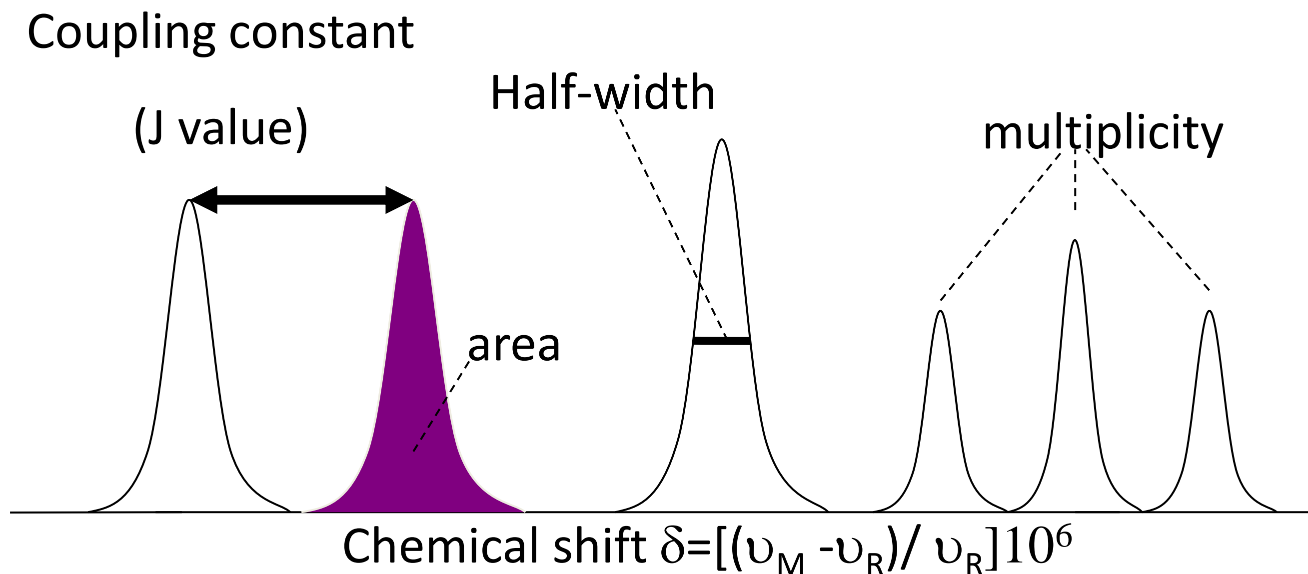
MEMO: The important parameters from an NMR spectrum



Where do we use this information? power of the technique

How do we get this information? personal skills

NMR IN ANALYTICAL CHEMISTRY

Qualitative analysis

Detection of one or more components

Requirements: the spectra of the compound should be available (database, previous determination)

Quantitative analysis, qNMR

Quantitative determination

- Calibration technique
- Standard addition

Ex: lab work ^{31}P phosphorus content determination

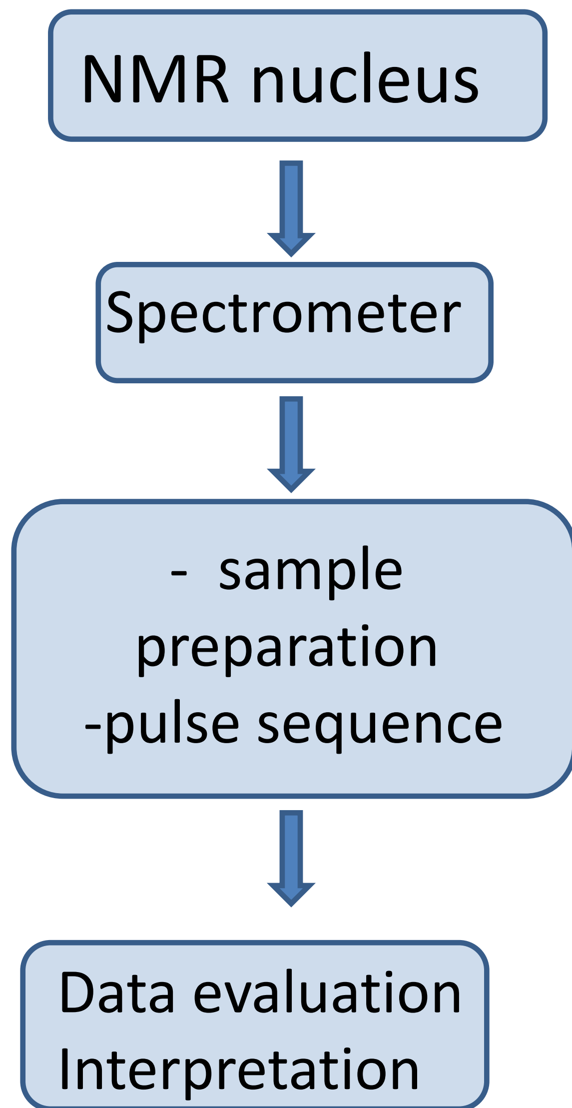
- Statistical analysis (PCA)

Error: < 2%

Complex samples: drugs, cell extracts, body liquids, natural products, food samples

Bharti, S.K, Roy R.; *Trends in Anal. Chem.* ***2012***

Steps of the determination – the NMR view

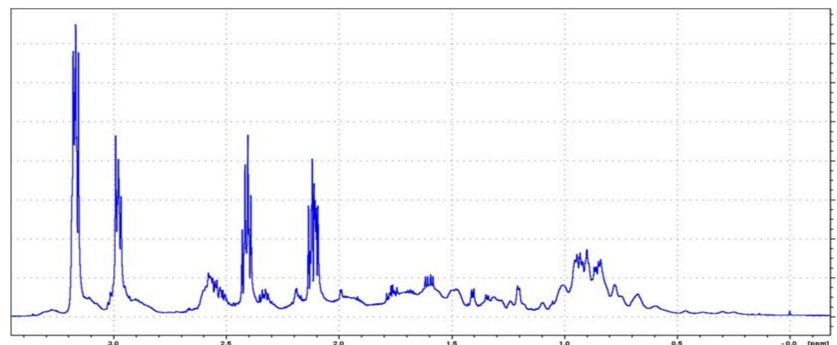


Technical knowledge:
NMR instrumentation
NMR active nuclei
Spectral parameters to be used
chemical shift
coupling constant
relaxation time (T_1, T_2)
Possible 1D, 2D techniques
Translational diffusion - Mw

Basic considerations

^{1}H, ^{13}C, ^{31}P

High-field applications > 400 MHz spectrometer



Sensitivity increase

Probe-head: BBI vs BBO

S/N increase

- RT probe-head < cryo probes
- sample volume: 5mm > Shigemi > 2mm NMR tubes

1% precision:

S/N > 250:1 (^{1}H), >600:1 (^{31}P)

Nr of transients (n) : $(S/N)_n = \sqrt{n}\,(S/N)$

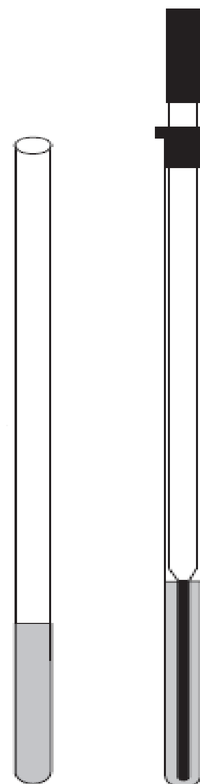
NS=64 and 16: 2x (S/N) increase

NS=128 and 64: 1,41x (S/N) increase

Sample preparation, referencing

Requirement: reproducibility, comparability

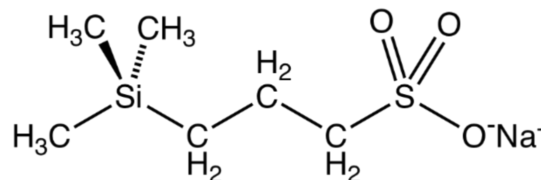
Parameters influencing the chemical shift value



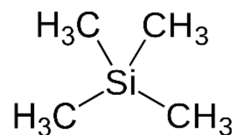
- Sample side: solvent/ deuterated solvent
  pH (buffer)
  ionic strength, metal ion presence
  temperature

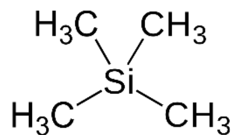
- Referencing (internal, external standards)
  H2O, dioxane, TMS, DSS
  in the sample, or in capillary

DSS: sodium trimethylsilylpropanesulfonate:



TMS: tetramethylsilane





Dioxane: ^1H NMR spectrum: singlet at 3.54 ppm

Holzgabe, U.; *ProgrNMRSpect.*, ***2010***

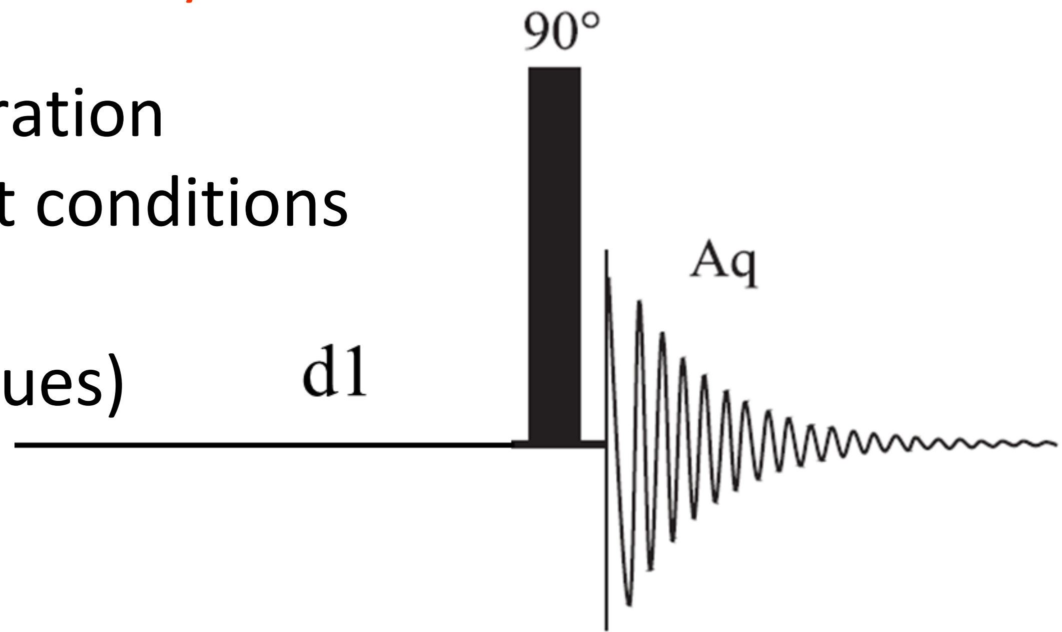
Parameters influencing quantitativity

Peak intensity ~ concentration

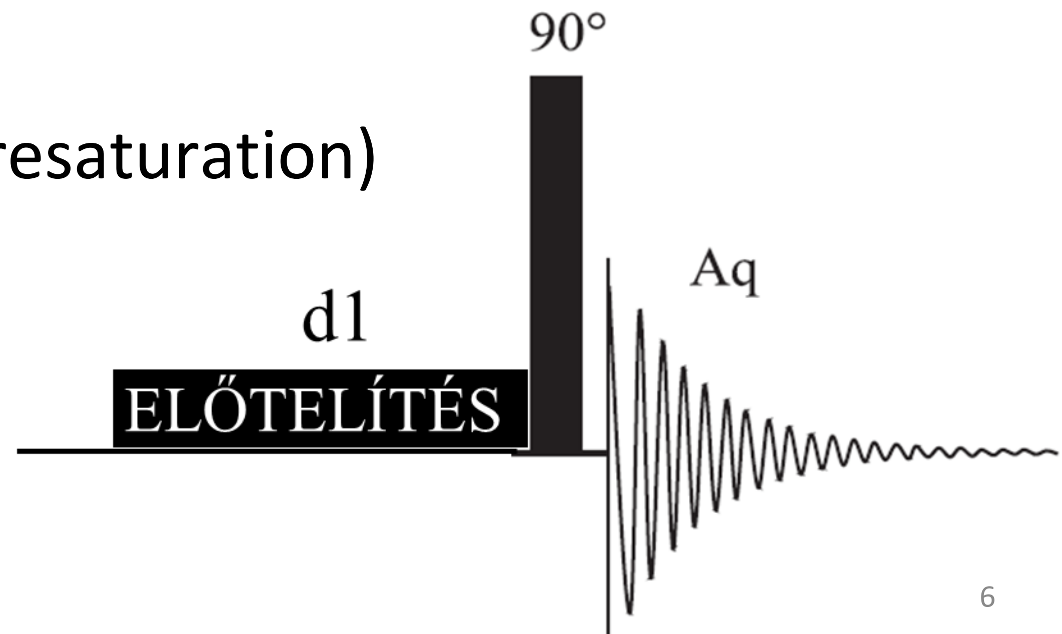
Optimising measurement conditions

Crucial parameters:

- d1 (depends on T_1 values)



- Huge solvent peak (presaturation)

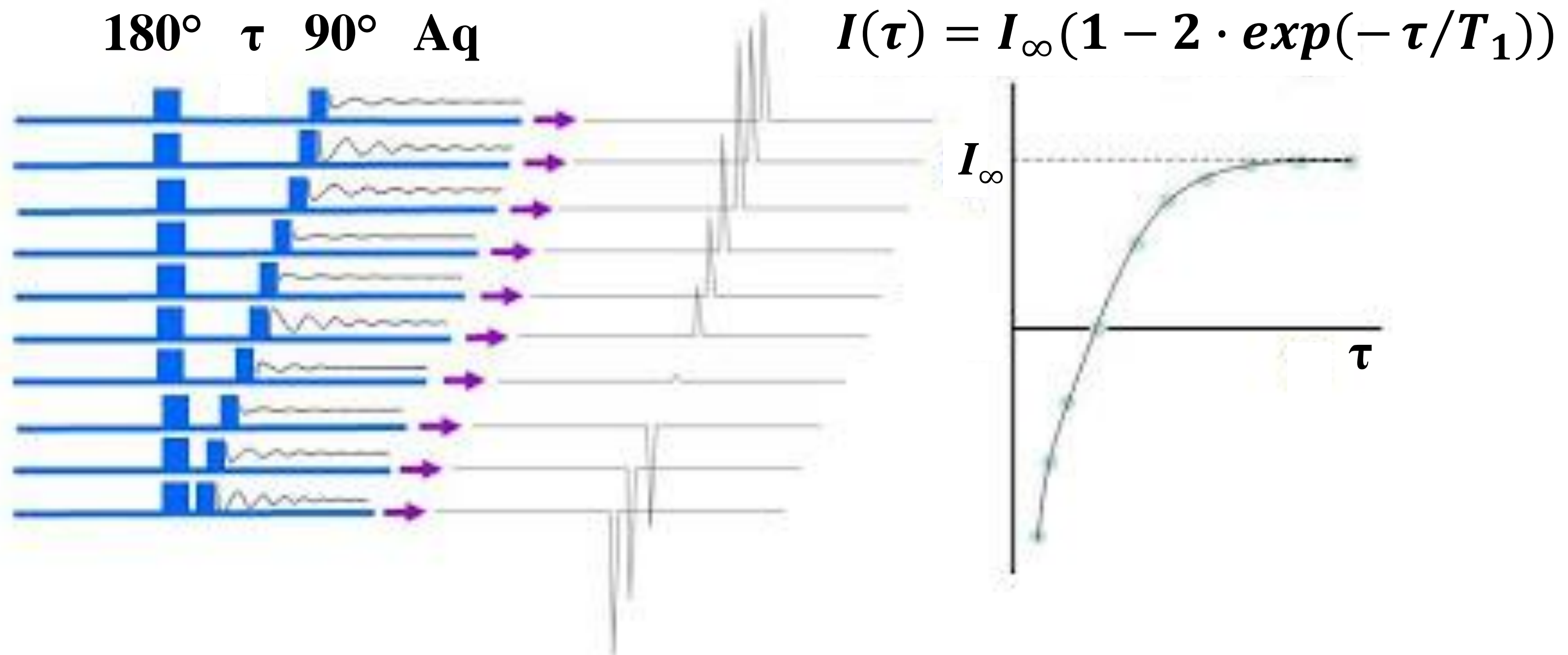


$$d1 \geq 5T_1 - aq$$

$$T_1 = 1{,}44\ T_{null}$$

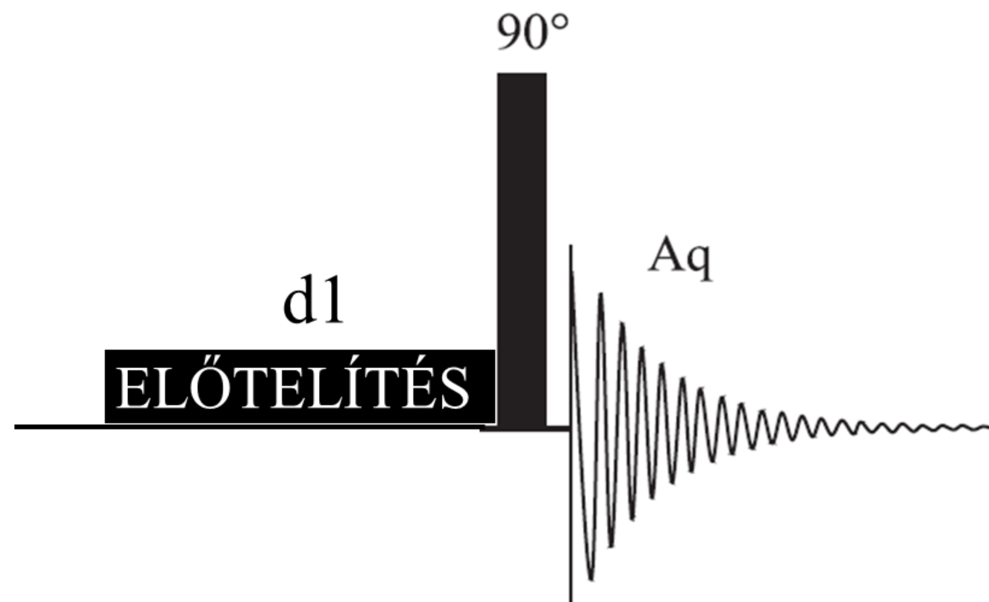
where T_{null} belongs to τ giving no signal

T_1 measurement



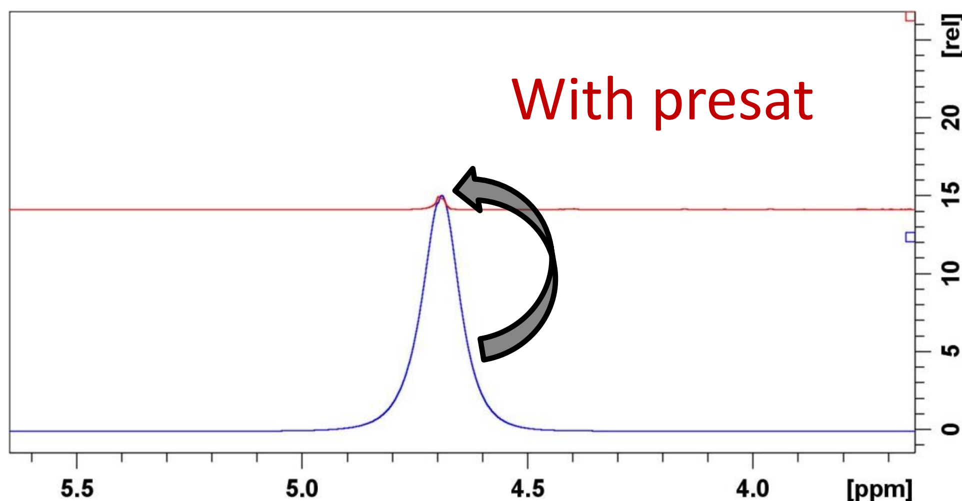
presaturation

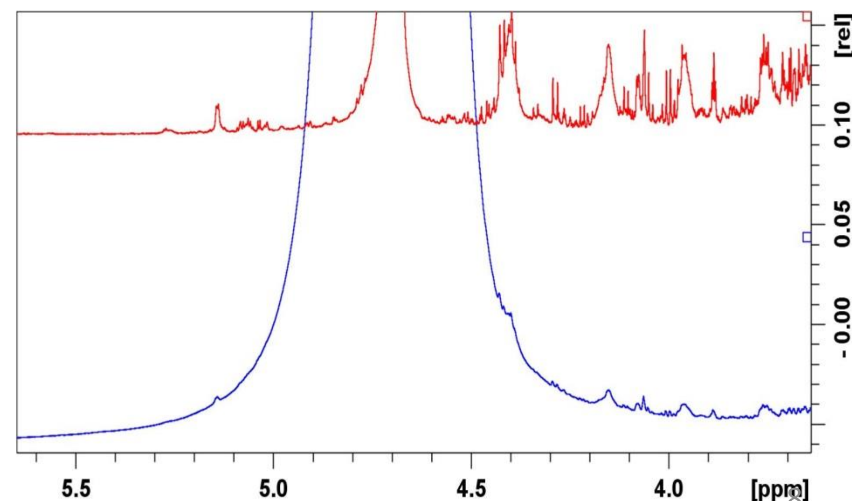
Solvent peak (H_2O) on-resonance
Selective iradiation with low power



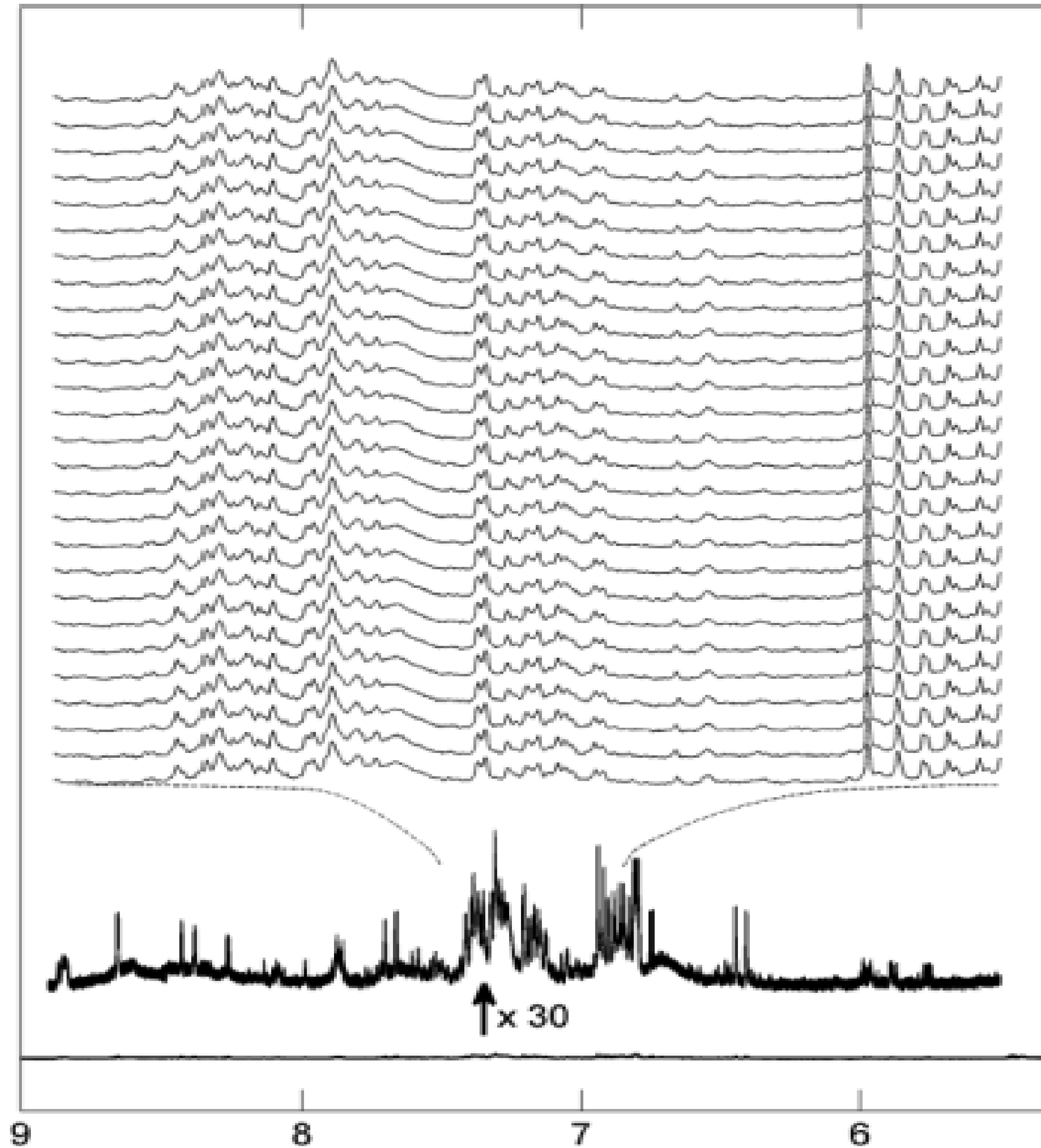
Note:

- Dissociable H^+ environments are affected (–NH_2, -OH)
- Distorted integral values for the peaks in the H_2O resonance proximity





reproducibility



30 separate samples

Intra- and intermolecular composition

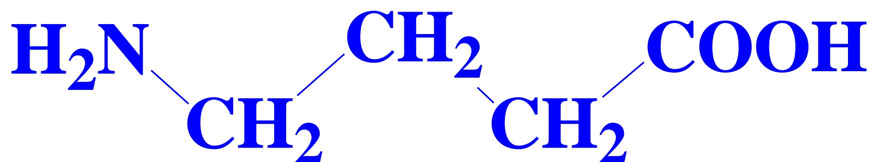
Relative ratio of the components:

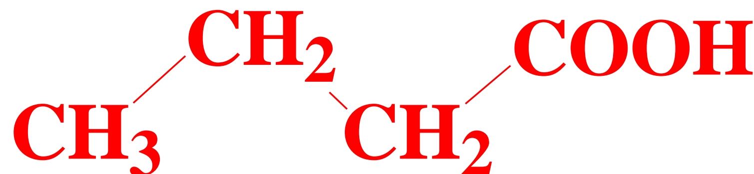
$$\frac{n_A}{n_B} = \frac{I_A}{I_B} \cdot \frac{N_B}{N_A}$$

I: integrated intensity,

N: belonging nr of proton environments

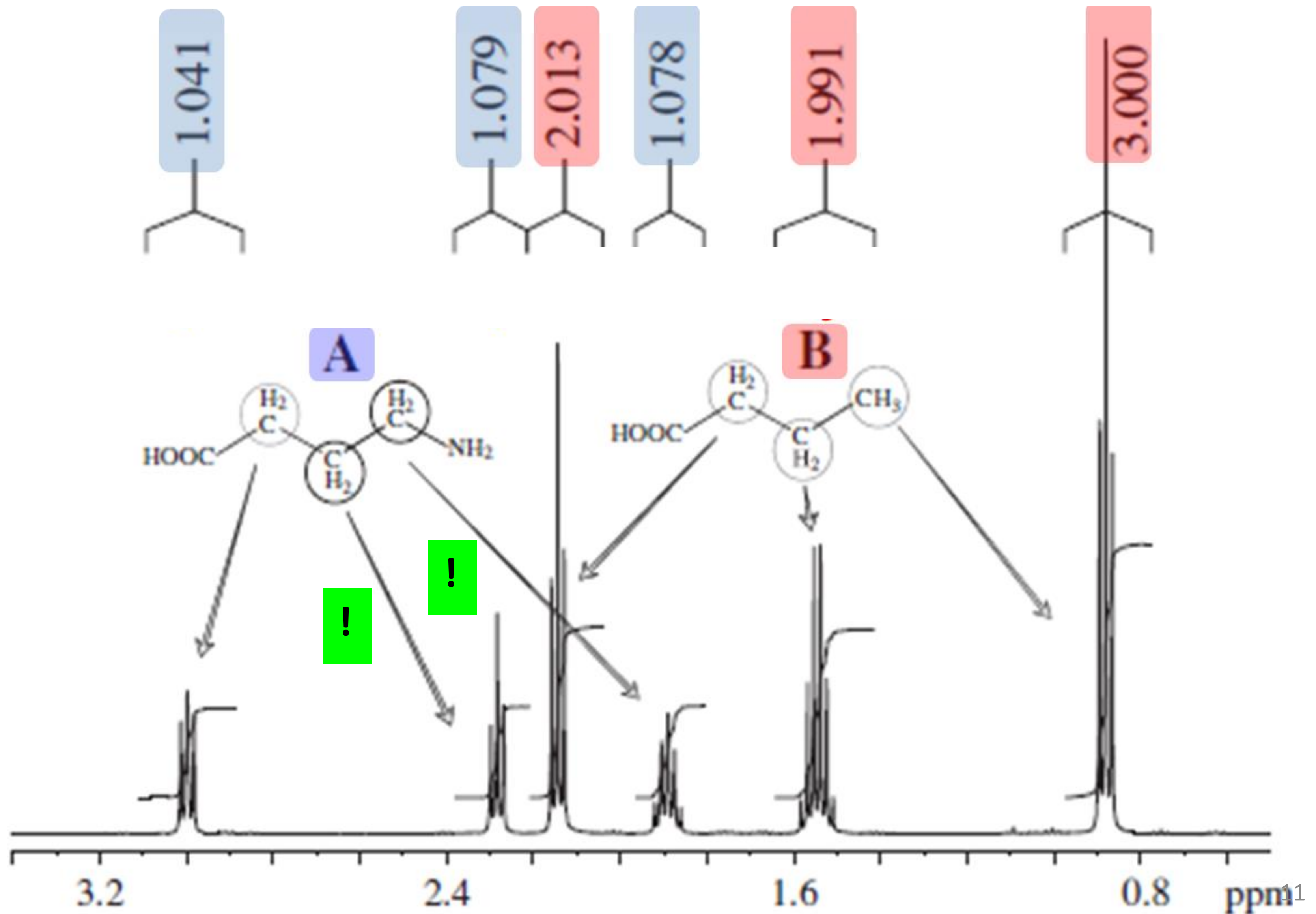
Determine the ratio of gamma-amino-lactic acid (A) and lactic acid (B) in a mixture!



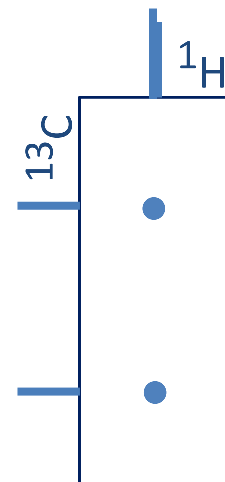


$I_A=1+1+1; N_A=2+2+2$ $I_B=2+2+3; N_B=2+2+3$

$n_A/n_B = 1/2$



Can the 2D spectra be quantitative?



Limitations of 1D: signal overlap
Solution: **^{1}H-^{13}C HSQC**

Drawbacks:

- Long measurement time (min, h – depends on the type of measurement and also on the skills/knowledge of the operator)
- Integral values depend on several factors:

  non-uniform excitation
  different environments have different (T_1,T_2) values
  the $^{1}J_{HC}$ coupling constant value is not the same for all

environments

Solution:

Error cca 2,7%

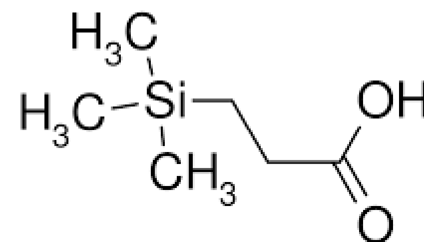
- Calibration method
- Standard addition method

Applicable only if the number of components is low

- Determination of T1, T2, J for a given system

Quantitative ^{1}H-^{13}C HSQC without a calibration curve

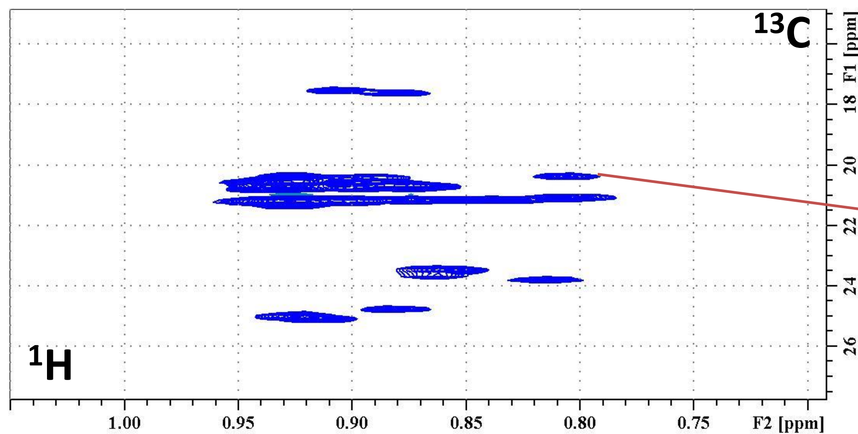
Inner reference: TSP: 0,0 ppm.
(trimethyl-silyl-propionic acid)

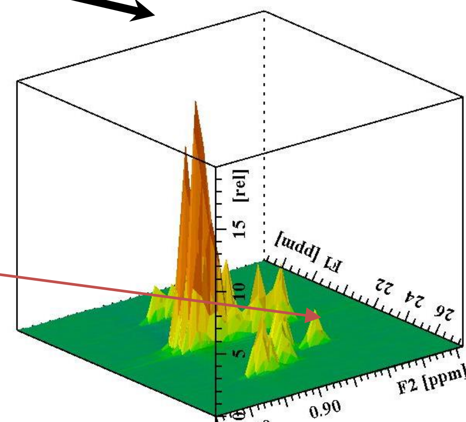


Volume integral
The 2D spectrum representation is usually the contour plot. When determining the integrated intensity we usually calculate the volume integral (see graphs)
Two possible ways of representation (TopSpin program contour and oblique mode)

^{1}H-^{13}C HSQC (the methyl region of a protein) the two representations

Contour plot





Quantitative ^{1}H-^{13}C HSQC without a calibration curve

$$V_0 \propto \varphi^0(T_1^H, T_2^H, d1) \cdot \varepsilon^0(J_{CH}^1) \cdot c^0$$

V volume integral

φ, ε correction factors

c concentration

0- inner reference (TSP)

V_0 / V_m *arány* => c_m

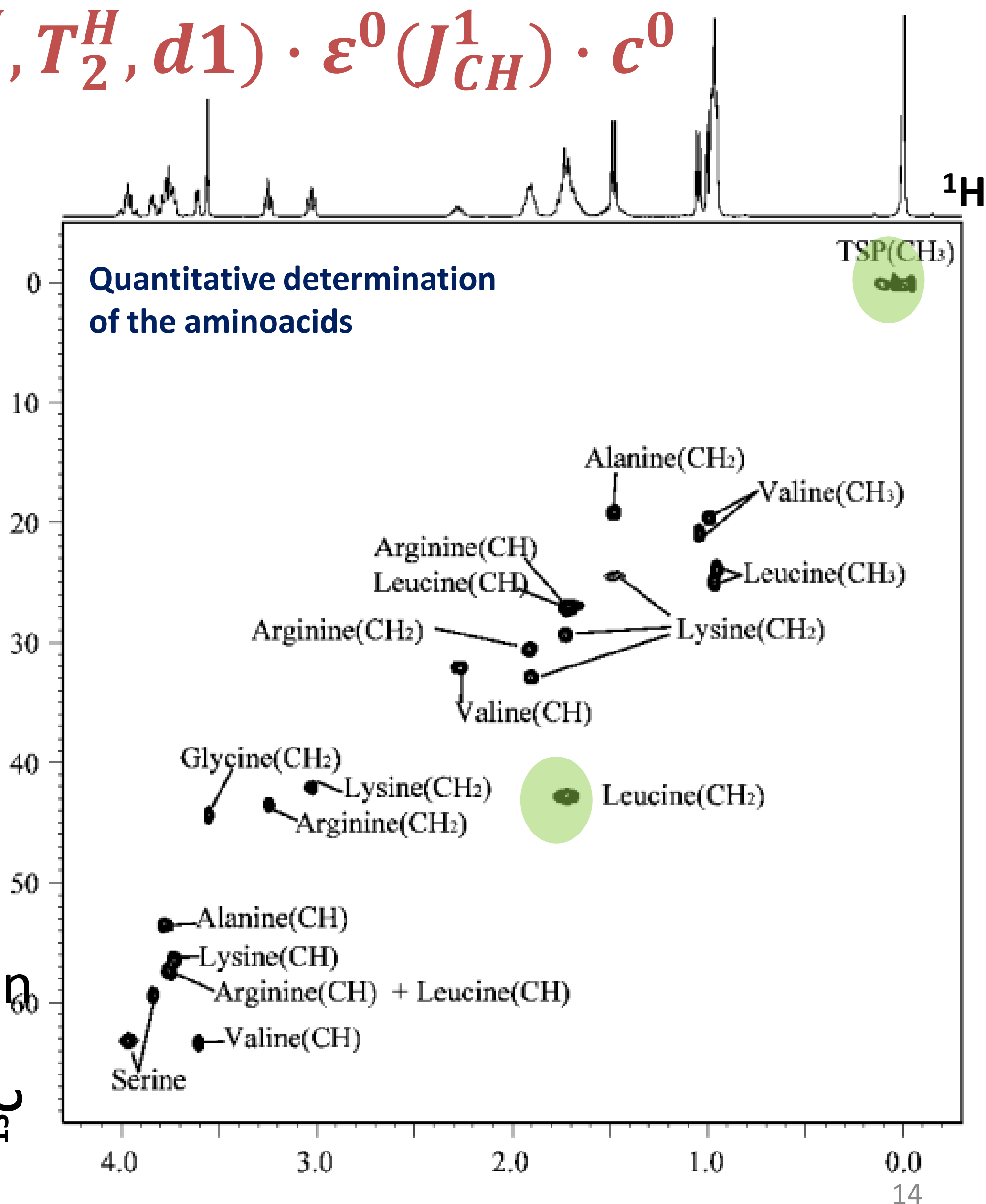
Separate measurement:

T_1^H, T_2^H, J_{CH}^1

Test: aminoacid mixture with known concentrations concentration-integral corelation

Real sample:

Metabolite determination from urine sample



Rai, R.K. *et al*; *AnalChem.*, ***2009***

Practical examples

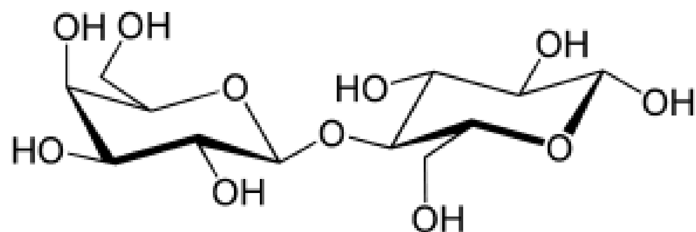
Think first about the solution. Answer questions beforehand, such as:

a) what is the molecular formula?
b) why would be NMR spectroscopy beneficial?
b) which other methods of investigation can you enumerate?
c) what type of NMR measurements would you run and why?
d) how could you ensure the quantitativity?

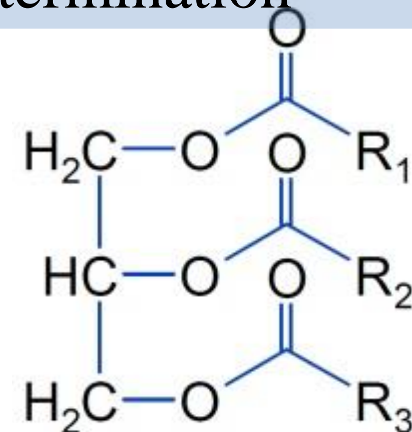
1. The analysis of milk

Goal: - lactose and milk-fat determination
- detection of other compounds (ex: trimethyl amine)
- determination of phosphorus content

Similar approach also for the lactose content determination



Laktose



triglicerid

^{1}H, ^{13}C measurements
500 MHz, 20°C

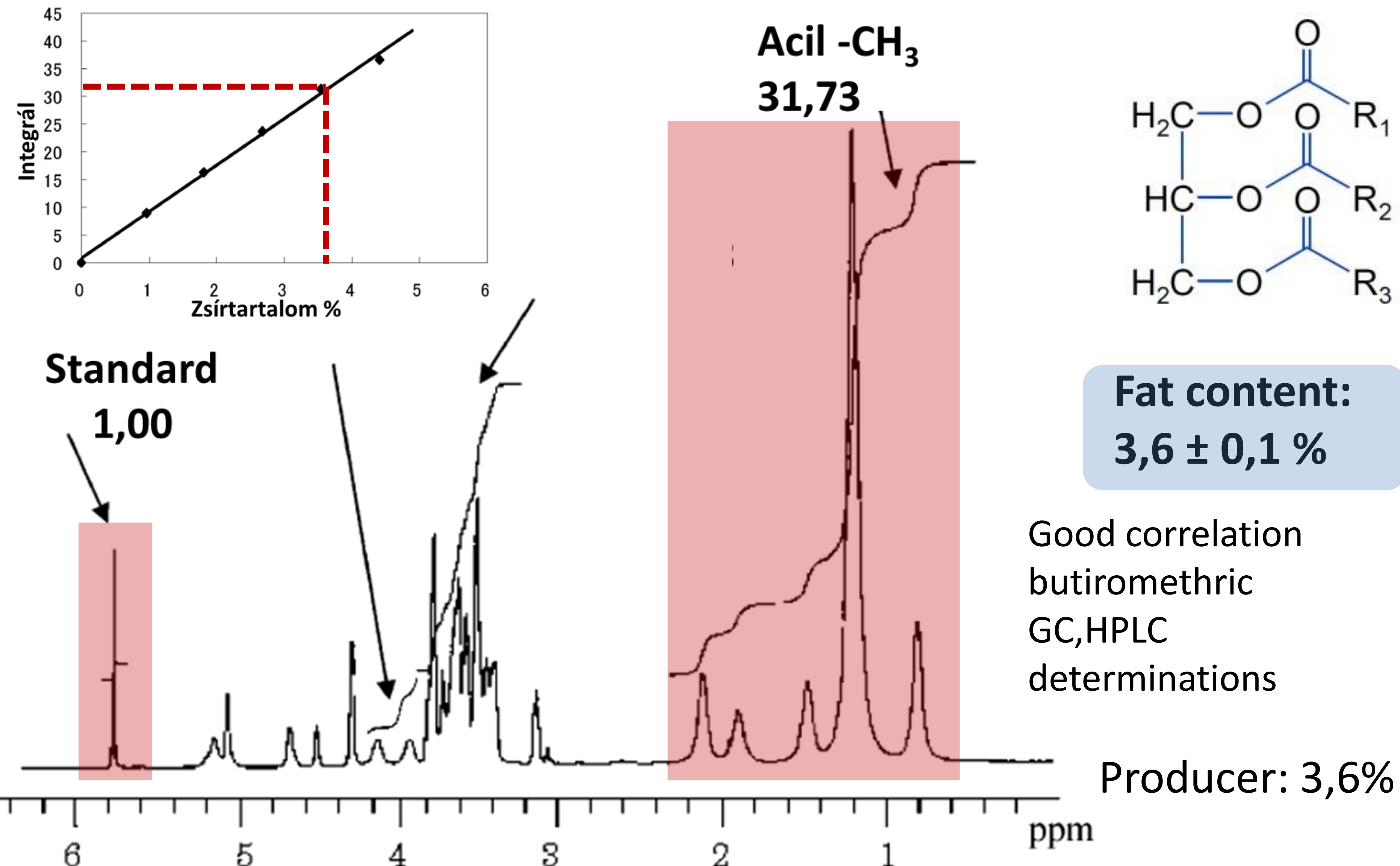
5 mm tube: homogeneous sample, non-invasive
capillary: concentration standard: $CHCl_2$-$CHCl_2$, $CDCl_3$-lock

For quantitative determination:
$T_{null}(CHCl_2\text{-}CHCl_2)=1{,}4s$
Relaxation enhancemnet: $Cr(acac)_3$:
$T_{null}(CHCl_2\text{-}CHCl_2)=0{,}28s$

Comment: the chosen reference was the water signal. Other solutions?

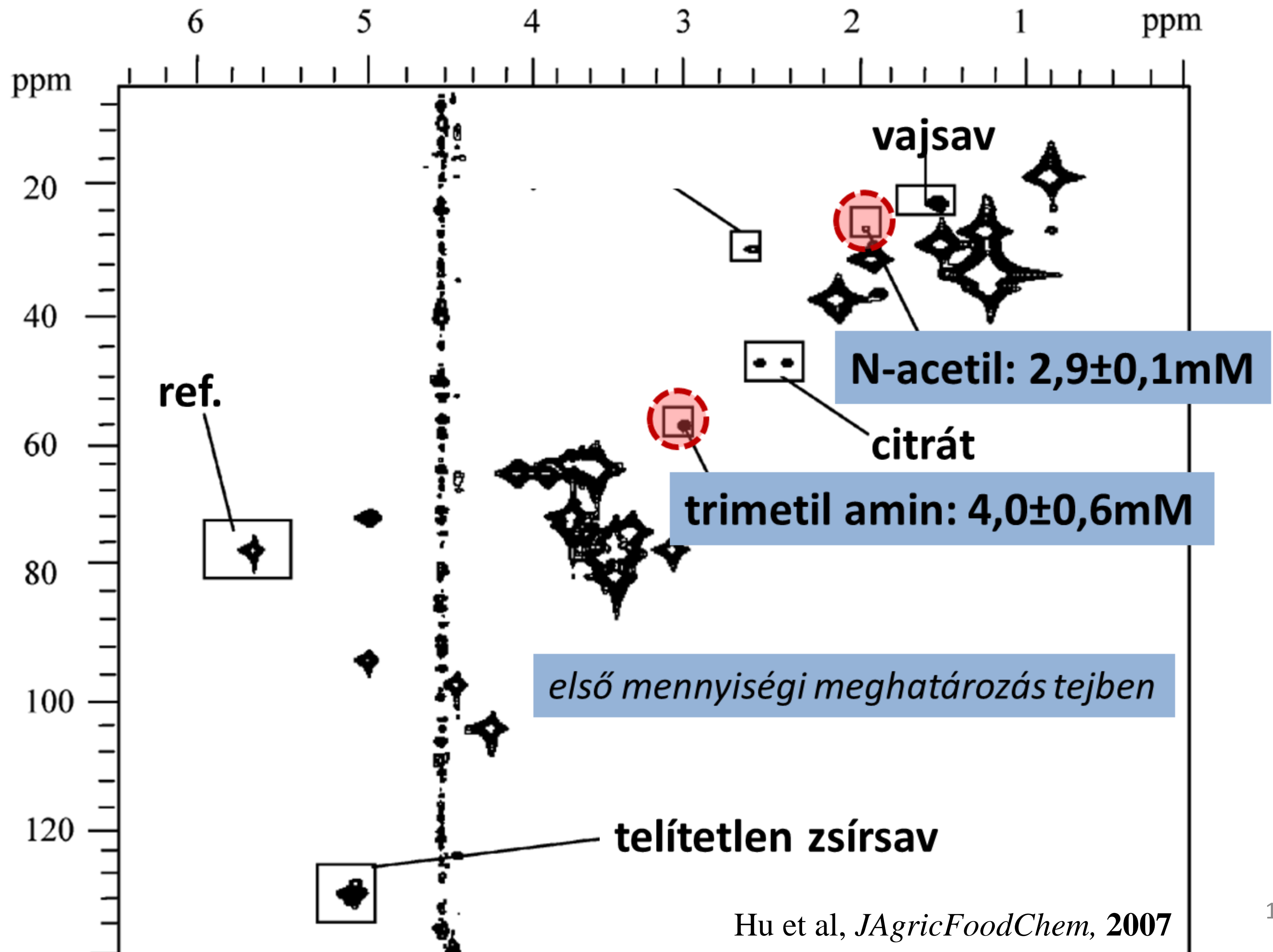
Hu et al, *JAgricFoodChem*, **2007**

1.1 Determination of milk fat conent



Comment: peaks originating from proteins or other components will not affect?

1.2 Determination of other components using the calibration method based on ^{1}H-^{13}C HSQC measurements



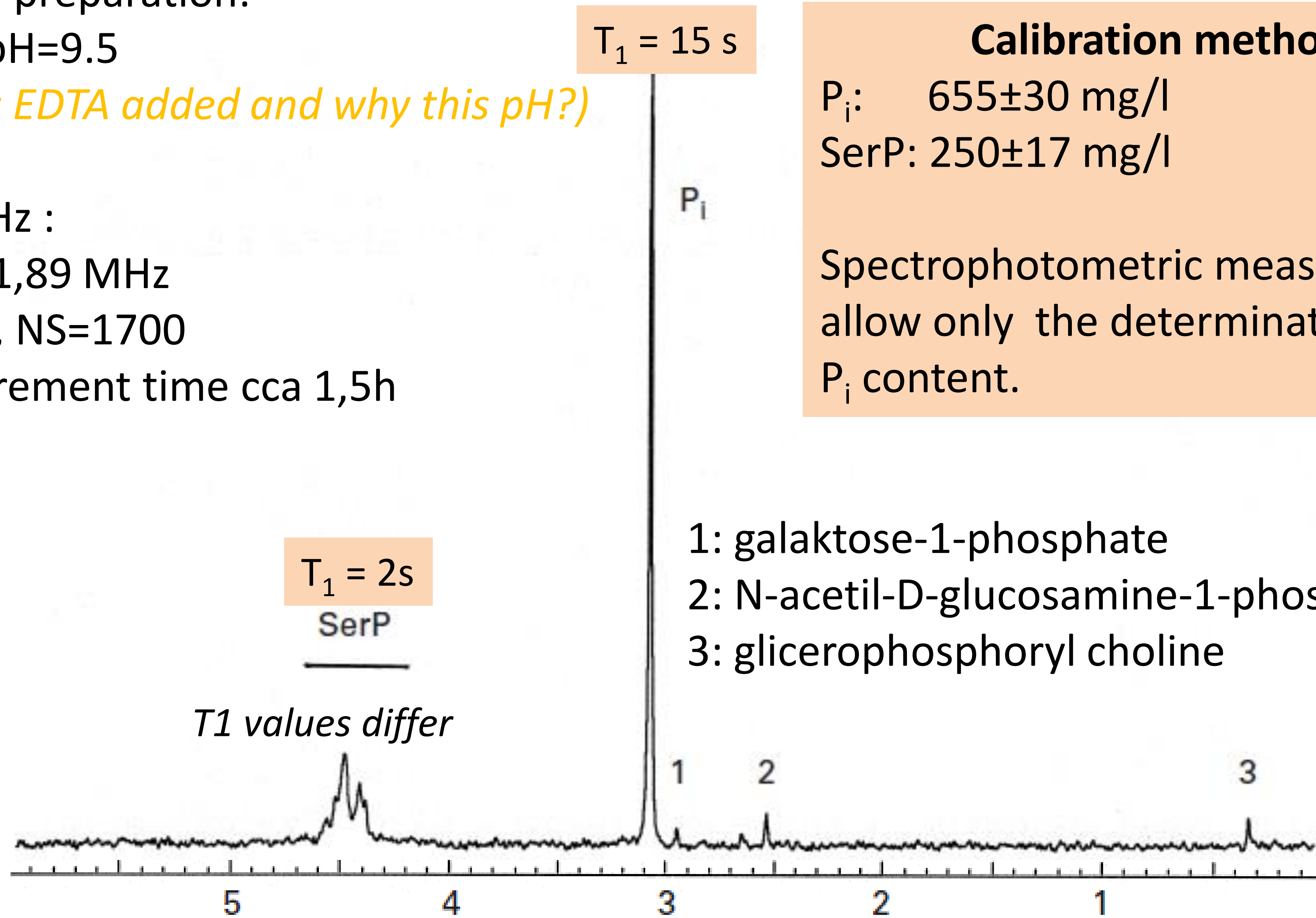
1.3 Phosphorus content determination by ^{31}P methods

Sample preparation:
EDTA, pH=9.5
(Why is EDTA added and why this pH?)

400 MHz :
^{31}P: 161,89 MHz
^{31}P{^{1}H}, NS=1700
Measurement time cca 1,5h

Calibration method:
P_i: 655±30 mg/l
SerP: 250±17 mg/l

Spectrophotometric measurements allow only the determination of the P_i content.



1: galaktose-1-phosphate
2: N-acetil-D-glucosamine-1-phosphate
3: glicerophosphoryl choline

Drawbacks: sensitivity, measurement length, quantitativity

Belloque et al, *JDairyRes,* **2000**

1. Conclusion

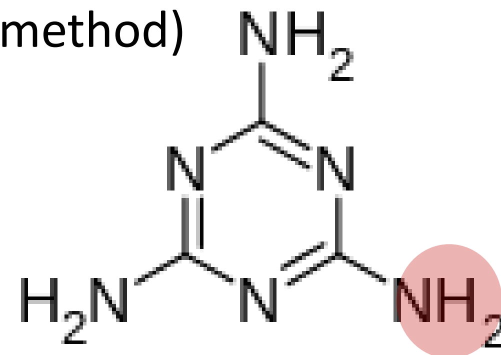
- Non-invasive technique
- Solution phase
- Qualitative determination by ^{1}H, ^{13}C, ^{31}P NMR
- Quantitative determination even for low concentration components.

2. Forgery: the melamine

What is the chemical formula of melamine?
Can you draw the 1H NMR spectrum in aqueous solution?
What is the Kjeldahl method?

2. Forgery: the melamine

1% melamine: increase in protein content by 4,16% (Kjeldahl method)



Analysis of baby food

Kimutatás: milyen közegben?

400 MHz,
^1H DMSO-d_6

Sample from Sanlu
Distributions of Reference Samples

Melamine
5,93 ppm
-NH_2

6.05 6 5.95 5.9 5.85 5.8

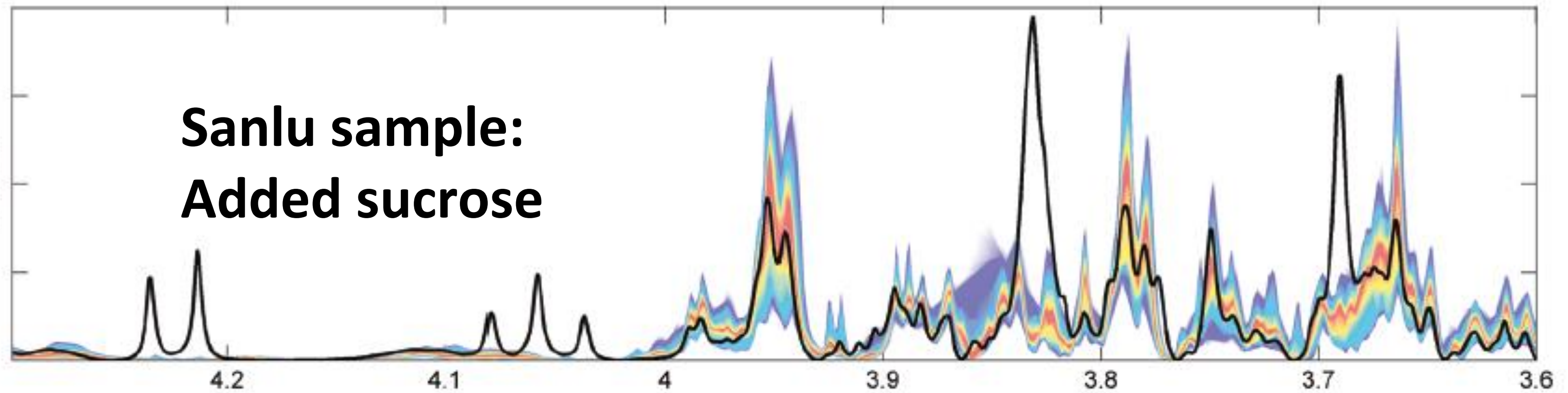
LOD: Limit Of Detection

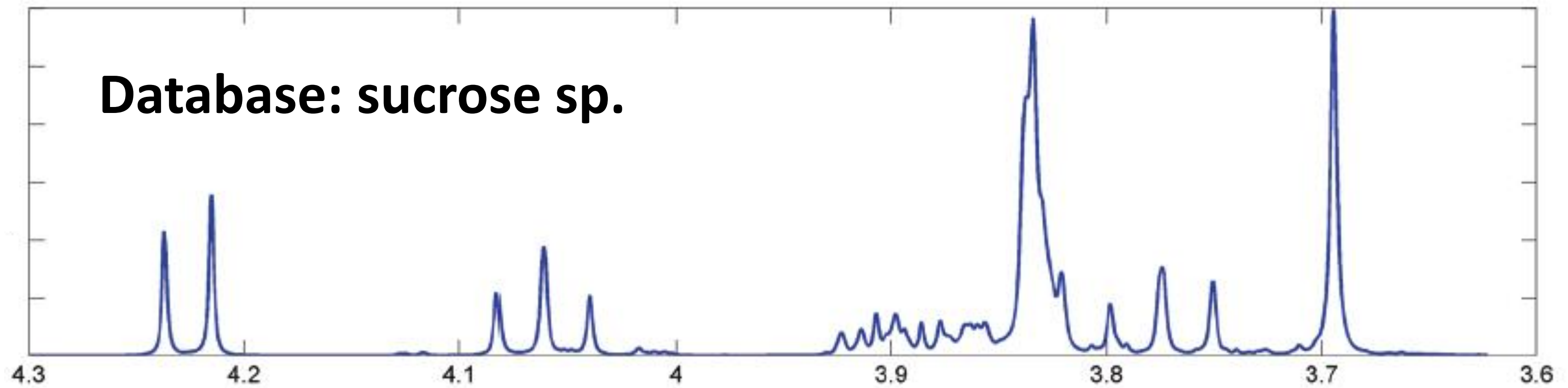
Method	**LOD (mg/l)**
NMR	0,33
SPE-LC/MS/MS	0,005
(better, but invasive)	

Lachenmeier, D.W: et al, *JAgricFoodChem*, **2009**

What else is in the baby food?

Solvent: H_2O





2. Conclusion

^{1}H NMR: fast qualitiative picture
Easy to apply for forgery detection

3. Analysis of wines

Origin determination and forgeries

- addition of sugar
- DOC (denomination of controlled origin) mixture of good quality wine with a lower quality

HPLC, GC, MS, nearIR, NMR methods

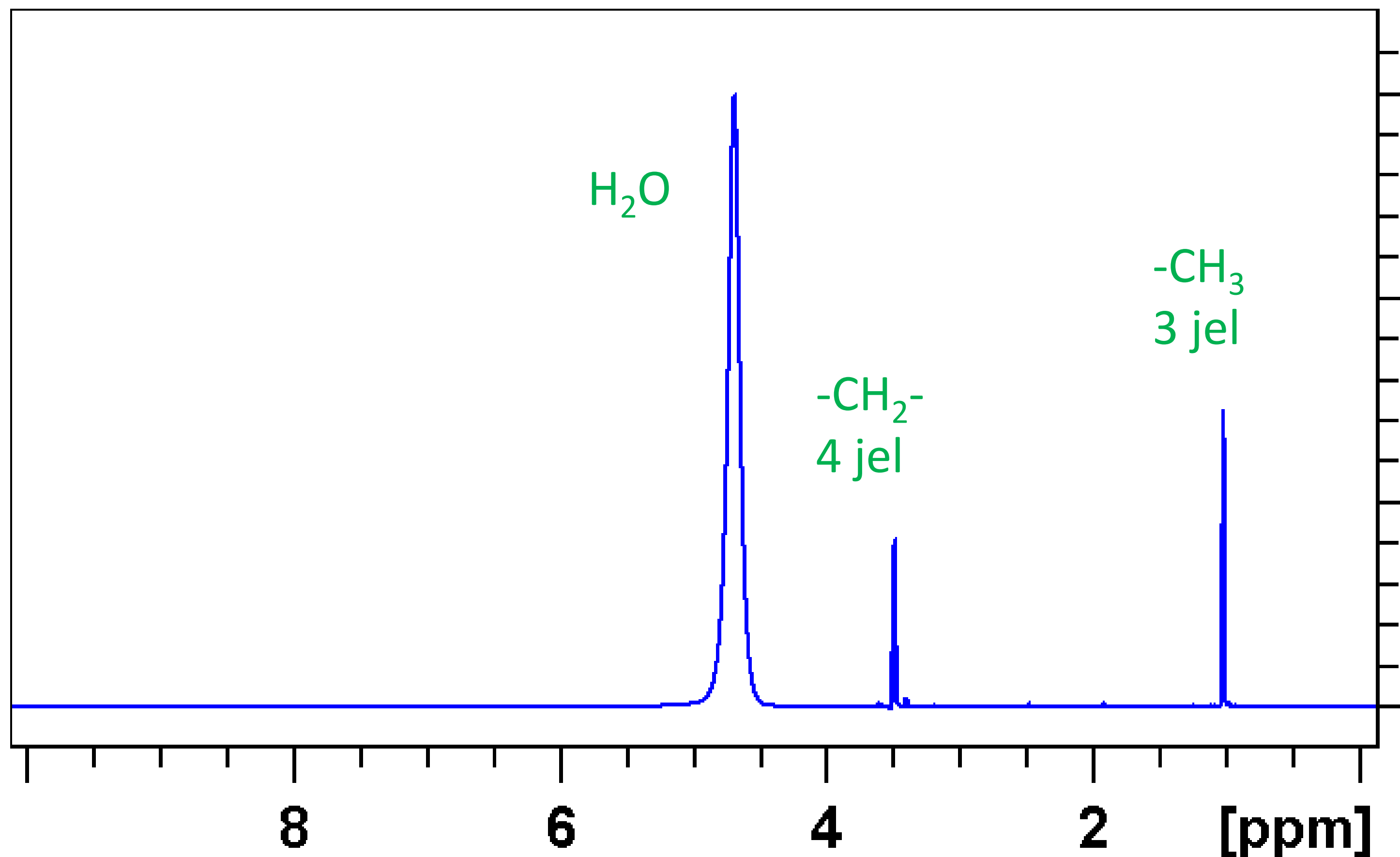
NMR: geographyical origin, type,

- Amino acid and sugar profile analysis – minimal effort for sample preparation
- Phenol compounds and metabolite composition = f(type, origin, climate, UV lights, weather, diseases, etc)

^1H NMR

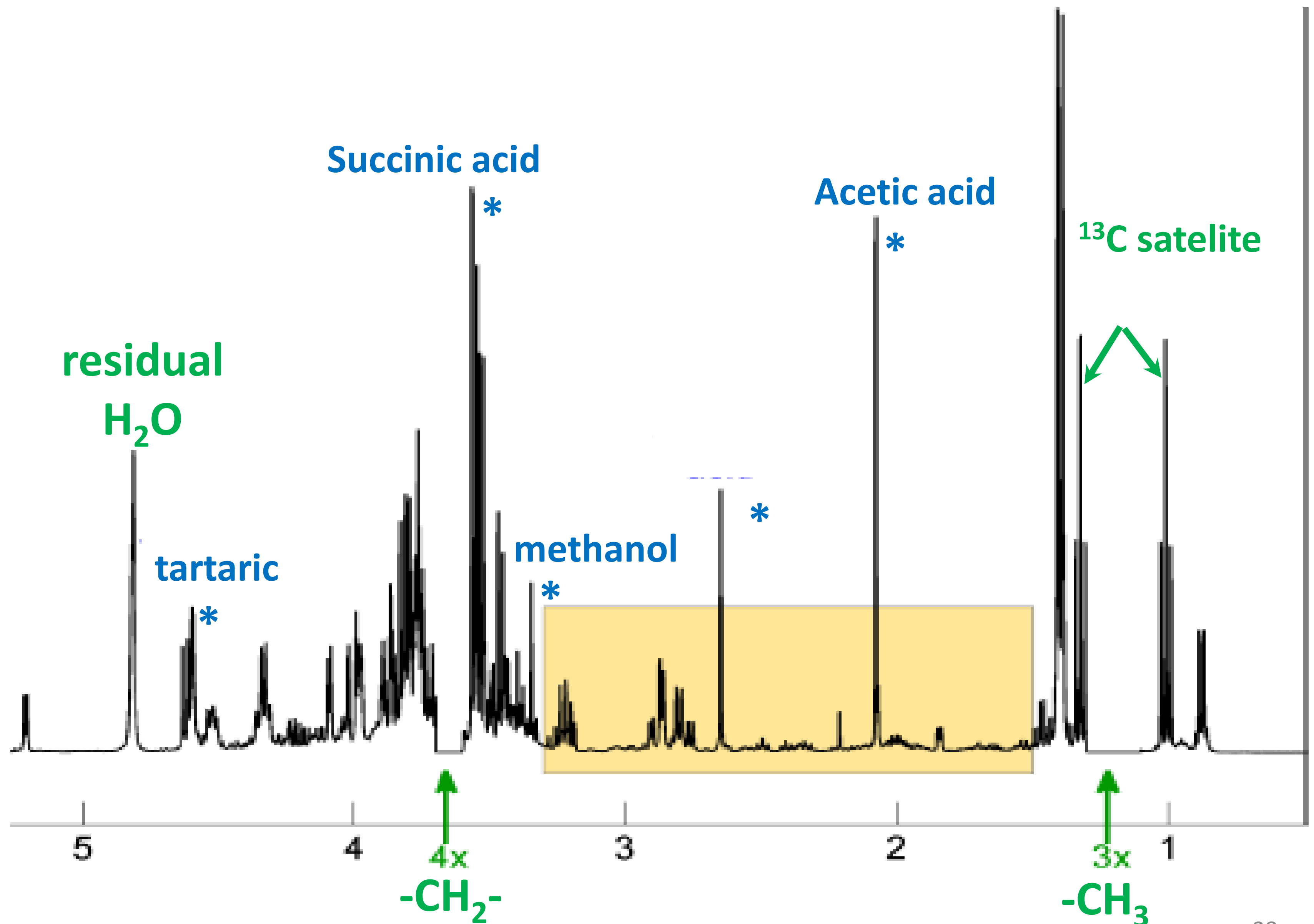
Wine : H_2O + CH_3CH_2OH mostly

Presaturation: H_2O signal removed, but huge resonances of CH_3CH_2OH stay

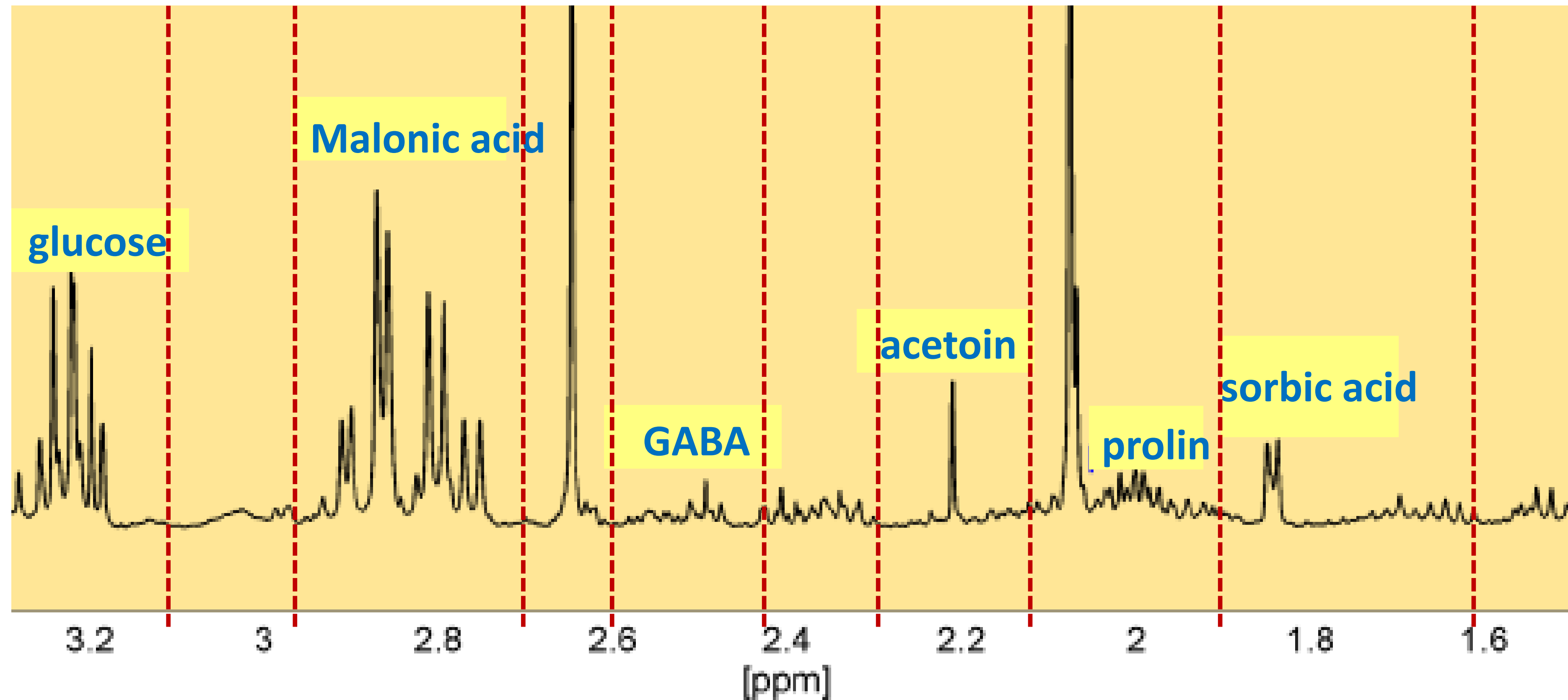


Solution: presaturation for all 8 frequencies!

Result



Bigger zoom

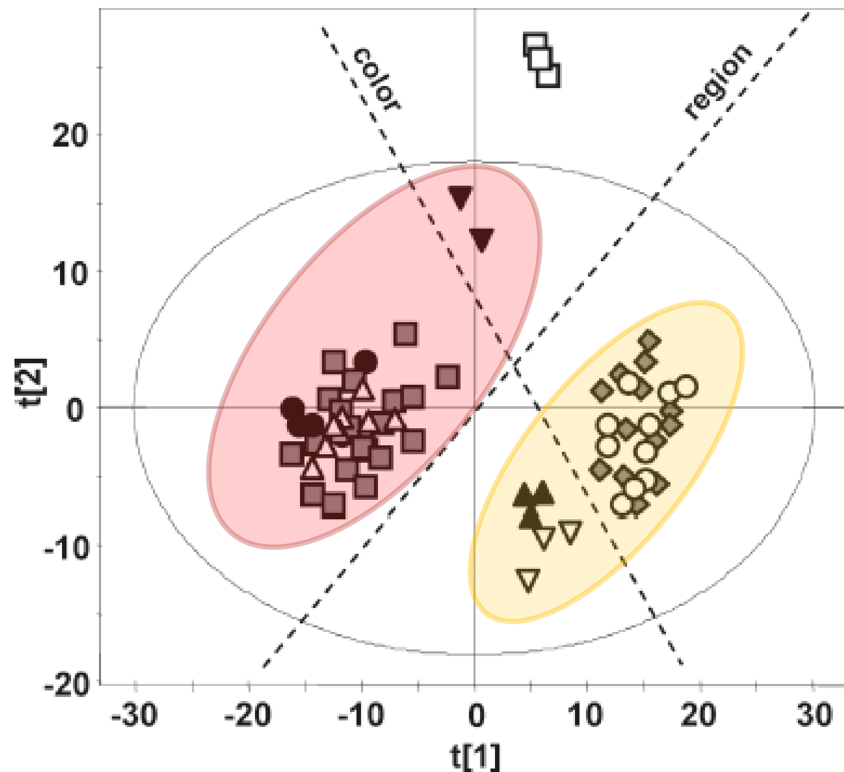


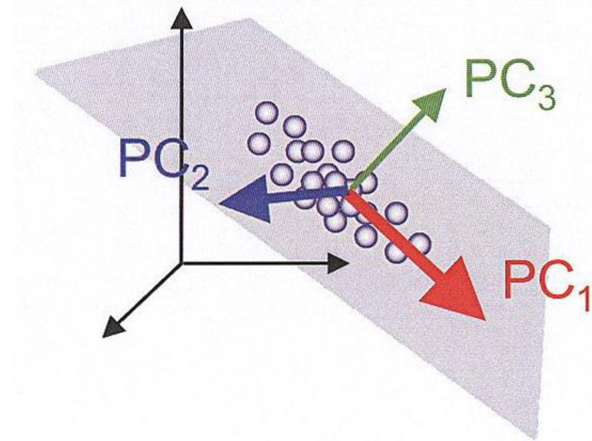
One takes the integral values of these incremented spectral ranges

N samples, x regions, y intensities

Principal Component Analysis (PCA): database analyis , statistical approach looking for patterns and the inner structure of the data

Analysis of red and white wines





Similar approach for:

Orange juice
Beer
Honey

Metabolites

Anastasiadi et al. J. Agric.Food

3. Conclusion

Forgery detection
Determination of origin, also year, etc

Pro and cons for NMR spectroscopy in analytical chemistry

Pro

- Non-invasive, non-destructive
- automatised
- Small sample quantity
- Short measurements (mins)
- Not expensive (if the spectrometer is available)
- High degree of reproductibility (between different labs)
- Sees everything

Cons

- conc > 10µM
- Signal overlap
- Detailed method development