the signal area which is not assigned to the molecule.

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