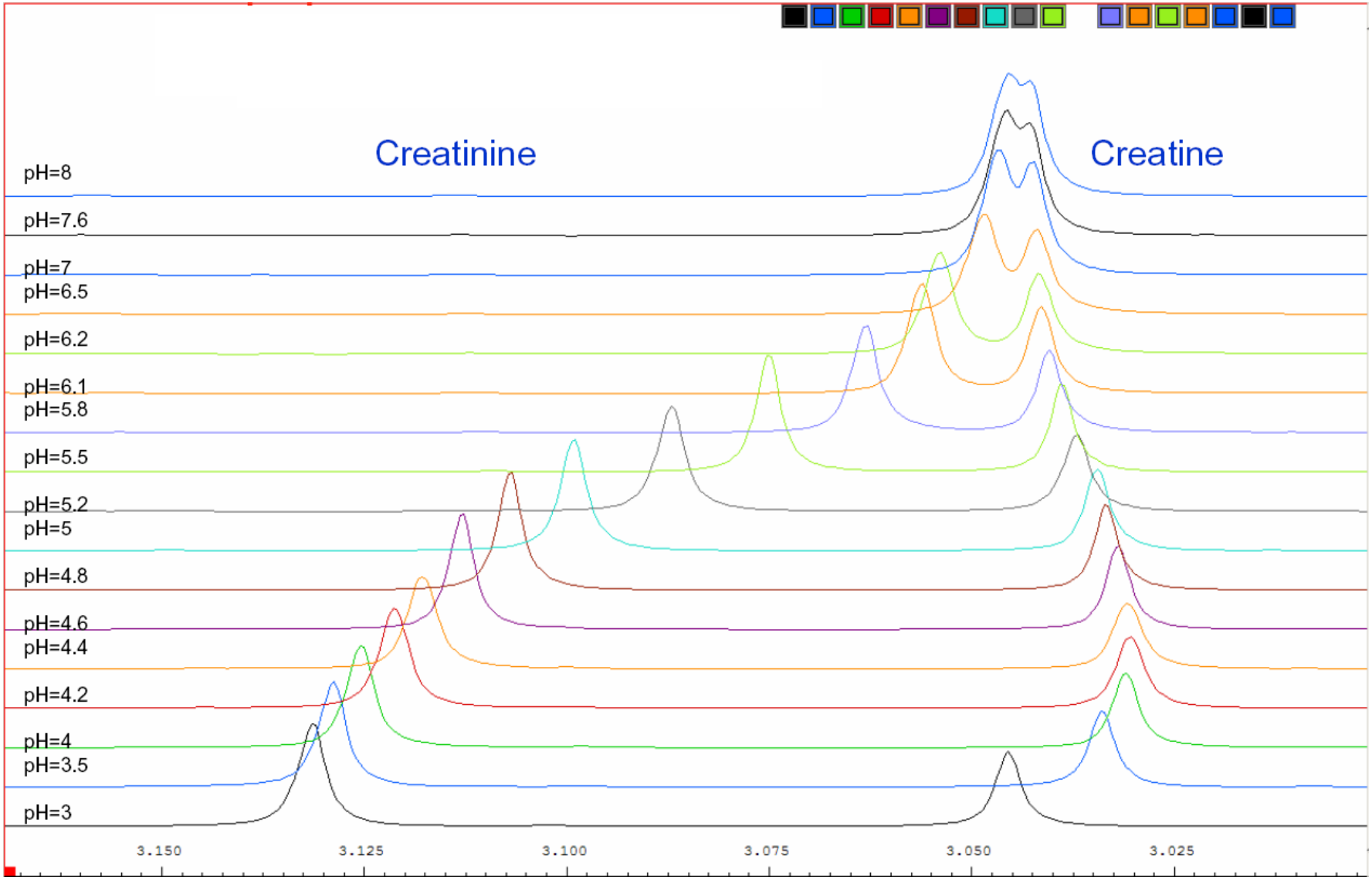
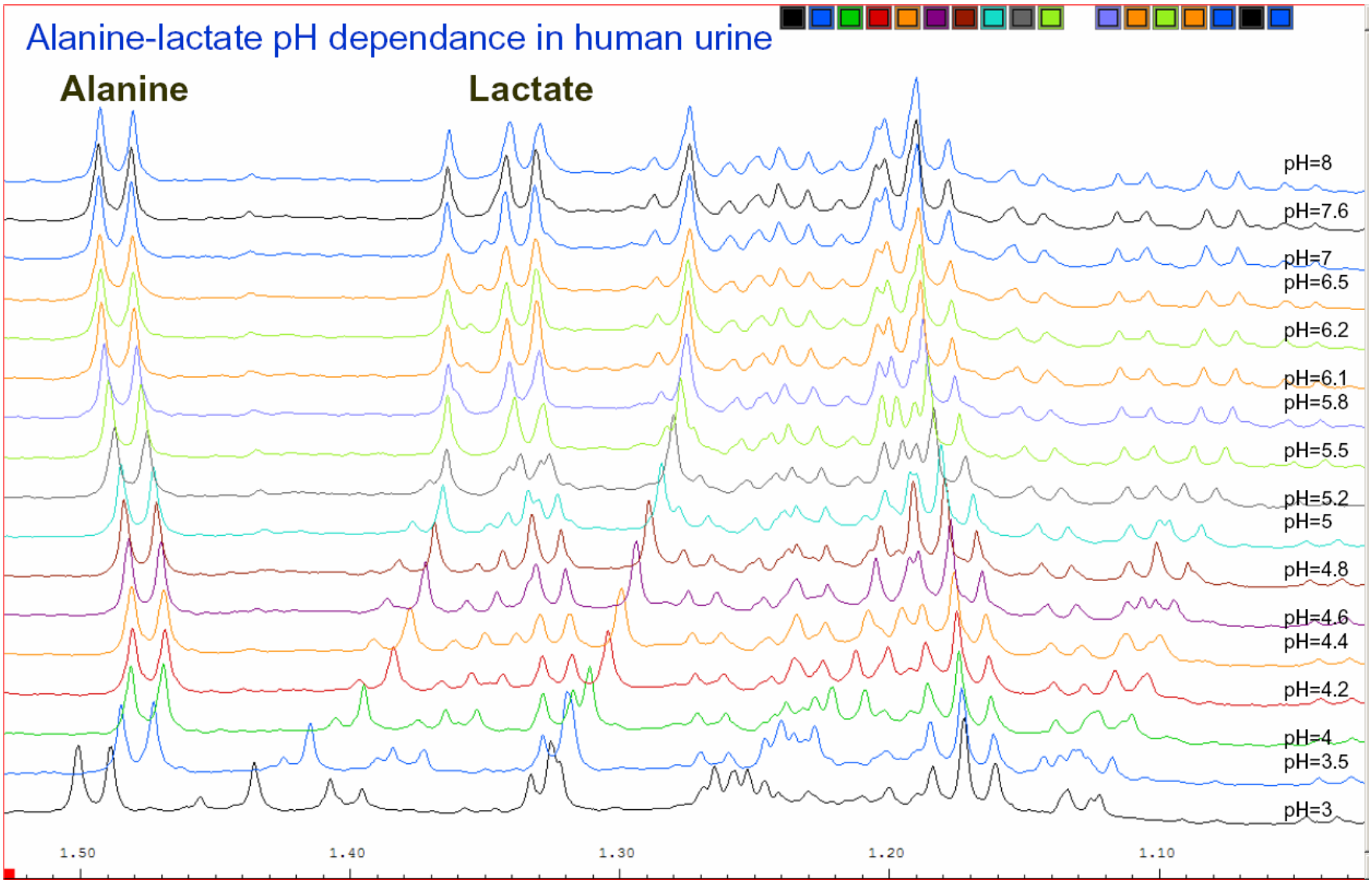
pH changes


Creatinine
Creatine
pH=8
pH=7.6
pH=7
pH=6.5
pH=6.2
pH=6.1
pH=5.8
pH=5.5
pH=5.2
pH=5
pH=4.8
pH=4.6
pH=4.4
pH=4.2
pH=4
pH=3.5
pH=3
3.150
3.125
3.100
3.075
3.050
3.025

pH changes (…)

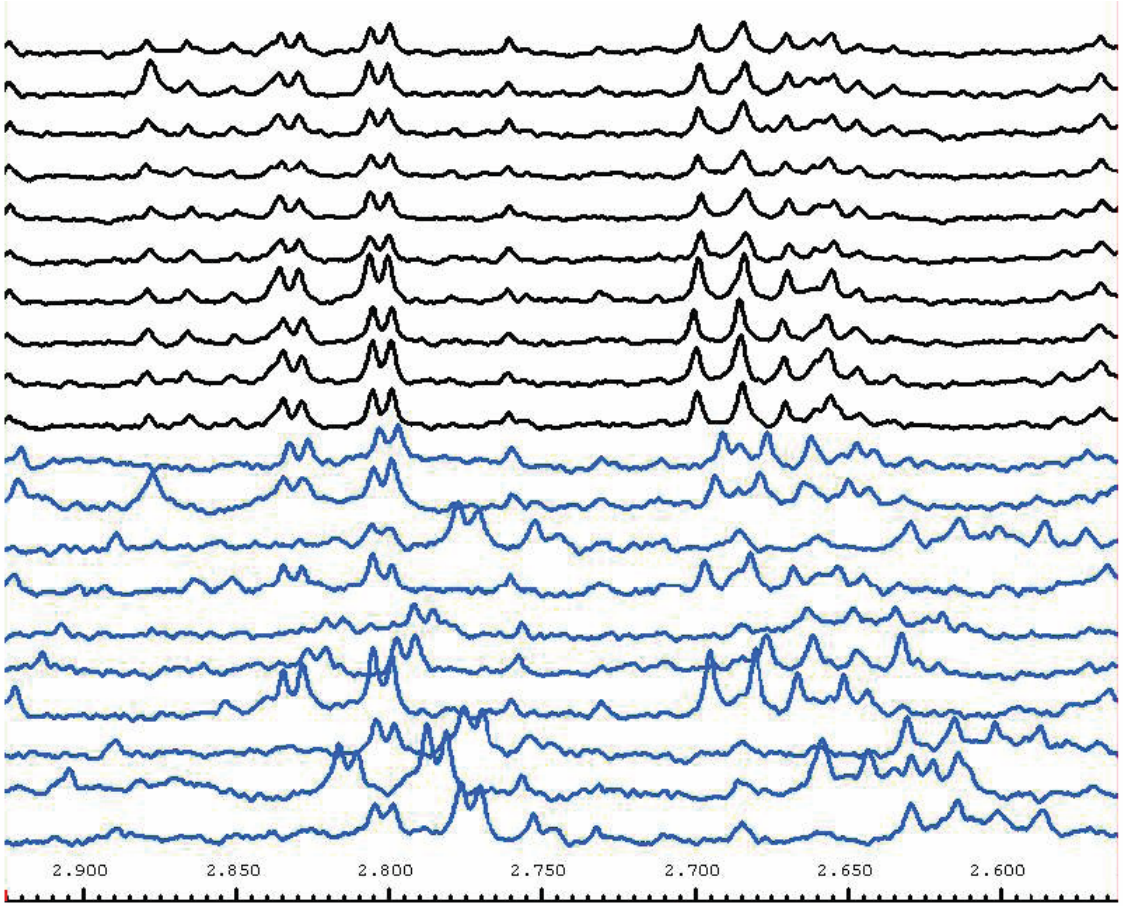

Alanine-lactate pH dependance in human urine
Alanine
Lactate
pH=8
pH=7.6
pH=7
pH=6.5
pH=6.2
pH=6.1
pH=5.8
pH=5.5
pH=5.2
pH=5
pH=4.8
pH=4.6
pH=4.4
pH=4.2
pH=4
pH=3.5
pH=3
1.50
1.40
1.30
1.20
1.10

pH adjustment

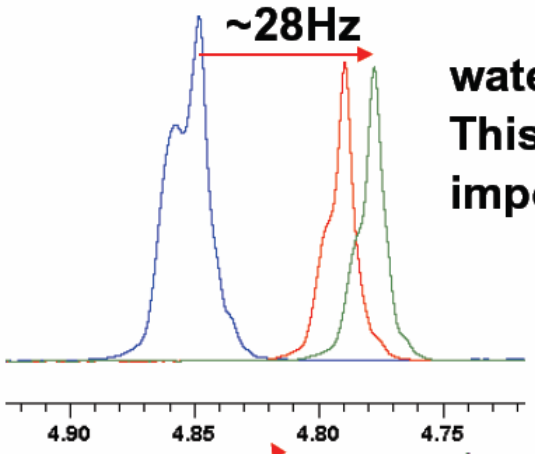
Buffer + pH adjustment
(pH = 7)

Buffer:
1.5M phosphat buffer
(KH2P04) in D20.
~0.01% NaN3 and
0.1% TSP is added.

extreme cases most affected region



Temperature effects

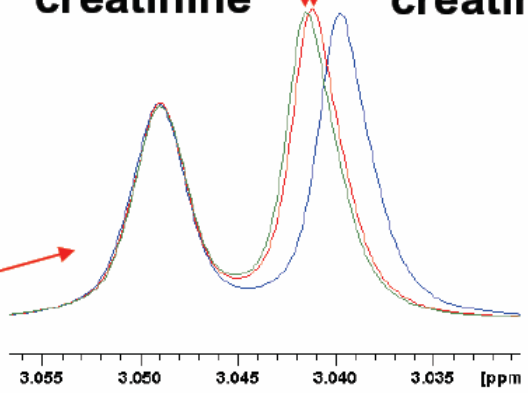


water: ~4.5 Hz / K!
This is the most important effect!!!

~0.1Hz / K

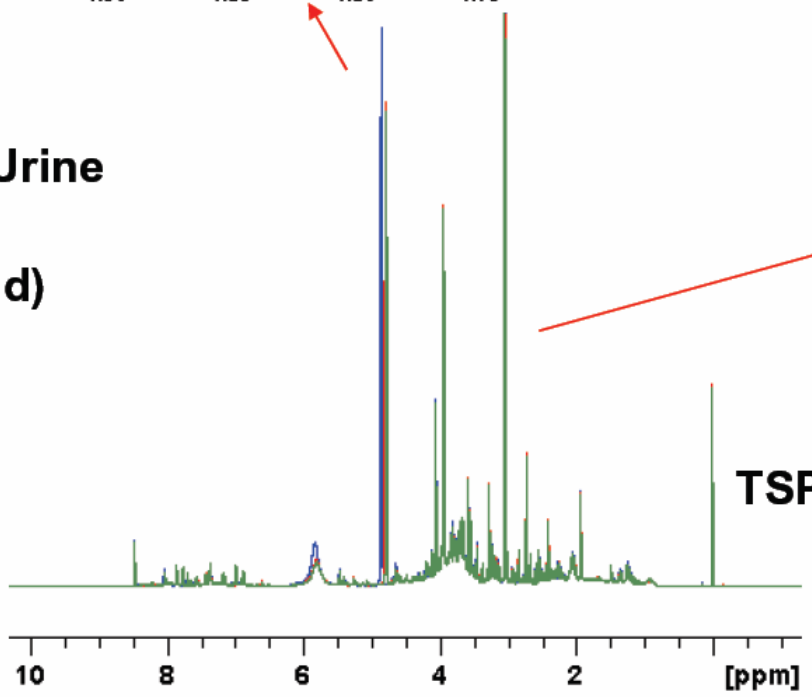
creatinine

creatine

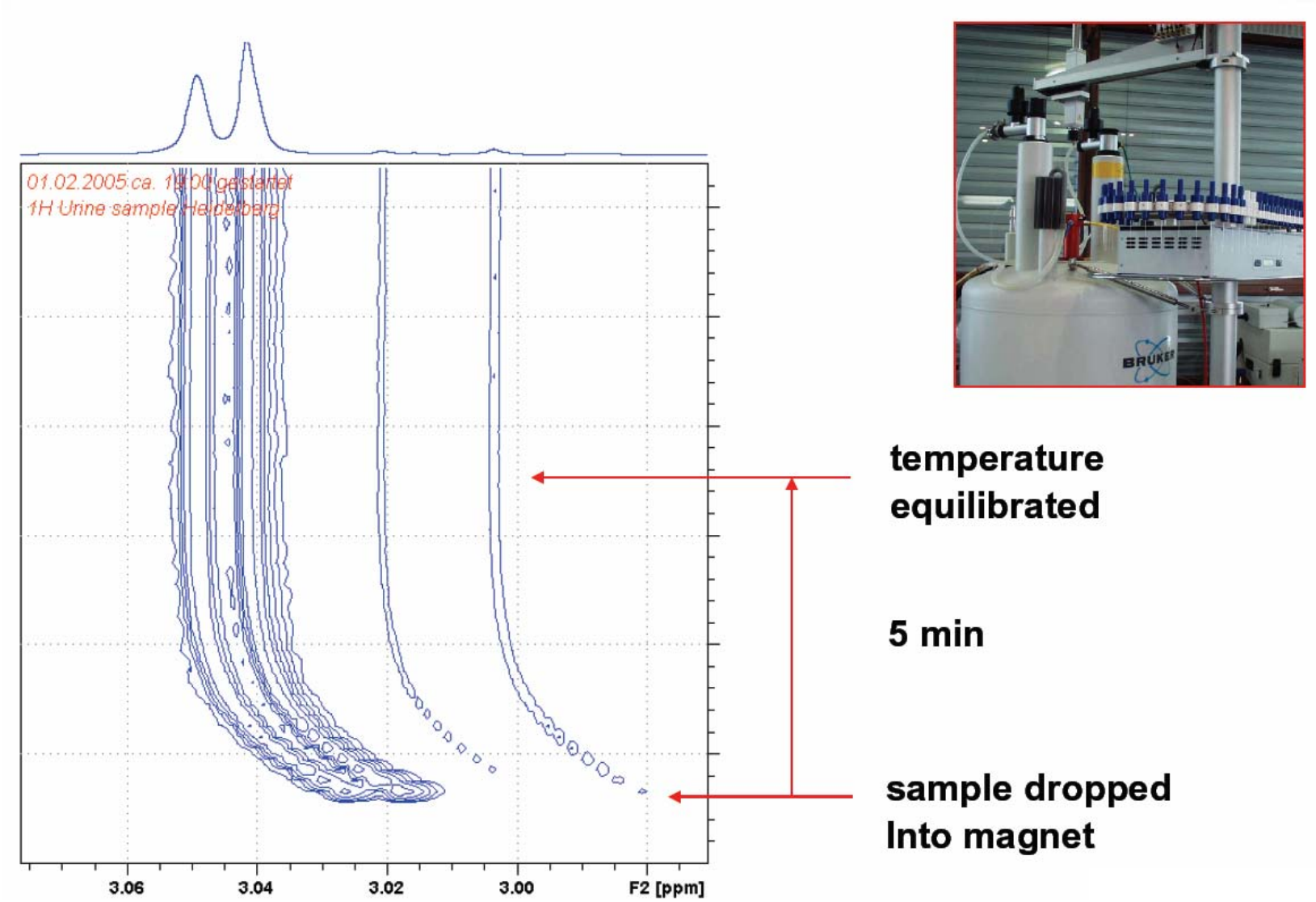


1H NMR on Urine
@ 400 MHz
(noesygppr1d)

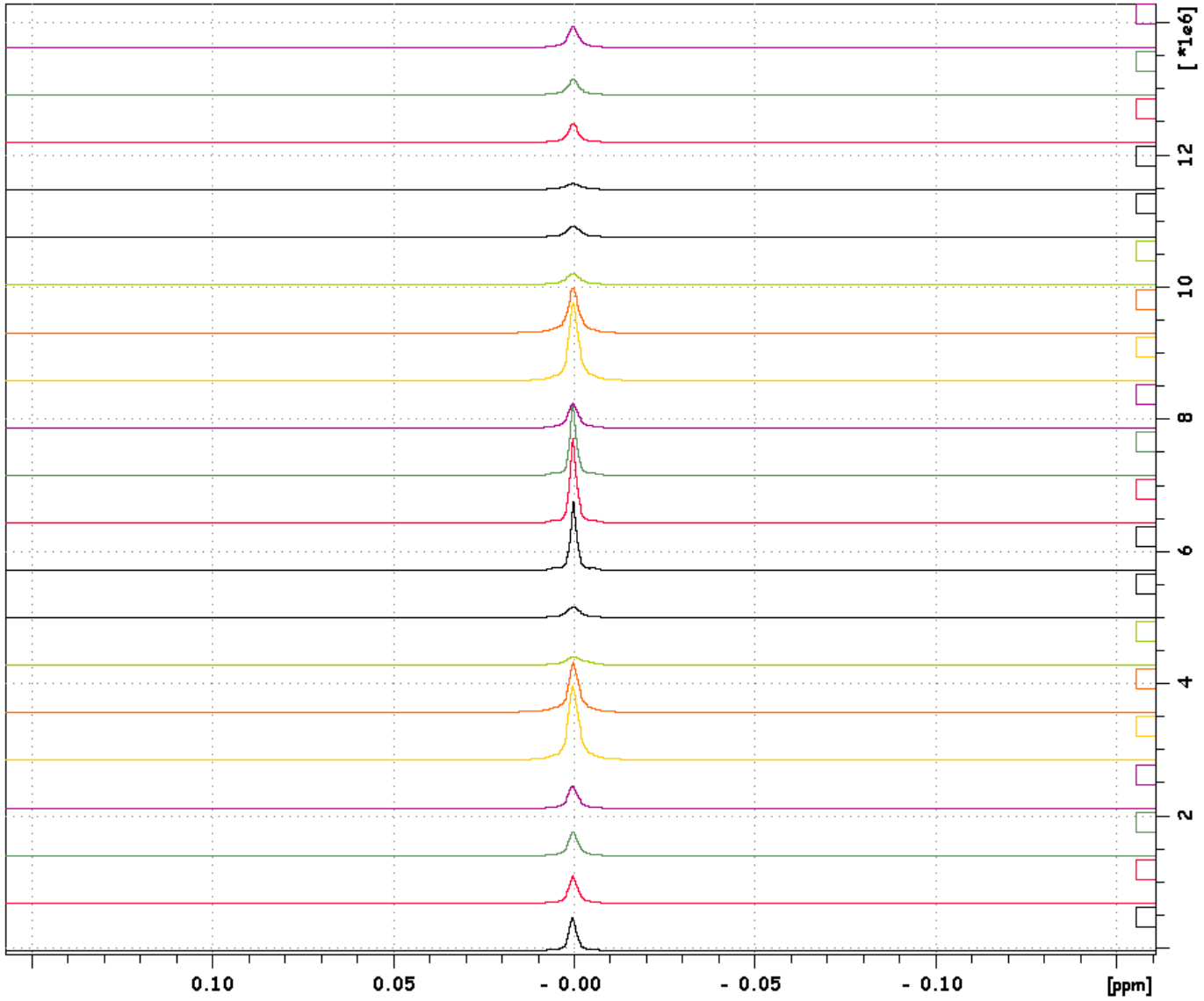
295K
300K
301K



Temperature equilibration

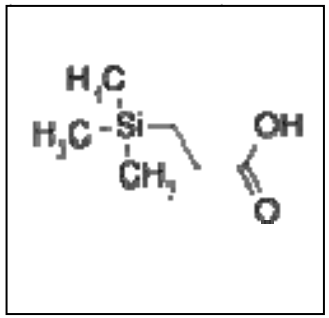


La concentrazione del riferimento è riproducibile?

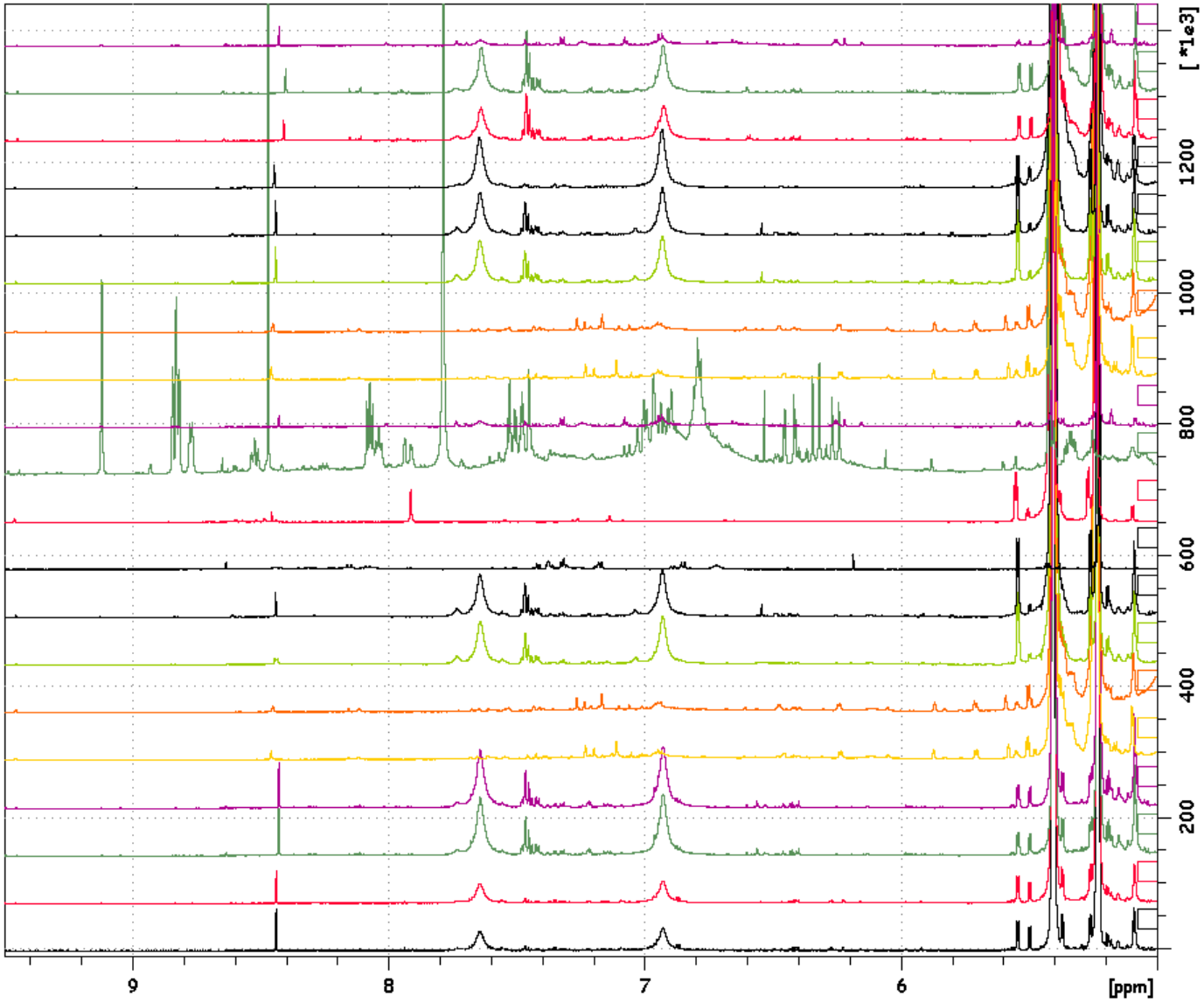


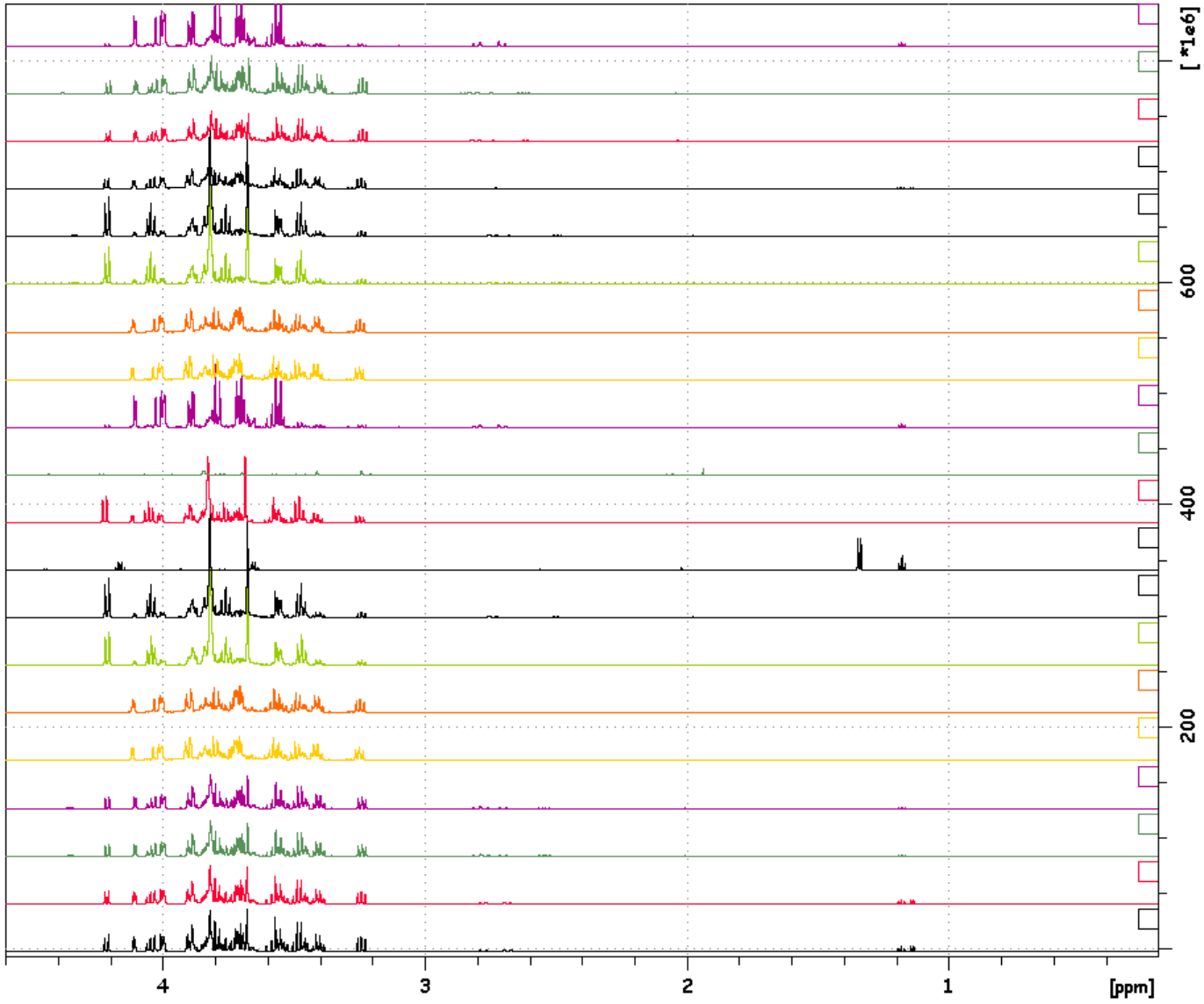
L’ omogeneità di campo è soddisfacente?

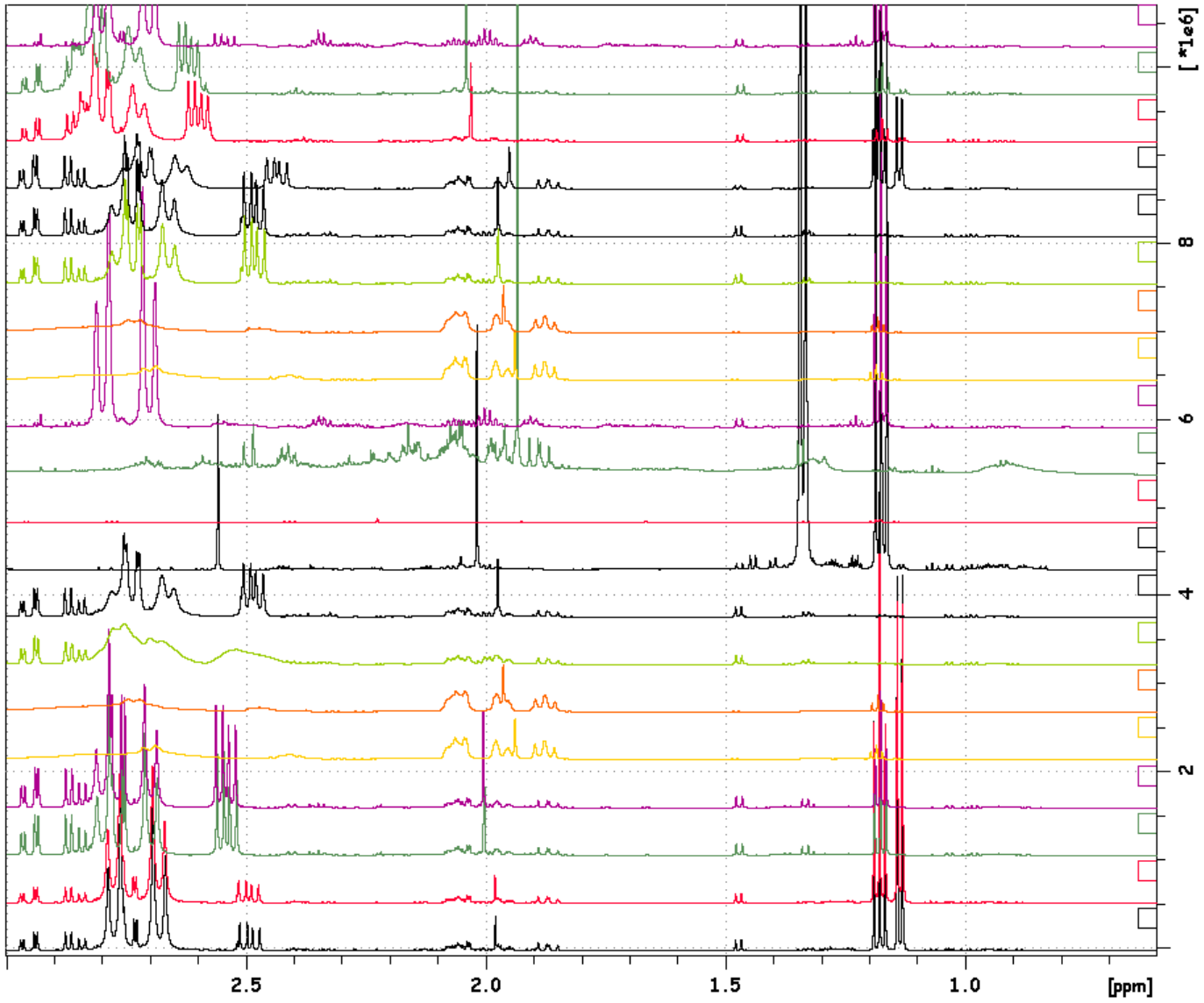
3-(Trimethylsilyl)propionic acid-d4 sodium salt



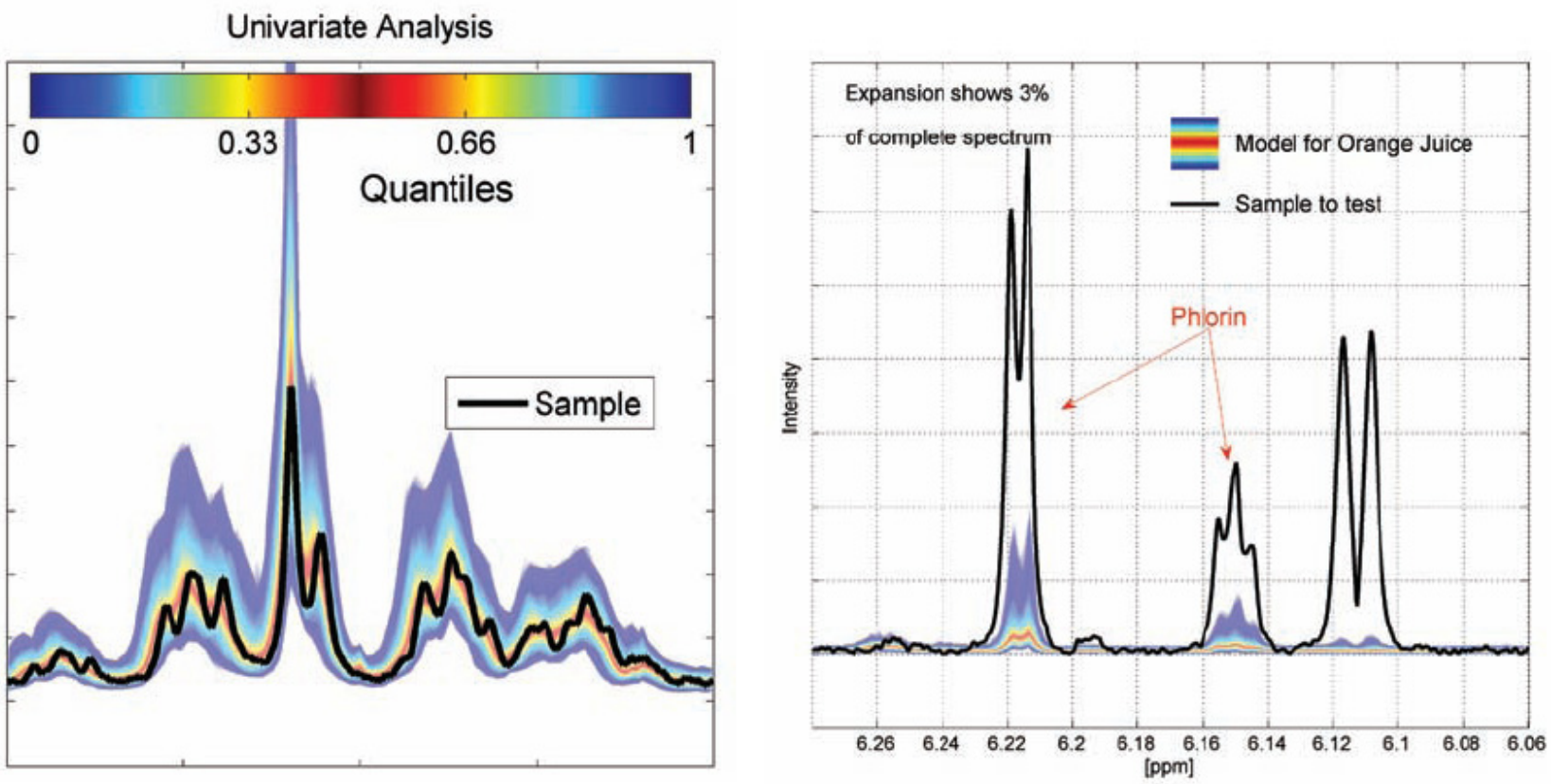
Il pH risulta omogeneo?




[*1e6]
600
400
200
4
3
2
1
[ppm]


[*1e6]
8
6
4
2
2.5
2.0
1.5
1.0
[ppm]

Juice Verification

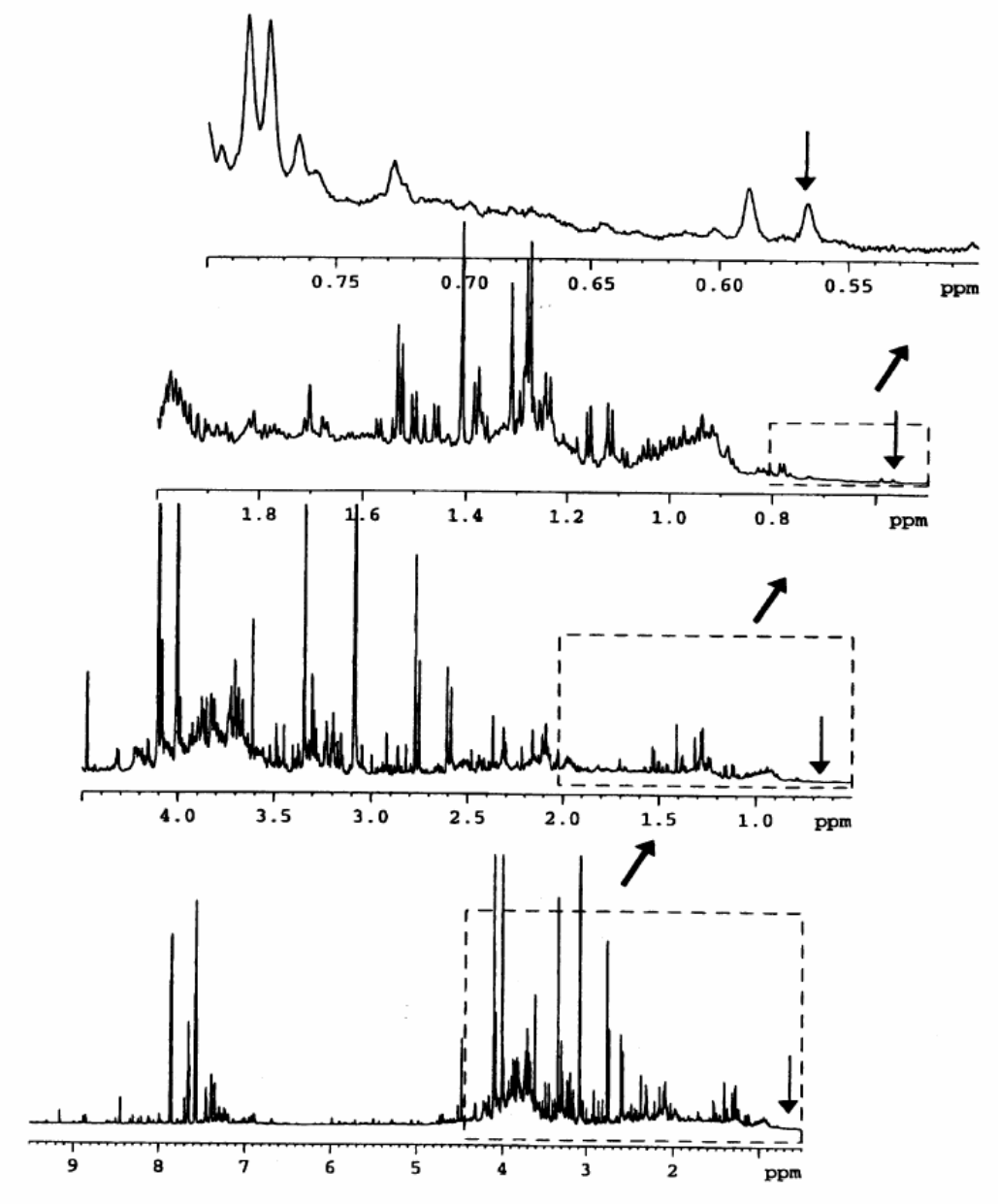


Verification of fruit juice samples. Left: apple juice, the 400 MHz ^{1}H spectrum in the region near 2 ppm (black trace) is plotted over a quantiles plot (color) of the model spectra set (univariate analysis of apple juice at 2 ppm). Right: orange juice, unusual high amount of phlorin indicates the usage of orange peel.

Recent NMR developments relevant to metabolomics studies

- Sensitivity and dispersion gains from ultra high field magnets – up to 900 MHz ^{1}H observation frequencies
- Sensitivity improvements from use of cryogenic probes
- High throughput from flow-injection NMR methods and robotics
- Simplification of profiles from spectral editing – e.g. coherence level filtering, editing based on molecular diffusion and spin relaxation properties, 2D NMR
- Sample sparing using micro-scale NMR probes - 5 nl - 30 µl range
- High resolution magic angle spinning NMR of intact tissues
- Biomarker identification using hyphenated NMR methods - HPLC-MS-NMR
- Identification of biomarker peaks using statistical methods across technologies

Spectral resolution



900 MHz NMR spectrometer

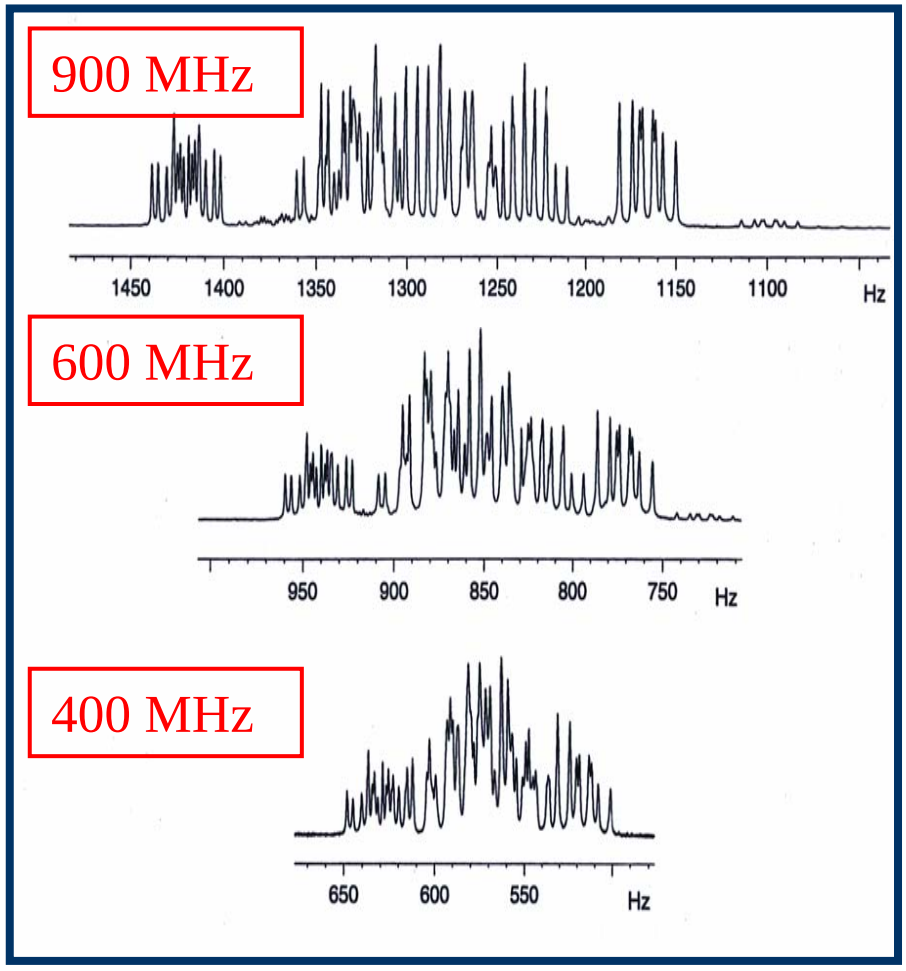
Actively shielded superconducting magnets

These contain a second coil to partly compensate for the magnetic field of the main magnet coil

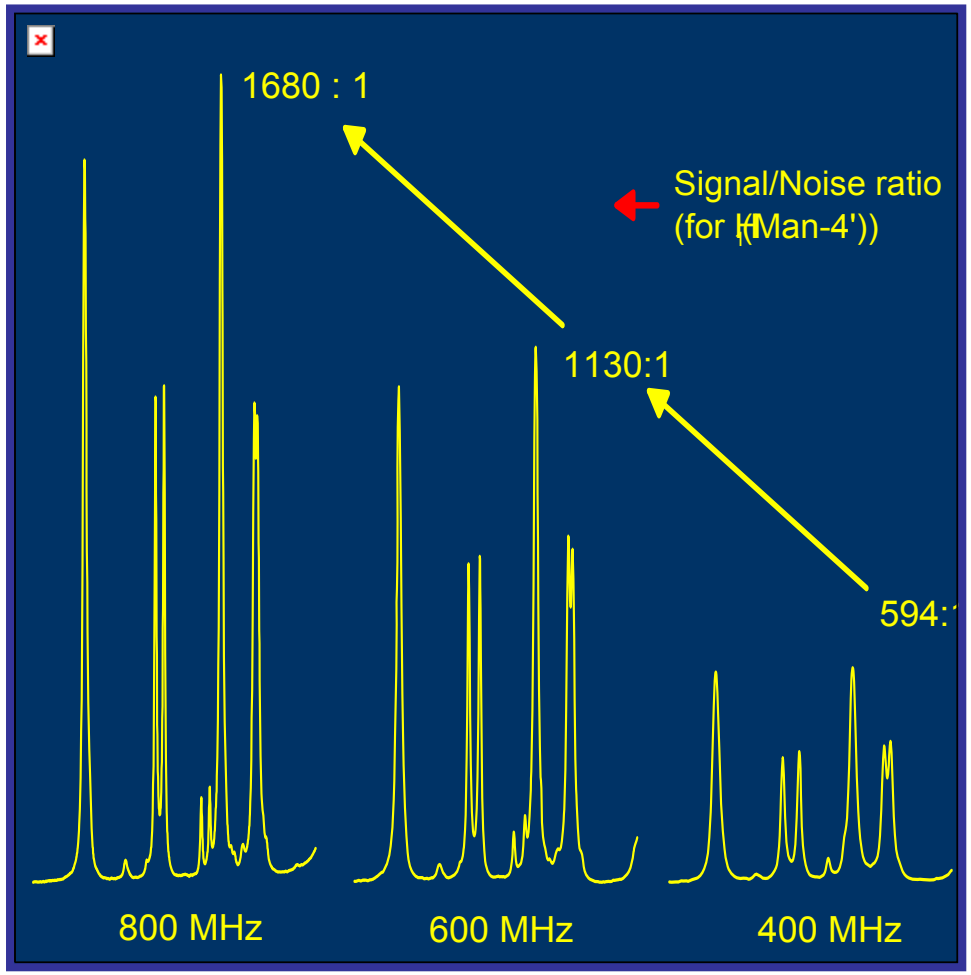
This results in a drastically reduced 5 gauss line, allowing ultra high field magnets to be located more easily

It also means that HPLC-NMR equipment and mass spectrometers can be moved much closer to the magnet, resulting in shorter transfer times and pathways

Sensitivity and dispersion gains by increasing field strength



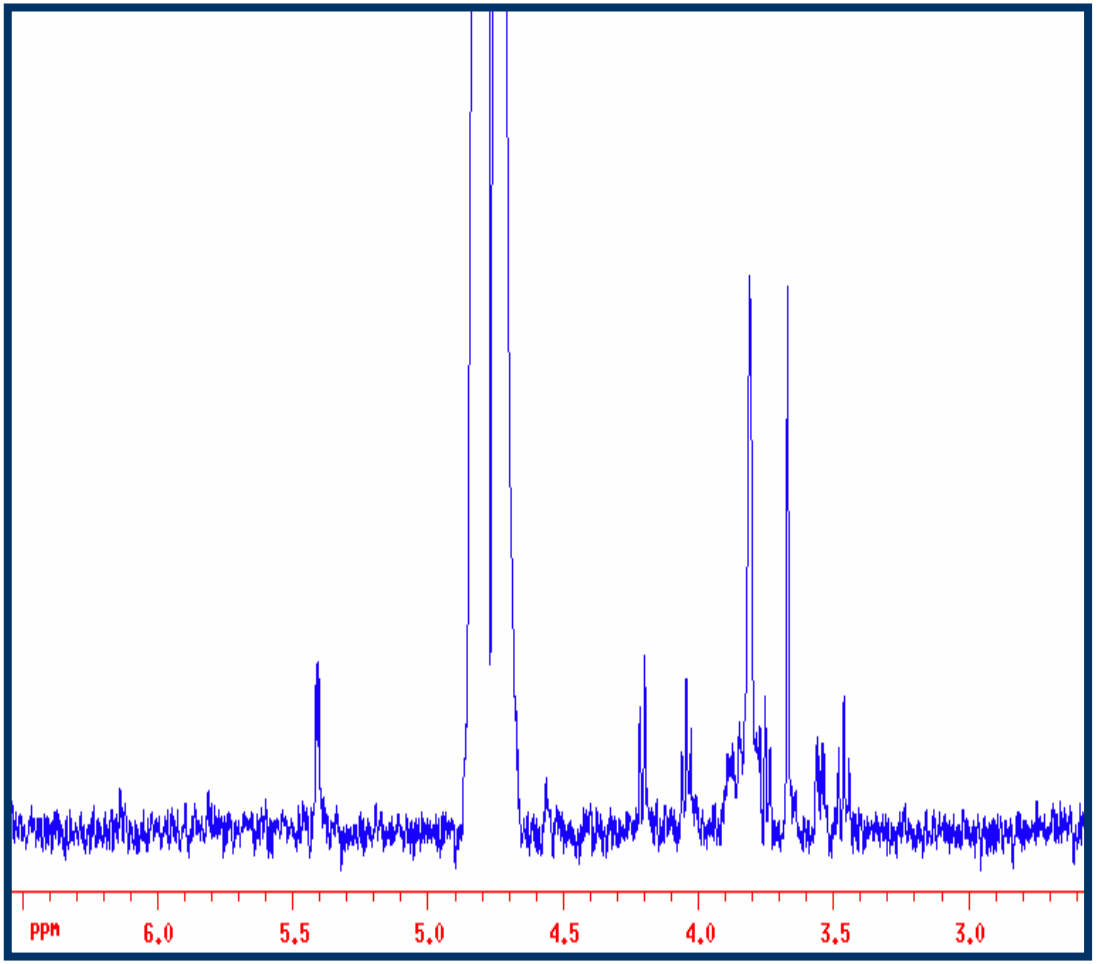
Oestradiol acetate in $CDCl_3$



Decasaccharide sample, 64 scans

Robotic sample preparation and NMR measurement

Cryoprobe technology



NMR detector coil and RF preamplifier cooled to around 20K

Reduces the thermal noise in the circuits by ca 4-5 times

Can detect 20 ng sucrose in 11.3 µl D_2O

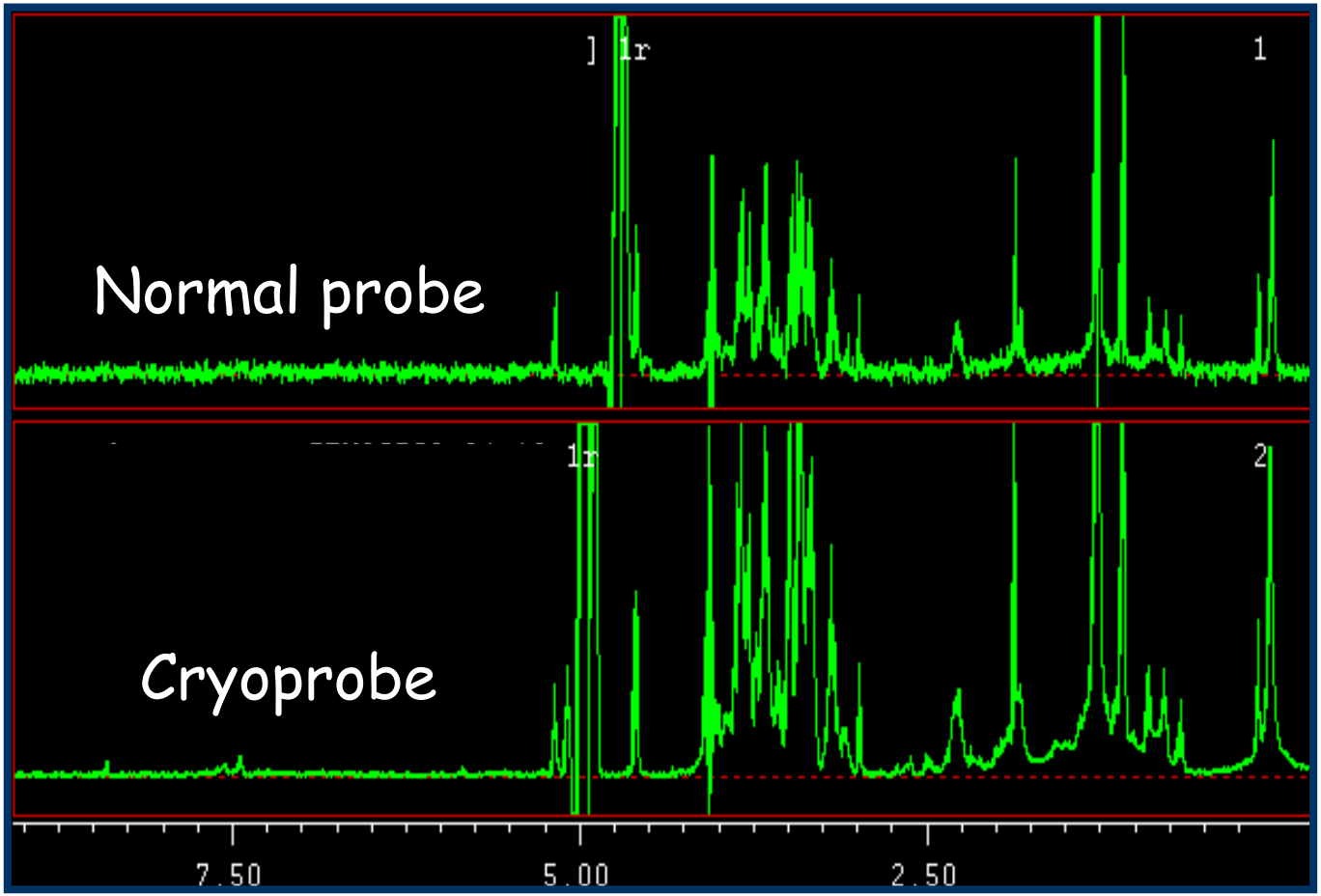
400 Scans LB = 1
1.7 mm capillary

~ 32 minutes

^{1}H NMR spectra of mouse CSF

Only 10 - 20 μl available

512 scans, 30 min, 20 ms echo time, 15 μl freeze-dried and dissolved in 25 μl D_2O, measured in a 1.7 mm capillary tube



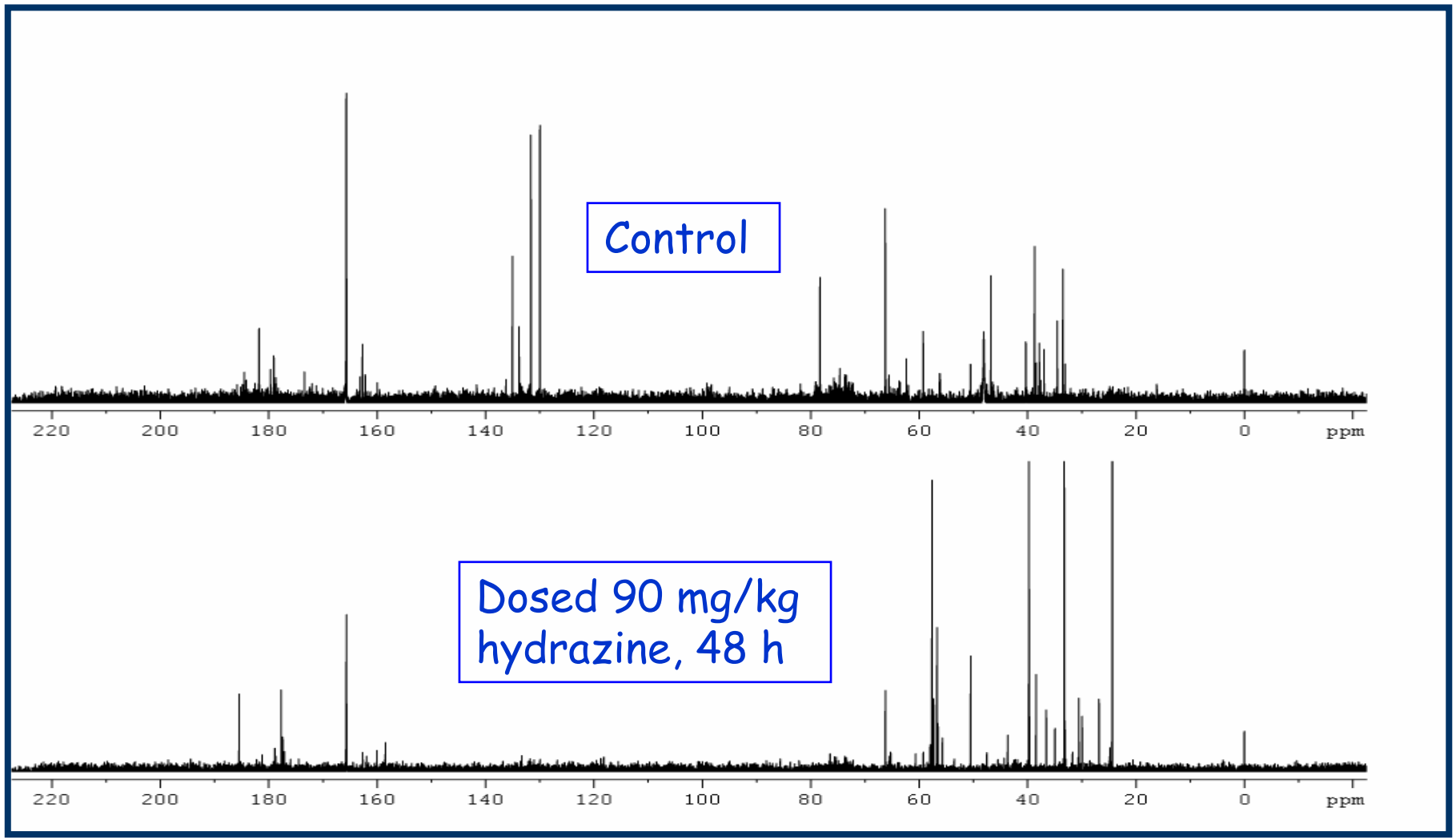
S/N = 7:1 normal

S/N = 32:1 cryoprobe

Cryoprobe ^{13}C NMR spectra

500 MHz $^{13}C/^{1}H$ dual probe

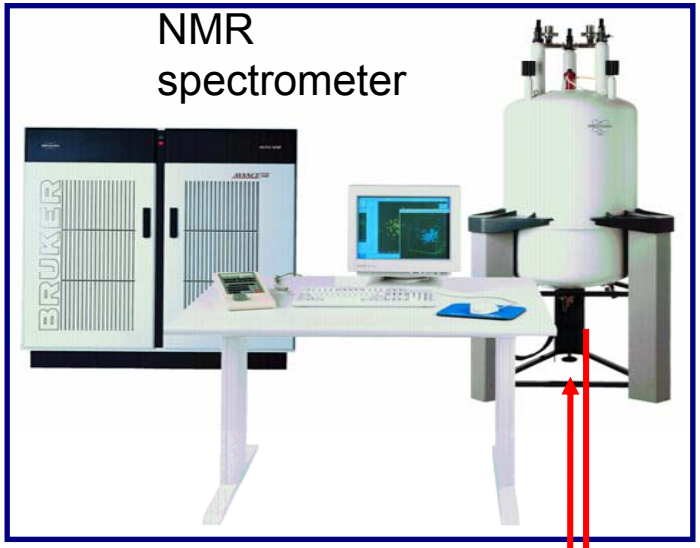
Rat urine diluted 2:1 with buffer, 512 scans, 30 minute acquisition



Directly-coupled HPLC-NMR-MS

HPLC, oven, diode array detector and loop collector

NMR spectrometer



Splitter

95%

5%

Mass spectrometer, usually ion trap

Fraction collector

On-flow, isocratic and using HPLC solvent gradients

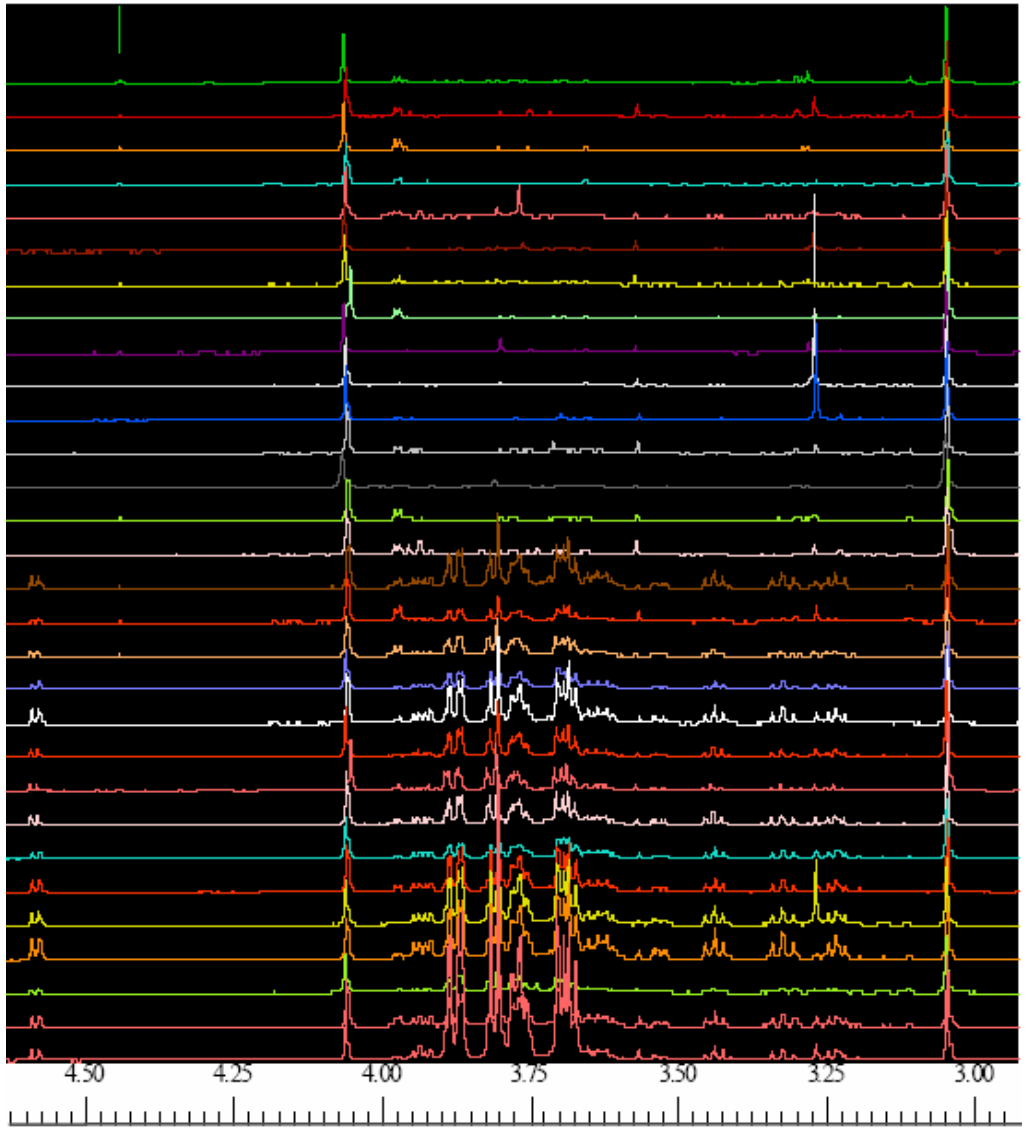
HPLC peak automatic detection, followed by:

Direct stop flow

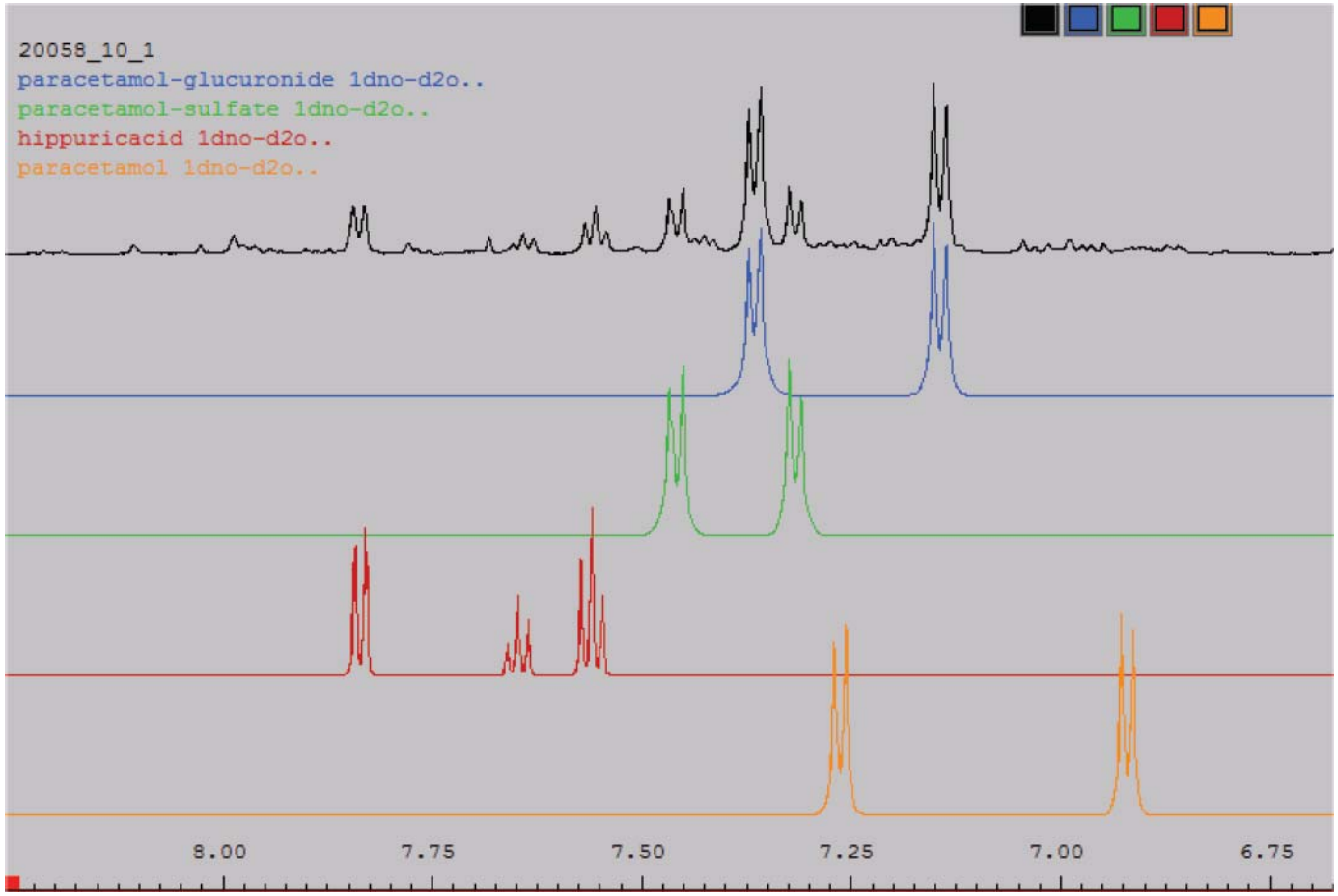
Time slicing

Loop collection, and later sequential measurement of loop contents

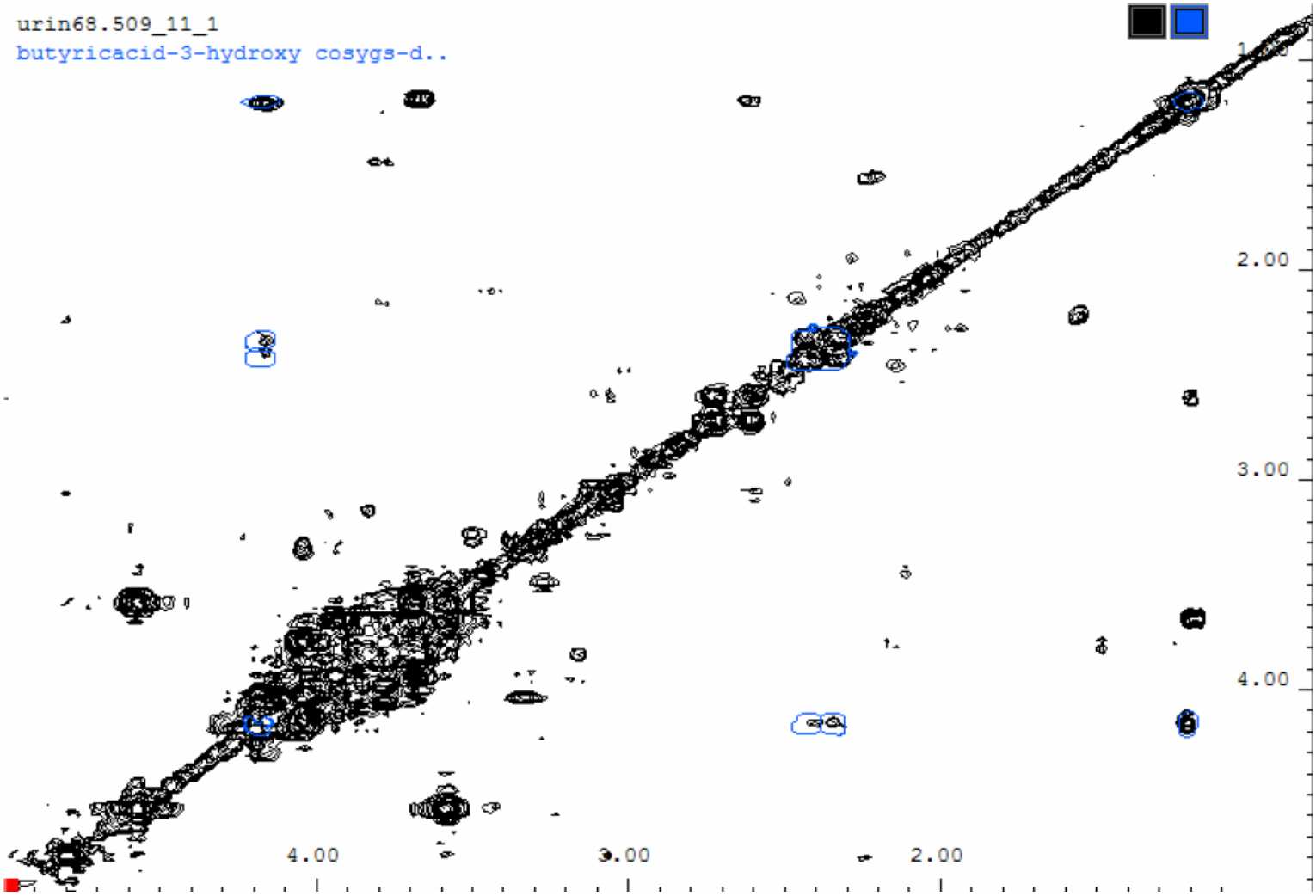
Visual inspection



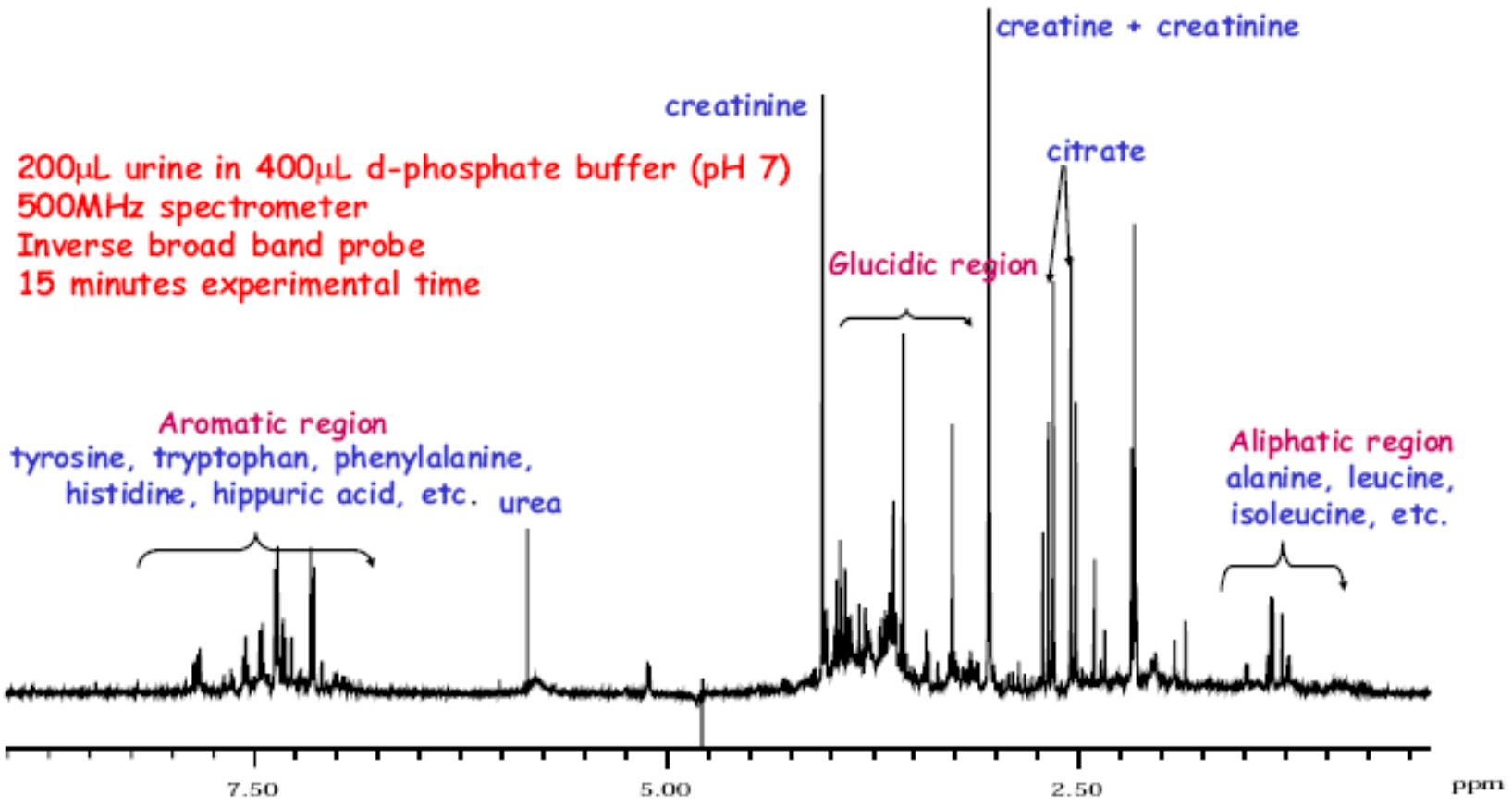
Peak assignment: reference databases



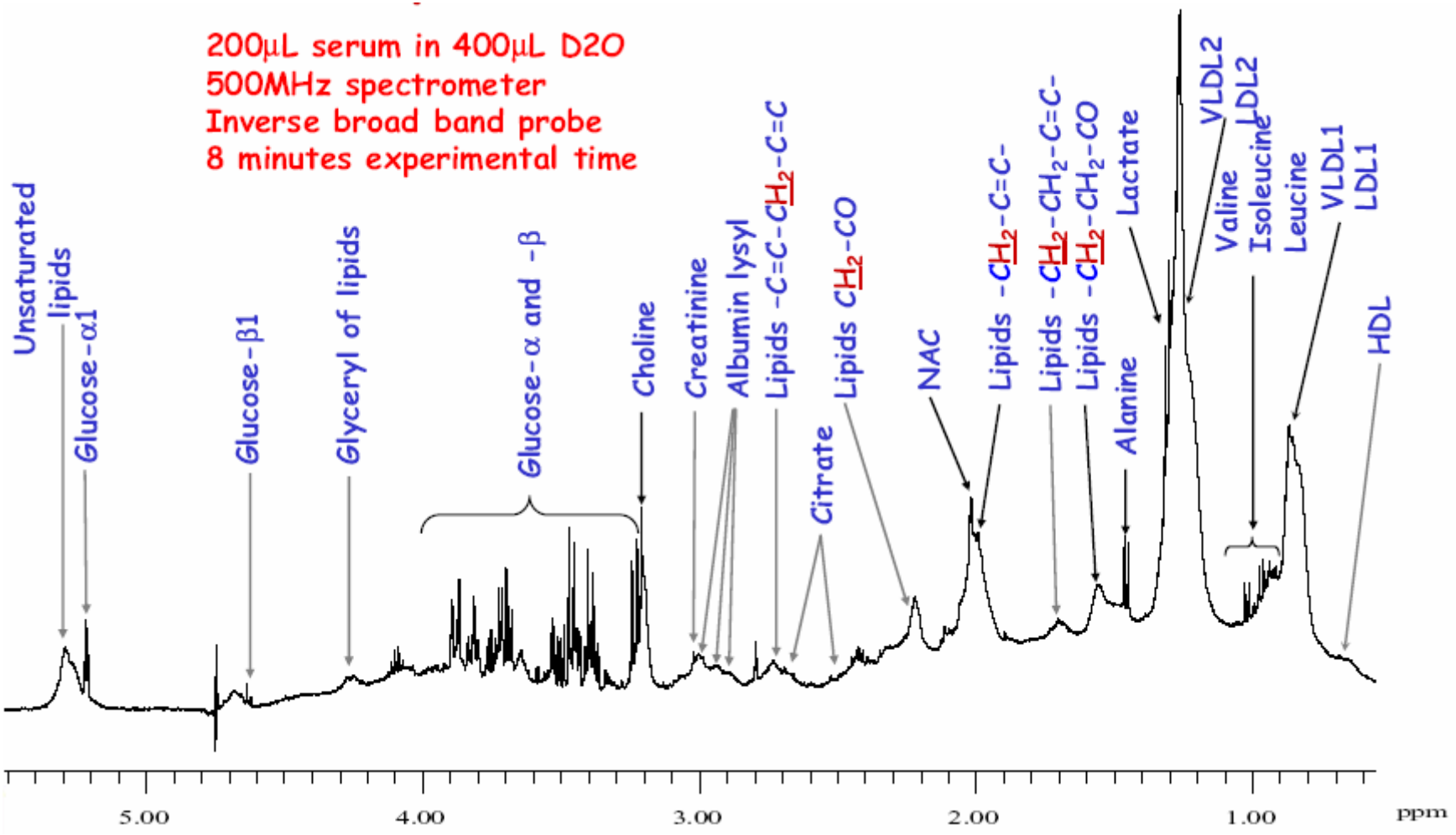
Peak assignment (...)



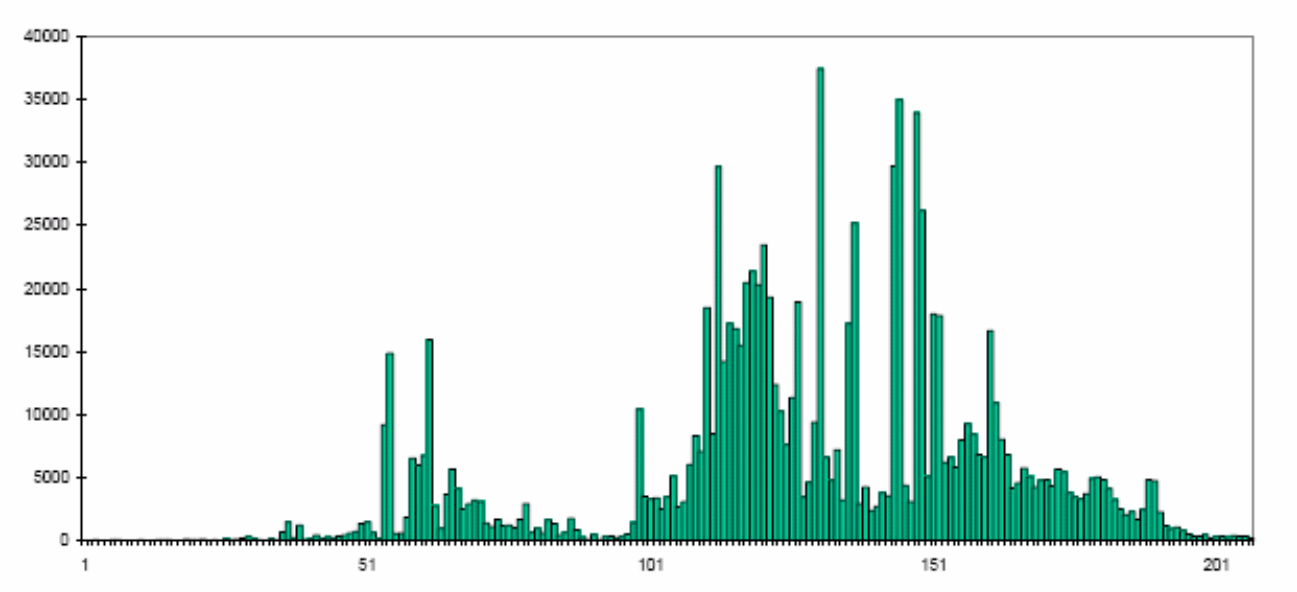
Assignment of major peaks/regions



1H NMR of human blood serum

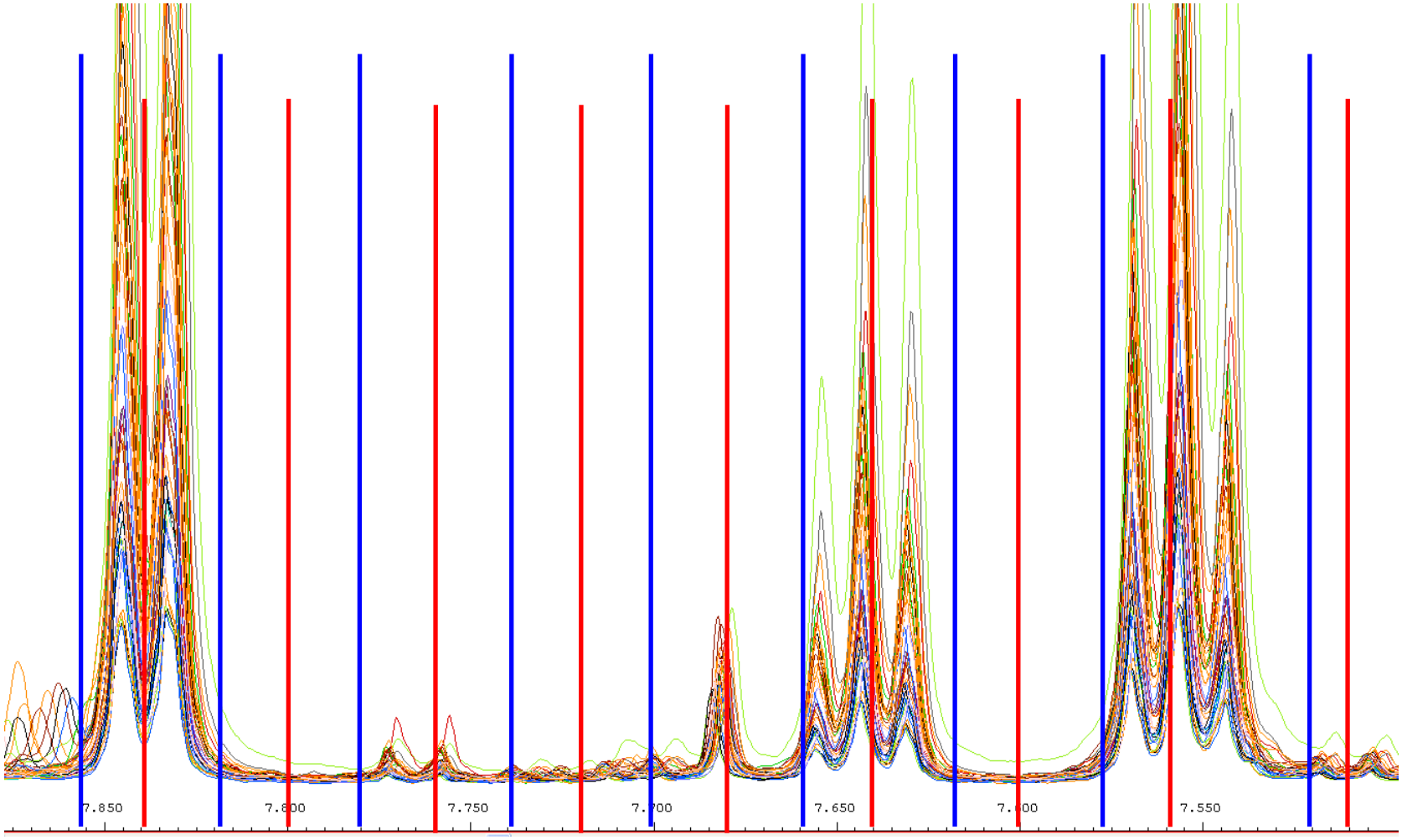


Data pre-processing



- Discretise x-axis into *n* equal sized bins, height = area under intensity (reduces impact of small variations in chemical shift e.g. due to pH)
- Normalise bars for constant total area (removes effect of differences in concentration across samples)
- Remove insignificant regions (e.g. water and urea resonances in urine spectra)

Fixed vs variable bucketing



Normalization