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Response Factors (RF) are not always one !

Reino Laatikainen[a], Pekka Laatikainen[a], Henri Martonen[a] and Tuulia Tynkkynen[b]

[a] Spin Discoveries Ltd., Kuopio, Finland

[b] University of Eastern Finland, Kuopio, Finland

RESPONSE FACTORS

RF = 1.000 if the area of NMR signal/proton = area of the reference signal /proton

Response Factors (RF)

- According to the theory the intensity of proton NMR signal is independent of the environment of the proton. Therefore, NMR is often assumed to be a **metric** method – like a metric measure which can be used anywhere without calibration. And many are those reports where the rule is proved, once, once and once again.
- The theory is close to correct if some conditions are fulfilled – the best known of which is that if the relaxation delay (RD) is long enough. Usually, total repetition time of 30-60 secs is assumed to be sufficient. Numerous are the experiments where this rule has been applied - and NMR spectrometer time is wasted.

If a nucleus obeys the theory, its RESPONSE FACTOR (RF) is defined to be 1.000.

- However, there are many common situations in which RF differs from 1.0: the more different pulses and phases (solvent suppression, T2-editing..) in the experiment, the more RF varies
    - For example, Table from *Tiainen & al, JMR 242(2014)67-78 – the RF's of glucose protons differ up to 20% from 100% - even with long RD's!*

BUT DON'T WORRY, RF's can be included and optimized in QMSA!

α-Glucose RF's with different experiments (*JMR 242(2014)67)*

	qH[a] D_2O	H[b] D_2O	qpresat[c] D_2O	presat[d] D_2O	qpresat[c] H_2O+D_2O	presat[d] H_2O+D_2O
H1	**0.962**	**0.875**	0.960	**0.880**	0.950	0.924
H2	**0.974**	0.993	0.965	0.993	0.904	0.909
H3	**1.000**	0.910	1.000	0.920	0.969	1.000
H4	**0.978**	0.953	0.990	0.990	1.000	0.978
H5	**0.965**	0.997	0.975	1.000	**0.850**	0.885
H6A	**0.977**	0.997	**0.953**	0.994	0.884	0.868
H6B	**0.975**	1.000	0.955	0.981	**0.811**	**0.840**

[a] **Basic proton spectrum** (zg): 128k data points (td), 4 dummy scans (ds), 8 transients (ns), AQ=7.7s, **RD(d1)=52.3s** and 90° pulse.

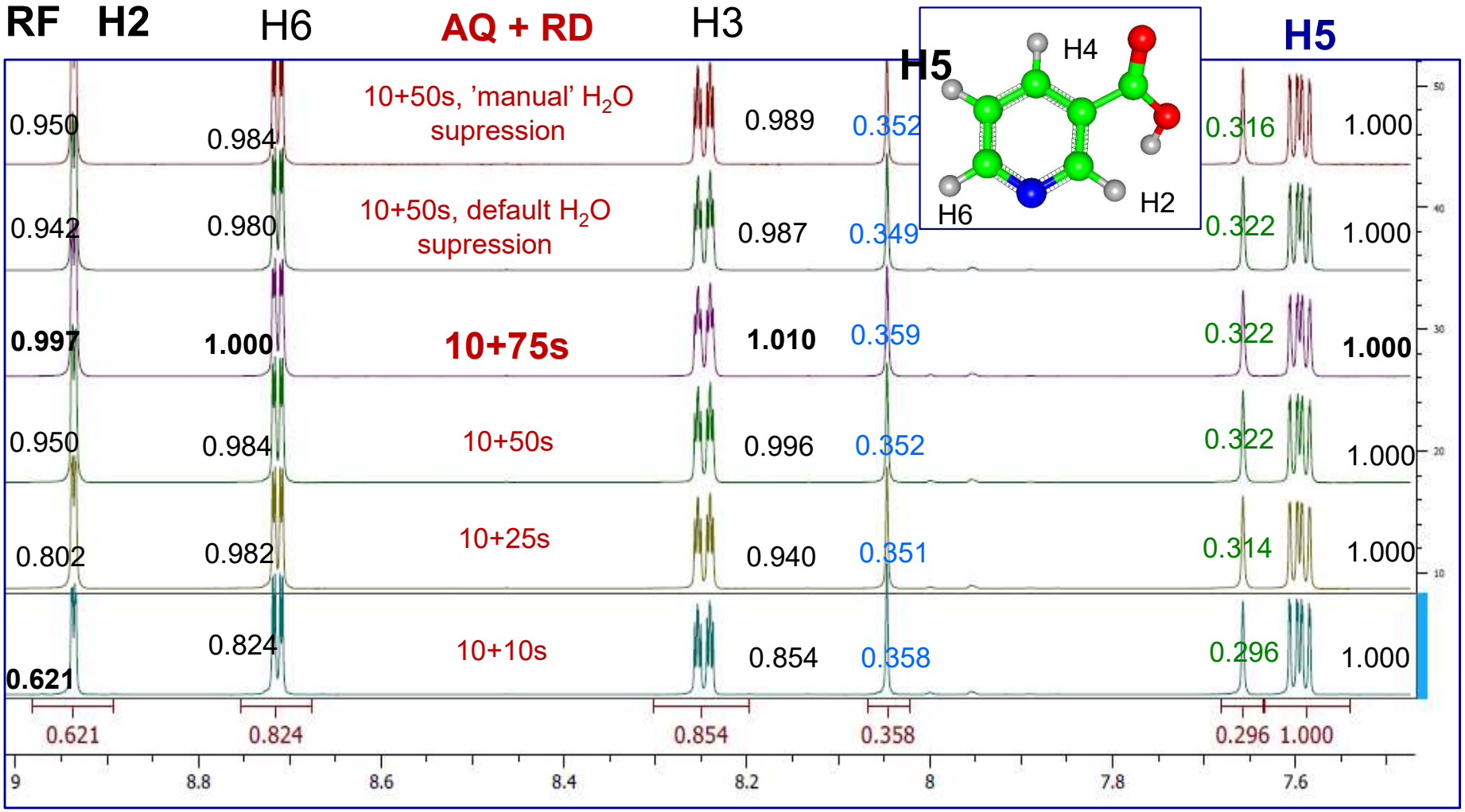
[b] Basic proton spectrum (zg): td=128k, ds=4, ns=32, AQ=7.7s, d1=2.3s and 90° pulse.

[c] Noesypresat pulse sequence (noesygppr1d): 10 ms mixing time, td=128k, ds=4, ns=8, AQ=7.7s, d1=3.0s, additional delay before suppression(d2)=49.3s and 90° pulse.

[d] As in c, but d2=0.

Nicotinic acid in ***B-FORCE tablets*** – the macromolecular (CMC = Carboxy Methyl Cellulose) effect to RF's!

RFs were obtained with the integration tool of ChemAdder, by setting the integral of H5-proton to 1.000



Conclusions

- The RF's are sensitive to the measurement, and therefore **solvent suppression** and **T2 editing** should be avoided.
- The **relaxation delays** should be long enough – in above 10s+75s was needed to equalize the response factors of nicotinic acids for the samples containing polymeric excipients - not only CMC.
- Even 1% deviation of RF from 1.0 may become significant in qQMSA analyses where the concentration range is large, and concentration components are hiding under peak jungle. Therefore, the RF's of the major component signals must be optimized if minor components are to be determined.
- **Spiking** is a solution – qQMSA protocol is under testing, follow the Letters. Spiking is also the method to detect and assign singlets in complex biofluids.