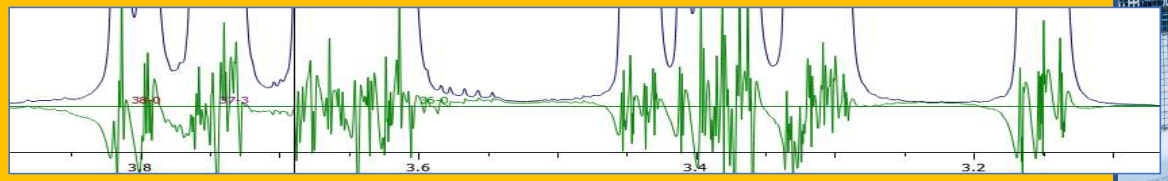
QMSA Letters 2025



The IT Supported QMSA for *pNMR* (*p*urity *NMR*)

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Integration bias

Integration is the common way for determination of NMR signal area. However, it suffers of serious problems:

- **Integration** is highly sensitive to phase and baseline imperfections. It has been demonstrated by Malz & Janck that, when the spectrum presents isolated signals, with **integration** is possible to achieve 95% confidence intervals as low as 1.5% of the compound concentrations. They have also shown that the **integration** of well-isolated peaks can lead to relative uncertainties of 11% when there are even slight phase and baseline errors. ***This makes the integration analyses easily manipulable through phase, baseline and integration range, which are subjective parameters, but fortunately, easily traceable from the original FID – in opposite to weighting and reference quality bias.***
- The most serious of problem of **integration** is the peak overlap. For the **integration** to be sufficiently precise, it is necessary for the signals to be integrated to be sufficiently isolated. If Lorentzian signals are considered, they decay very slowly to infinity. Griffiths has shown that for a maximum error of 1%, **integration** limits of 25 times the line width in both directions must be employed. If errors < 0.1% are desired, the integral width should be $\pm$76 times the peak width. For example, in a 500 MHz NMR spectrum with a peak width of 1 Hz, the integrated region should be $\pm$152 Hz ($\sim\pm$ 0.30 ppm), which is out of question in the most cases. Increasing integration width makes the integrals vulnerable to impurity bias.
- The risk that an impurity signal is hiding under the integrated signal, increasing thus the integral – and purity – is serious with **integration**. The essential impurities can be expected to resemble to the target compound. The same problem but more serious, is with the MS and chromatographic methods.
- Despite these facts, **integration** is claimed to prove that a sample purity is >99.9%.

Malz, F.; Jancke, H., Validation of Quantitative NMR. Journal of Pharmaceutical and Biomedical Analysis, 2005, 38, 813–823. https://doi.org/10.1016/j.jpba.2005.01.043

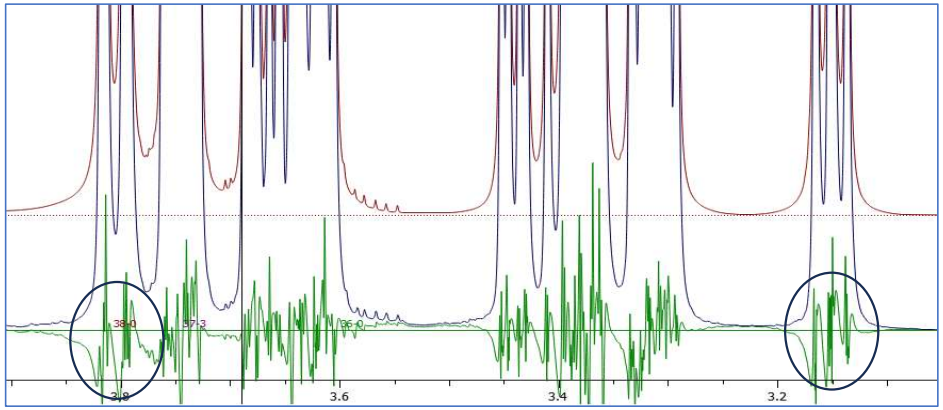
Griffiths, L., Assay by Nuclear Magnetic Resonance Spectroscopy: Quantification Limits. The Analyst, 1998, 123, 1061–1068. https://doi.org/10.1039/a800625c

TLS bias

In principle, QMSA is a perfect tool for qNMR – if it was not sensitive to line-shape. To be perfect, the line-shape should be completely described - which is not easy or is clumsy!

- The line width and line shape depend on compound, spectrometer and weather**.**
- In ChemAdder, the line shape can be described using asymmetrical Lorenzian, Gaussian and Dispersion functions, adding out-of-coil corrections, virtual long-range couplings and isotope shifts for ^{13}C and S, Cl.
- Despite the multi-term models, the fitting usually leads to typical **observed-calculated difference spectrum**:

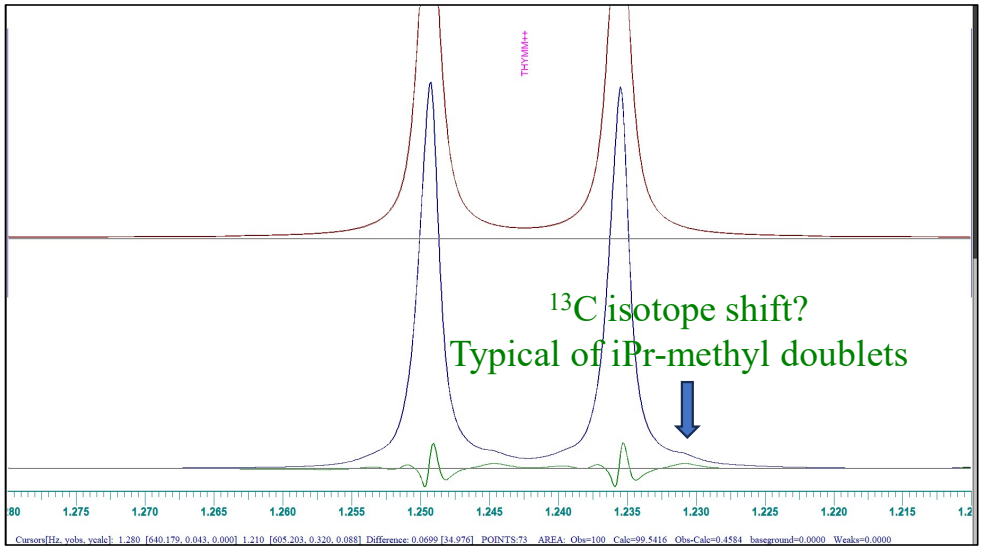
The '*mysterious*' difference arises from line-shape artefacts, which are compound- and proton-specific (see inserts, similar for the two multiplets). The artefacts cannot be removed by line-shape tools or the approaches are clumsy.



- The **area of the observed-calculated difference spectrum** is not necessarily ZERO, <u>as it should be</u>, which leads to a corresponding small bias in calculated spectrum and, thus, in concentration. Unfortunately, the area is sensitive to the line-shape model!
- The **area of the observed-calculated difference spectrum** can be stabilized using '**pNMR Options**' *(pNMR),* which minimizes the bias, while not disturbing the parameter fitting.
- ***NMRPO*** usually also smoothens the convergence.

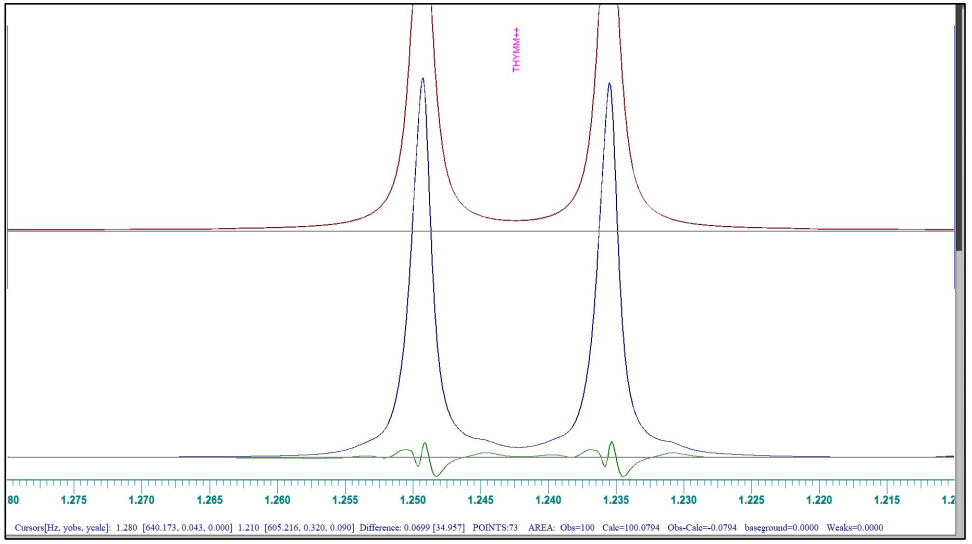
pNMR Options: the TLS is reduced by 83%

iPr-methyl doublet fit without the ‘pNMR Options’:



The observed-calculated area = 0.46% of the calculated area

iPr-methyl doublet with the ‘pNMR Options’:



The observed-calculated area = 0.08% of the calculated area

The IT Supported QMSA for *pNMR* - **pNMR Options**

N = no constraint
Y = for all chemical shifts and **integrals**
A = Automatic: reference signal, singlets, but not impurities, not broad or overlapping multiplets

The constraint can be defined also (with STAT=YI) for individual signals (shifts)

Solves the TLS bias, isotope artefacts and minor Out of Coil effects!