

# qNMR ROUND ROBIN TESTS AND ESTIMATION OF UNCERTAINTIES OF TOTAL SAMPLE PREPARATION PROCESS

Tanja Beyer and Bernd Diehl, Spectral Service AG, Emil-Hoffmann-Str. 33, 50996 Köln

## INTRODUCTION

Besides the well-known application of NMR spectroscopy in structural elucidation, nowadays this technique is of growing importance in the field of quantitative analysis. Surprisingly, although a multitude of earlier studies on this technique demonstrated its potential as a quantitative methods – in fact, a technique which is accurate and precise enough to rival chromatographic methods – qNMR has only recently gained the wider acceptance that it warrants.

In order to become accepted as a valid quantitative method also by public authorities, our laboratory has started to organise round robin tests for instance to check the operator-to-operator and instrument-to-instrument variabilities on the content values obtained by qNMR spectroscopy, to ensure the ability of other NMR laboratories to provide accurate results as well as to establish an uncertainty budget for this technique which is an important factor of ISO/EC guideline 17025.

## OPERATOR-TO-OPERATOR VARIABILITY

An intra-laboratory round robin test was performed to show the influence of sample preparation and integration procedure on the accuracy and precision of qNMR spectroscopy. 8 laboratory technicians prepared 6 independent sample solutions with accurate weighing of the sample analysed (BHT) and the internal standard (TCNB). The integration procedure was carried out by 10 professional qualified evaluators.

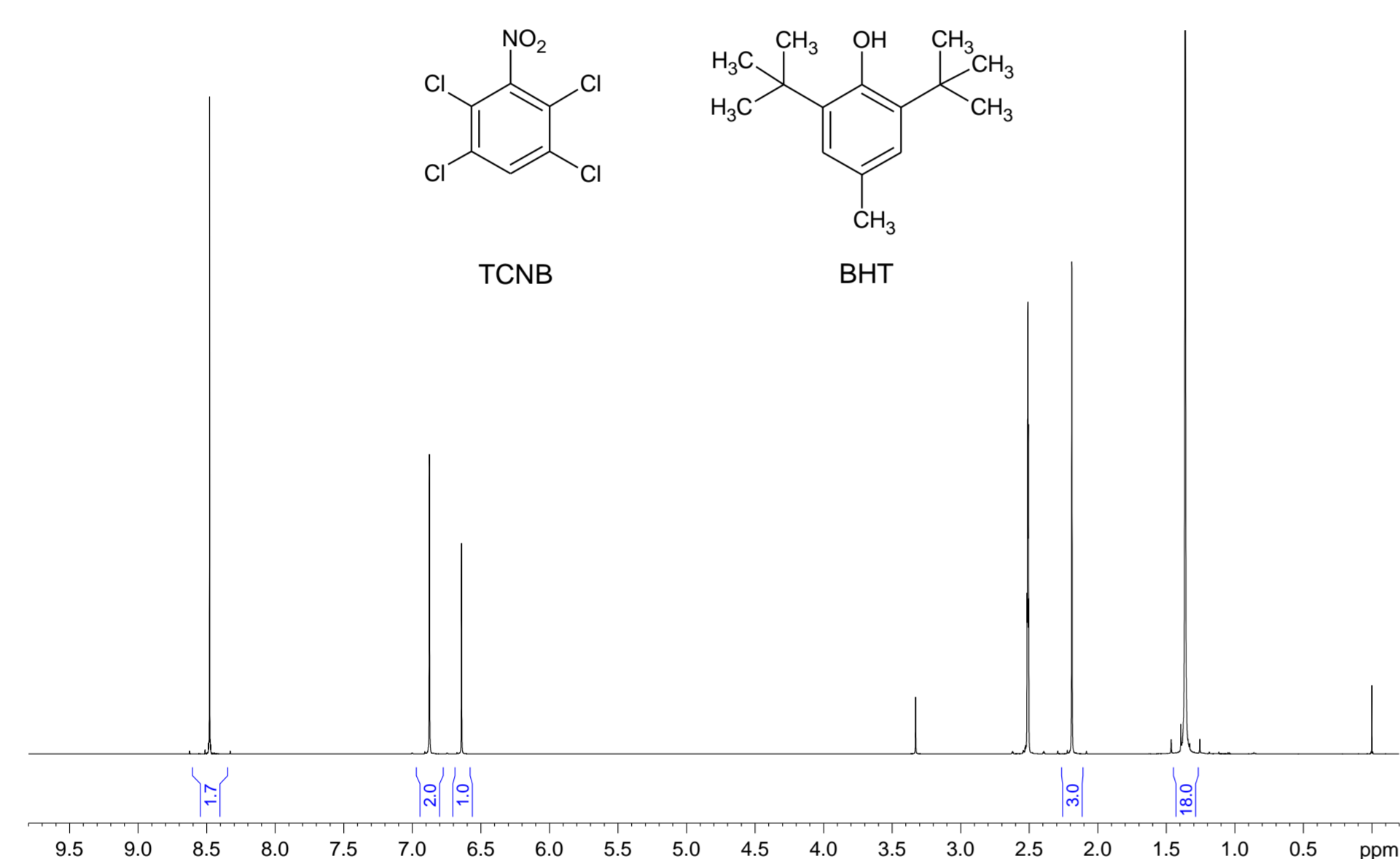


Fig. 1: <sup>1</sup>H NMR spectrum of the sample solution analysed containing BHT and TCNB

**SAMPLE PREPARATION.** The accuracy of the results obtained is highly dependent on the sample weighing process. As shown in Fig. 2a significant variations in the content and relative standard deviation are observed in relation to operator, environmental conditions, and weighing dishes. Thus, for accurate sample weighing the balance should be located in a room that is temperature and humidity controlled and antistatic weighing dishes should be used to eliminate static electricity.

**INTEGRATION.** In contrast, the integration procedure does not show a significant effect on the accuracy and precision. Comparable results were obtained if the NMR spectra were analysed by both an individual and predefined integration protocol (see Fig. 2b). Relative standard deviations well below 0.2 % were observed when the same signals were used for content calculation. In fact, manual and automatic correction routines of phase and baseline as well as integration limits which include or exclude <sup>13</sup>C satellites do not influence the accuracy of integrals and its reproducibility.

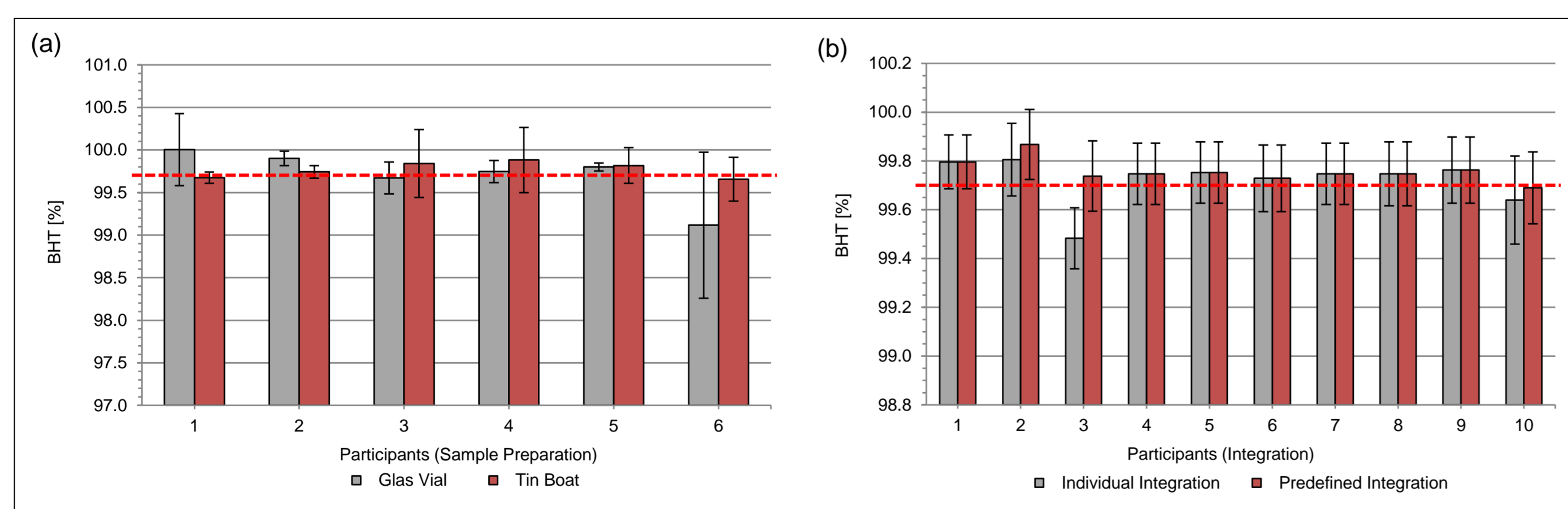


Fig. 2: Results of intra-laboratory round robin test: (a) sample preparation, (b) integration. Red line: true content

## CONCLUSION

By definition, NMR is a quantitative spectroscopic tool because the intensity of a signal is directly proportional to the number of nuclei being observed. Until today, there is still the prejudice that qNMR is not accurate and precise enough to rival chromatographic methods. Inter-laboratory tests have demonstrated the high metrological quality of this technique by good agreement of results. The method is comprehensively validated and can be applied in every laboratory on each NMR spectrometer using a validated protocol (for details: Malz et al. (JPBA 38 (2005) 813-823)). Round robin tests have shown the significant influence of the operators quality on the accuracy. Thus, the successful participation in round robin tests – an essential element of quality assurance for laboratories – is a very suitable opportunity to ensure the ability of NMR laboratories to provide accurate and precise results.

## INSTRUMENT-TO-INSTRUMENT VARIABILITY

An inter-laboratory round robin test was performed to verify the reproducibility of quantitative NMR spectroscopy. Identical model mixtures were measured by different laboratories using the same method by different NMR equipment (e.g. difference in magnetic field strength and type of (cryo) probe).

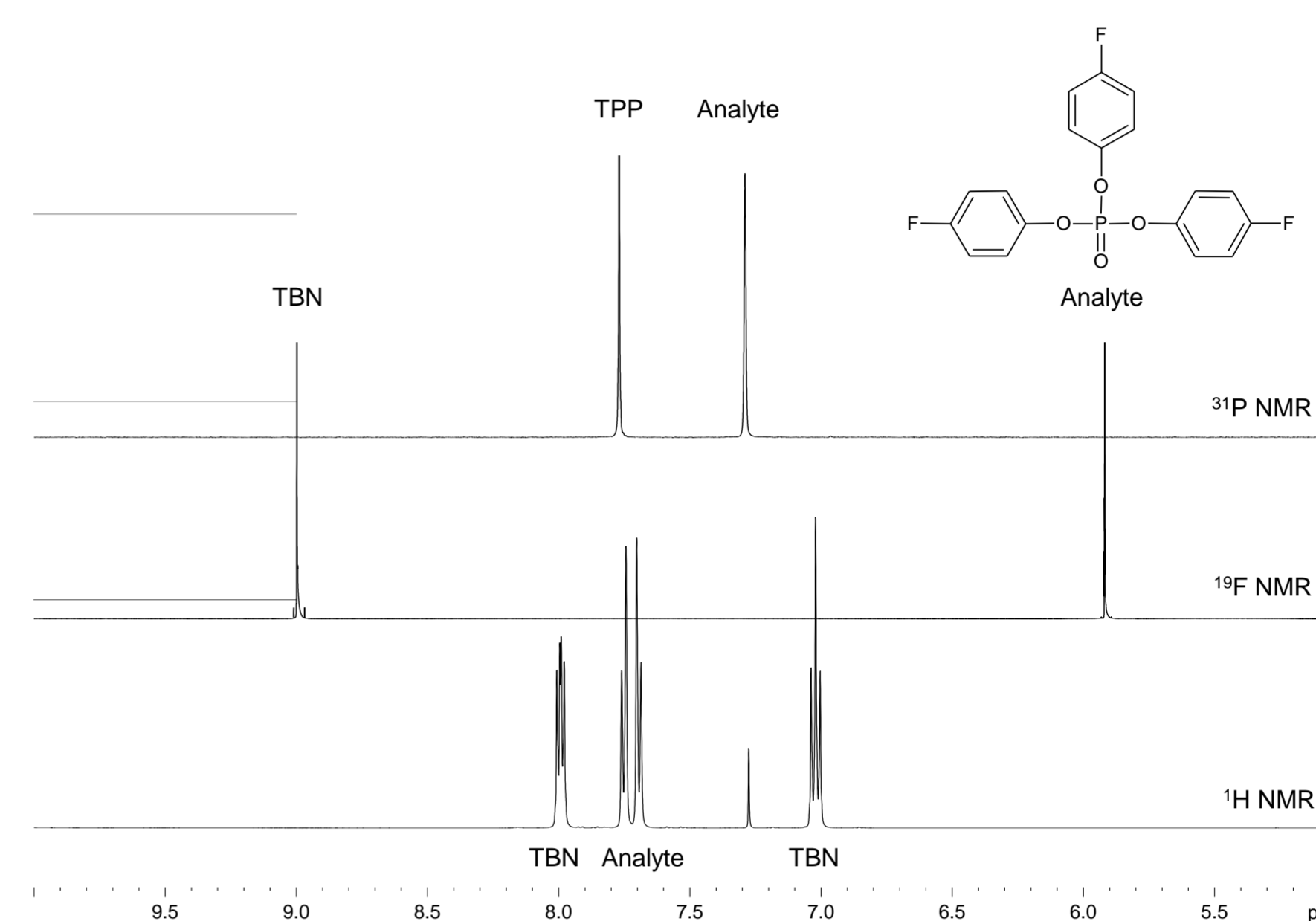


Fig. 3: Model mixtures analysed by <sup>1</sup>H, <sup>19</sup>F, and <sup>31</sup>P NMR spectroscopy

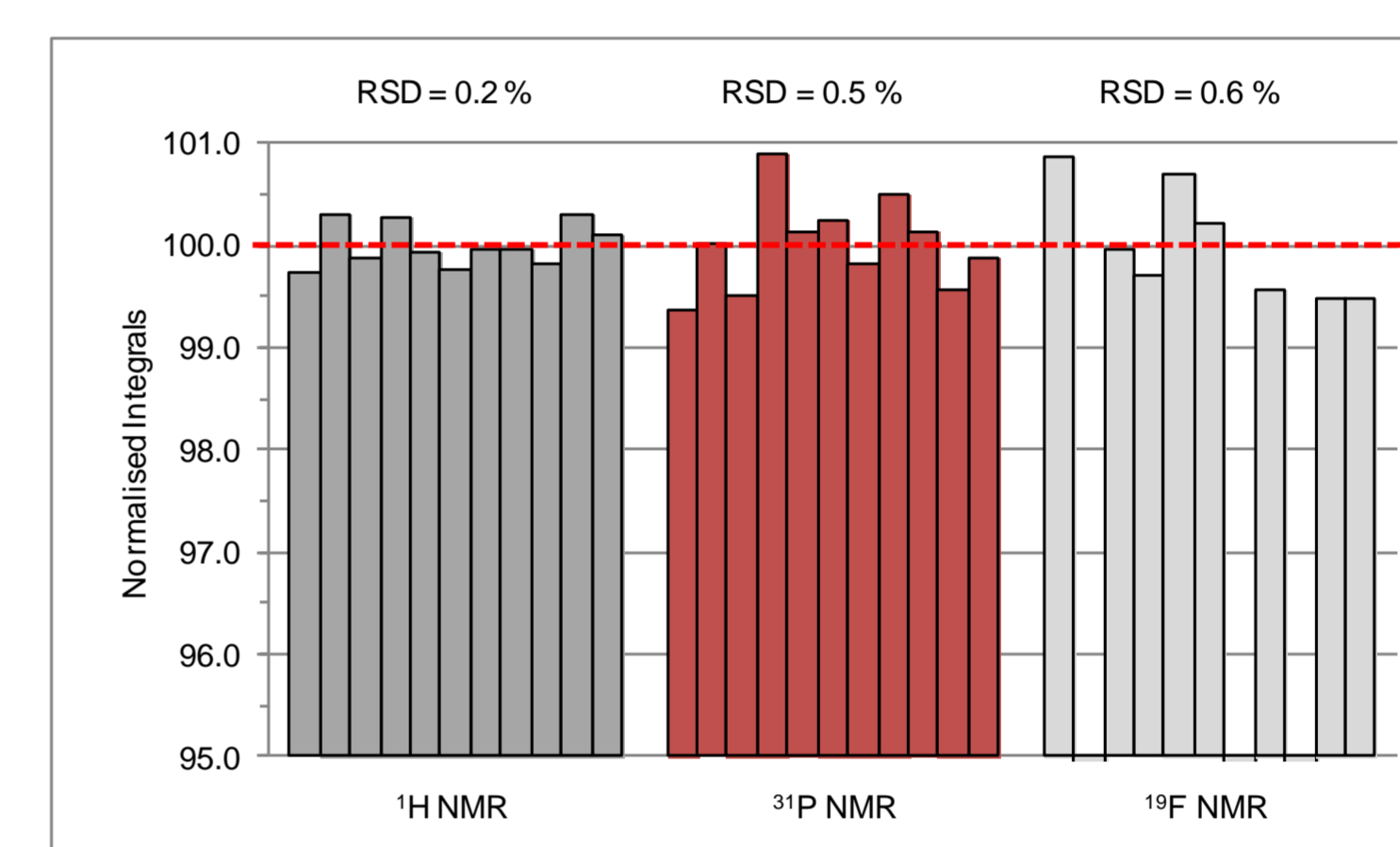


Fig. 4: Results of inter-laboratory round robin test. Comparison of normalised integrals

**INSTRUMENT.** This study clearly shows that qNMR spectroscopy provides a very reliable determination of purity in main component analysis. The experimental precision of <sup>1</sup>H NMR spectroscopy is 0.2 %, which is comparable or even better than results obtained by chromatographic techniques. Parameters such as magnetic field strength and cryo probe do not affect the accuracy and precision when relaxation effects and a minimal signal-to-noise ratio of S/N = 250:1 are taken into account.

## UNCERTAINTY BUDGET OF QUANTITATIVE NMR

The uncertainty of quantitative NMR measurements by using the internal standard method mainly depends on the purity of the internal standard ( $P_{IS}$ ), the uncertainty in the sample weights ( $m$ ), and the uncertainty in the integration procedure ( $I_X/I_{IS}$ ).

$$\frac{u(P_X)}{P_X} = \sqrt{\left(\frac{u(I_X/I_{IS})}{I_X/I_{IS}}\right)^2 + \left(\frac{u(m_X)}{m_X}\right)^2 + \left(\frac{u(m_{IS})}{m_{IS}}\right)^2 + \left(\frac{u(m_X)}{m_X}\right)^2 + \left(\frac{u(m_{IS})}{m_{IS}}\right)^2 + \left(\frac{u(P_{IS})}{P_{IS}}\right)^2}$$

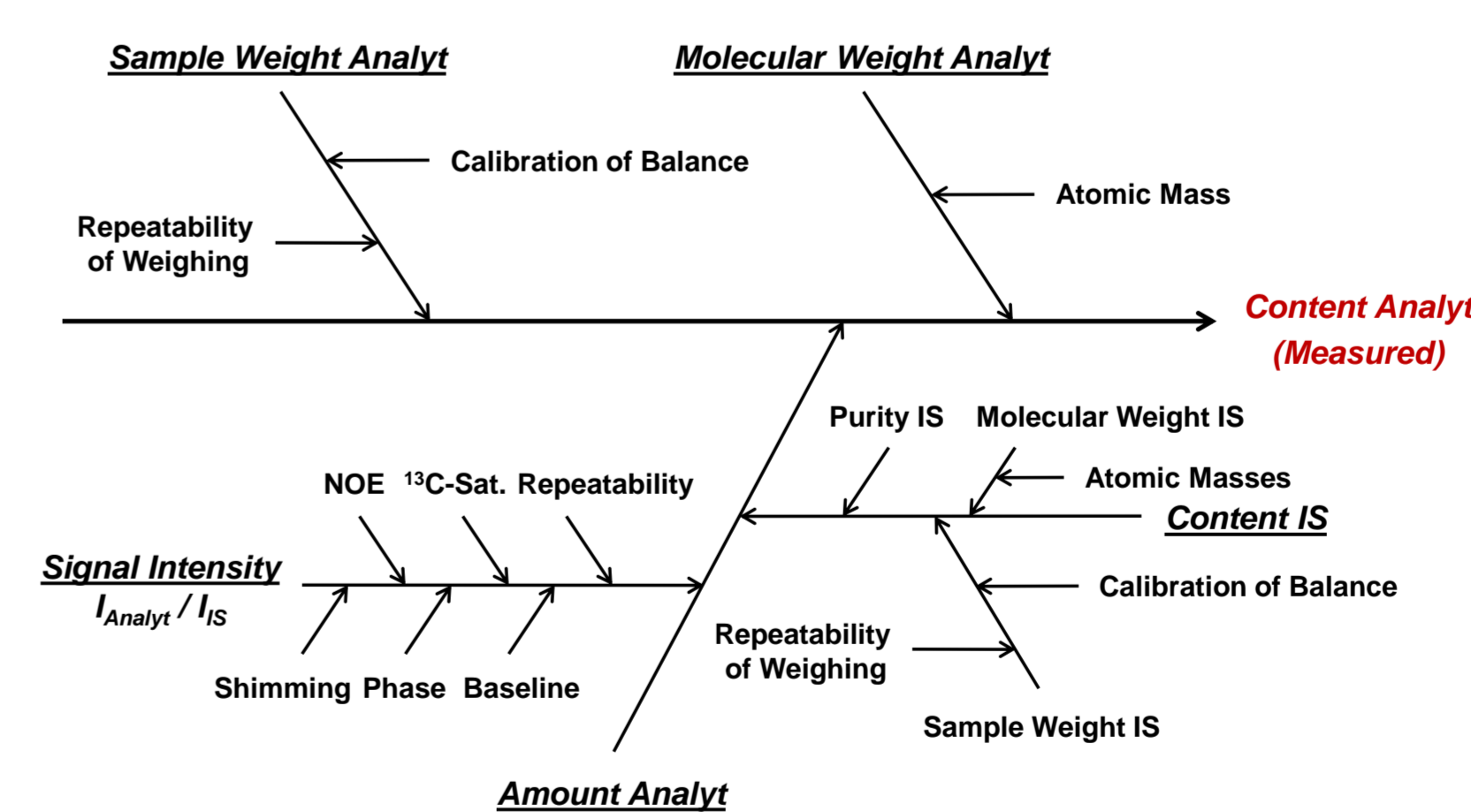


Fig. 5: Cause and effect diagram for the determination of the content of a substance by qNMR

The greatest uncertainties resides in the purity of the reference standard and the operator precision of weighing; the uncertainty in signal intensity plays only a subordinated role. For qNMR a total uncertainty well below 1 % is expected.