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Anatomy of a 60 MHz Benchtop NMR Spectrum - Dissection

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Experimental

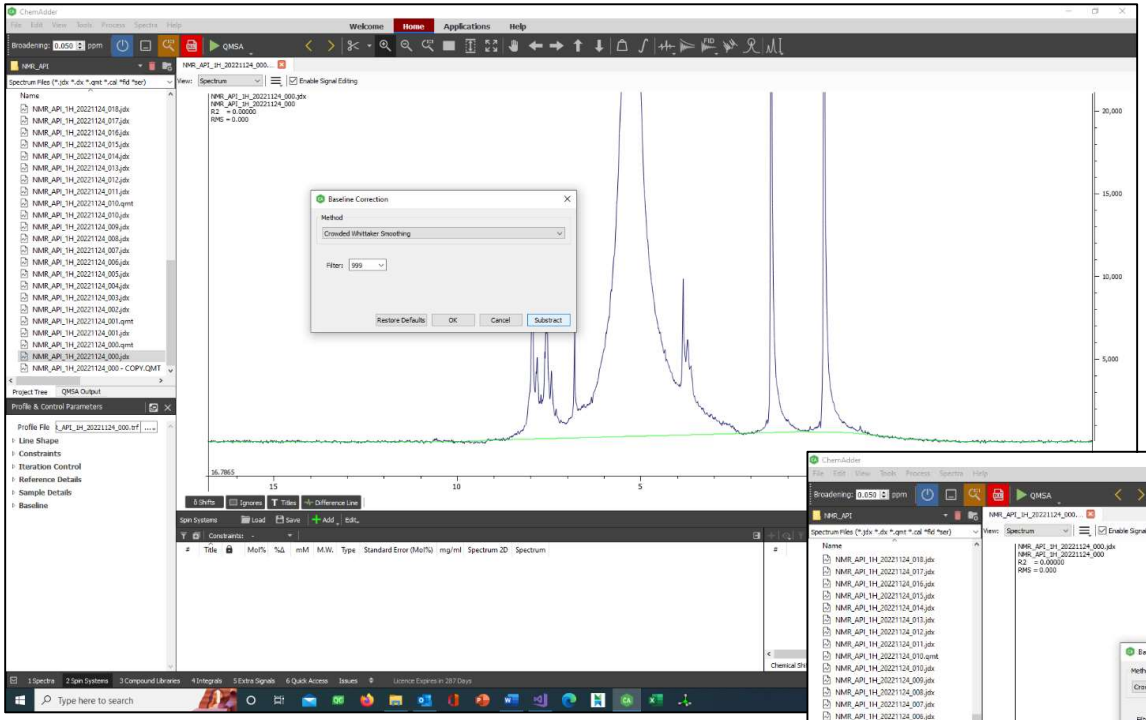
The spectrum was obtained from a study designed for an assessment of qNMR in kinetics using benchtop technology.

Data: Low field benchtop dataset were collected on Nanalysis 60 MHz (NMReady-60e). Also a high field dataset was collected on Bruker Avance 400 MHz, for the spectral parameter analysis of the Target compound. Reagents were mixed in a 5 mm NMR tube and transferred to the instruments where occasional ^{1}H spectra were acquired over a period of 4 hours. TMS was added as internal standard for quantifications.

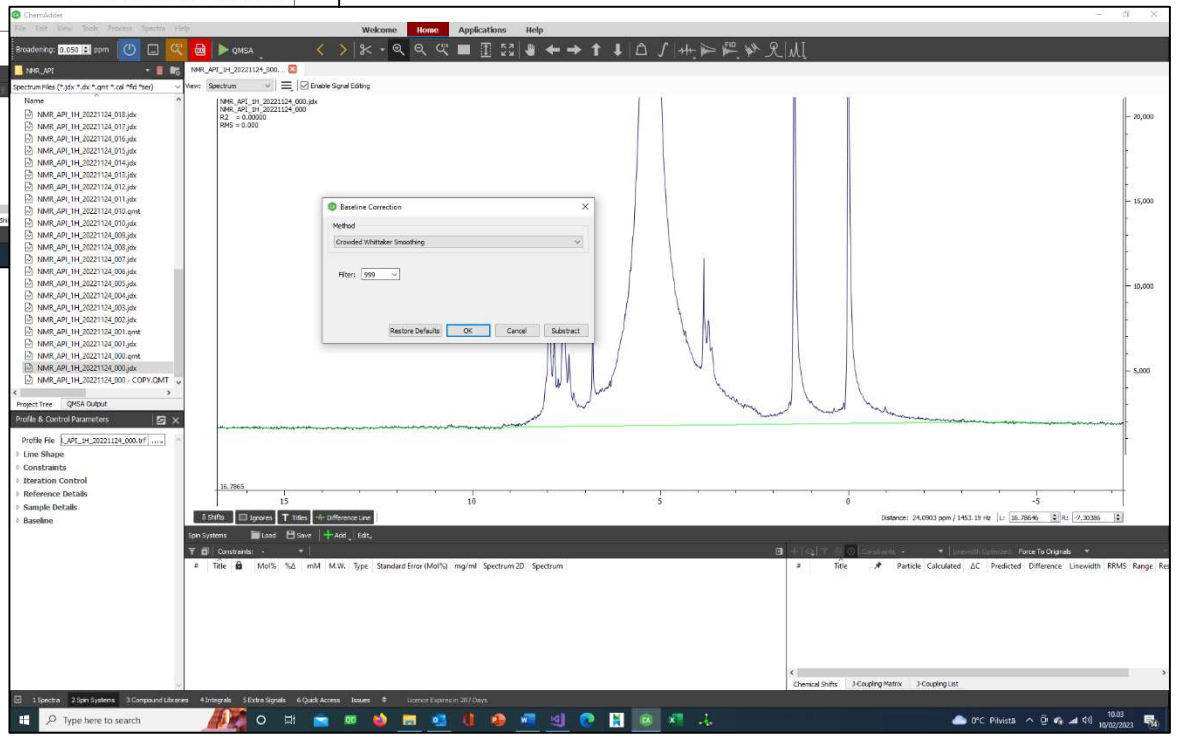
Chemistry: The epoxidation reaction of Allyl Carbamate with mCPBA was selected as the model reaction. This reaction is of interest for two reasons. First, it is a relatively simple reaction system with components that can be readily isolated. Second, the poor signal dispersion seen in the low field ^{1}H reaction mixture spectra make it an ideal test case for the benchtop spectral analyses.

qQMSA: All the spectral preparations and analyses were performed using **ChemAdder** software, using **QMSA** (**Q**uantum **M**echanical **S**pectral **A**nalysis).

Raw spectrum before baseline Whittaker correction with 1000 filter points:

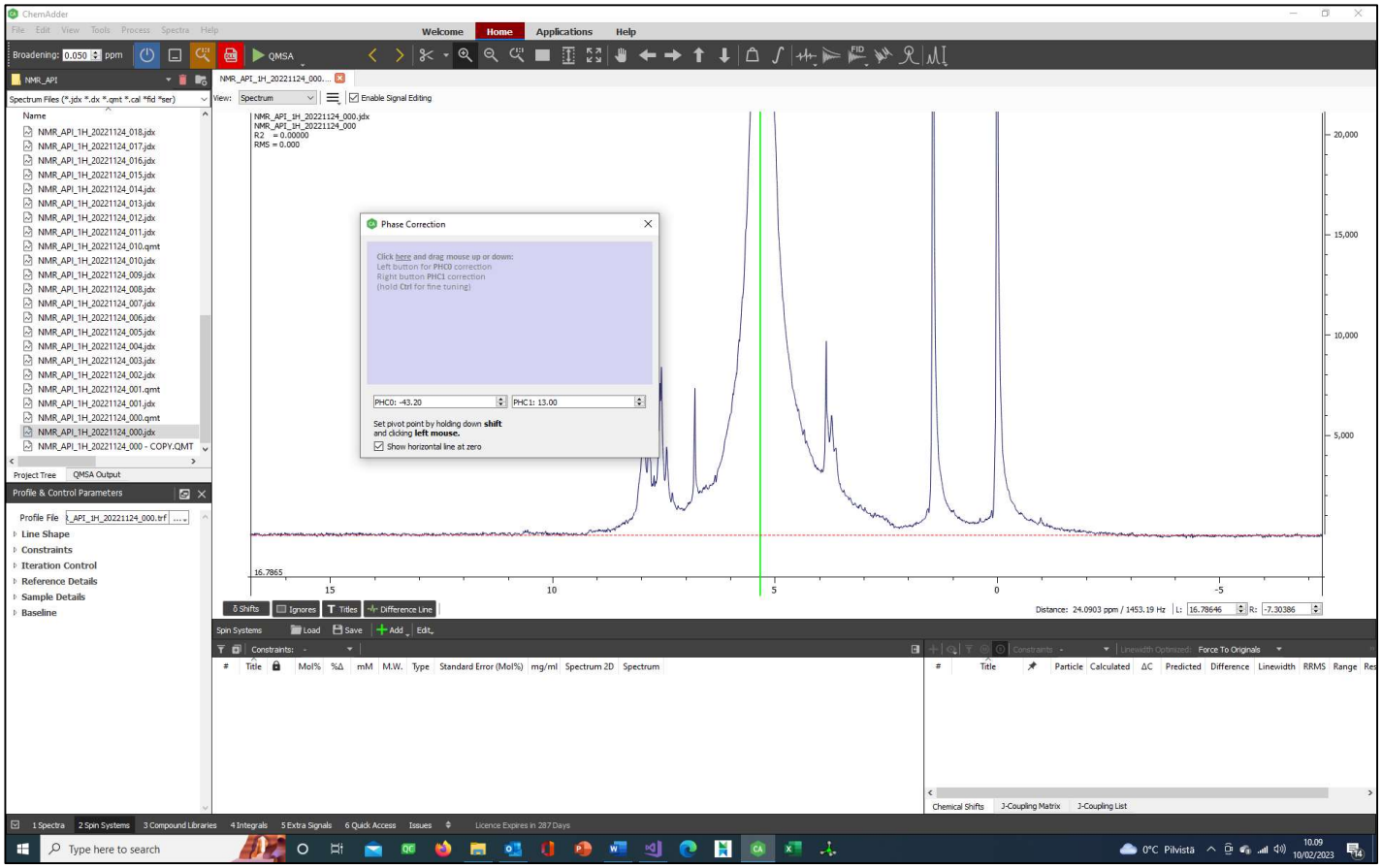


Whitaker baseline corrected spectrum:



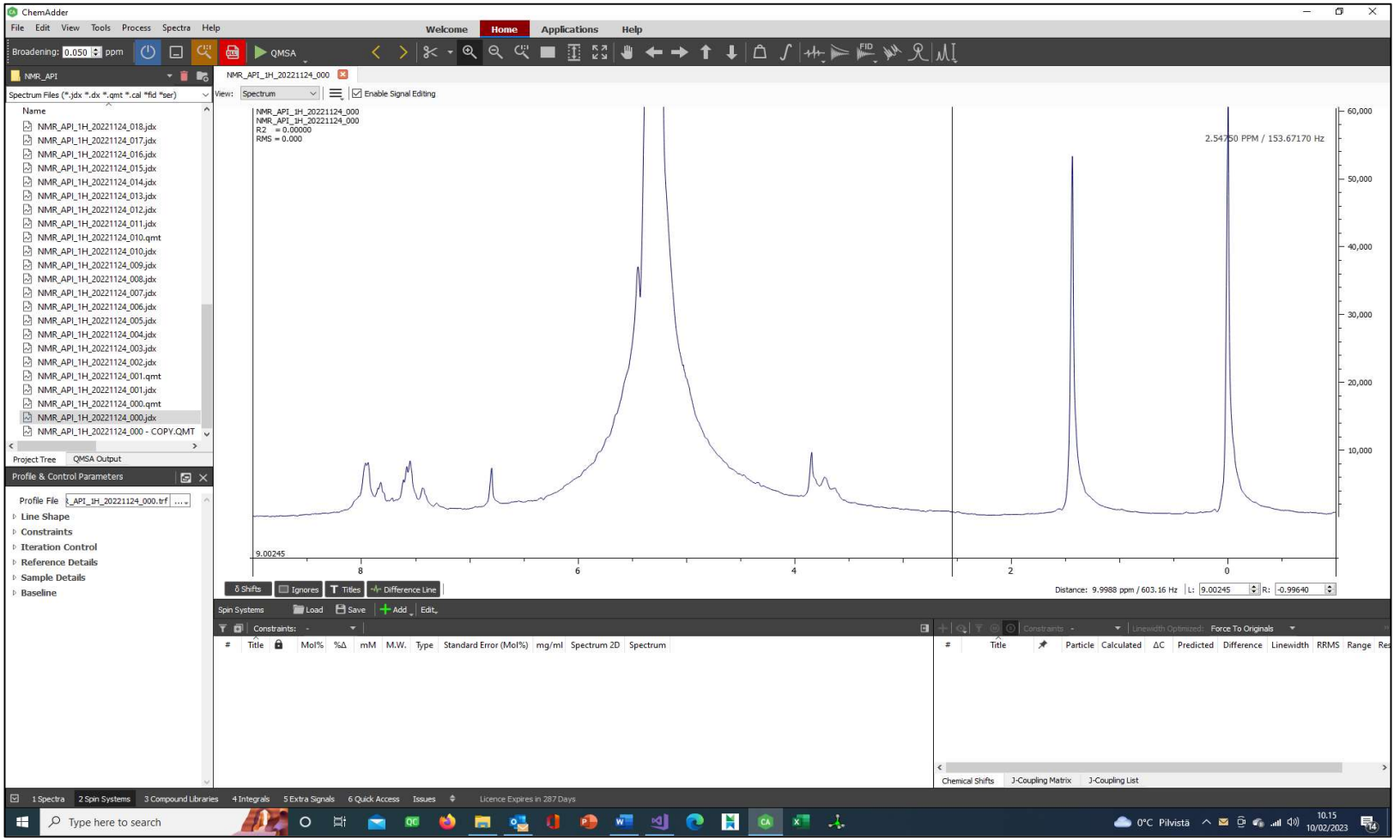
- The zero-level (green) is too low: the tails of DCM extends beyond 10 ppm. The spectrum from 9-10 to the left side should be positive!

Baseline after adjusting phase0:



- The zero-level (red) is too low: the tails of DCM extend beyond 9 ppm and -3 ppm (where the baseline hits zero).
- The Whitaker correction yields a (slightly) biased (but obviously the best possible?) baseline, which needs to be considered in fitting.

Ready for QMSA:



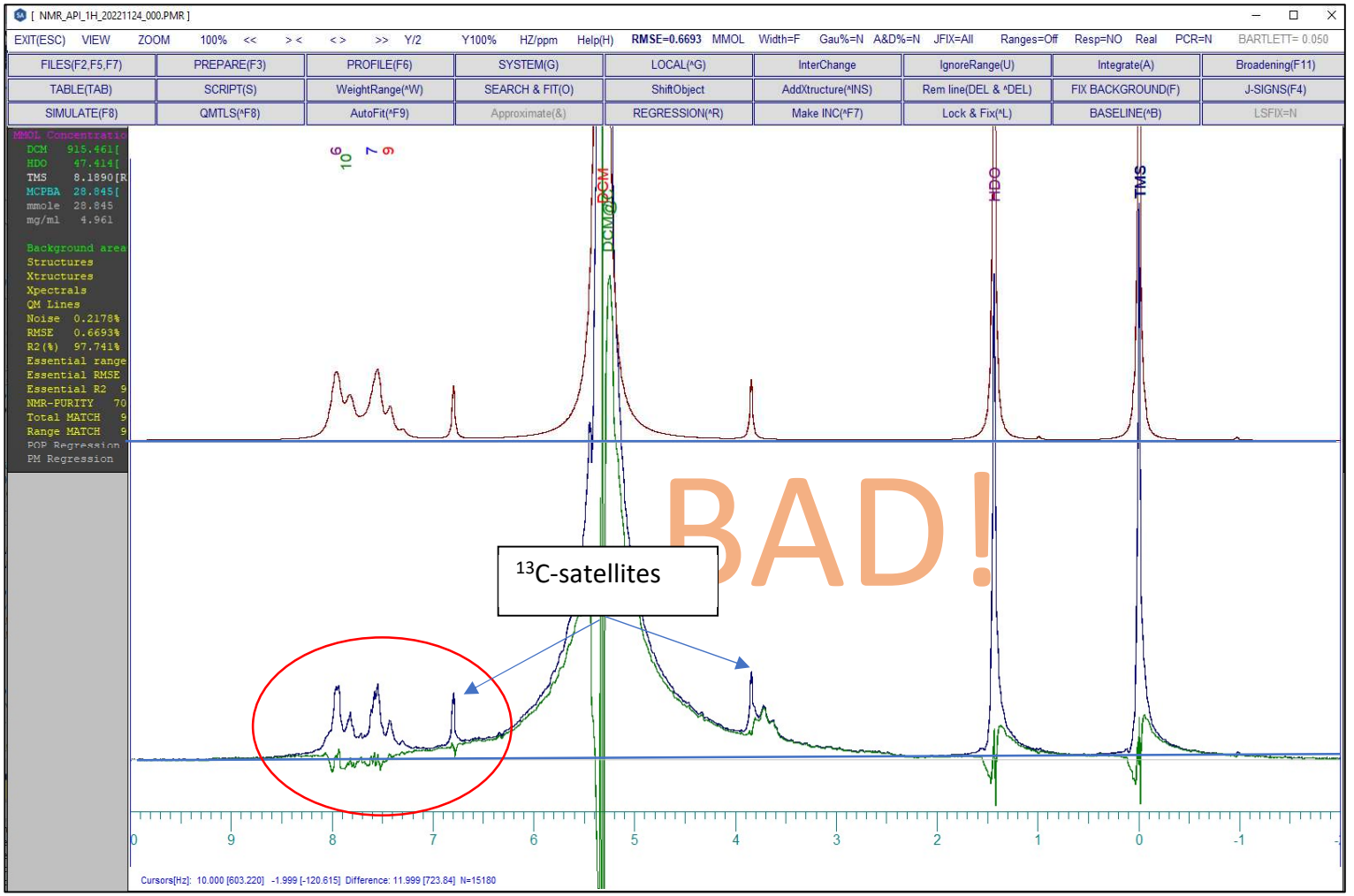
- LineShapes of DCM, HDO and TMS are similar, with strong right-side ‘low-intensity asymmetry’.
- The objective is to get the correct area of Target signal and compare it to a reference signal (TMS or DCM) to get the concentration of Target.

The very basic (raw) fitting:

With G%, D% and Line-Asymmetry=0: the target signal tries to fit the tail of DCM signals, therefore the **target** concentration **28.8** mmol is far too large. The target linewidths grow for the same reason, too. The reason is obvious when the difference is examined:

Release of G% and Line-Asymmetry does not help.

Rrms = **0.669%**

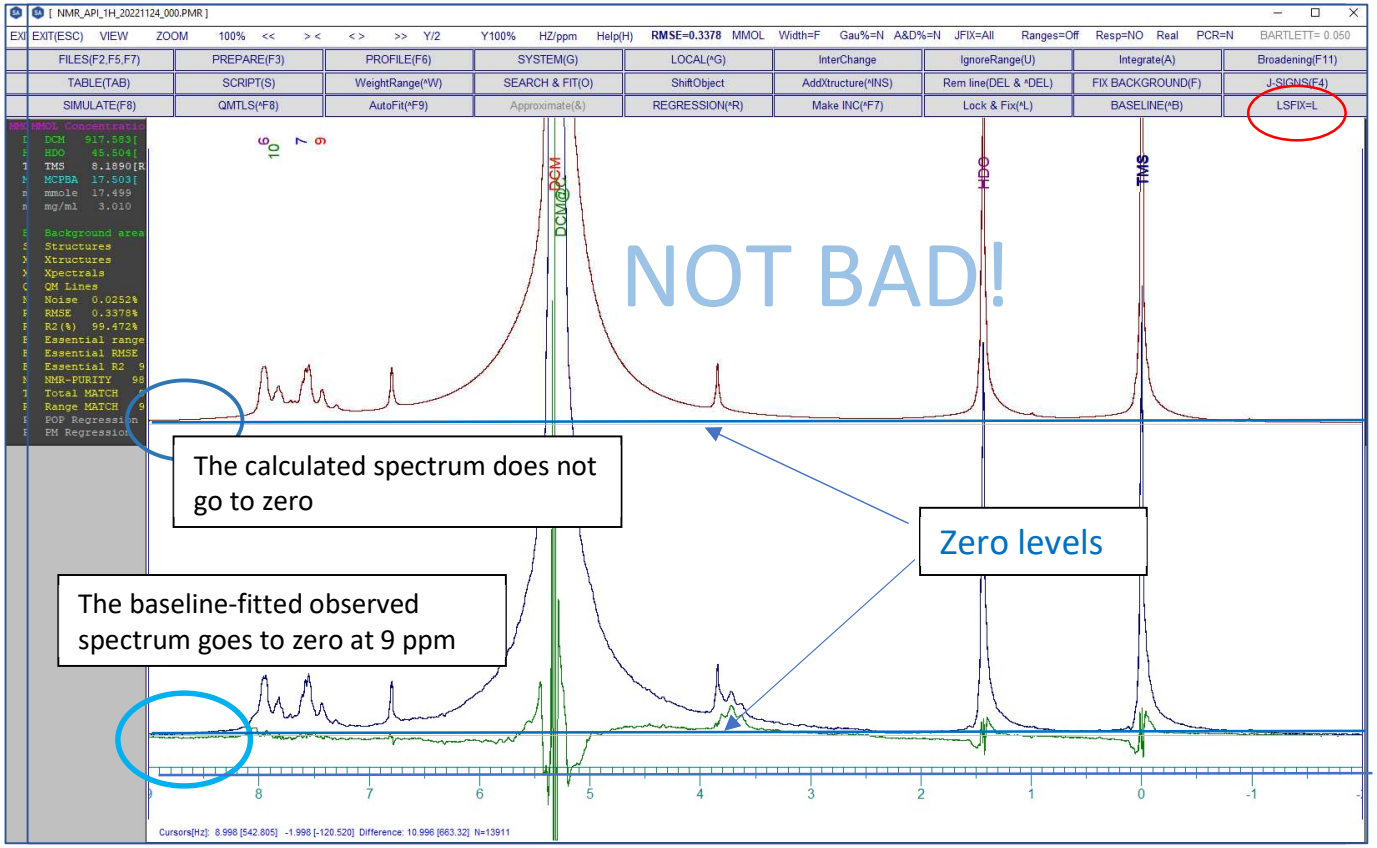


A better line-shape with broad asymmetric low-intensity components:

The **difference** spectrum is flat and close to zero-level so that *DCM shadow* range is only ca. 1 ppm (from 4.7 to 5.7 ppm).
The target concentration is now **17.5** mmol.

Base-line fitting bias: The signals are very broad (>3-5 ppm), so that the DCM, HDO and TSP signals do not decay to zero the within the display range, while the observed spectrum decays – as pushed by the Whittaker (or any) baseline correction and/or phase correction. The intensity zero-levels are shown for both the spectra. The line-shape fitting gives a fair estimate for the correct base-line.

Rrms = **0.338%**

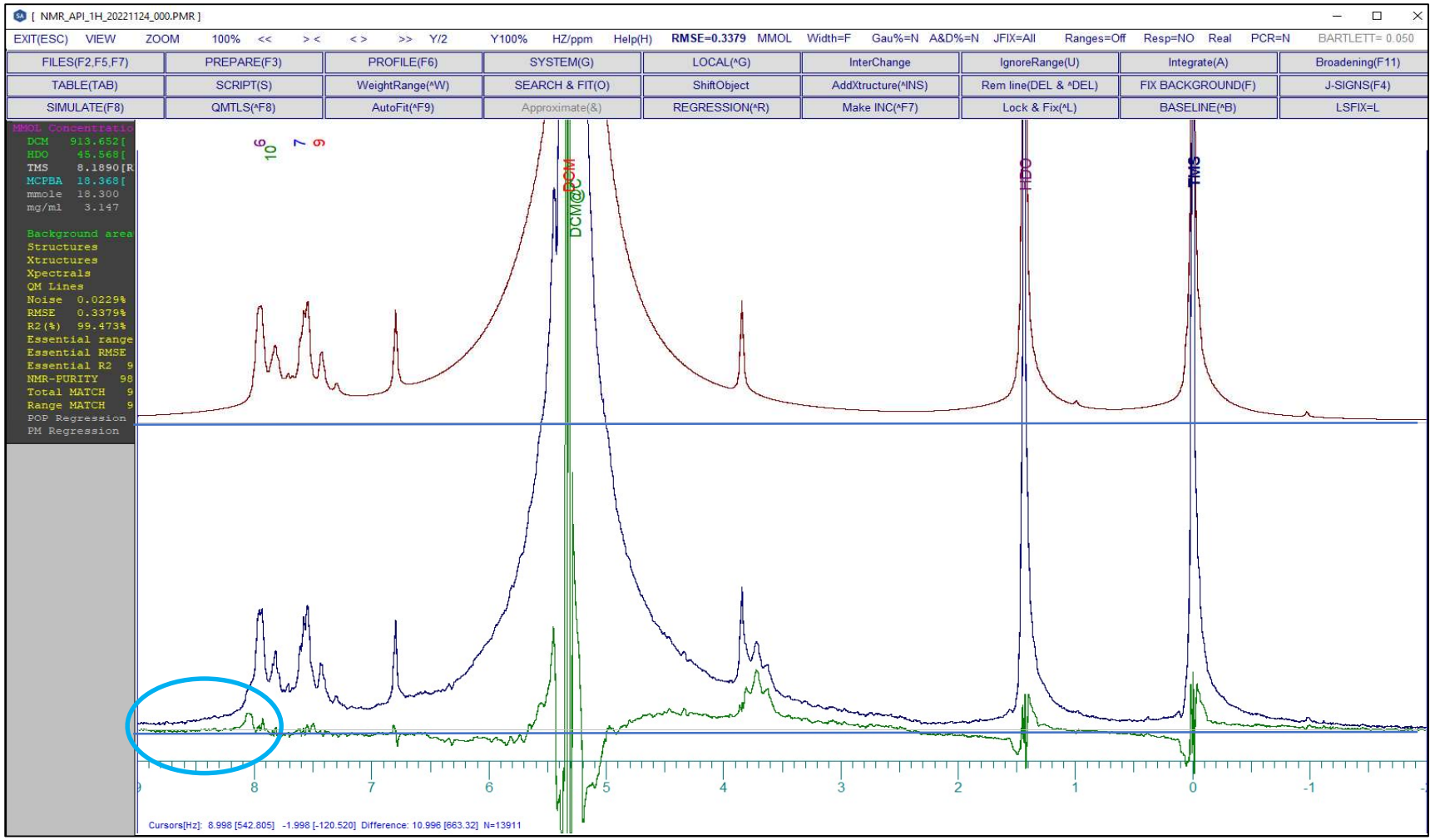


It was assumed that the same line-shape correction, being an instrumental artefact, can be used for all the signals.

This one step protocol can be considered as the easiest model for the target!

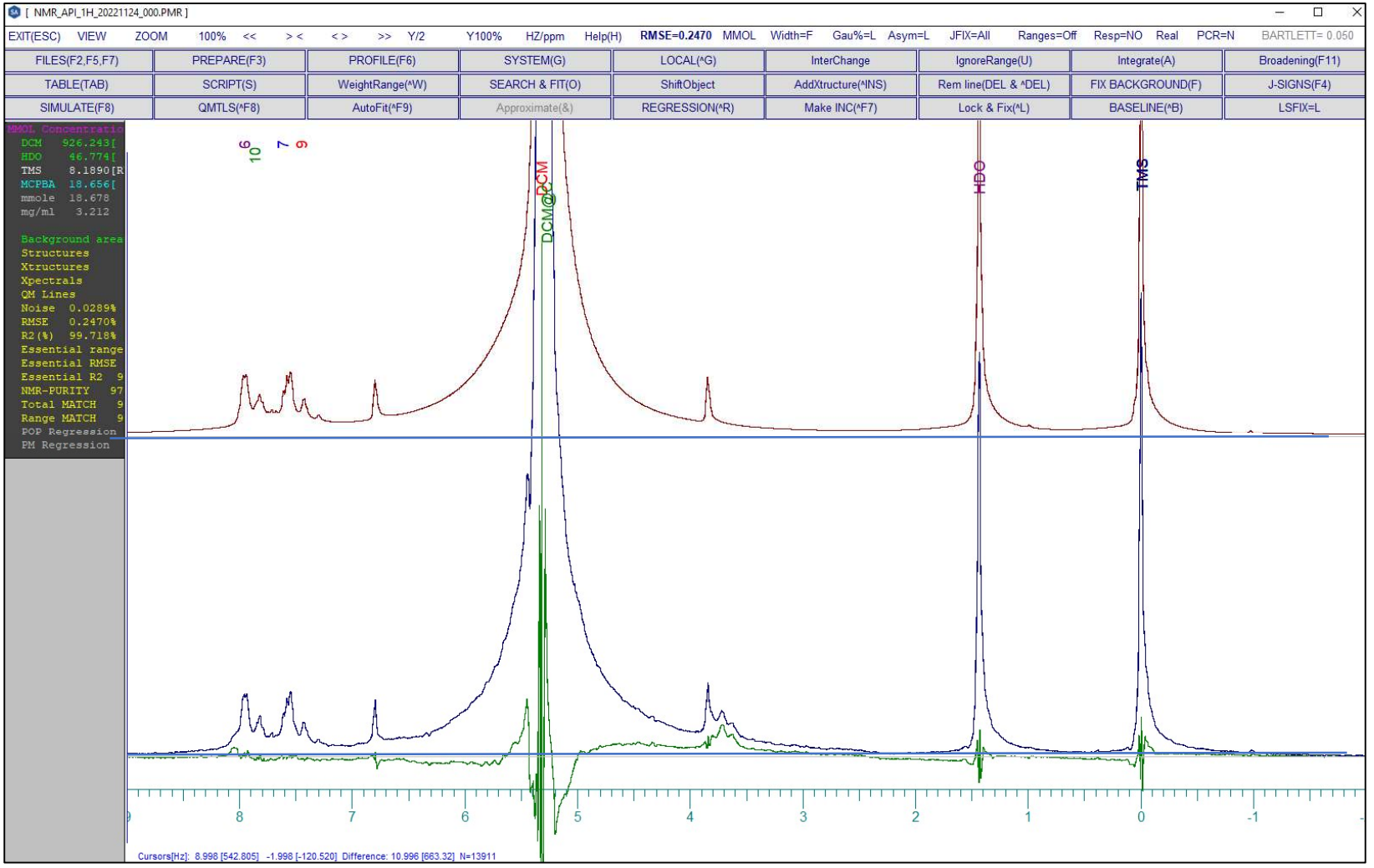
The base-line fitting bias can be reduced by adding a correction (y = A + B y) to the observed spectrum.

After optimization, the target concentration is **18.3** mmol and rrms = **0.338%**

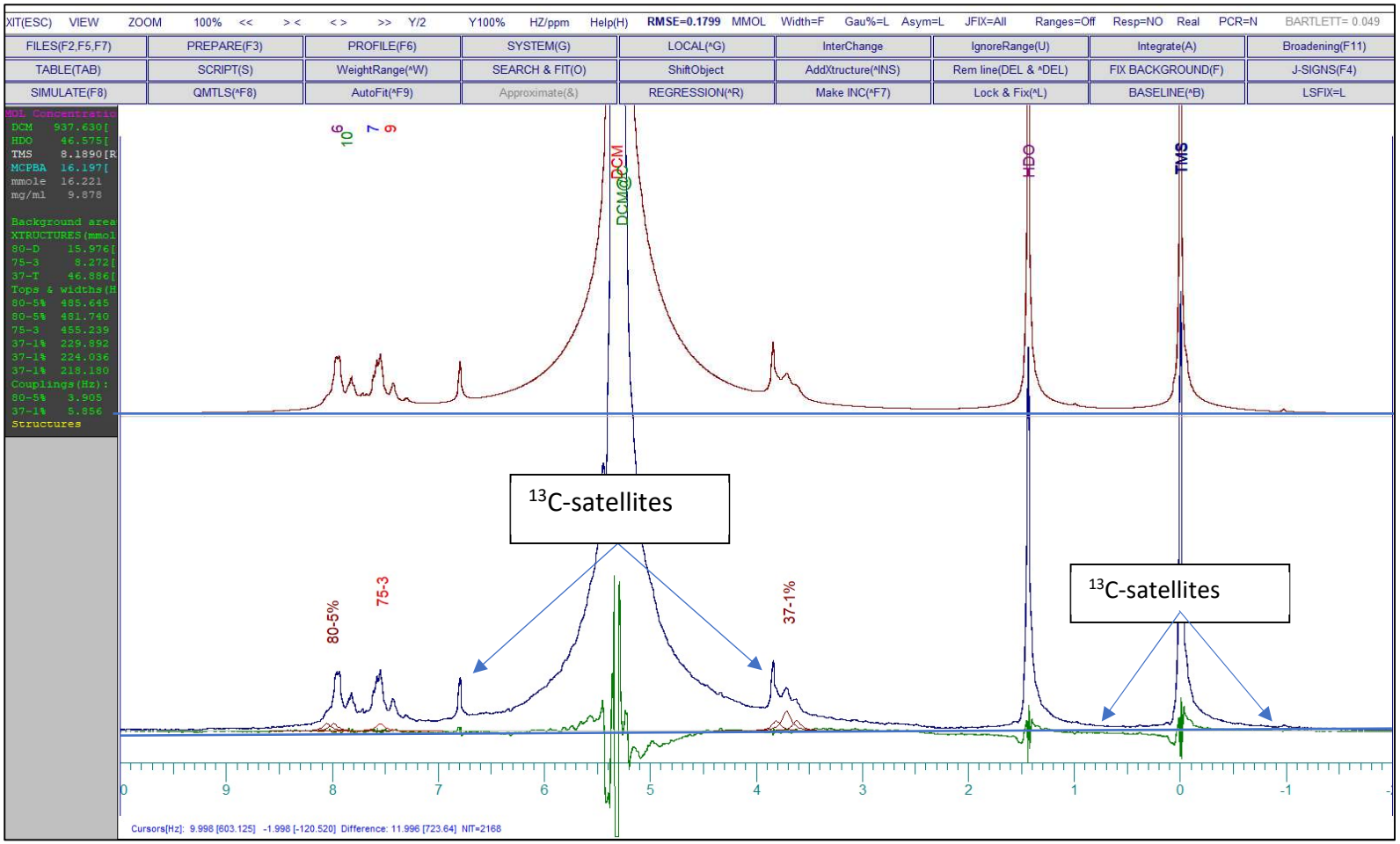


Signal specific G% and asymmetries

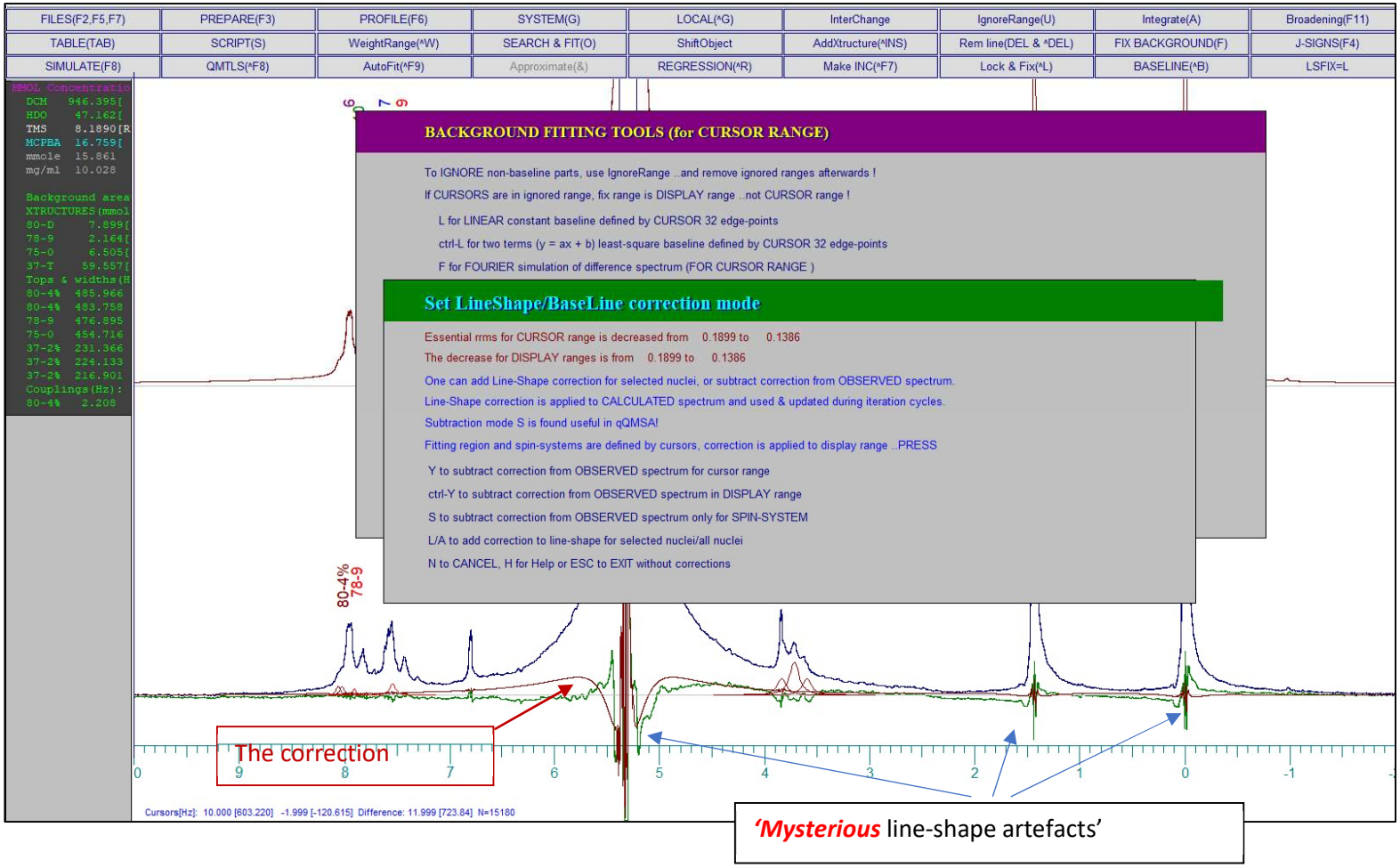
Rrms drops to **0.247%,** the target concentration is **18.6** mmol



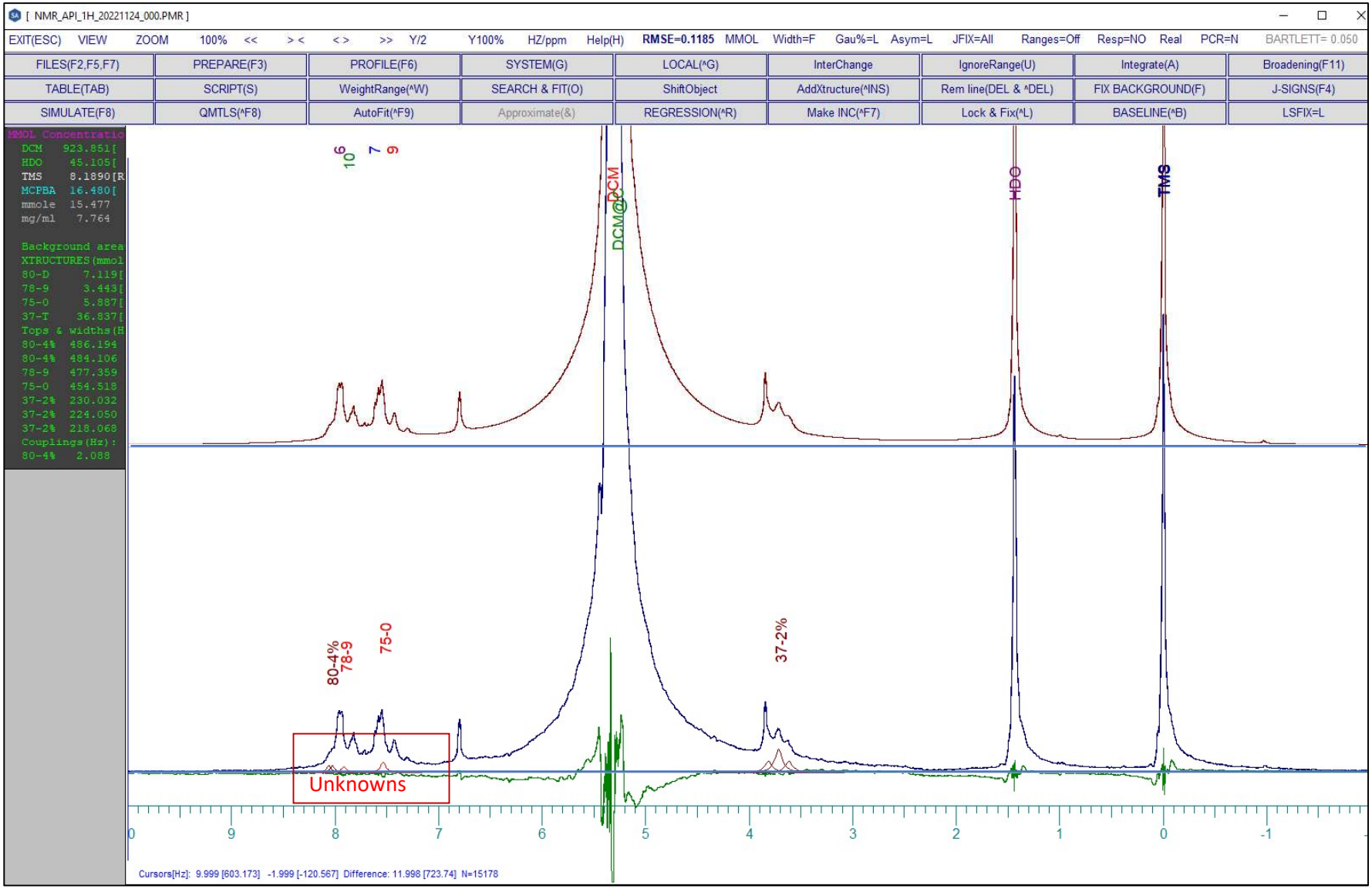
When three **xtructures** (doublet 80-5%, singlet 75-3 and triplet 37-1%) are added to the model, rrms is dropped to **0.180%,** and they decrease the target concentration to **16.2** mmol:



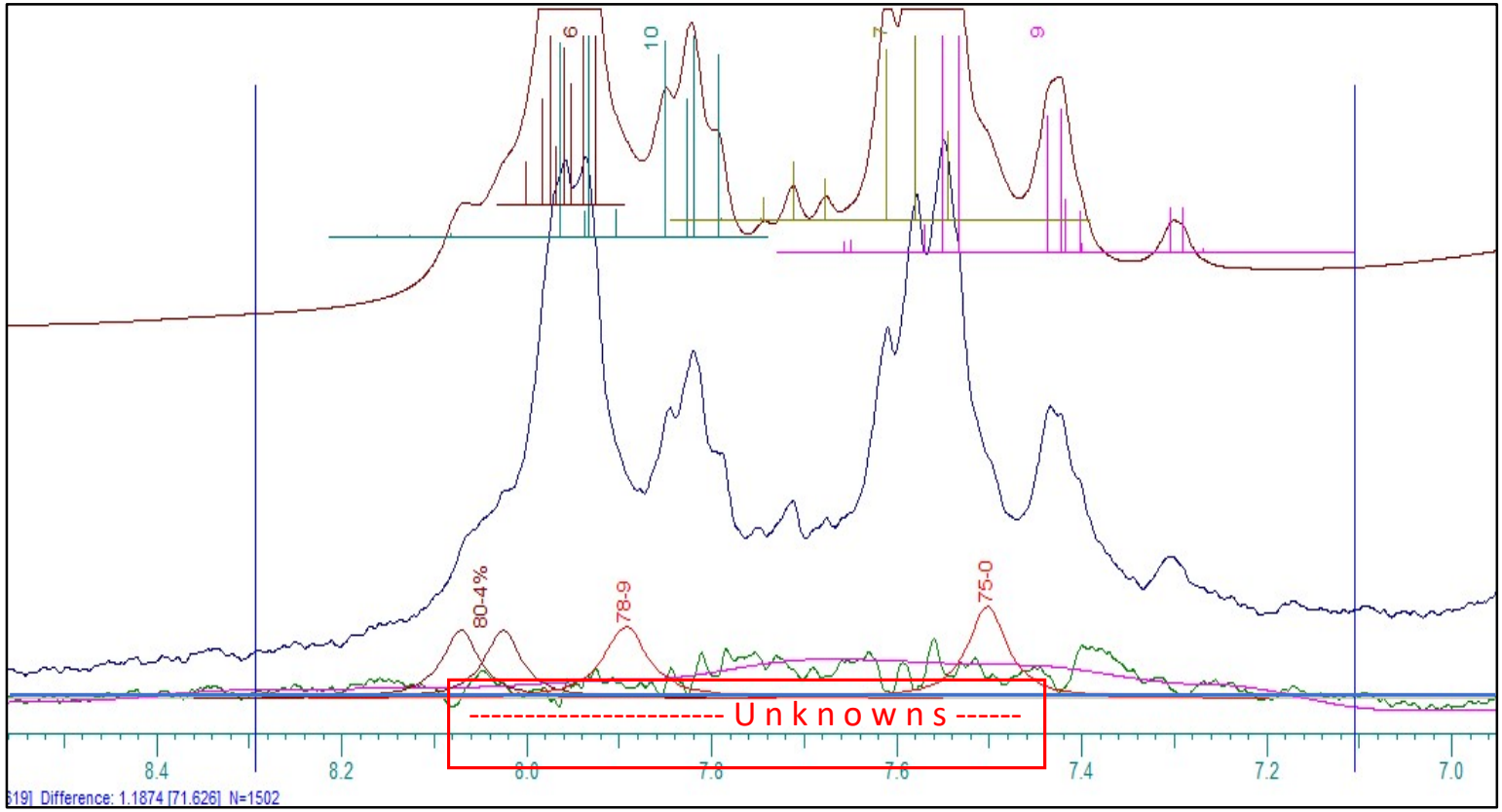
The line-shape can be fixed also using N (def. 33) terms (Local Fourier) correction function which is the same for all the lines:



After the Fourier correction (followed by basic optimization), rrms drops to **0.119%:**



The target signal stands on a broad pedestal and there are <u>three signals</u> which cannot be explained by the spin-system – giving a hint about by-products of the reaction followed:



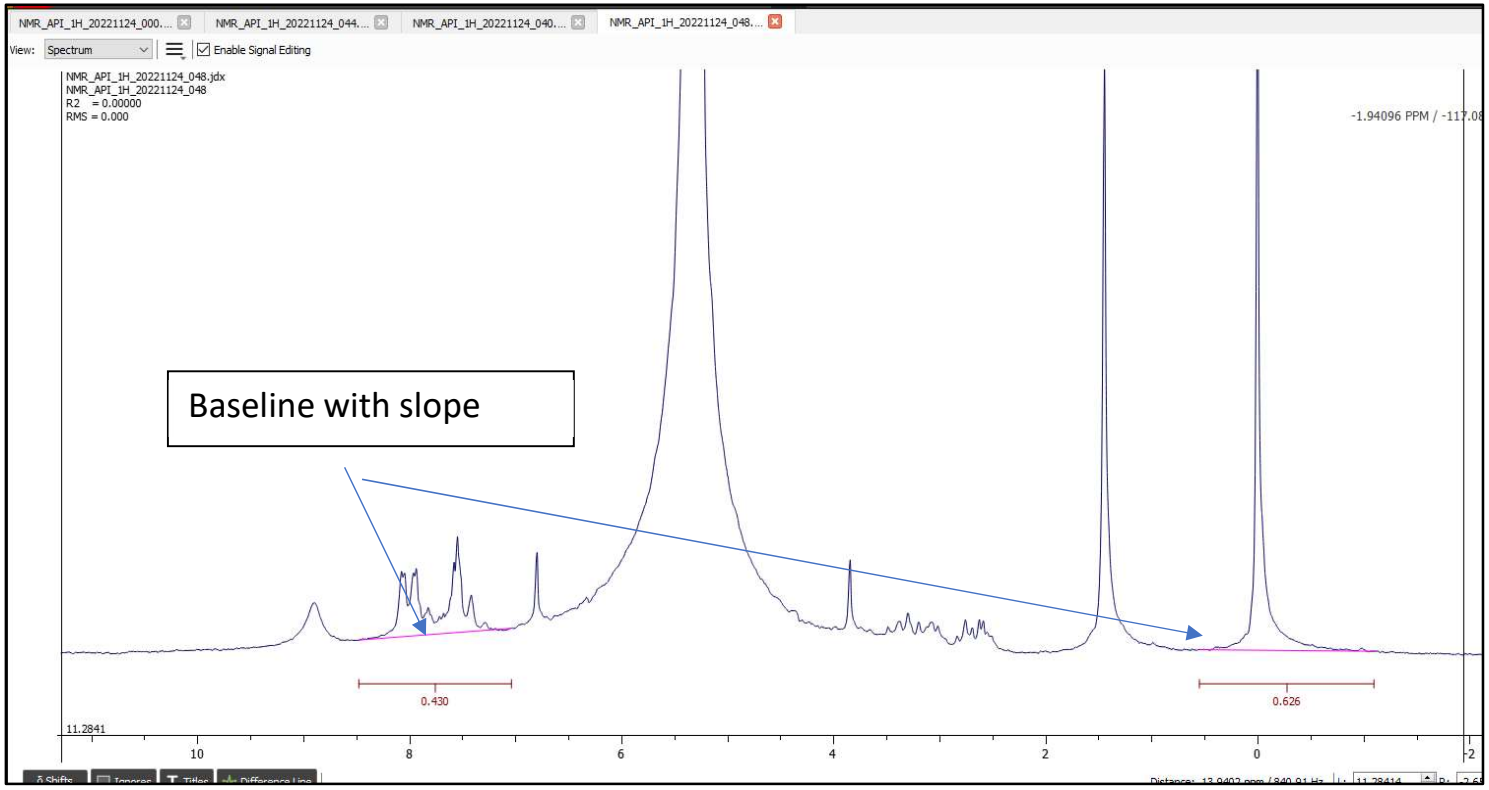
Conclusions:

- Benchtop spectra suffer from poor line shape (more than high field spectra), obviously(?) due to out of coil information. Because the signal overlap is more significant than at high field, the line shape and overlap form a challenge for qQMSA.
- A good total fit of the benchtop spectral lines is obtained by adding *low-intensity, broad and asymmetric line-shape correction*. In this way, all the major features of the spectrum are described quantitatively.
- The Whittaker baseline fitting (or as any functions not based on the natural Lorentzian line-shape of NMR signals) yields a bias that can be removed by the new line-shape.
- A good fit may demand adding some impurity signals ('Xtructures') – revealing important information about the sample.
- The *mysterious line-shape artefact's* can be reduced by using Local Fourier fitting.

The protocol is rather robust and straightforward.

P.S. The easy way: the traditional integration

- The values of integrals depend on the integration width, which leads to a considerable bias in their values.
- If the baseline can be set in the same way for all the spectra of an experiment, the bias does not matter?
  Good for kinetic studies – not for purity analyses?



The approach tells nothing about the impurities hiding under target signal!