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Removal of ^{13}C Effects for Impurity qQMSA. How Quantitative is DISPEL – Comparison with Gentle ^{13}C Decoupling Method?

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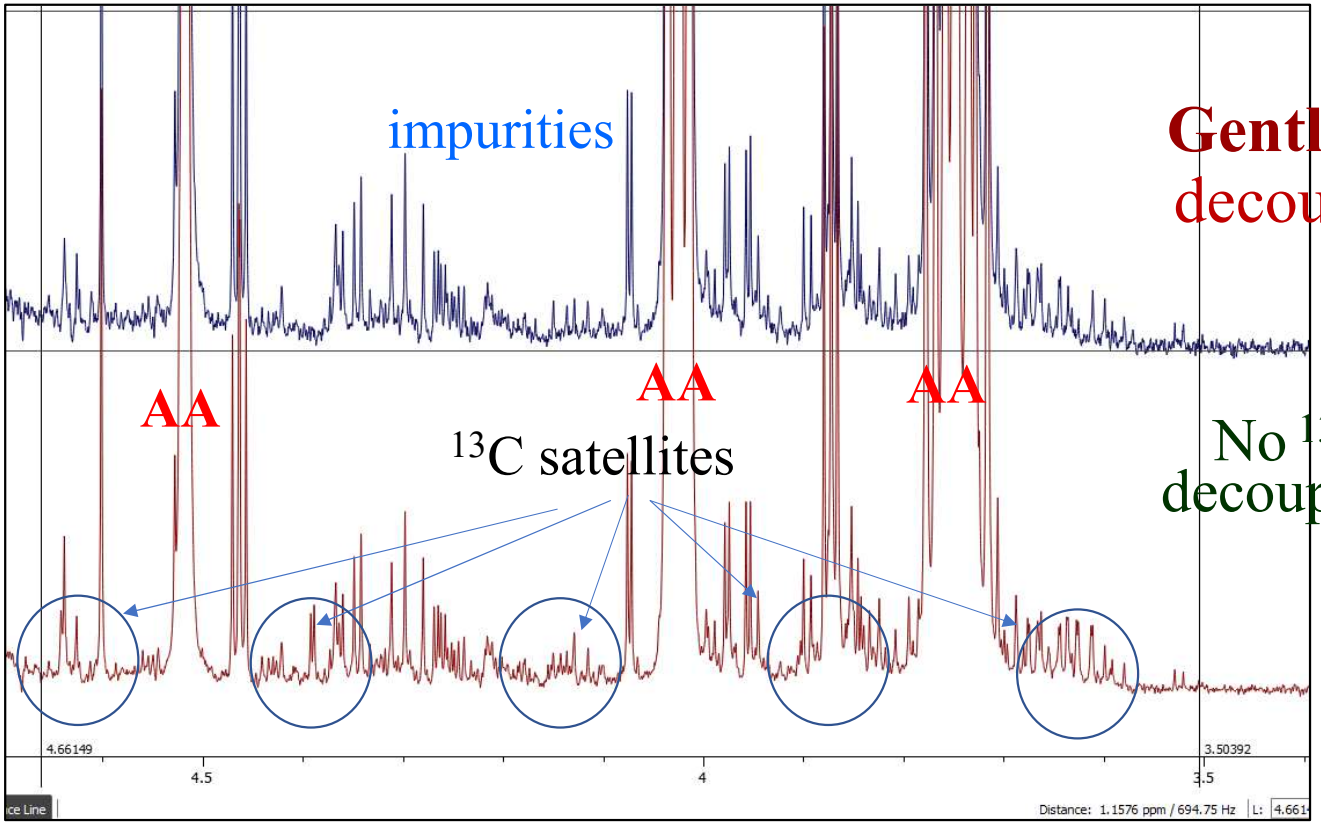
Announcement

After a discussion with Jari we decided to change **RANDOM Decoupling** to **Gentle Decoupling**, because the variation of the decoupling frequency can be done systematically, but always GENTLY

^{13}C satellites may disturb analysis of small impurities:



If signals << ^{13}C satellites can be detected and quantitated, it means concentrations << 0.55 mol%.
Here it means < 0.2 mol%.

Toward ^{13}C cleaned quantitative spectra

- As discussed in QMSA Letters 2(2022), the ^{1}H,^{13}C coupling effects disturb QMSA analysis and should removed in hunt for very small impurities, see also [1].
- The DISPEL-method [2] is a good for removal of ^{13}C satellites, but for qQMSA it demands an additional ^{13}C decoupling of ^{13}C,H long-range couplings. In the original report [2] it is told that the method is not fully quantitative and the typical bias are about the magnitude of noise.
- An alternative method for the ^{13}C effect cleaning is the '**Gentle ^{13}C decoupling**' (S13D) method where the ^{13}C satellites are dissolved into baseline and the ^{13}C,H long-range couplings decoupled [3].
- In this express letter we report large, up to 13% bias in our DISPEL measurements.

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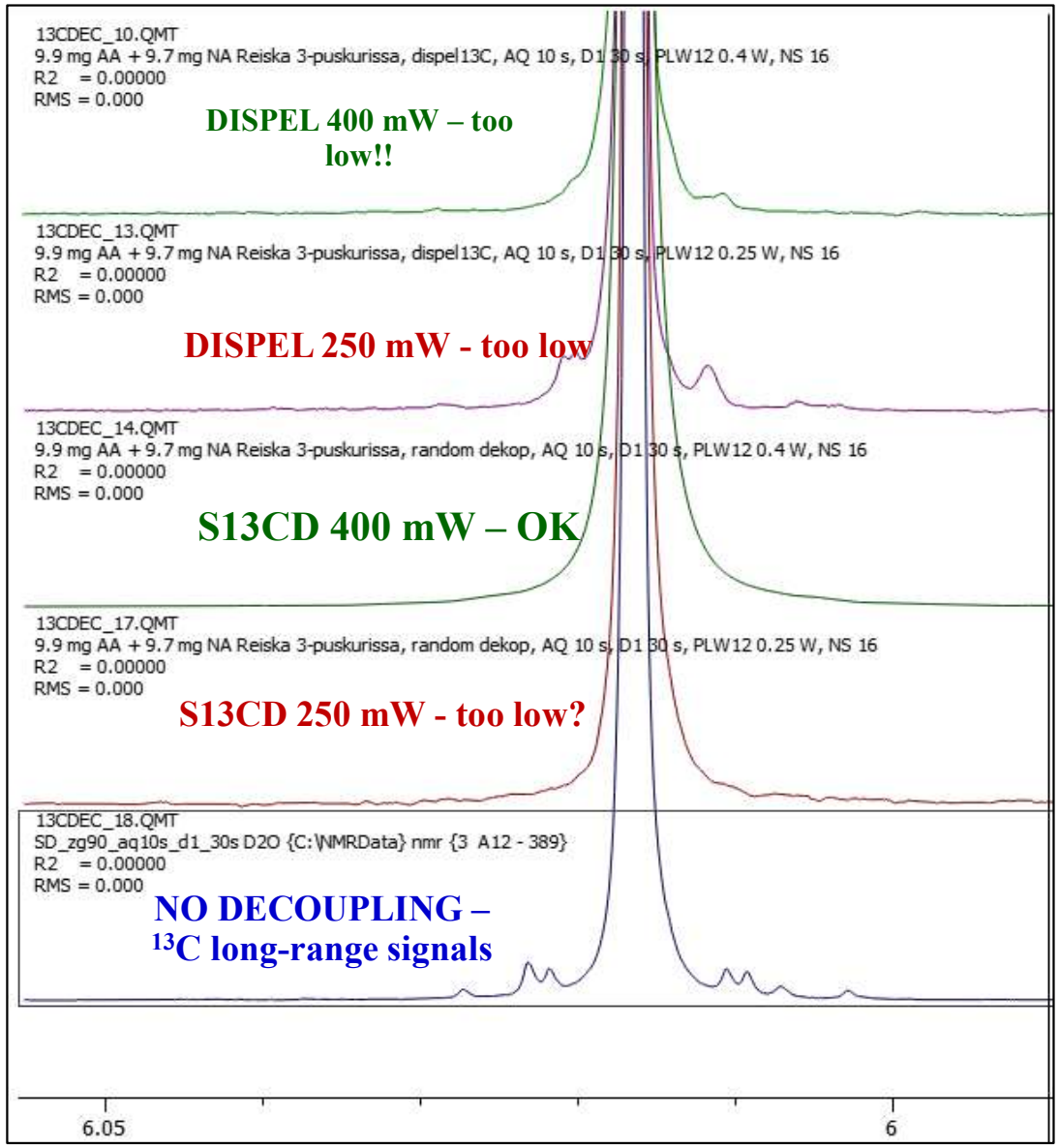
1. A. Bahadoor, A. Brinkmann, and J.E. Melanson,*13C-Satellite Decoupling Strategies for Improving Accuracy in Quantitative Nuclear Magnetic Resonance*, Anal. Chem. 2021, 93, 851−85.

2. P. Moutzouri, P. Kiraly, A.R. Phillips, S.R. Coombes, M. Nilsson, and G.A. Morris, *13C Satellite-Free 1 H NMR Spectra,* Anal. Chem. 2017, 89, 11898−1190, DOI: 10.1021/acs.analchem.7b03787

3 P. Soininen, J. Haarala, J. Vepsäläinen, M. Niemiz, and R. Laatikainen, *Strategies for organic impurity quantification by ^{1}H NMR spectroscopy: Constrained total-line-shape fitting, Anal.Chim.Acta, 542, 178-185 (2005).*
PasiSoininen[a]JormaHaarala[b]JoukoVepsäläinen[a]MatthiasNiemitz[c]ReinoLaatikainen[a]

^{13}C decoupling power:

- The long-range coupling effects are visible in maleic signal, if the power is low.
- The **Gentle** *Decoupling* yields a better decoupling with a lower power.



Is DISPEL quantitative ?

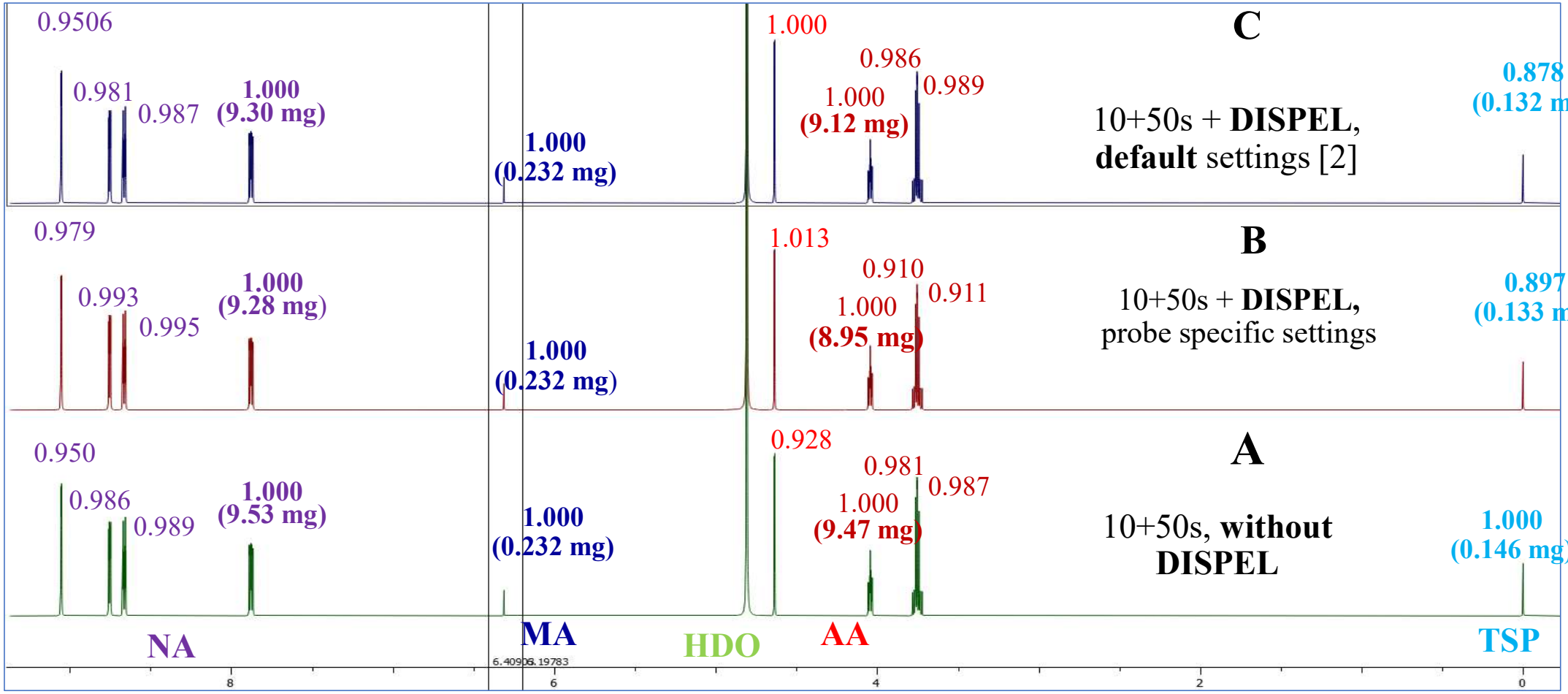
- The experiments were done with phosphate buffer solution containing 1 mmol TSP (0.1461 mg/ml), 2 mmol maleic acid (0.232 mg/ml, MA), 9.87 mg/ml ascorbic acid (AA) and 9.99 mg/ml nicotinic acid (NA).
- The 600 MHz Bruker spectra (next page) were measured using **(A) standard SD-zg90 sequence**, the **SD_dispel4stage sequence, (B) with probe selective settings**[&] or **(C) the defaults given by Morris et al [2]**. In all cases AQ=10s and d1=50s were used.
- In qQMSA, the response factors (RF) were optimized by setting the RF=1 for the largest RF (NA) or the best resolved proton (AA). The RF of TSP was set to calculated/given weight. The RF's and qQMSA estimates for mg/ml are given below.
- The standard measurement (A) gave expected values TSP, but for DISPEL (both RF and mg/ml) they were > 10% too low!
- The NA and AA concentrations were all too low, obviously *due to too short d1 delay*[$]. The DISPEL concentrations were 2-5% smaller than given by the standard measurement .
- The default DISPEL parameters [2] gave slightly better results.

[&] Our *probe specific values* were d16=200 and p18=100 ms, while the defaults [2] were 500 and 1 ms.

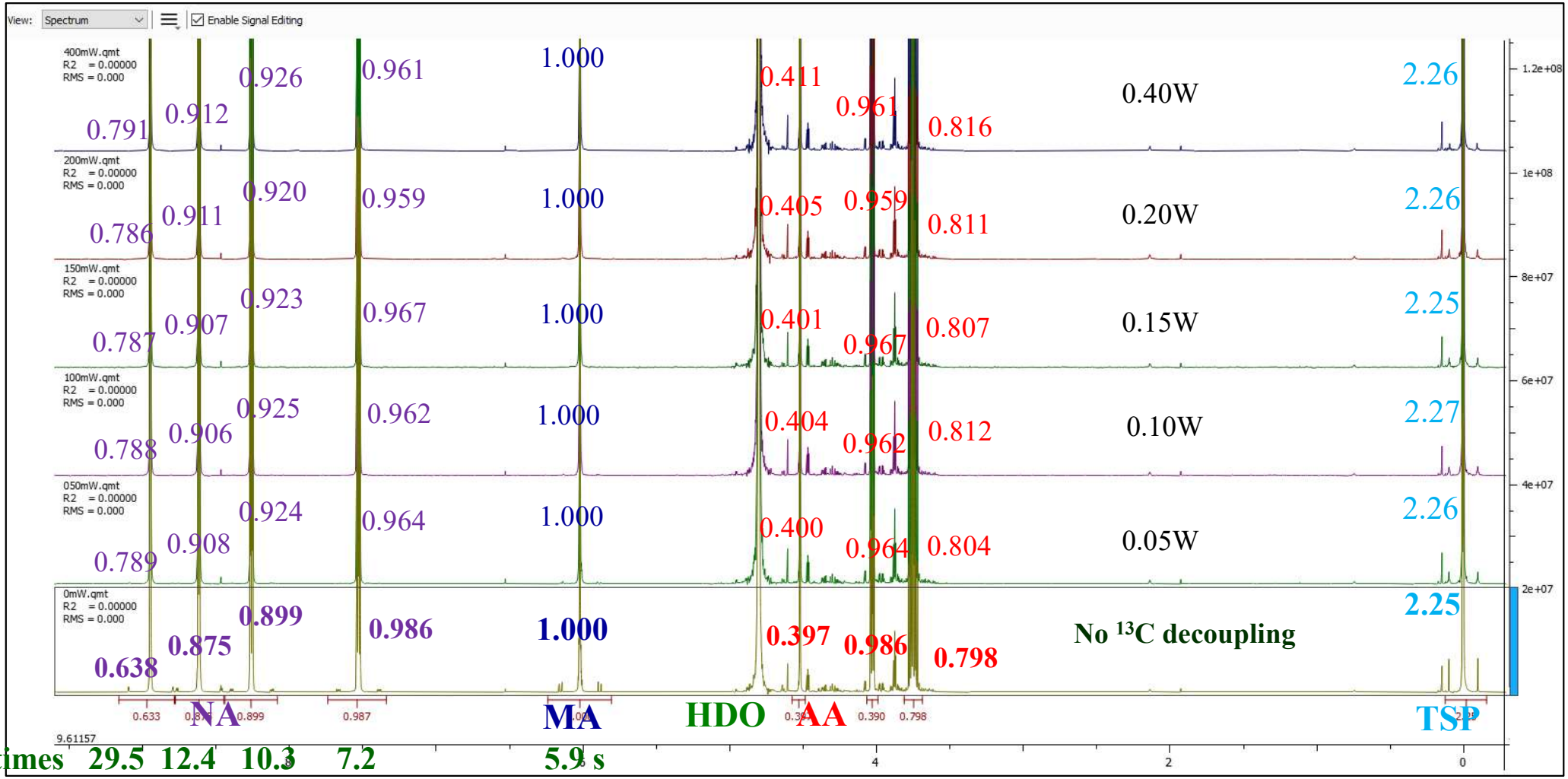
[$] The NA response factors vary remarkably (the 2-proton RF = 0.95, see QMSA Letters 4(2022)), and RF=1.000 would demand longer relaxation (d1) delay – proved before.

Response factors* for TSP (0.146 mg) + Maleic Acid (0.232 mg, MA), Nicotinic acid (9.87mg, NA) and Ascorbic Acid (9.99 mg, AA)

*The largest RF of each compound was set to 1.000

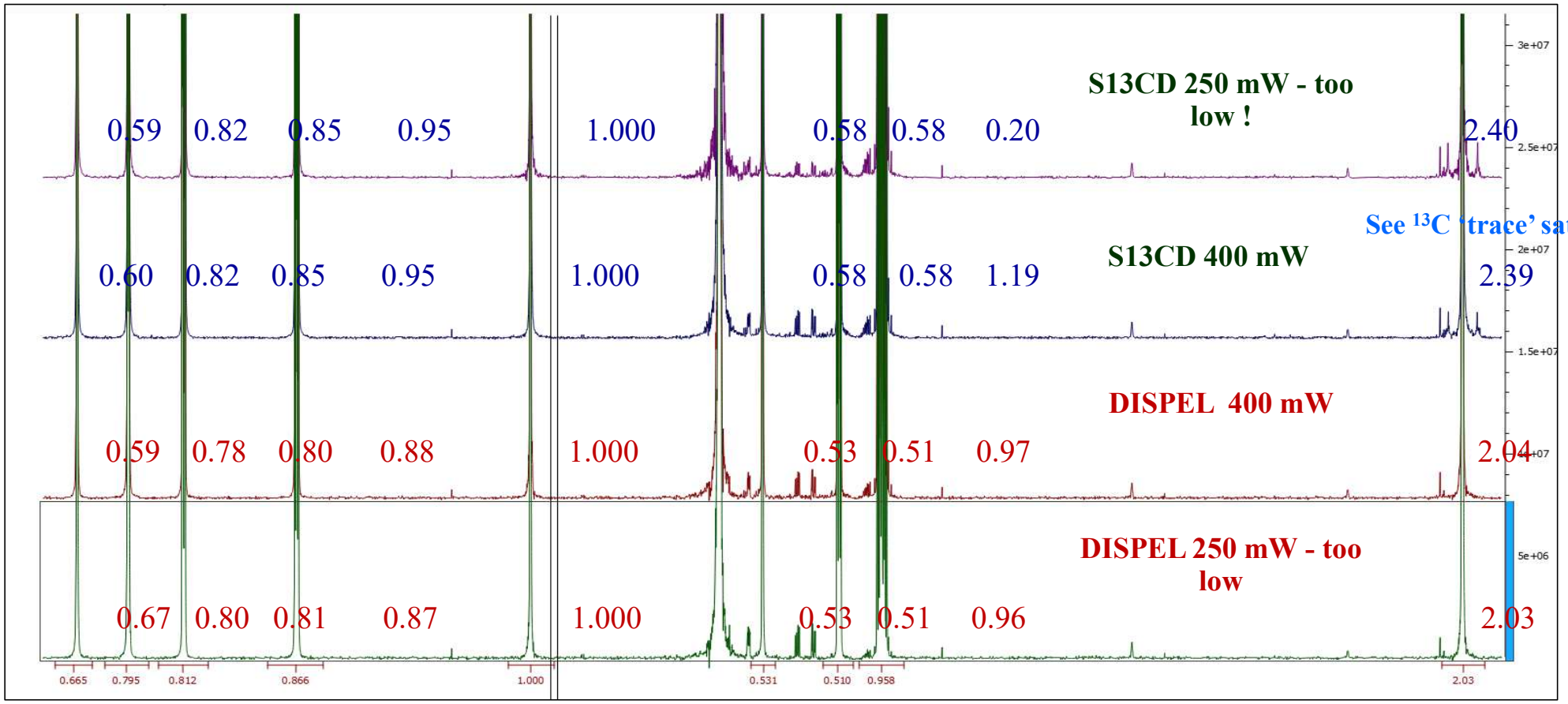


Gentle ^{13}C decoupling power has no significant effect to **integrals** of AA and TSP, but the NA integrals differ with and without decoupling – as related to their T_1-times:



T_1-times 29.5 12.4 10.3 7.2 5.9 s

S13CD and DISPEL+^{13}C decoupled spectra of NA & AA with decoupling powers of 250 and 400mW. - See Conclusions.



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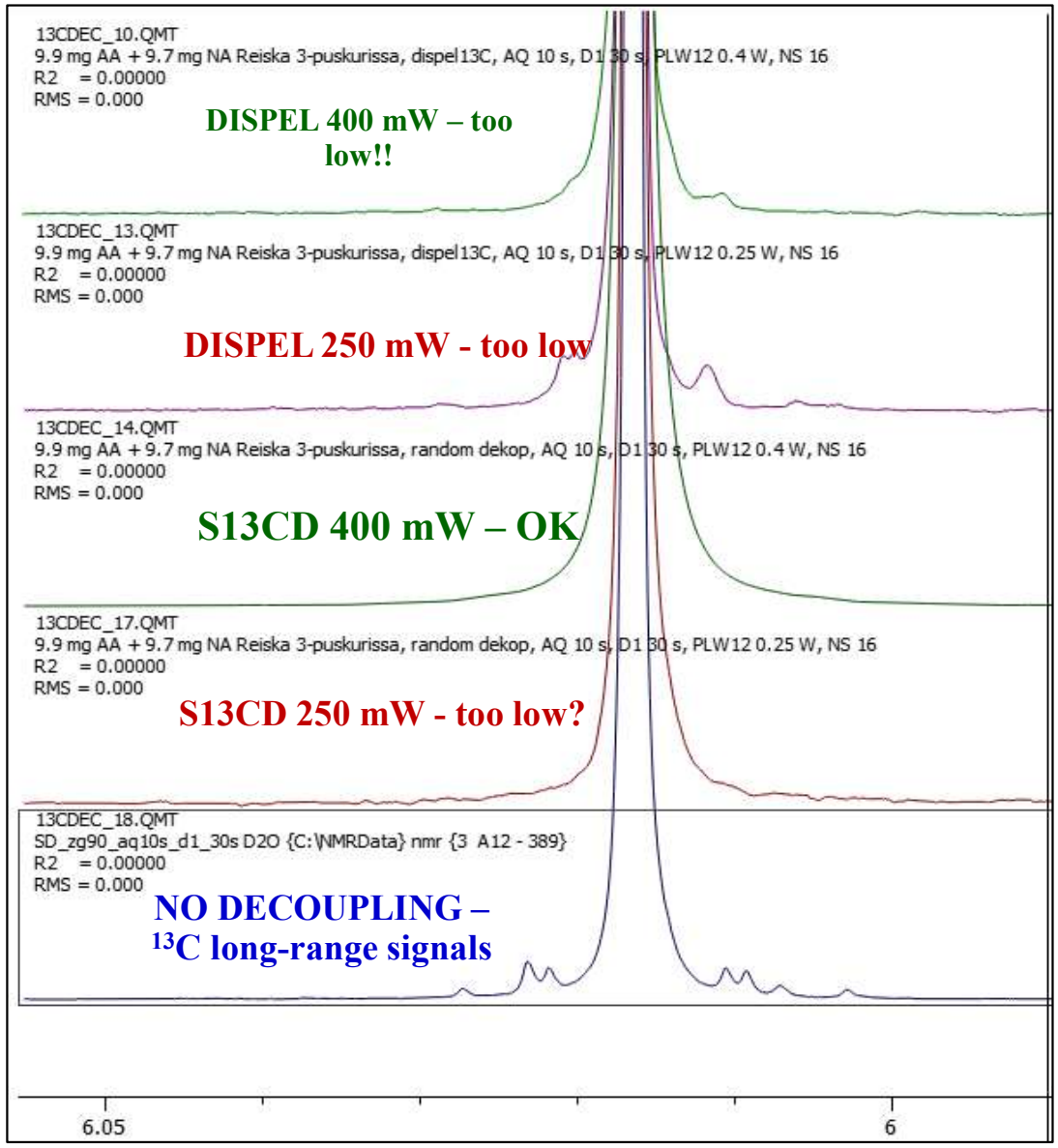
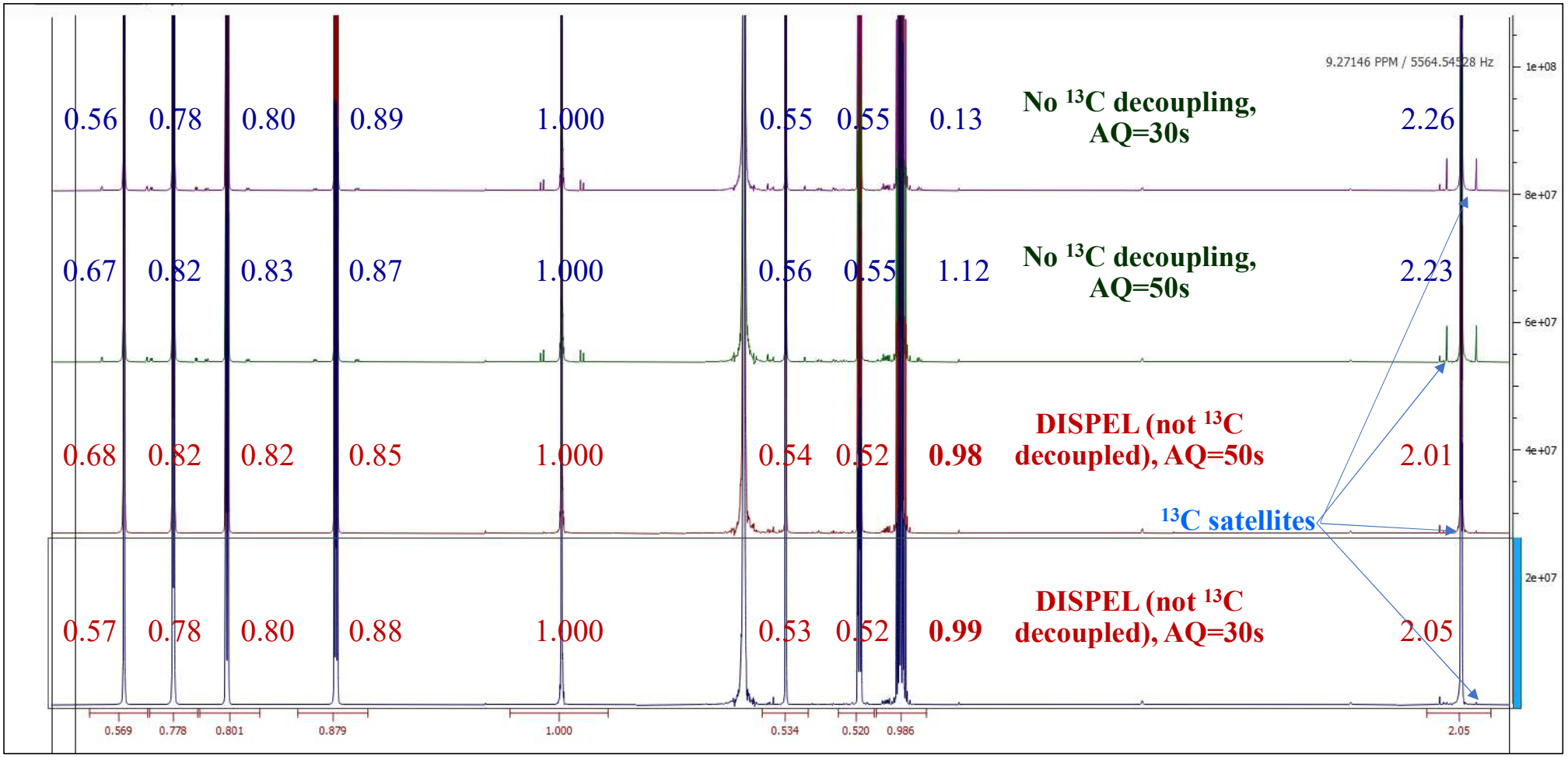


Not ^{13}C decoupled **Basic** and **DISPEL** spectra of NA & AA with AQ's of 30 & 50s:

The AQ has little effect, the **Basic** integrals ratio are near to expected



Glucose

To explore the ***DISPEL bias***, also RF's of glucose were analysed (at 298K in D_2O, at 600 MHz). The RF's of the 5-protons were set to 1.000. For spectra and assignments, see ***QMSA Letters 2(2022).***

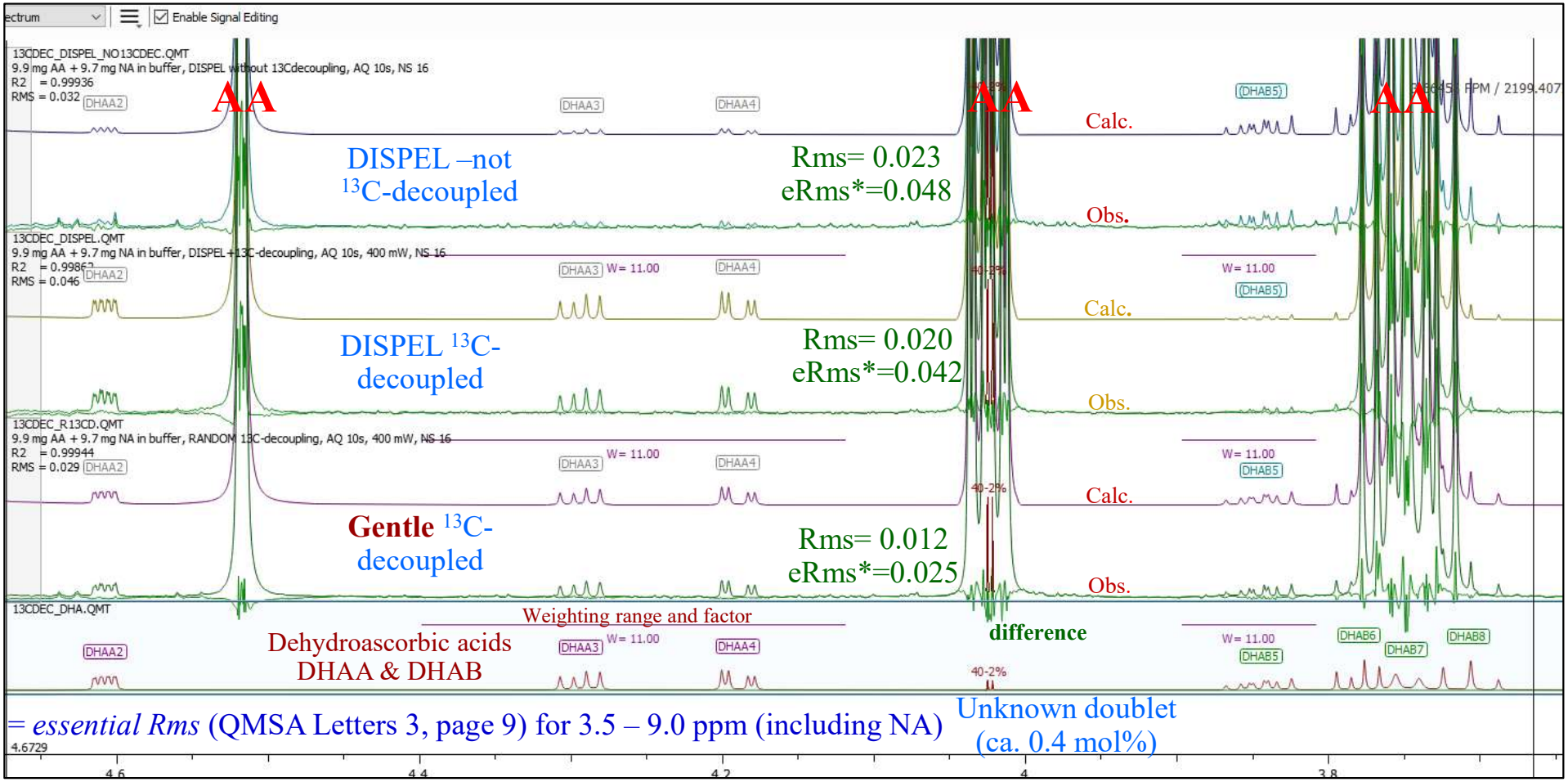
	No DISPEL		**DISPEL-1**[1]		**DISPEL-2**[2]	
B-Glucose	*RF*	*RMSE*	*RF*	*RMSE*	*RF*	*RMSE*
B1	**0.9604**	1.069	**1.0372**	0.568	**0.9960**	0.965
B2	0.9612	0.279	0.9791	0.451	0.9798	0.506
B3	0.9652	0.235	0.9744	0.385	0.9514	0.454
B4	0.9737	0.174	0.9721	0.363	0.9572	0.373
B5	1.0000	0.608	1.0000	0.684	1.0000	0.729
B6A	0.9904	0.515	0.9580	0.488	0.8794	0.443
B6B	0.9970	0.296	0.9146	0.410	0.8439	0.338
A-Glucose						
A1	**0.9306**	0.244	**1.0126**	0.197	**1.0086**	0.345
A2	0.9487	0.313	0.9411	0.329	0.9568	0.356
A3	0.9485	0.134	0.9653	0.135	0.9574	0.181
A4	0.9816	0.103	0.9472	0.112	0.9846	0.200
A5	1.0000	0.285	1.0000	0.257	1.0000	0.131
A6A	0.9457	0.239	0.9409	0.207	0.9057	0.254
A6B	0.9728	0.390	0.9151	0.464	0.9007	0.506

[1] **DISPEL-1**: with default d16=500 and p18=1 ms [2]; [2] **DISPEL-2**: with probe specific parameters d16 = 200 and p18=100 ms.

QMSA of spectra of NA + MA + two isomers of dehydro-ascorbic acid

The isomer populations (0.1-0.5 mol%) vary with time - but not in straightforward way!?

Clearly the best fit is obtained with **Gentle** ^{13}C Decoupling!



*eRms = *essential Rms* (QMSA Letters 3, page 9) for 3.5 – 9.0 ppm (including NA)

Unknown doublet (ca. 0.4 mol%)

Conclusions & Consequences

- The MA/TSP ratio is a good indicator of the DISPEL quantity.
- In the **MA+TSP+NA+AA** measurement, DISPEL led to up to 13% (TSP) bias in the integrals.
- The largest DISPEL effect on **glucose** RF factors was 7%. Also, the (local) RMSE-values for protons were significantly larger with DISPEL.
- If the bias cannot be fixed, we recommend **Gentle ^{13}C decoupling**' (S13CD) [2] for the '^{13}C-cleaned' quantitative spectra. *As shown, the method does not disturb quantitation!*
- The DISPEL method is excellent for analyses of ASL (=Adaptive Spectral Library) data, and **impurity** analysis where a small bias is less significant than the disturbing satellites.
- In qQMSA of DISPEL spectra, the bias can be handled by optimizing **Response Factors**.
- If DISPEL is used and the bias cannot be removed, SPIKING is the way to solve it – and other bias in qNMR. It multiplies the measurements but allows shortening AQ times. - One should know that also the **spiking bias** can be significant if the system contains macromolecular excipients.
- In NA+AA the T_1 times vary from 6 to 29 s, which might traditionally mean 5 x 29 = 145 s AQ time! In qQMSA the T_1's are handled with response factors, and thus, AQ can be based on the shortest time – here 30 s.