QUANTITATIVE NMR IN PROFILING OF BIOREFINERY PRODUCTS

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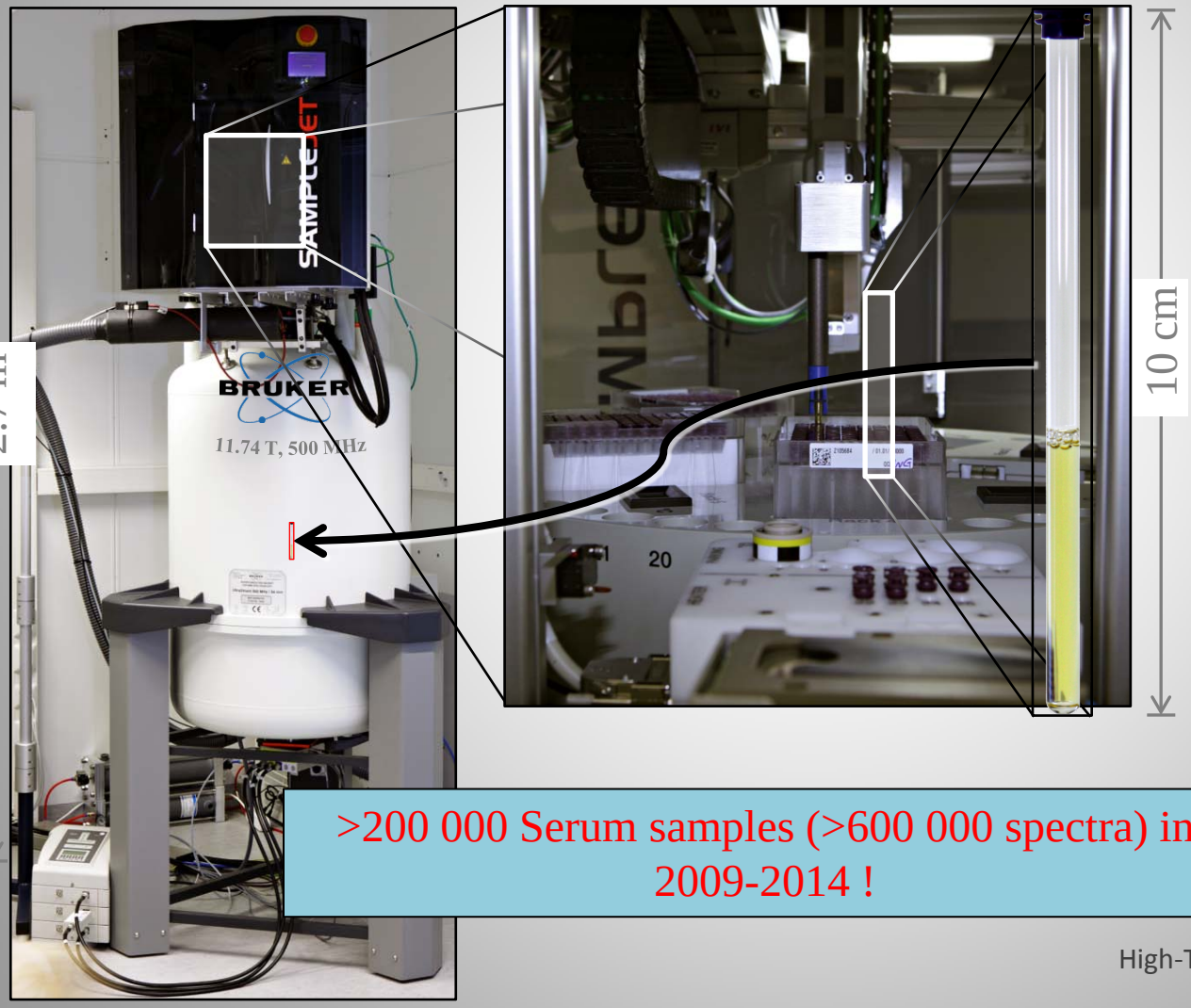
NMR METABONOMICS LABORATORY (MLAB)

HIGH-THROUGHPUT NMR METABONOMICS

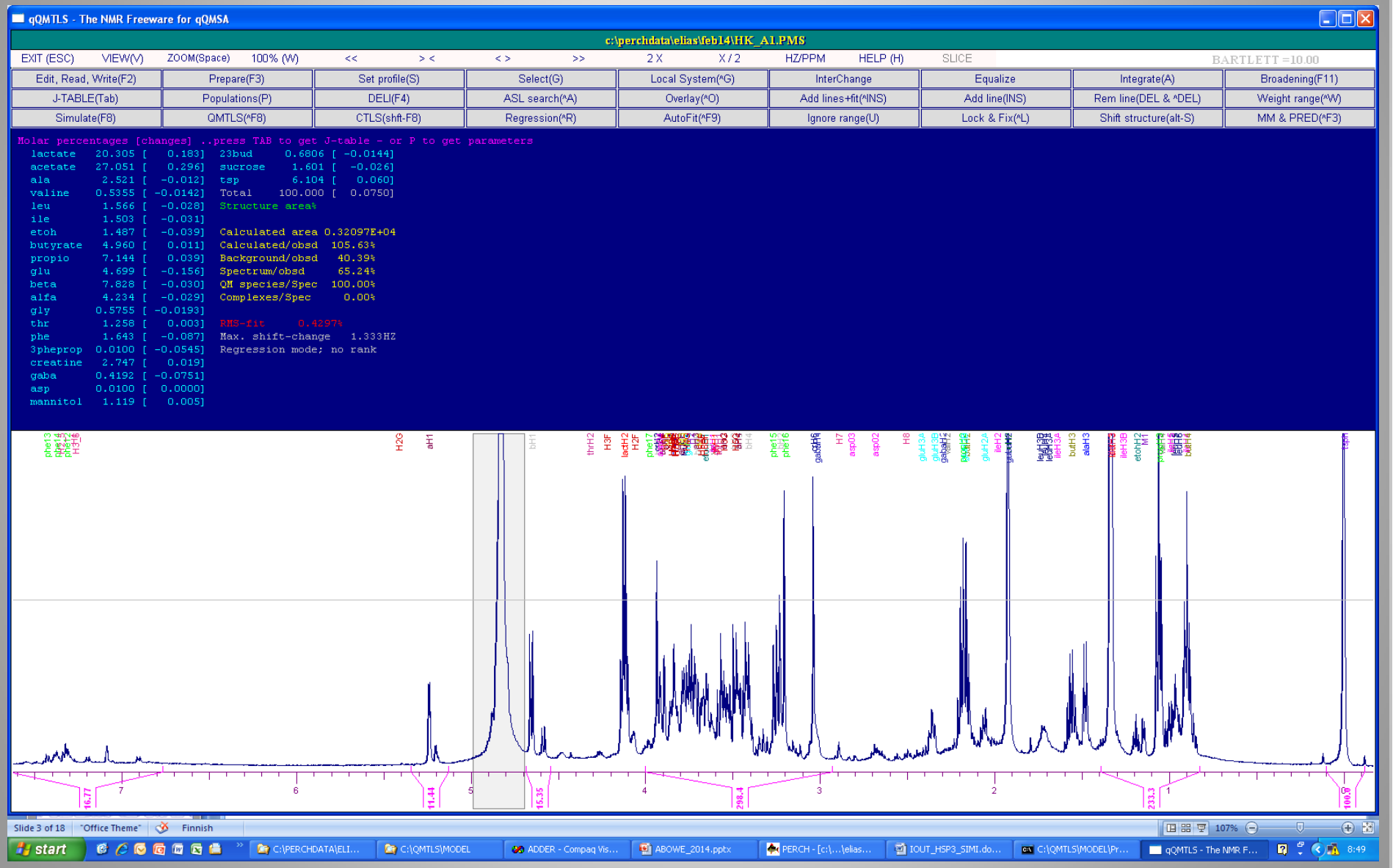


- Sample into magnet
- Heat sample to +37°C
- Tune &Homogenise magnetic field
- Measure data
- Analyze data
- Make conclusions

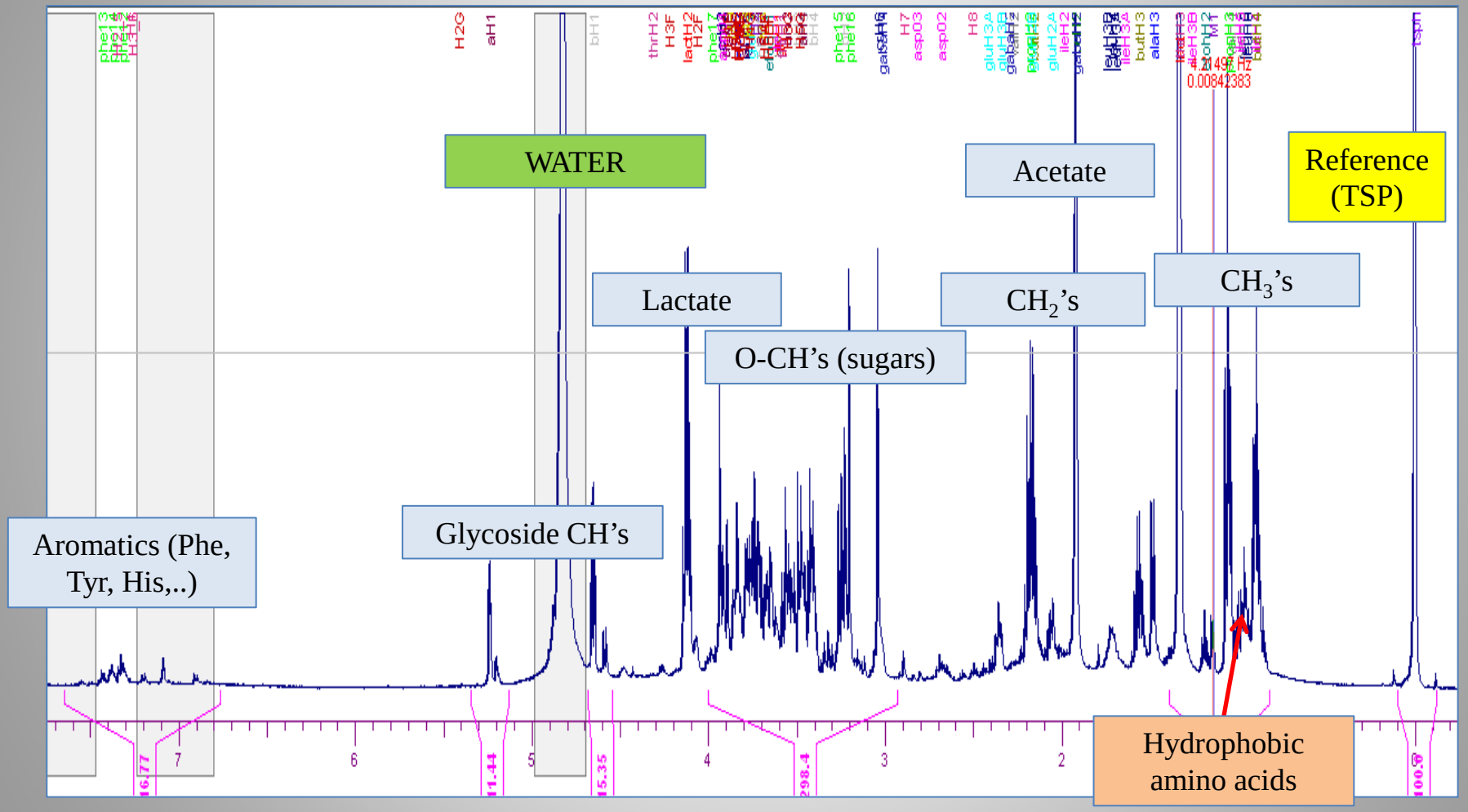
>200 000 Serum samples (>600 000 spectra) in 2009-2014 !

QMTLS (Quantum Mechanical Total-Line-Shape) Analysis

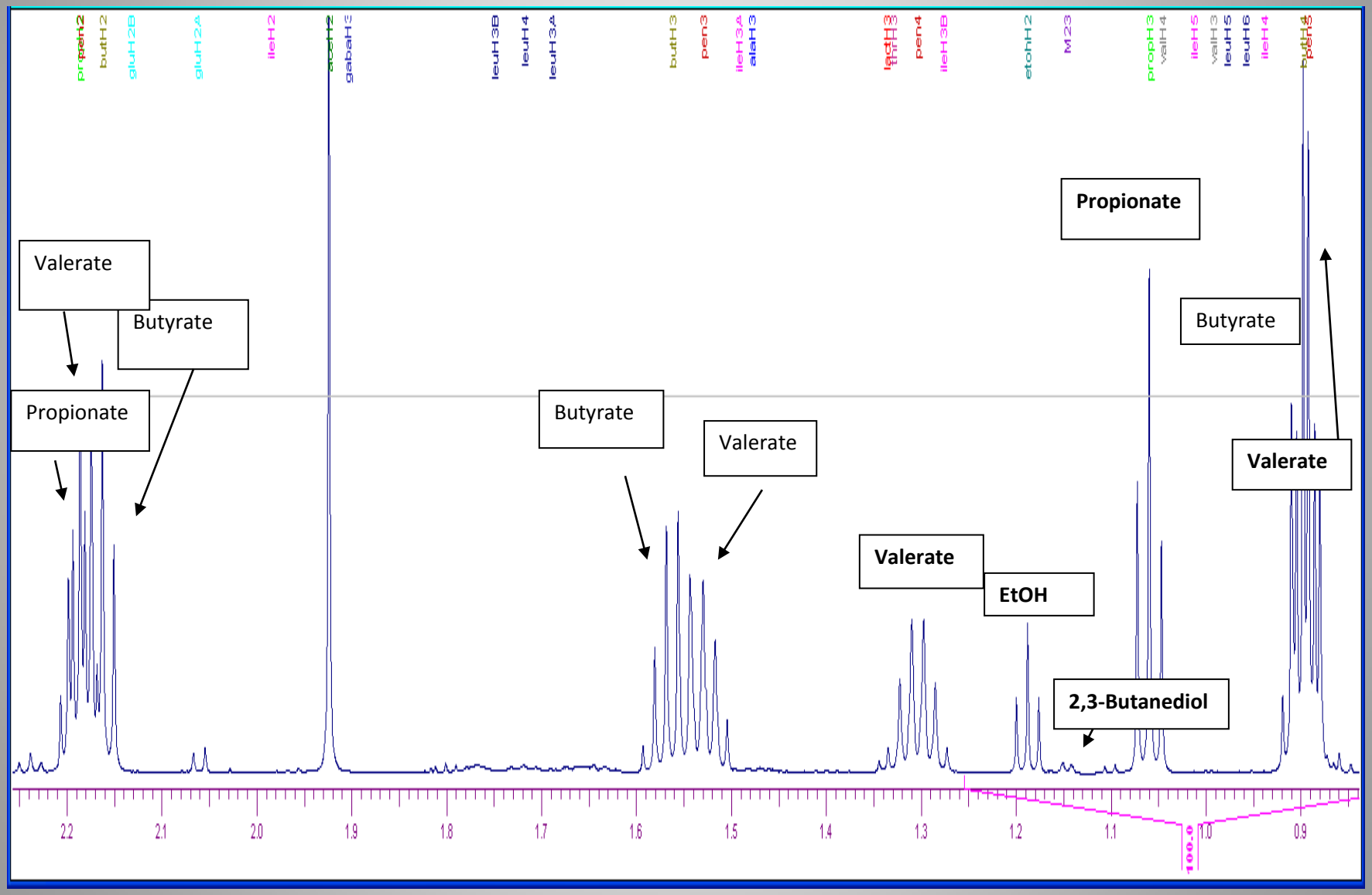
User interface of program qQMTLS



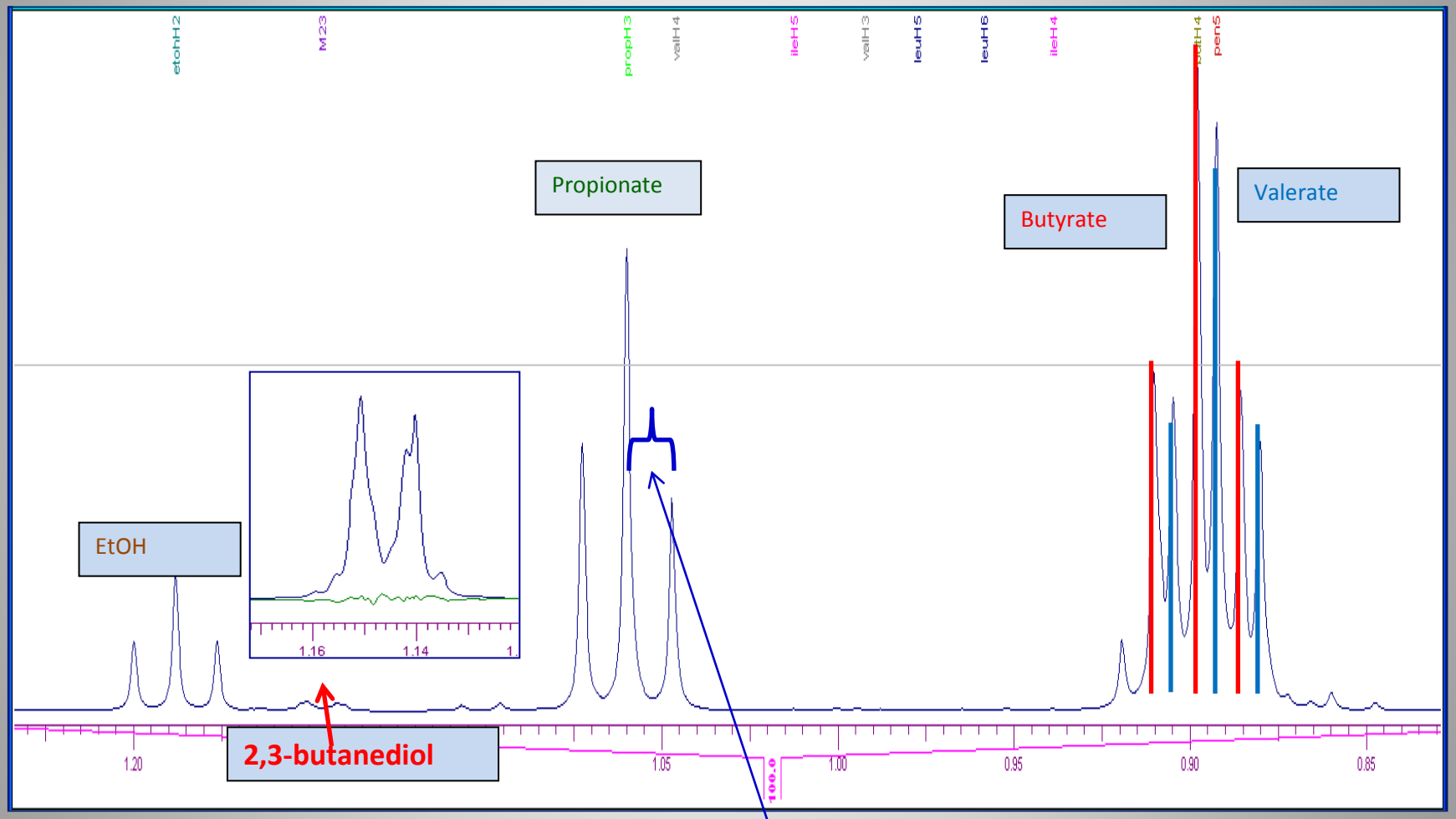
NMR Spectrum (at 600 MHz) of an ABOWE sample



Aliphatic Region

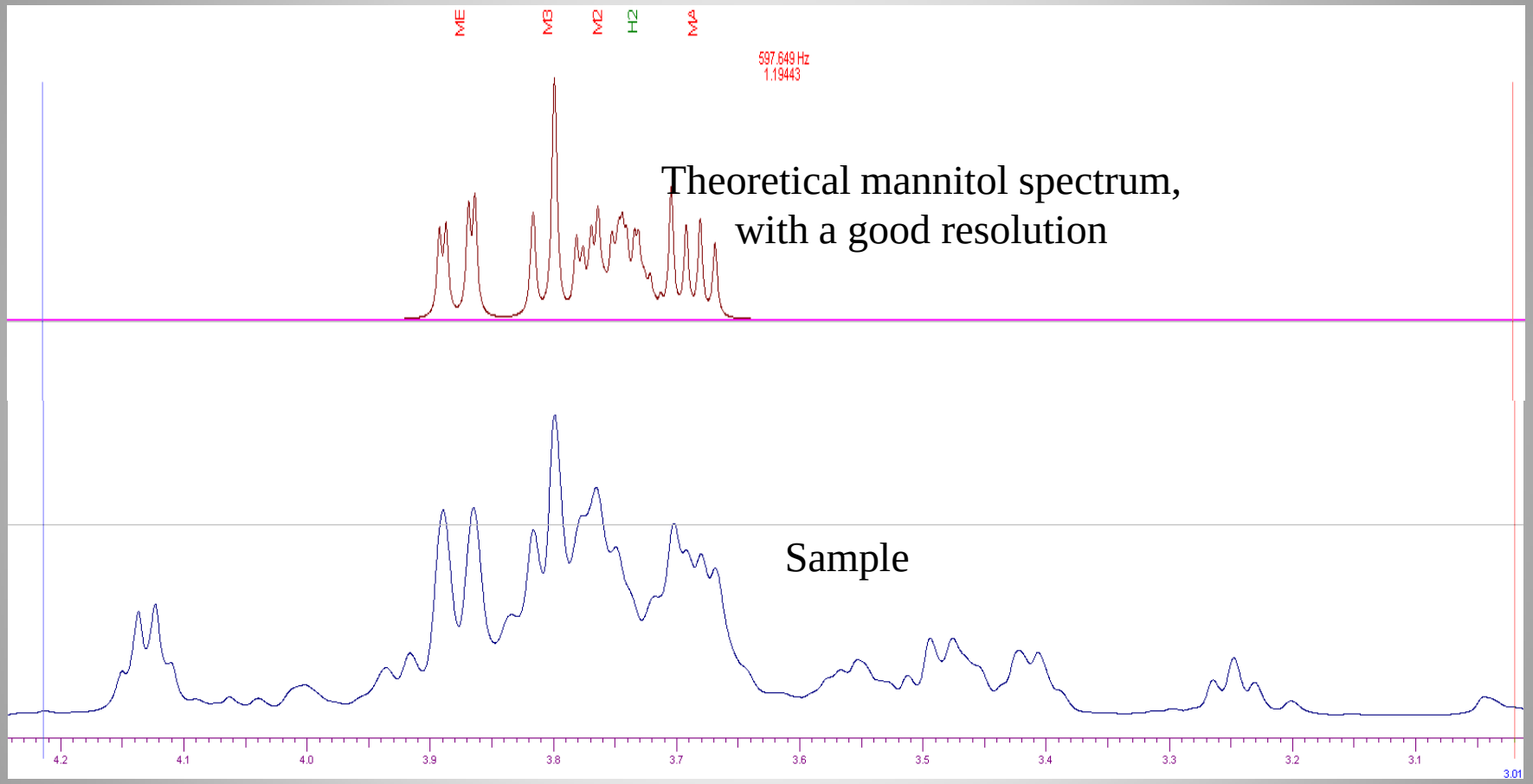


2,3-Butanediol has a unique signal



A compound can be identified also from splittings (coupling constants) of multiplets: couplings do not depend on instrument or sample

Detection of mannitol



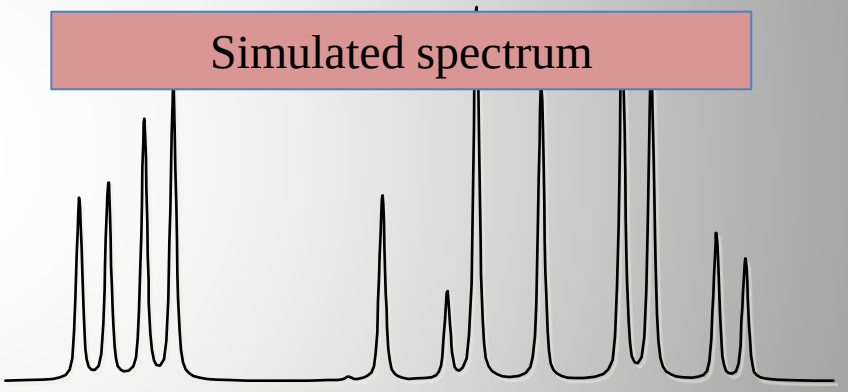
Spiking is sometimes used to ensure identification a component in complex samples

If chemical shifts, coupling constants & line-shape are given, spectrum (even the smallest details) can be simulated quantum mechanically !

ν_A, ν_B, ν_C

J_{AB}, J_{AC}, J_{BC}

QMTLS

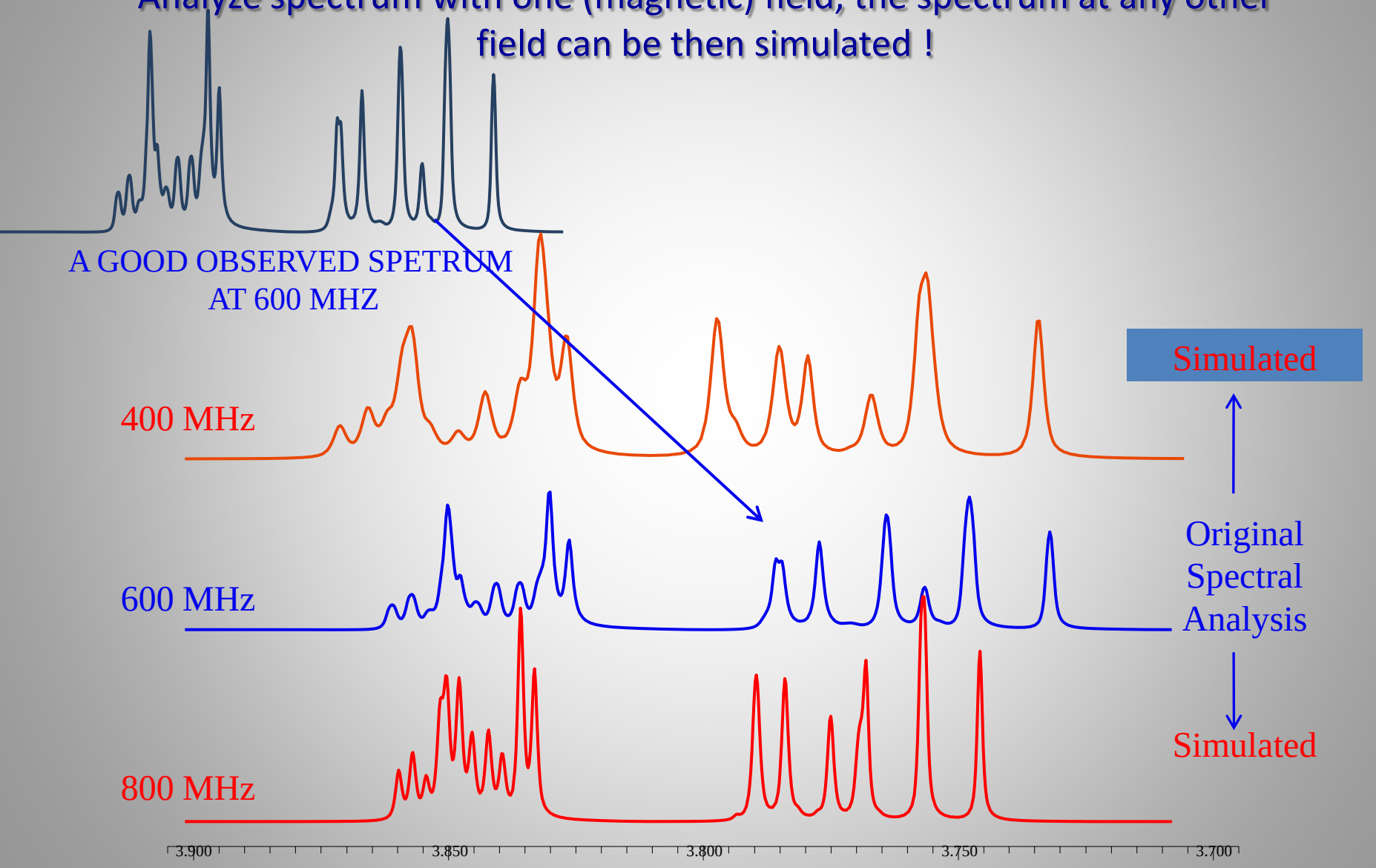


=> Model spectra (for quantitative analysis)

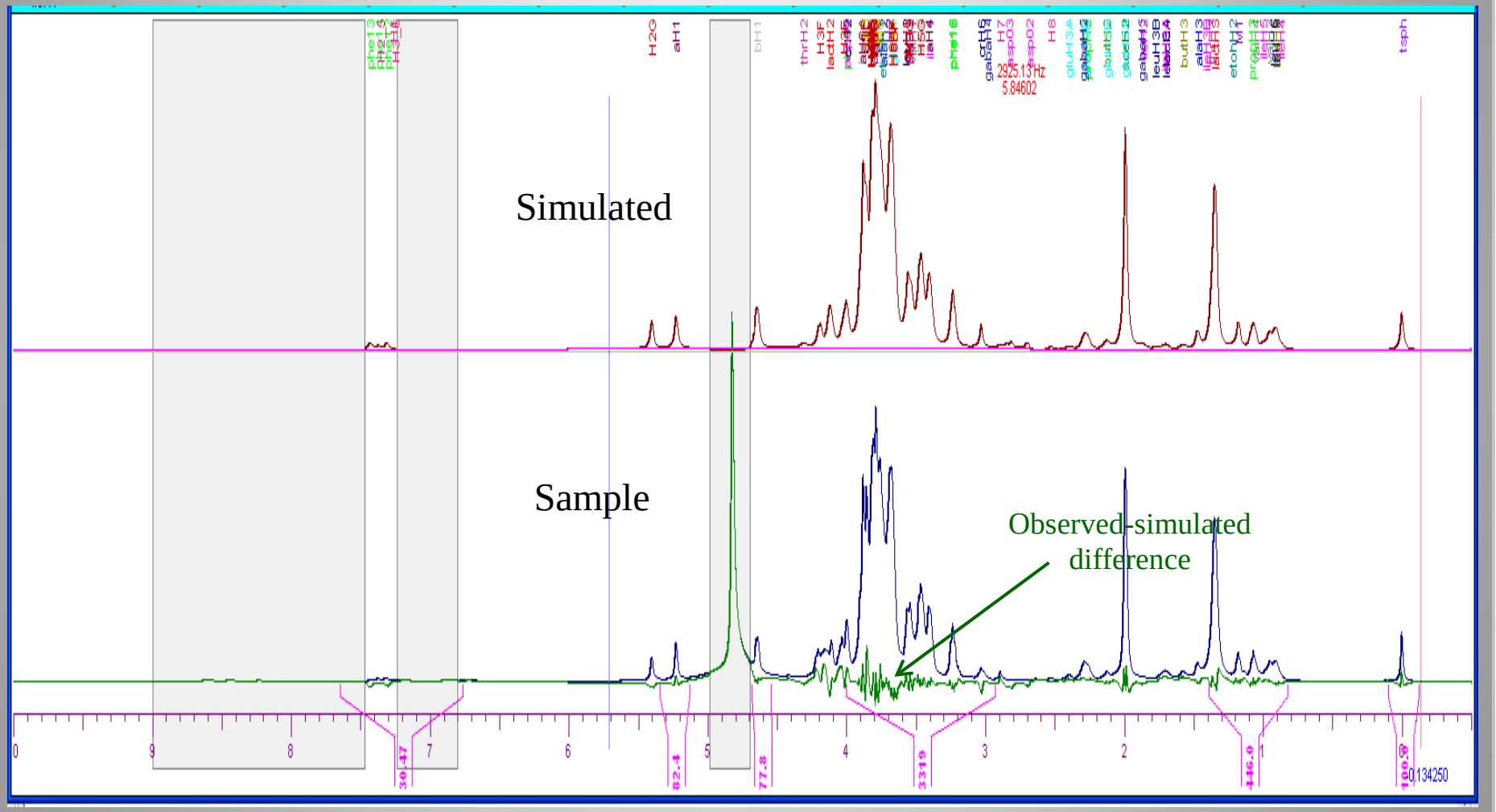
A problem: line-widths and chemical shifts (less) depend on sample, which means that a sophisticated software is needed for accurate quantification, …a simple regression analysis does not work.

Adaptive Spectrum Libraries:

Analyze spectrum with one (magnetic) field, the spectrum at any other field can be then simulated !



Quantitative QMSA of an ABOWE sample using 23 metabolites:



Sometimes spectral lines are broadened by Fe & Mn-ions, like above.

REPORT

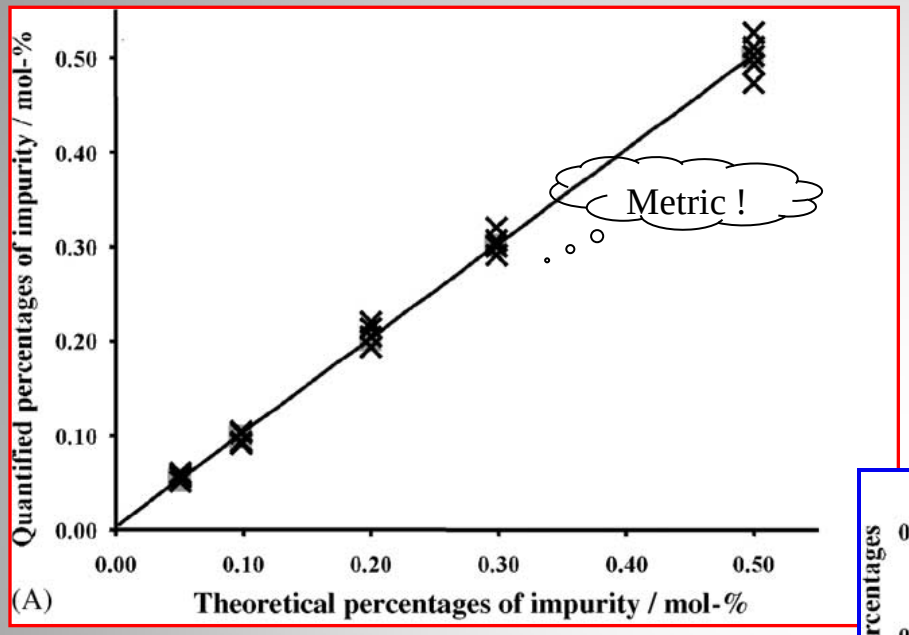
&QM	NAME	N	PROTONS	POPULATION	MOL%	mMOL	Weight(mg/ml)
%Q	**lactate**	**1**	**4**	**0.9004E+01**	**9.0918**	**86.3675**	**7.7731**
%Q	**acetate**	**2**	**3**	**0.1680E+02**	**16.9621**	**161.1321**	**9.6679**
%Q	ala	3	4	0.6009E+01	6.0681	57.6442	5.1303
%Q	valine	4	8	0.2383E+01	2.4059	22.8546	2.5140
%Q	leu	5	10	0.2505E+01	2.5291	24.0256	3.1474
%Q	ile	6	10	0.1930E+01	1.9491	18.5155	2.4255
%Q	**etoh**	**7**	**5**	**0.7797E+01**	**7.8735**	**74.7941**	**3.4405**
%Q	butyrate	8	7	0.7870E+00	0.7947	7.5494	0.6643
%Q	propio	9	5	0.1874E+01	1.8922	17.9750	1.3661
%Q	glu	10	5	0.9932E-02	0.0100	0.0953	0.0141
%Q	**beta**	**11**	**7**	**0.8427E+01**	**8.5091**	**80.8322**	**14.5498**
%Q	**alfa**	**12**	**7**	**0.5282E+01**	**5.3338**	**50.6687**	**9.1204**
%Q	gly	13	2	0.1311E+01	1.3236	12.5734	0.9430
%Q	thr	14	5	0.9269E+00	0.9360	8.8912	1.0581
%Q	phe	15	8	0.1850E+01	1.8678	17.7434	2.6793
%Q	3pheprop	16	9	0.2500E+00	0.2524	2.3980	0.3597
%Q	creatine	17	5	0.1144E+01	1.1552	10.9735	0.9766
%Q	gaba	18	6	0.9932E-02	0.0100	0.0953	0.0098
%Q	asp	19	3	0.1473E+01	1.4870	14.1258	1.8787
%Q	**mannitol**	**20**	**8**	**0.9783E+01**	**9.8790**	**93.8454**	**17.0799**
%Q	**23bud**	**21**	**8**	**0.1504E+02**	**15.1904**	**144.3013**	**12.9871**
%Q	**sucrose**	**22**	**14**	**0.4436E+01**	**4.4791**	**42.5497**	**16.0838**
%Q	tsp	23	9	0.9685E+00	0.9779	9.2900	1.3573
TOTAL(excl. reference) =				**99.0315**	**100.0000**	**949.9510**	**113.8694**

QMTLS - APPLICATIONS

- Up to 100 metabolites in one sample?
- Dynamic range of 0.01-100 mol%
- Concentrations > 0.01-M

- Applications:
  - Any mixtures and impurity analysis
  - Biofluids: plasma, CSF, lipid extracts of serum, urine, ….
  - Bioextracts, juices, ...

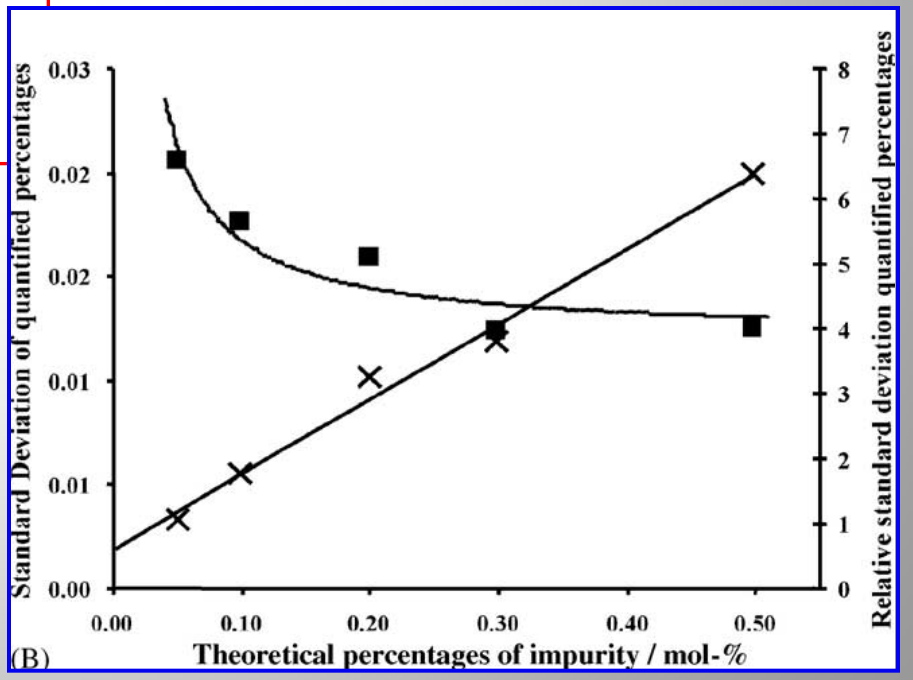
Linearity & confidence limits



Calculated vs. real impurity concentrations (in mol%)
$R^2 = 0.995$

NO CALIBRATION NEEDED !!

Standard deviation vs. mol% %



CONS & PROS

+ Sample preparation, ..just filter and add reference
+ No calibration
+ Semiquantitative analysis of sample at one glance
+ **Chemical confidence** (identification of components directly from spectrum),..also carbohydrates (not with MS)
+ ASL (Adaptive Spectrum Libraries)
+ Almost automatical analysis

- Some expertise needed
- Not very sensitive, sample size > 0.3 ml
- Instrumentation (ca. 20€/sample, depends on n), ..liquid Helium and Nitrogen

NEW GENERATION OF NMR INSTRUMENTS

No liquid He or N_2 !!

Model	Magnetic Field (MHz)	Bore size (mm)	5 Gauss (mm) Axial/Radial	Uniformity (PPM)[1]	Magnet Height (mm)	Magnet Width (mm)
MR 4T7-54	200	54	<100/50	<1	400	360
MR 9T4-54	400	54	<200/100	<1	600	600
MR 18T8-54[2]	800	54	<2000/2000	<1	800	800

[1] *Bare magnet uniformity, no electrical shims*
[2] *Under development*

COCLUSIONS and answers to questions presented after speech

Is ON-LINE possible with NMR ?

- In principle, yes, in fact NMR could allow automatic follow-up of the process once in a few minutes.
- Unfortunately, not yet feasible with the presently available instruments, ..but probably in near future with the new instruments (previous slide).

Are the new low field (40-100 MHz) instruments useful?

- Not for the water solutions! The minimum useful field is probably 200-400 MHz and demands far better water suppression and sensitivity than in the new < 100 MHz instruments.

RECOMMENDATION:

- NMR is invaluable in checking composition and detecting metabolites (especially sugars) of fermentation products, whenever starting materials or protocols are changed.
- NMR suits perfectly to calibration of methods like GC and HPLC; it is not necessary to prepare the calibration samples containing accurate known concentrations of metabolites (which may be unavailable).

QMSA PROJECT

- UEF: Prof. Reino Laatikainen, PhD Pasi Soininen, PhD Mika Tiainen & PhD Tuulia Tynkkynen
- Univ. of Jyväskylä: BSc Pekka Laatikainen & BSc Henri Martonen

- **See also JMR 242 (2014),67-78:**

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Quantitative Quantum Mechanical Spectral Analysis (qQMSA) of ^{1}H NMR spectra of complex mixtures and biofluids

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