*QMSA Letters 2025*

# Why QMSA and ChemAdder

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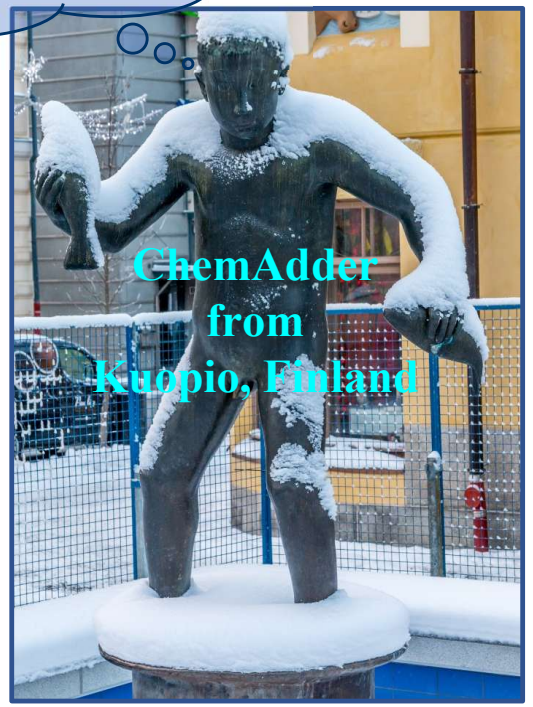
Last update July 1st, 2025

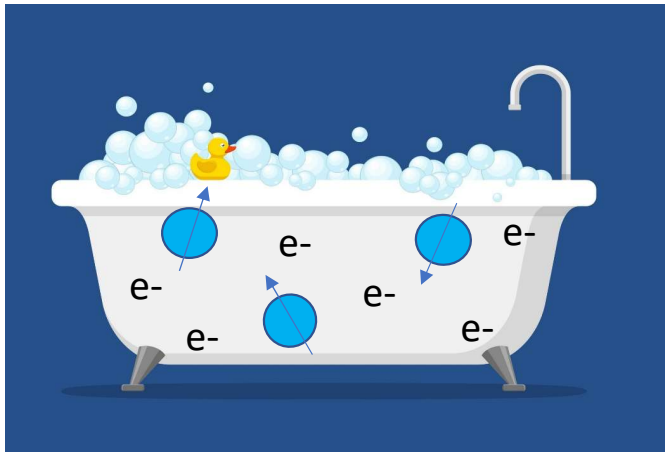
8 spins, Yess!

***LAOCOON group***, which gave the name for the early QMSA program

**>1000 spins, jeah!**
**No more PERCH !**




# From LAOCOON to ChemAdder




# qQMSA – quantitative Quantum Mechanical Spectral Analysis

- Nuclear spins hang or float in molecular electron cloud. They interact only weakly with the environment, so that the life-times of the spin-states in magnetic field are very long, seconds...even minutes. If the molecules are in isotropic environment, rotating quickly, a high-resolution NMR spectrum can be observed. The magnetic interactions (couplings) between the floating spins lead to the diagnostic fine-structure of the signals.
- The **energetics** of the spin-states of coupled protons, and the **relative transition intensities within multiplets** obey the laws of Quantum Mechanics, perfectly .
- The $^{1}$H NMR spectrum of a compound can be calculated into *very details*, using the only NMR parameters (chemical shifts and coupling constants, which do not depend on the instrument) and parameters describing line-shapes.
- The rest of the spectral area, not explained by the model, represents to other compounds (solvent, reference, and impurities) which are not in the model.

# WHY QMSA ?

Second-order spectra
Very accurate diagnostic couplings
Symmetric systems
Large systems and very large systems
Holistics: QMSA + CTLS + Xpectra + ..Prior knowledge
ASL (Adaptive Spectral Libraries)
qQMSA: no calibration – no pure reference compounds – minimal amounts - minimal bias!

# The frequency-based methods (like LAOCOON3) are impractical for systems of more than 8 coupled spins !



The dimension of Hamiltonian matrices, numbers of energy levels, and observable and allowed transitions for spin-½ systems

| Spins | Largest submatrix | Energy levels | Observable transitions[a] | Allowed transitions |
|---|---|---|---|---|
| 1 | 1 | 2 | 1 | 1 |
| 2 | 2 | 4 | 4 | 4 |
| 3 | 3 | 8 | 12 | 15 |
| 4 | 6 | 16 | 32 | 56 |
| 5 | 10 | 32 | 80 | 210 |
| 6 | 20 | 64 | 196 | 792 |
| 7 | 35 | 128 | 448 | 3003 |
| 8 | 70 | 256 | 1024 | 16 340 |
| 9 | 126 | 512 | 2304 | 59 670 |
| 10 | 252 | 1024 | 5120 | 1 67 960 |
| 11 | 462 | 2048 | 11 264 | 6 46 646 |
| 12 | 924 | 4096 | 24 576 | 24 96 144 |

The number of allowed and observed transitions for first-order spectra. For example, the transition between the ααβ and ββα states is allowed ( the total spin number changes by 1, from ½ to -½), but because all the three spins must flip simultaneously, the intensity of the *combination* transition is usually negligible, and the number of observable lines is close to the first-order number.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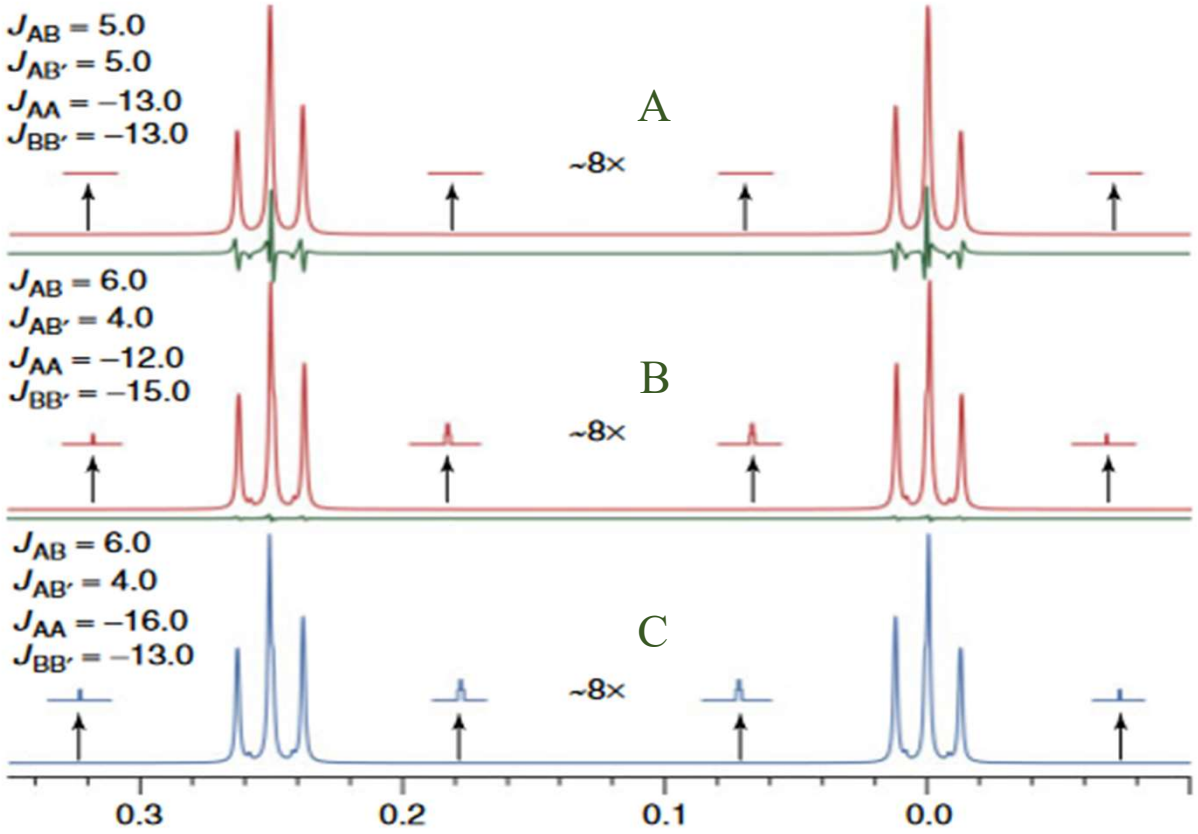
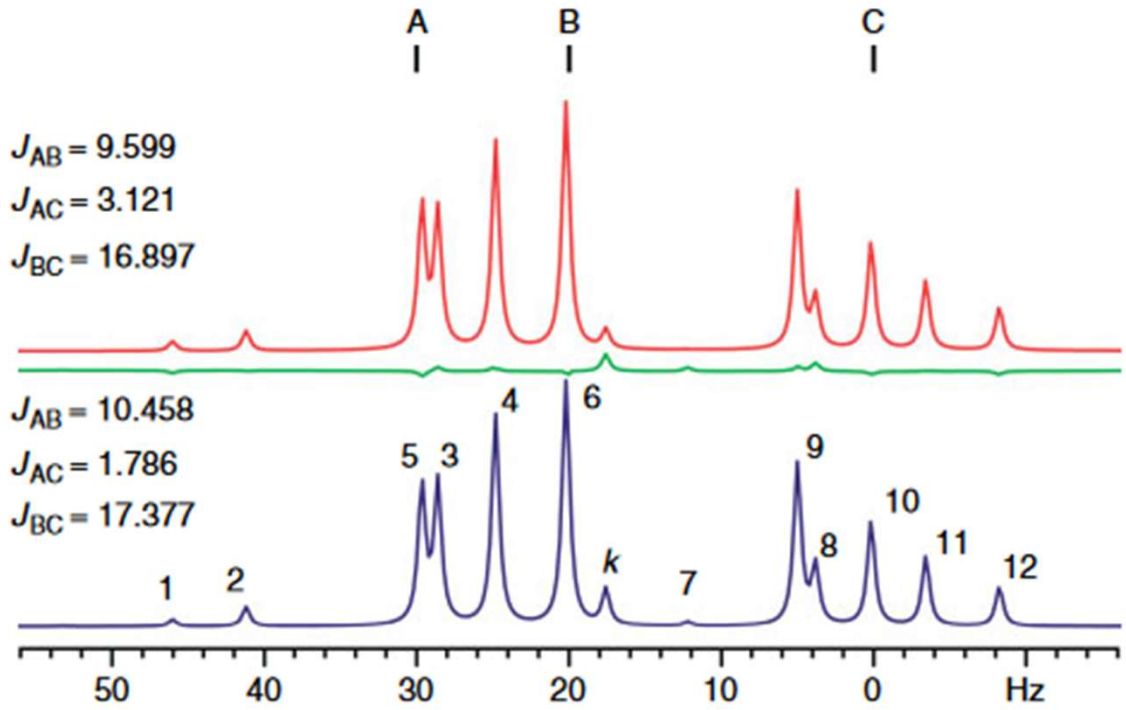
# Symmetric systems:




For example, the information about the $J_{AA'}$ and $J_{BB'}$ couplings of and AA'BB' system arising from X-$CH_2$-$CH_2$-Y is carried by the weak satellite lines, which are nonvisible in $A_2B_2$-systems (A). Only $J_{AB} + J_{BA'}$ is well defined, the information on $J_{AB}$-$J_{BA'}$ is in the small spectral details. A peculiar property of the system is that, if the iteration is started from $J_{AA'} = J_{BB'}$, the degeneracy is not broken by the iterator. The type of the substituents X and Y can be deduced from the coupling information..

Another example of useful analyses of AA'BB' system is formed para-substituted benzenes: the coupling information is substituent specific.

# Second-order spectra:




Two theoretical, strongly second-order ABC spectra are identical if only the positions of lines are compared but differ substantially with respect to the couplings. However, the intensities differ so much that they can be used to decide which one is the correct solution for acrylic acid. The spectrum consist of three quartets and one combination line (k).

# Why ChemAdder ?

Preparation of spectra for QMSA, 3D spectral parameter prediction

The spin-network size is not a limiting factor !

Spin-system packing: ChemAdder allows overlap of spin-systems !

Response factors - increase dynamic range

Line-shape artefacts (Lorentzian-Gaussian-Asymmetry + $^{13}C$ isotope shifts, virtual couplings, species specific line-shape,..)

Broadening: poor trial parameters allowed.

Essential RRMS, R2 and range

NMR-purity

**Holistics**: QMSA + CTLS + Xpectra + **prior knowledge** …

Impurity analysis & NMR purity: no calibration – no pure reference compounds – minimal bias!

ASL's & benchtop analyses

Biofluids

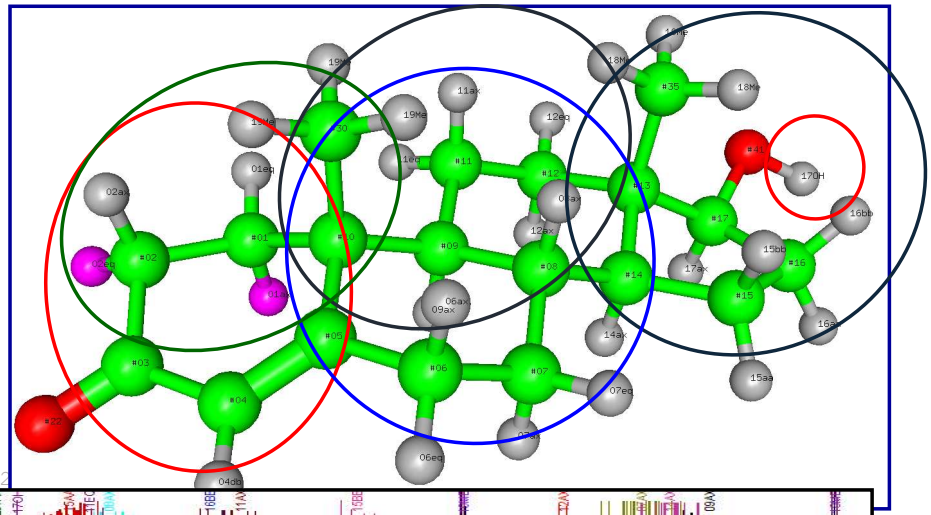
2D (HSQC)

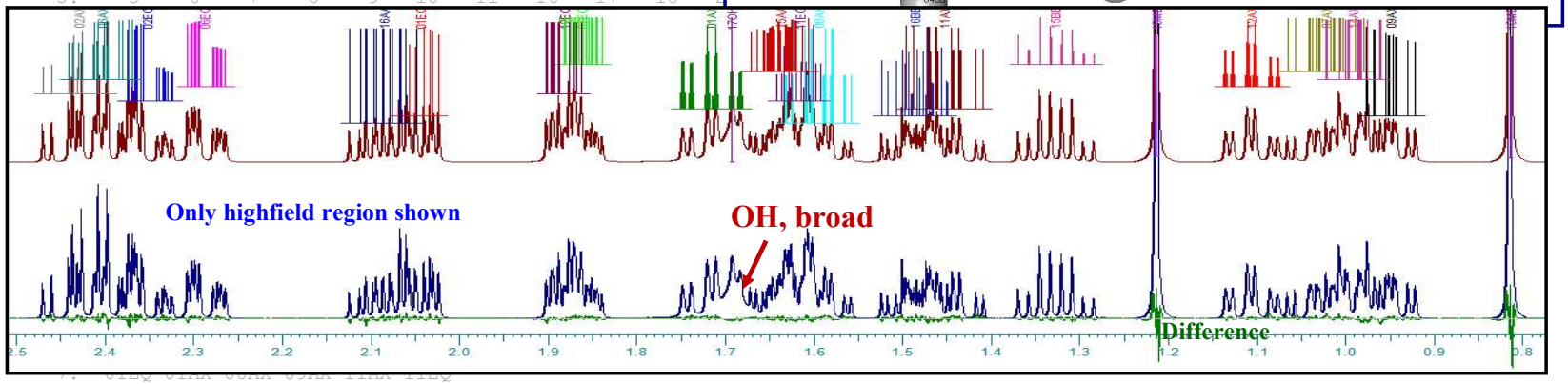
Python controlled

See more at https://www.chemadder.com/presentations/ChemAdder_Large_Systems.pdf

# Large spin-networks: Testosterone

*All the protons coupled with each others through the spin-network !*





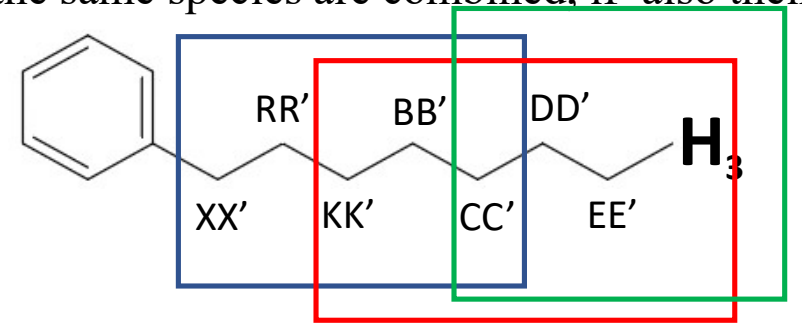

28 protons → 24-spin particles → 7 sub-systems → 25205 transitions → 1043 lines.

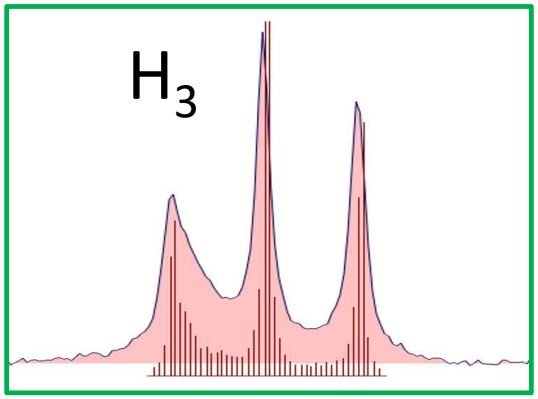
Simulation time < 1 sec, with different line-width for each particle.

# Spin-system packing: ChemAdder allows overlap of spin-systems

## A good simulation of the $H_3$ signal demands that the CC'DD' couplings are included although $H_3$ is not coupled with CC':

- Packing of spin-networks
  - XX'RR'KK'BB'CC'DD'EE'$H_3$ → **XX'RR'KK'**BB'CC' + KK'**BB'CC'DD'EE'**$H_3$ + CC'DD'EE'**$H_3$**
  - Transitions separated by < 0.01 Hz and belonging to the same species are combined, if also their derivatives are similar.
  - 58 000 000 → 24 000 Transitions, from 64 to **4** sec
- Multithreading offers a further speed up in calculations.




"Spin dust"

*$H_3$-signal is composed of thousands of non-degenerate transitions – which yield its diagnostic outlook.*

*The effect is not rare, for example leucine methyl signal has a similar shape.*

***How to describe the shape in another way than QMSA, we ask?!***

# FELIX of MILLIONS TRANSITIONS*

**Experimental:** normal $^1$H spectrum,

*Spin-system of **74** protons :*

XX'RR'AA'BB'CC'DD'EE' $H_3$(Ar-octyl) +

XX'RR'ZZ' BB'CC'$H_3$ (O-hexyl) +

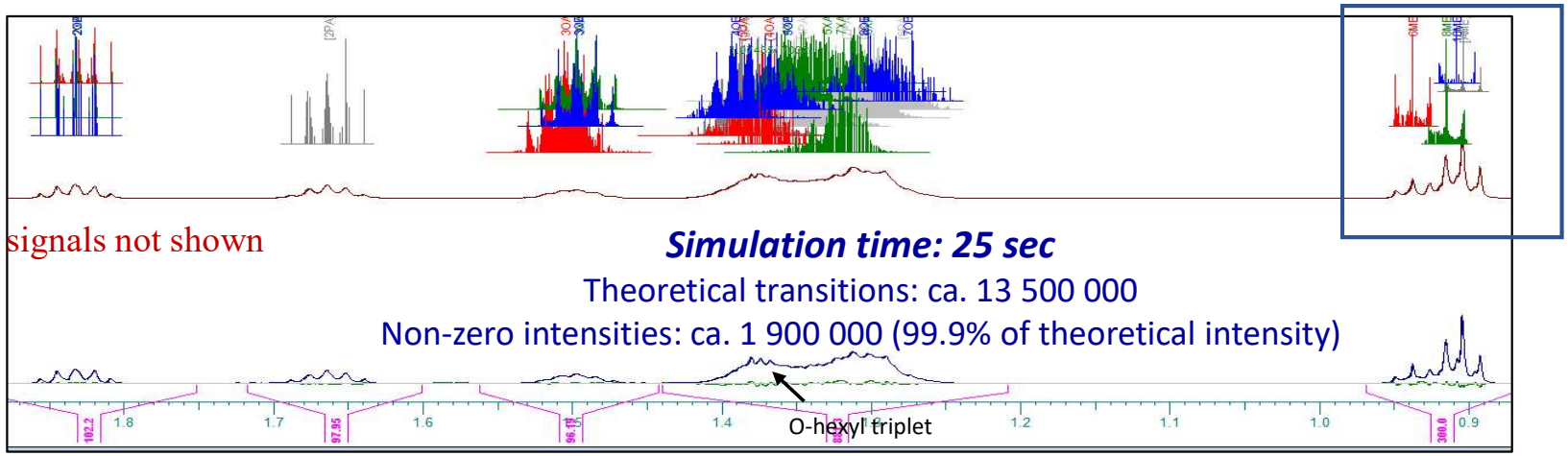
XX'RR'ZZ' BB'CC'DD'EE'$H_3$ (O-octyl) +

XX'RR'ZZ'BB'(CC')$_3$DD'EE'$H_3$ (O-decyl) +

XX'YY'ZZ'

(5 mol% *chiral incredient* with F was not included in QMSA)

*See S.K. Ahola, L.P. Ingman, R. Laatikainen, J. Sinkkonen and J.P. Jokisaari, *$^{21}$Ne and $^{131}$Xe NMR study of electric field gradients and multinuclear NMR study of the composition of a ferroelectric liquid crystal.* J.Chem.Phys. **149**, 234901 (2018). https://doi.org/10.1063/1.5052499




$OCH_2$ signals not shown

***Simulation time: 25 sec***

Theoretical transitions: ca. 13 500 000

Non-zero intensities: ca. 1 900 000 (99.9% of theoretical intensity)

Non-zero intensities: ca. 1 750 000 (93% of theor. intensity)

Lines after packing: ca. 135 000

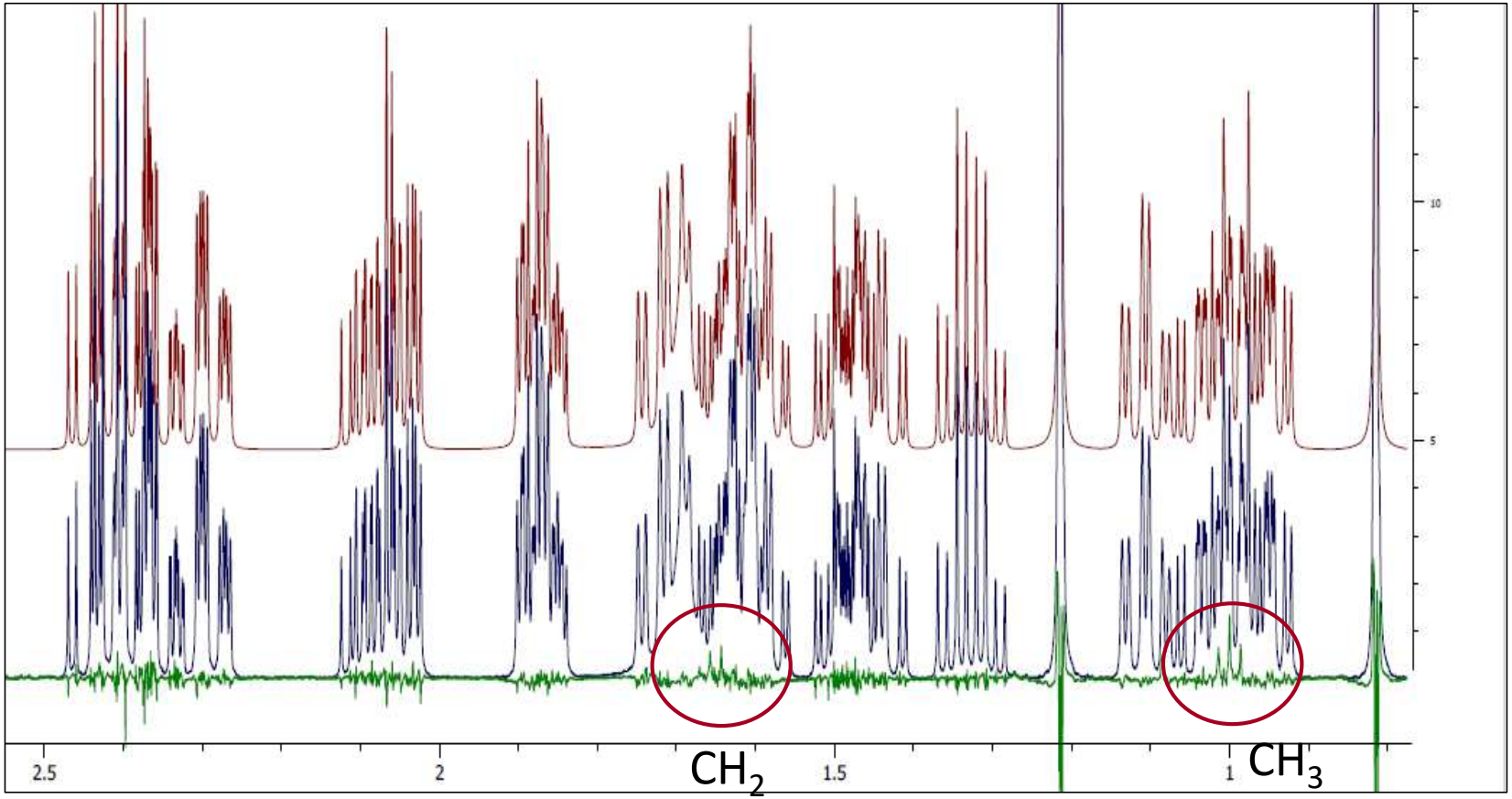
*Although the $CH_2$-shift order is somewhat unclear, the analysis gives the alkyl lengths with a fair confidence*

# *QMSA reveals impurity signals which are hiding in peak jungle*



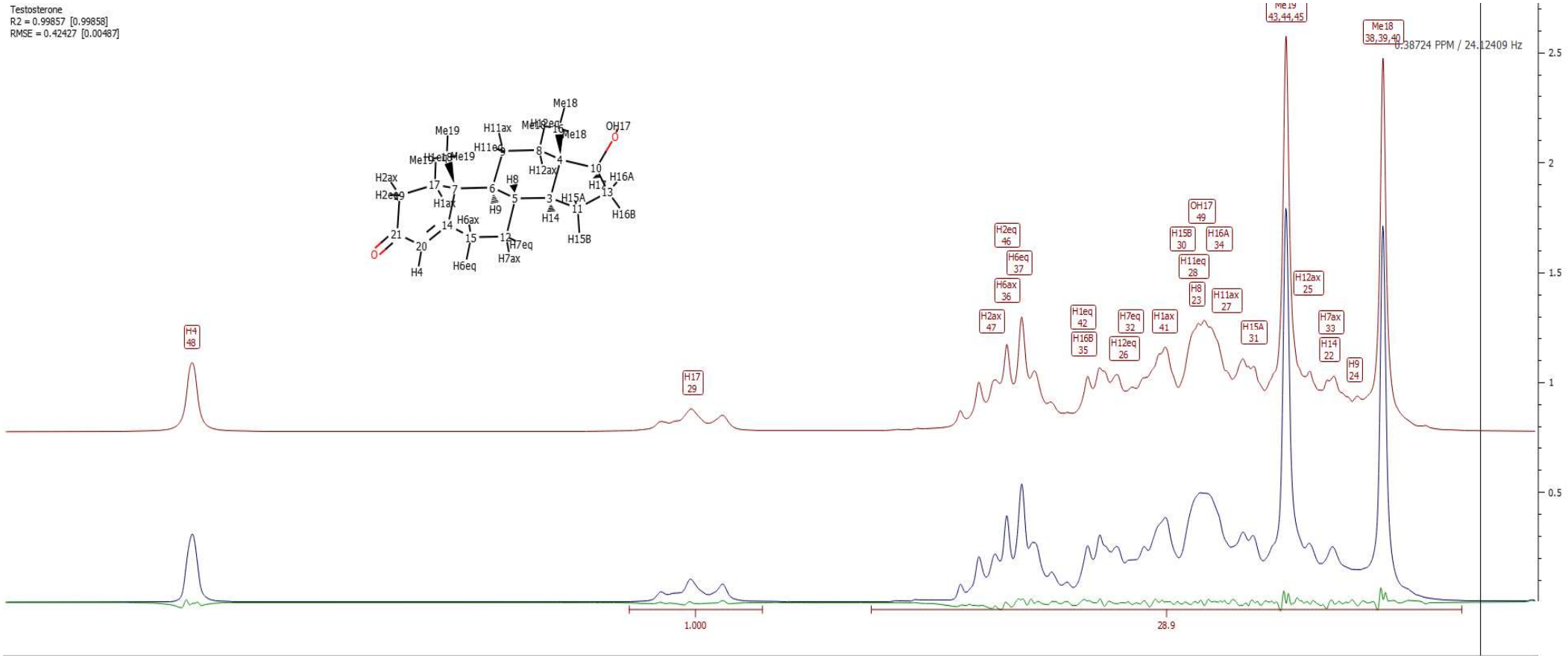
QMSA of Testosterone + ca. 0.40 wt% $CH_3CH_2R$-impurity

**fitted by ignoring the impurity:**

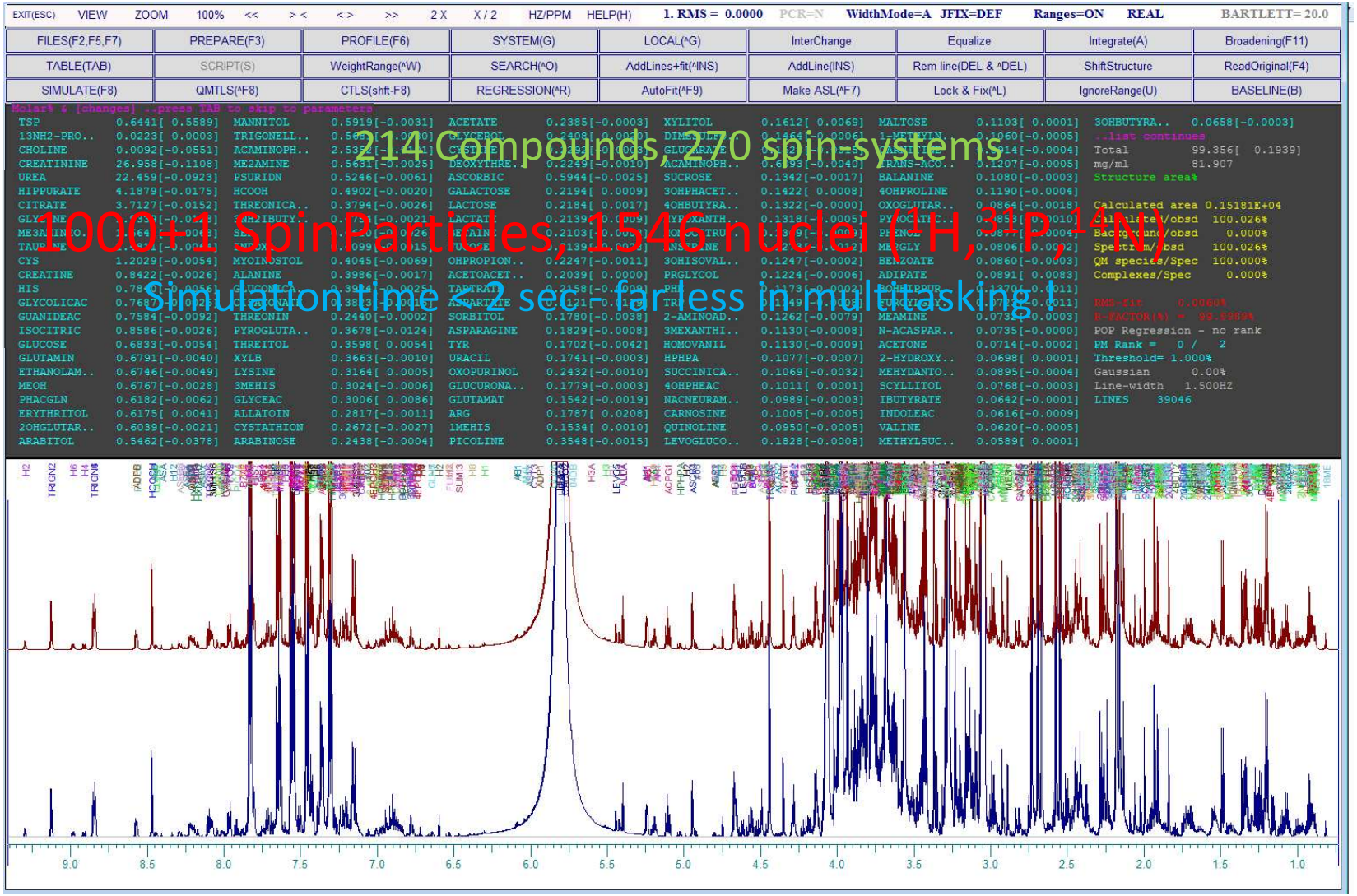


# The parameters from the analysis of high-field spectra can be used to analyse the testosterone 60 MHz Benchtop spectrum.

The coupling constant are field and concentration independent, but chemical shifts may need refinement.

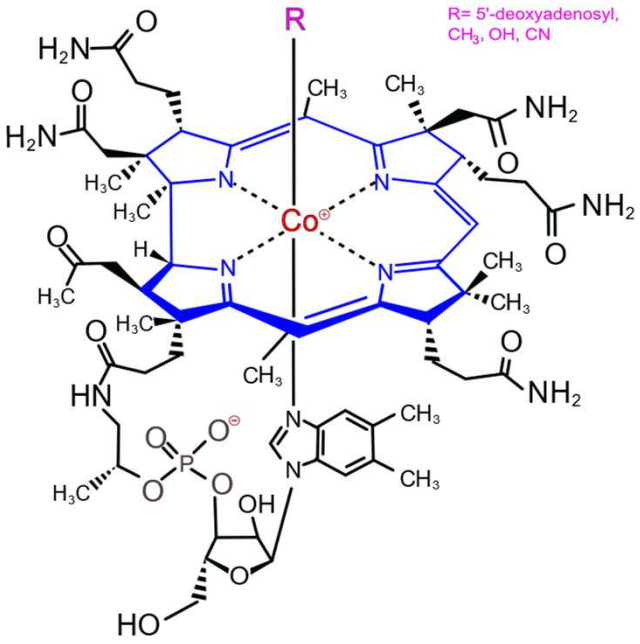


# Biofluid analyses

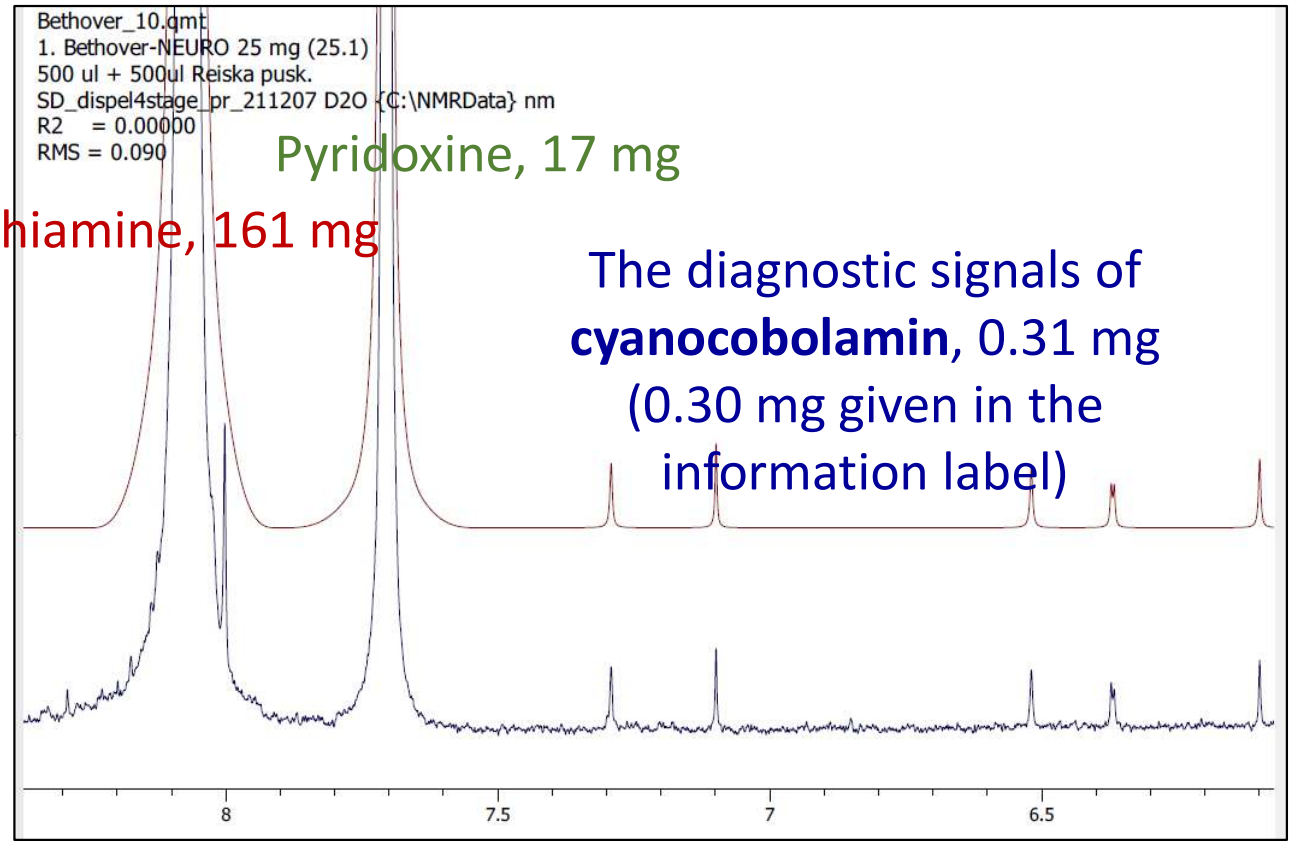


# Analysis of $B_{12}$-vitamin in *Bethover NEURO* capsules for B-vitamin supplementary






Cyanocobolamin ($B_{12}$vitamin):
MW =1355,
52 chemical shifts



# QMSA at BENCHTOP(40-80 MHz)

# QMSA at BENCHTOP(40-80 MHz)



Solve parameters at high field – apply at low field

PROS:

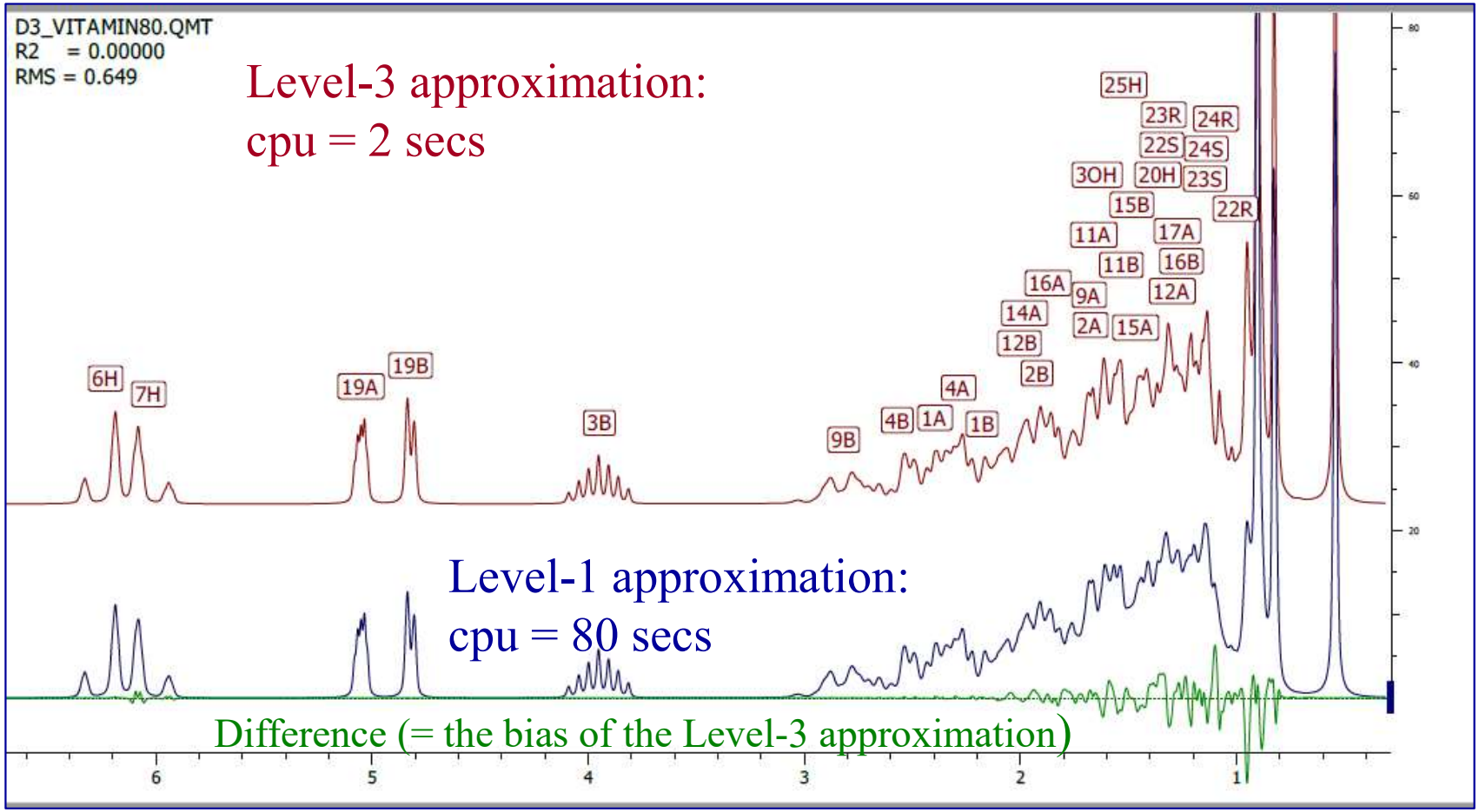
- Chemical shifts variations less significant – recalculation of spectrum is not needed so often – if at all – during QMSA
- Costs ??

CONS:

- Sensitivity – higher concentrations – longer measurement times – concentration effects to shifts
- Simulation times for large spin-systems
- Overlap of signals
- Limited number of analyzable components – simple mixtures of simple compounds

# $D_3$-vitamin 80 MHz simulation quality and time depend on *level of approximation* (1-3)



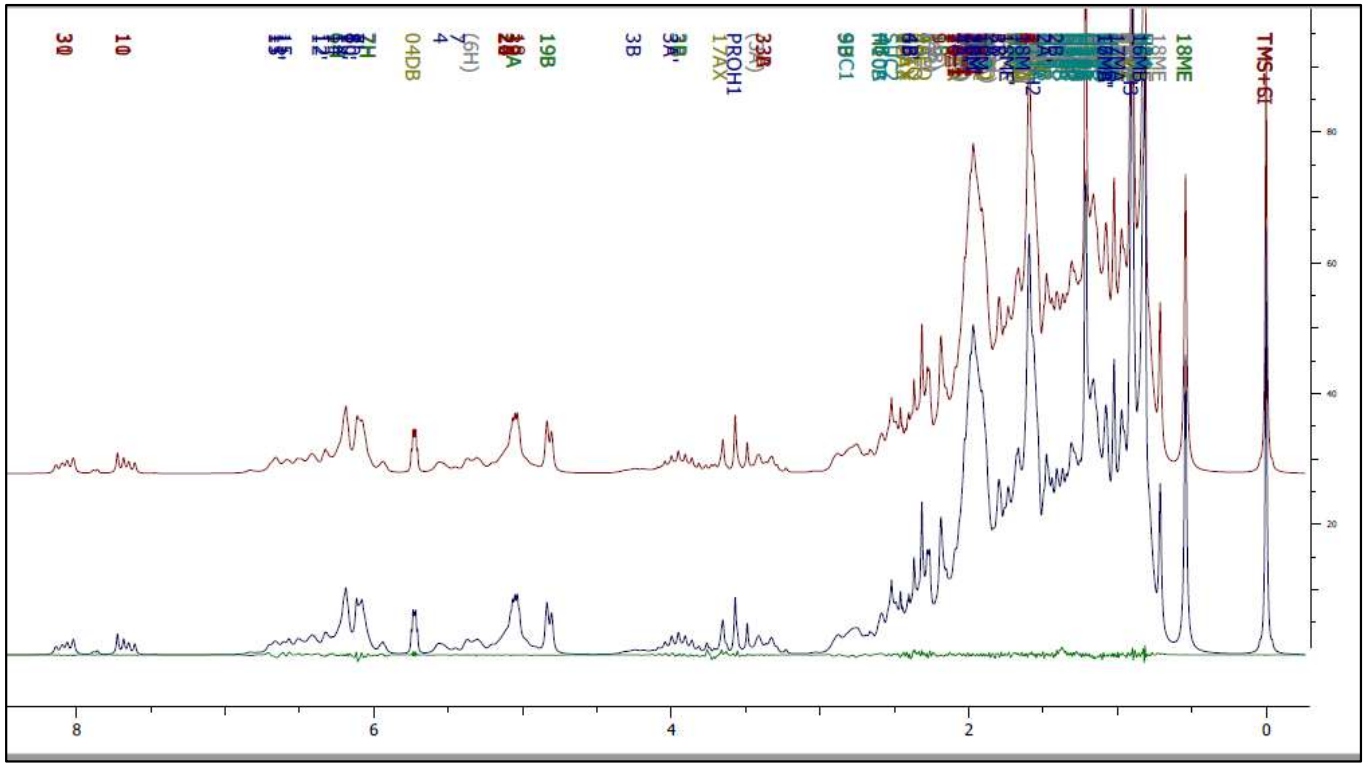


**ANALYSIS of MIXTURE of D3-VITAMIN, CALCIFEROL, TESTOSTERONE, CHOLESTEROL, A-TOCOPHEROL, MENAQUINONE-7, LUTEIN, DOCOSAHEXAENOICACIDETHYL ESTER (10 mg each) + 1 mg of PROPANOL, SYNTHETIC SPECTRUM at 80 MHZ**

The spectrum was simulated at approximation level 2 (5 min), then analyzed with highest approximation level 3 (< 5 sec/cycle).

When the 250 chemical shifts & the line-widths were optimized (to compensate the approximation bias), RMSE dropped from 0.42 to 0.14% and gave the concentrations within bias < 3%.

**Conclusion:** benchtop analyses of large molecule systems can be done in acceptable time.

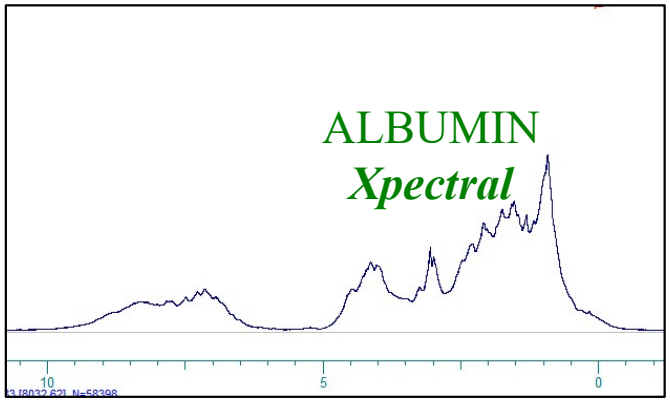


# Biofluids

## QM + CTLS

# Biofluids.. Xpectrals

- $^{1}$H NMR of serum = *QM-spectra* + *Xtructures* + *Xpectrals*
  - QM-spectra: glucose and other small molecule metabolites
  - Xtructures: lipoproteins
  - Xpectrals: albumin, cholesterol
  - ***Spiked QMSA (sQMSA)*** …to confirm assignments of singlets and to compensate RF bias!

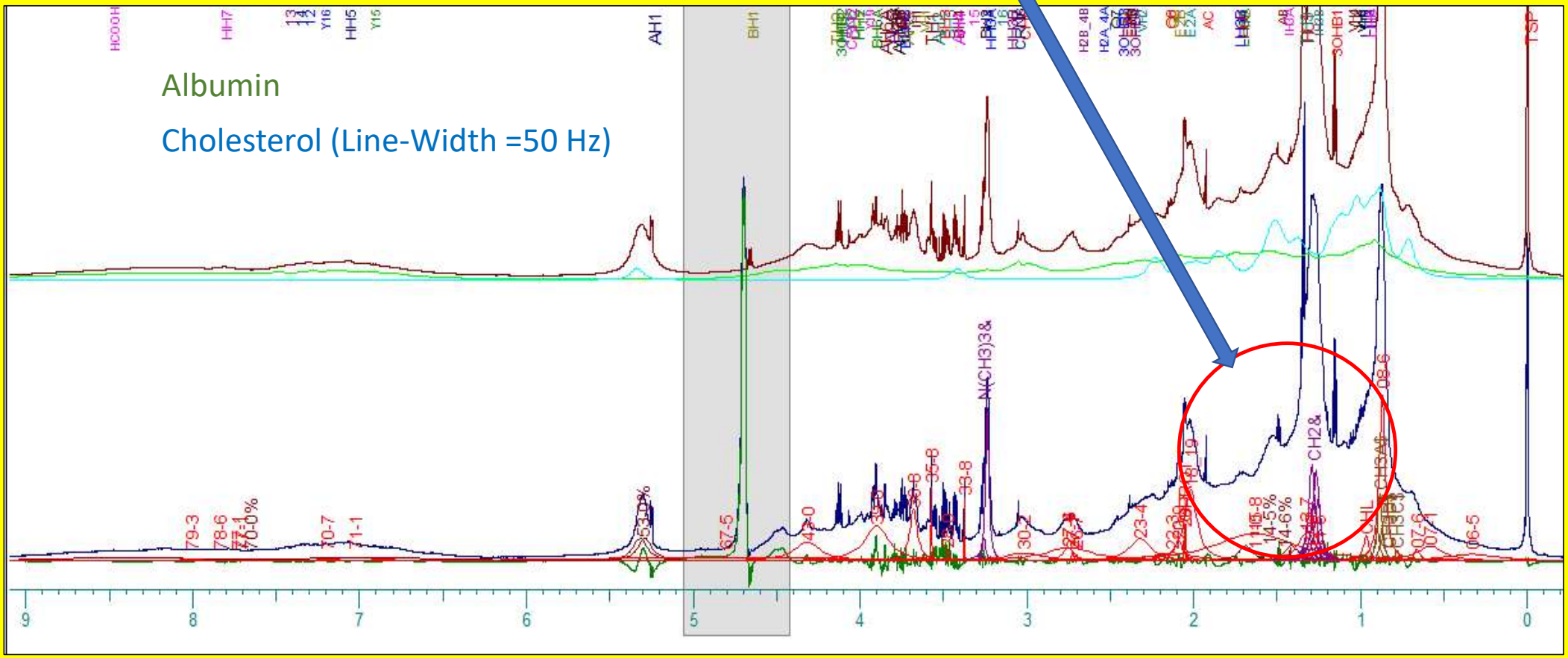



The **spectral xtructures** can be singlets, regular or less regular (several options) multiplets. In the less regular multiplets, either line-spacings, intensities and/or line-widths are allowed to vary.

# *Holistics:* QM-spectra + Xtructures + Xpectrals

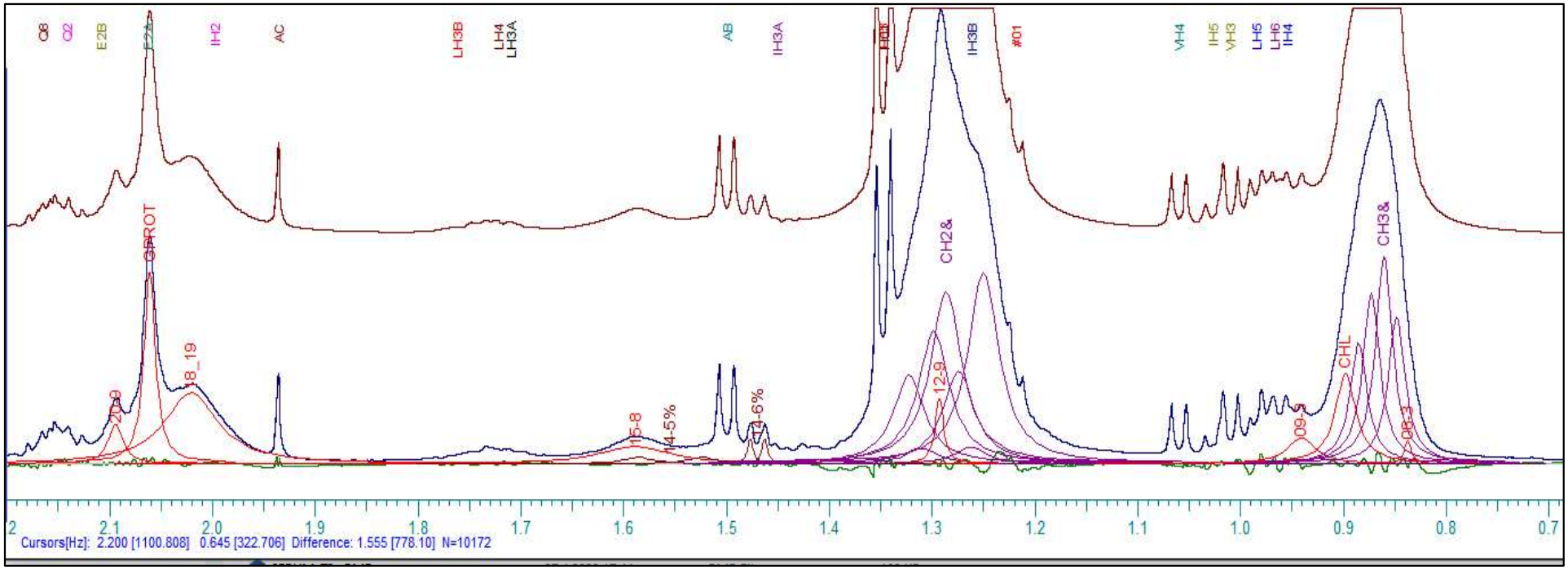
## Yields more pure lipoprotein signals ?





# T2 edited serum spectrum with QM & three types of ***xtructures***

- Singlets
- Regular doublets (X%)
- Multiplets with varying line-intensities, constant line-spacings and line-widths (lipoproteins, CH2& and CH3&)



# Analysis of 1001 spin particles: urine



1001 spin particles, 1546 nuclei ($^{1}H$,$^{31}P$,$^{14}N$)

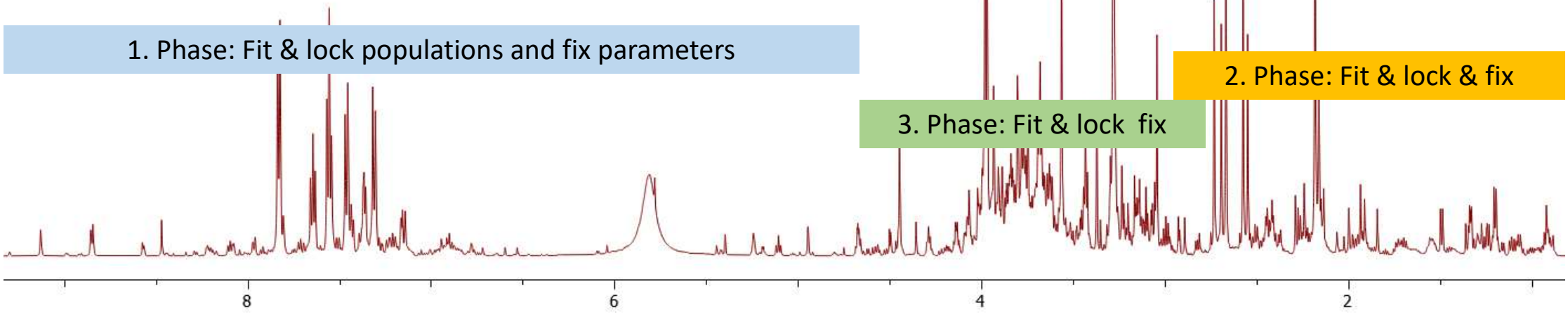
214 compounds with 270 spin systems

Simulation time < 2 s, << 2 s with multithreading

1. Phase: Fit & lock populations and fix parameters

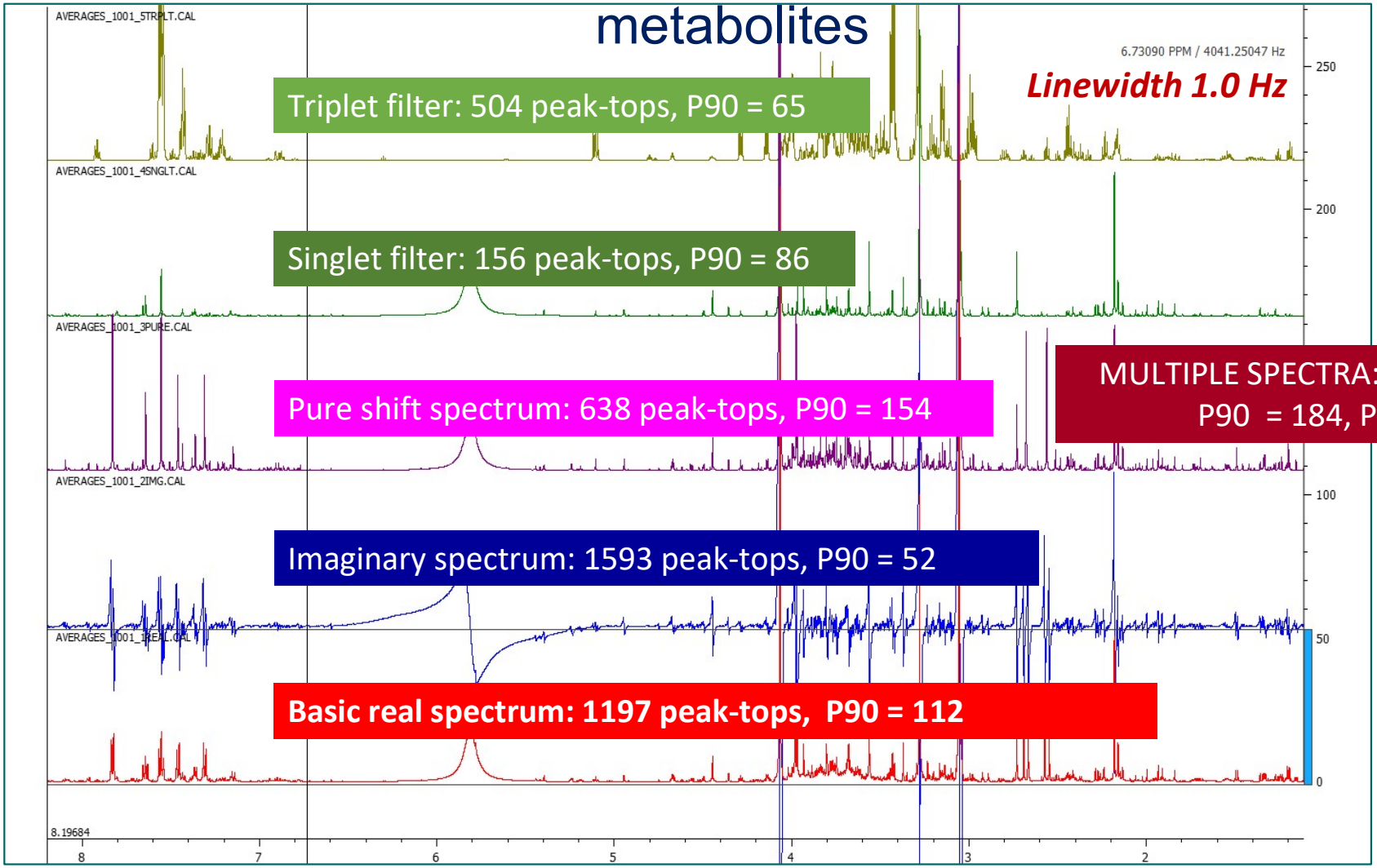
2. Phase: Fit & lock & fix

3. Phase: Fit & lock  fix



# URINE 1001 particles, multiple spectra QMSA (mQMSA) of 214 metabolites






P90 = No. of compounds having at least one **DIAGNOSTIC** 90% purity signal

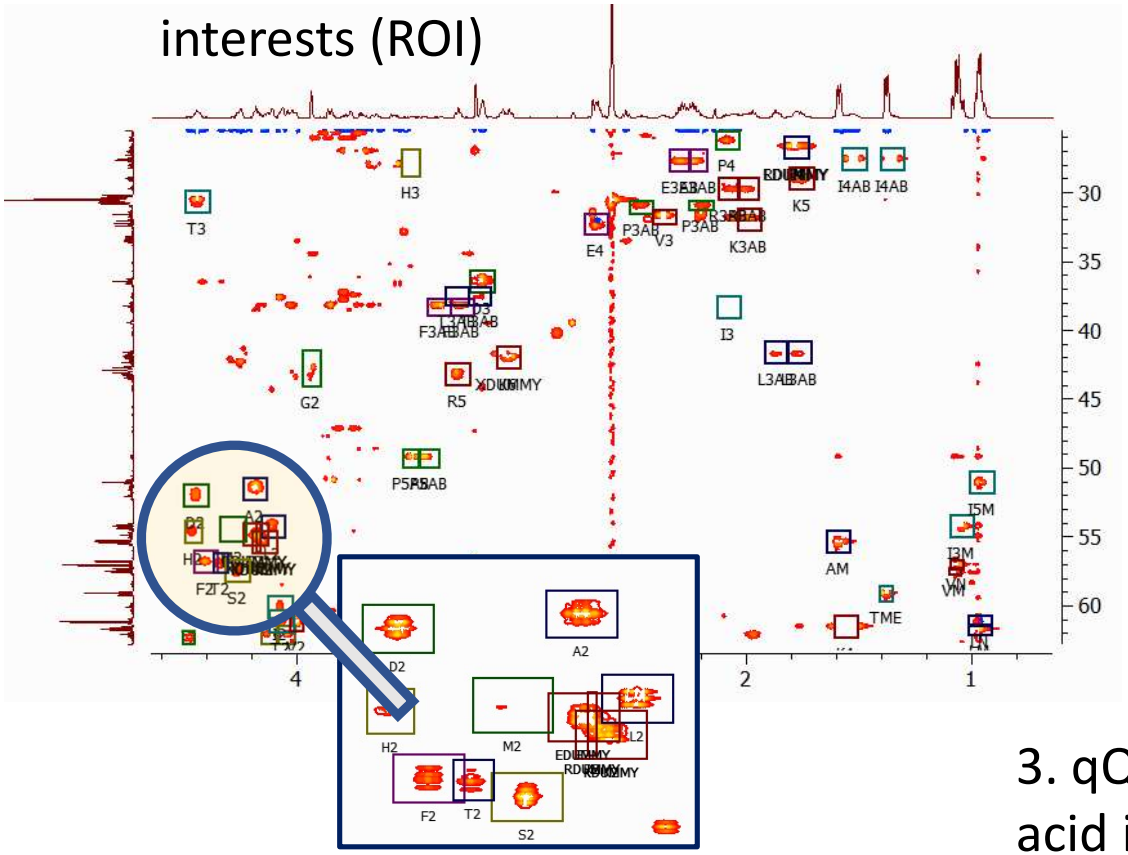
# QMSA of 2D spectra

# HSQC of amino acid $^{13}C$ isotopomers 2D spectrum to VIRTUAL 1D spectra: metabolic flux analysis 1/2

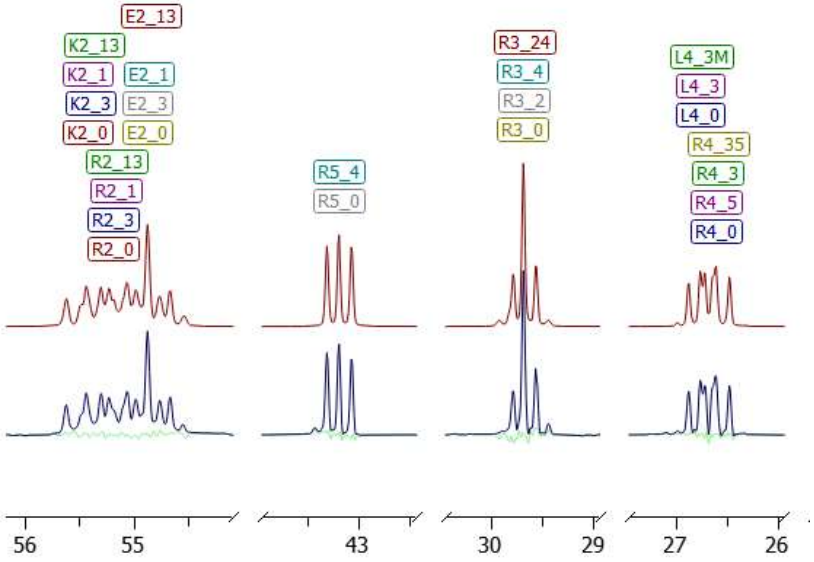


Collaboration with Technical Research Centre of Finland (VTT)

1. Extraction of regions of interests (ROI)




2. 2D → 1D F2 Projections

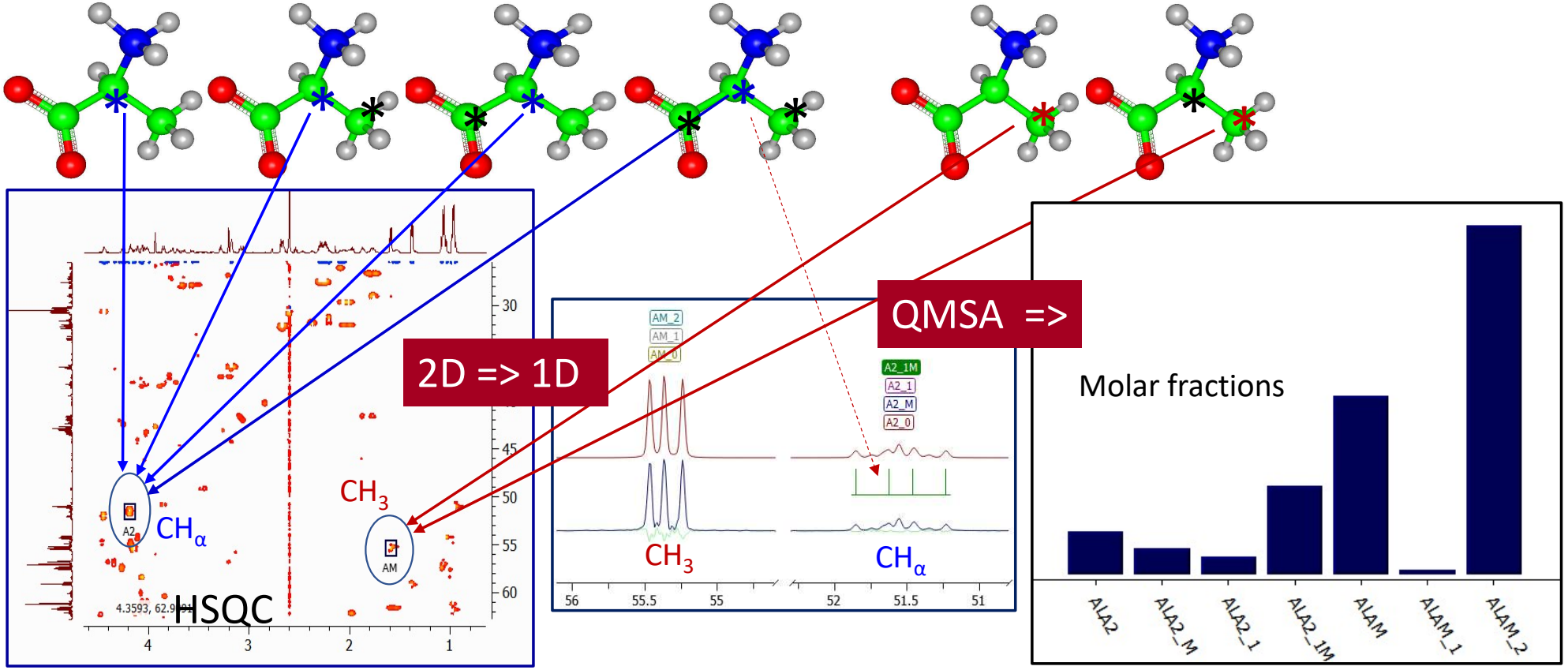



3. qQMSA → molar fractions of amino acid isotopomers

# HSQC of amino acid $^{13}C$ isotopomers 2D spectrum to VIRTUAL 1D spectra: metabolic flux analysis 2/2



## Alanine $^{13}C$ isotopomers:



# QMSA - pros



- Complete QMSA in a few minutes!
- Overlapping signals and variation of shifts – a challenge for integration protocols.
- Complex or second-order spectral structures – a challenge for deconvolution protocols.
- From spectral storage to qNMR and special applications.
- ASL's: one spectrum – one file – any field & line-shape & shifts – even from poor spectra and mixtures - no experimental artefacts – compression factor of > 90% - prior knowledge
- Chemical confidence – not only concentrations – also unknown compounds can be characterized.
- $^{13}C$ Satellites can be defined in ASL files – like creatinine in urine.
- Accurate peak-lists – pattern search, etc..
- Integral transforms – iteration of poor trial parameters – fast screening for maximum amount of a compound.
- Achieving and export of NMR data to journals and their supplementary... instead of raw spectra – an opportunity!

Maximum amount of information with minimum number of parameters!

# Holistic QMSA(hQMSA)

A spectrum data may contain different type NMR signals, needing different models:

- Quantum Mechanically modellable signals
- Xtructures (singlets, multiplets), like polymer and lipoprotein signals
- Xpectrals, like albumin spectrum
- *Xpurities ('WeakPeaks')*
- Signals defined by Integral ranges
- The common point is that the signal area/nucleus is the same:

  Total area = QM + Xtructures + Xpectrals + Xpurities + Integrals

All the species can be handled in one model by ChemAdder !

# Acknowledgements

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Ben Shapiro, US Pharmacopeia, Rockville, MD, USA
Sunil Paudel, US Pharmacopeia, Rockville, MD, USA
Yang Liu, US Pharmacopeia, Rockville, MD, USA
Yrjö Hiltunen, UEF