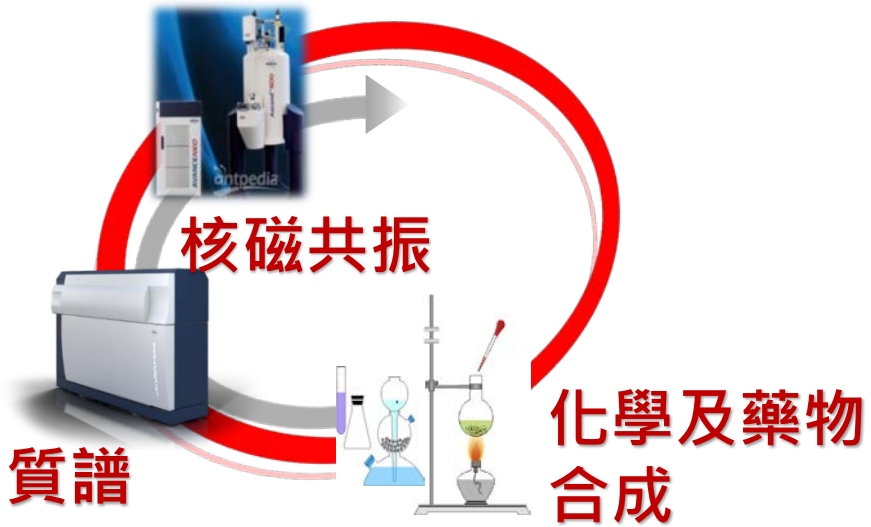
Metabolomics NMR using IVDr



藥物合成及分析核心設施
Medicinal Chemistry and
Analytical Core Facilities

NMR Core Facility
核磁共振子核心

羅元超

2023/10/25

TIGP-Course: NMR Spectroscopy-based Metabolomics

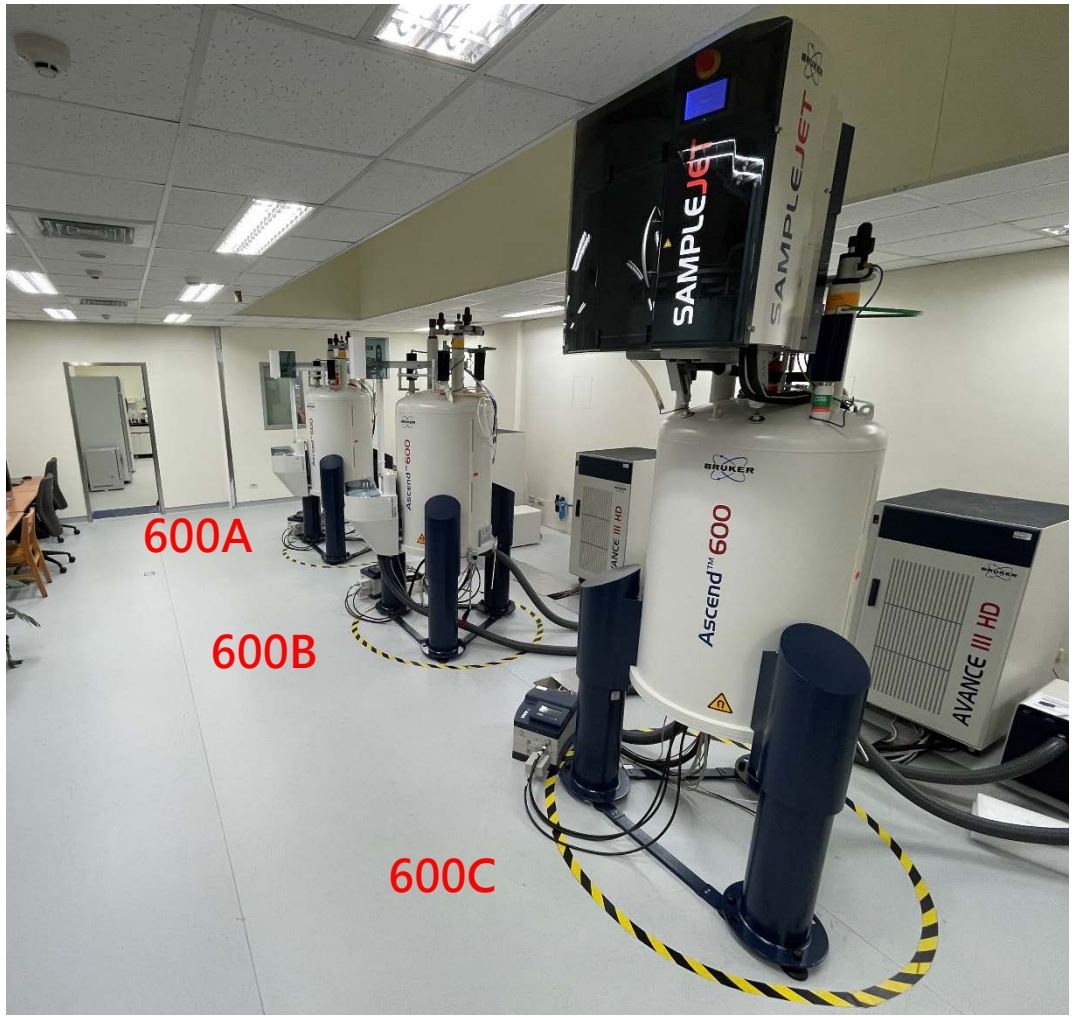
Date	Topic	Lecturer
10/26	Applications of NMR-based metabolomics I - Preface	M-S Hsiao 蕭明熙教授
11/02	Applications of NMR-based metabolomics II - Cancers	M-S Hsiao
11/09	Applications of NMR-based metabolomics III- Diabetes	M-S Hsiao
11/16	Applications of NMR-based metabolomics IV - Cardiovascular and neurodegenerative diseases	M-S Hsiao

Class hours: Thursday, 10:00-12:00

Classroom: A507, Institute of Chemistry, Academia Sinica

NBRP國家生技研究園區-核磁共振核心

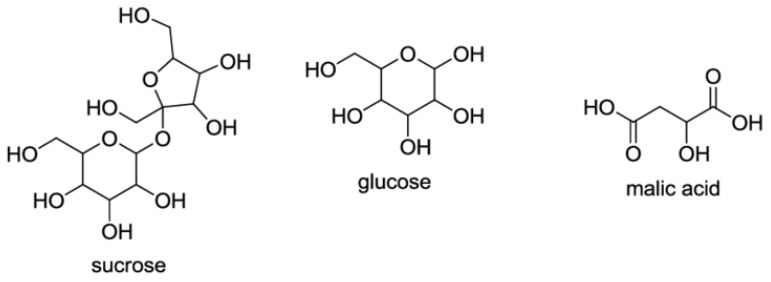
核心人員: 羅元超、袁茂嘉、王瓊萩

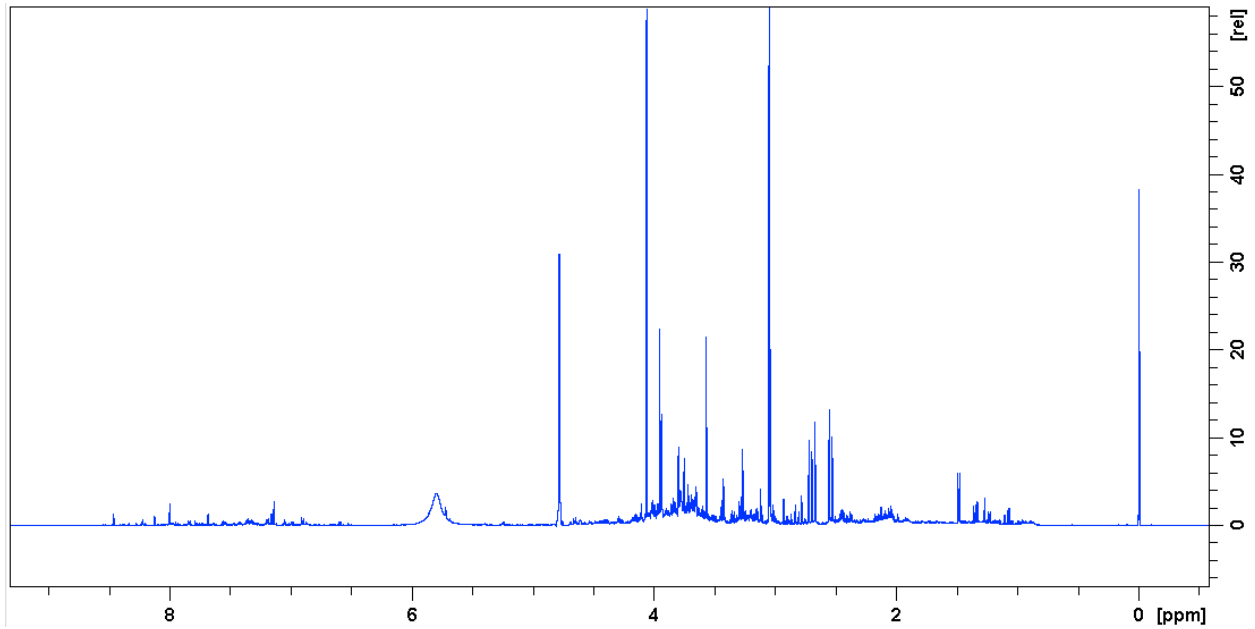


600A	• NBRP users • Reservation < 1hr • Acquire ^{1}H, ^{13}C, ^{19}F, ^{31}P signals • Auto-sampling for 24 samples • Open for self-operation
600B	• NMR collection services by core staff • Open for long reservation • Acquire ^{1}H, ^{13}C, ^{19}F, ^{31}P signals • Cooled auto-sampling for 24 samples • Open for self-operation
600C	• Bruker IVDr system • Automatic metabolic profiling for human plasma/serum and urine samples • High-throughput cooled auto-sampling for 96 x 5 samples

Outline:

- What is Metabolomics
- NMR Metabolomics
- Bruker IVDr Platform for Automatic Metabolic Profiling





For Beginners!!

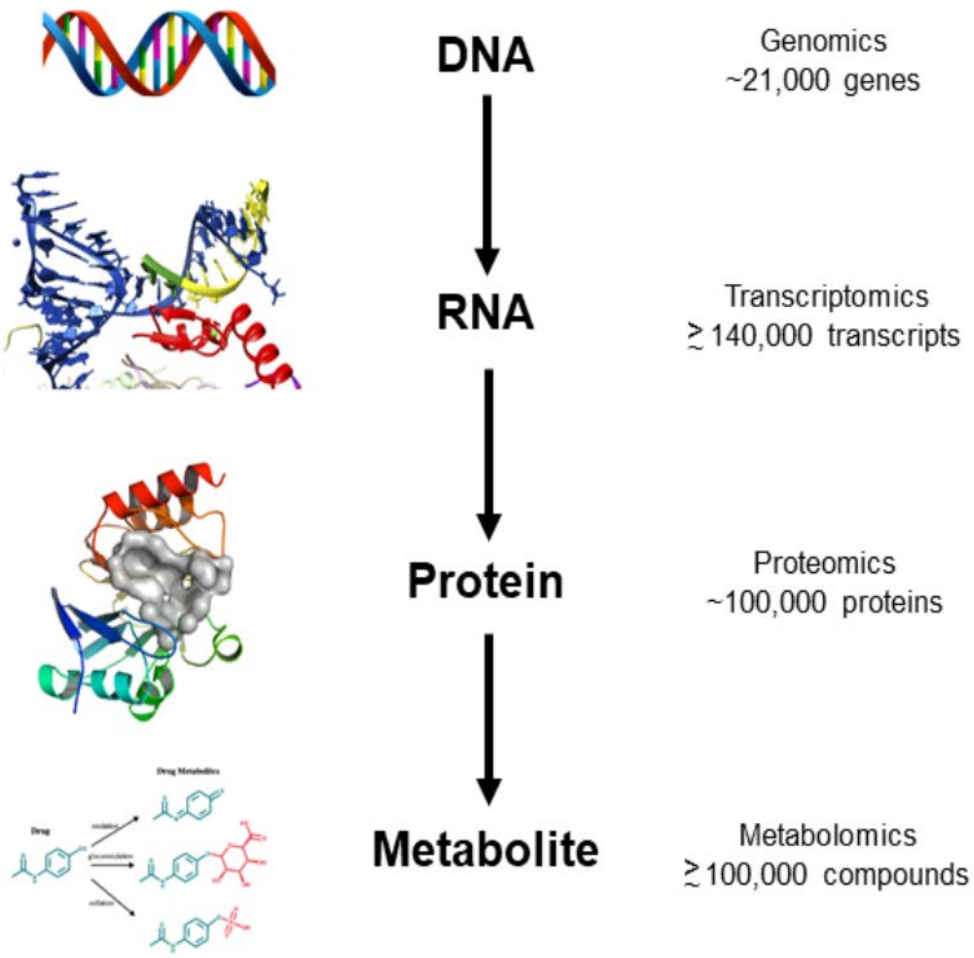
- Two Researchers: David Wishart & Jeremy Nicholson
- Websites:
  - https://www.tmicwishartnode.ca/
  - https://hmdb.ca/
  - https://www.metaboanalyst.ca/
- References: SO MUCH !!!

What is Metabolomics

Metabolomics is the study of small molecules called metabolites. Metabolomics is an emerging field of "omics" research specializing in the near-global analysis of small-molecule metabolites (1.5 KDa) found in living organisms.

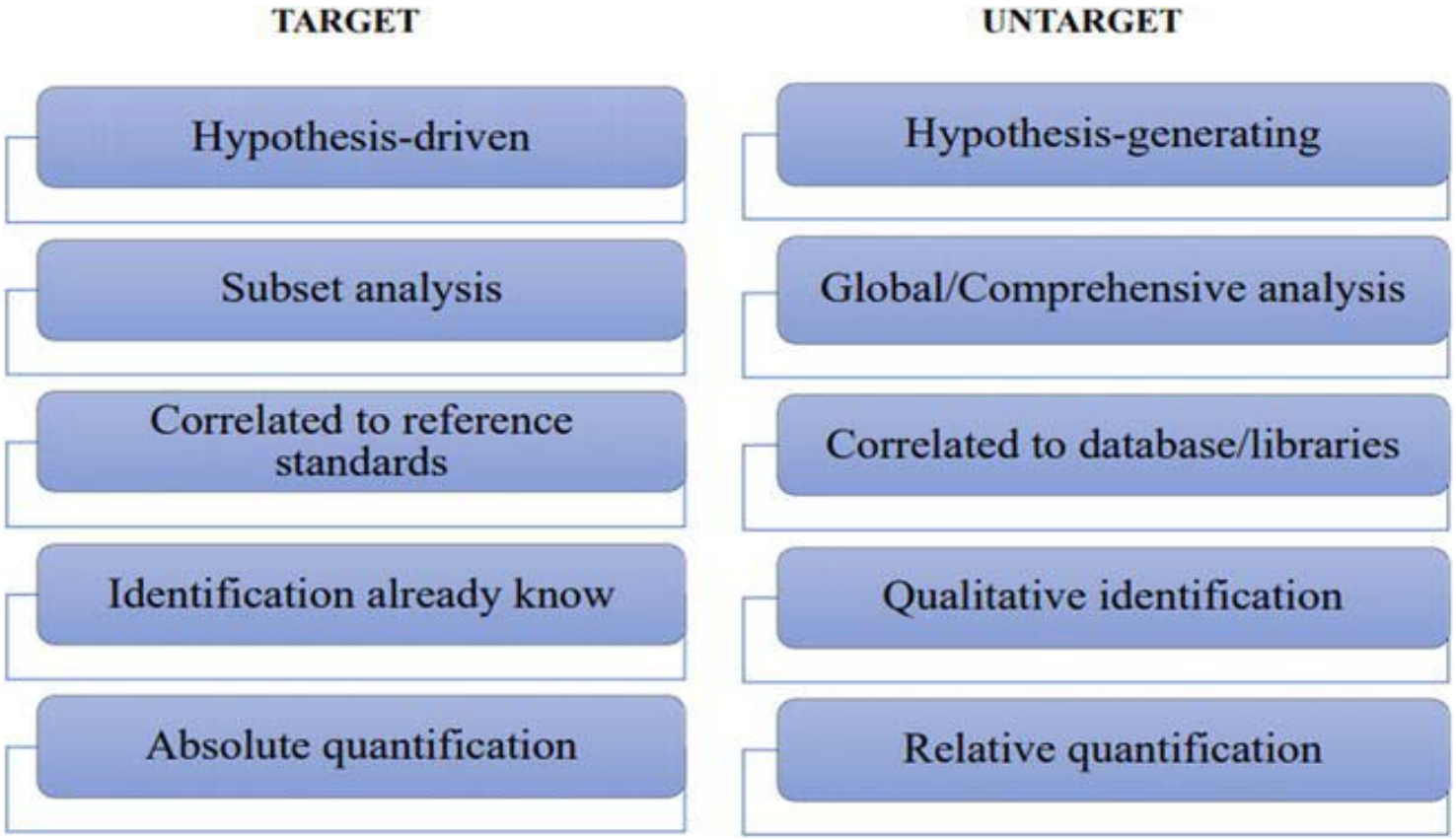
Metabolomics is applicable in many disciplines including disease diagnostics, agriculture, food and safety, and pharmaceutical research and development.

The use of metabolomics allows for the discovery of biomarkers, which in turn leads to the development of improved screening methods.



https://www.tmicwishartnode.ca/metabolomics-services/

Targeted vs Untargeted Metabolomics



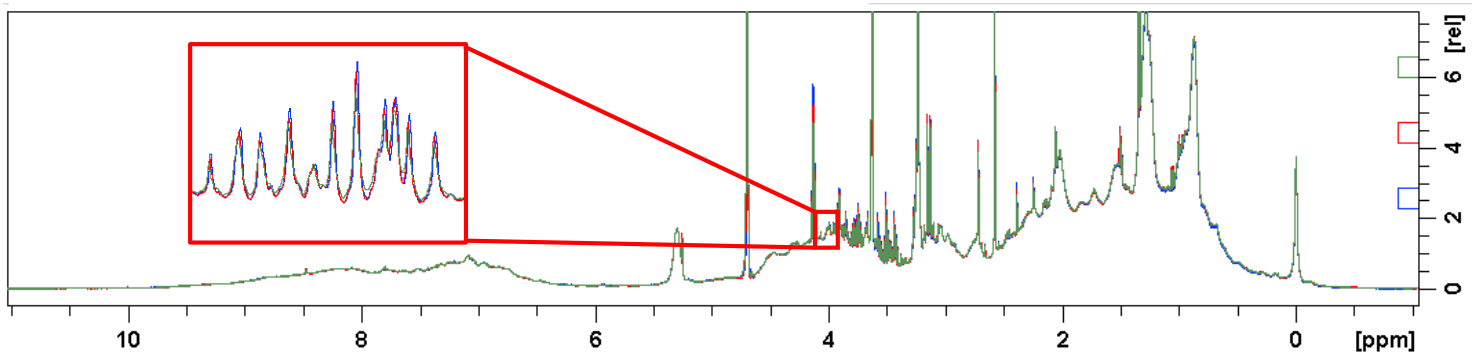
Targeted: Metabolites, known a priori, are defined by the biological problem and may represent a class of molecules or a specific pathway.

Untargeted: This type of analysis involves identification and quantification of as many metabolites as possible.

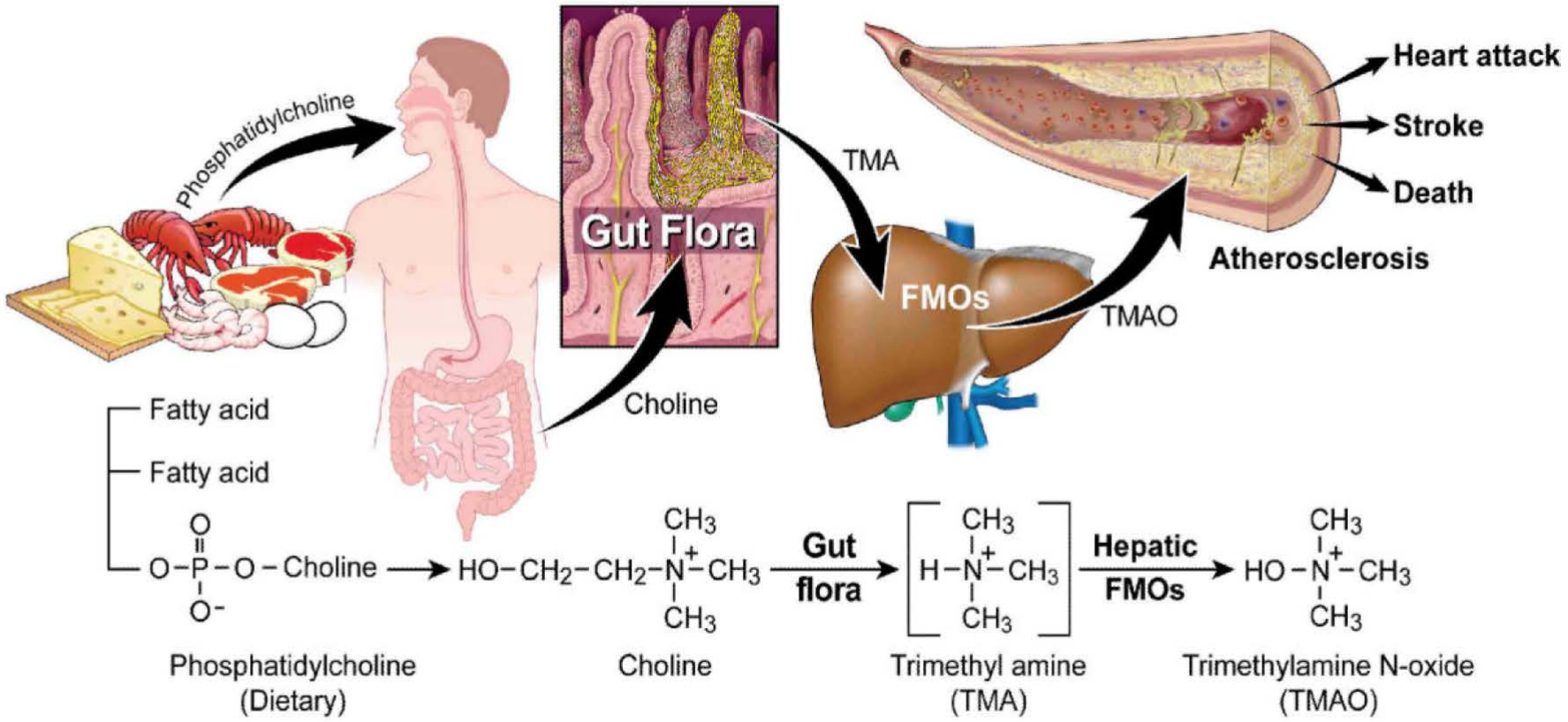
Equipment Used for Metabolomics

NMR		LC-MS		GC-MS	
Pros	Cons	Pros	Cons	Pros	Cons
• Non-invasive, non-destructive • High specificity • High resolution • Qualitative and quantitative analysis of metabolites	• Low dynamic range • Low sensitivity • High capital investment	• High sensitivity • High resolution	• Unstable • Derivatization difficult • Sparingly volatile metabolites • Databases incomplete • Many artifacts	• Good resolution • Good selectivity • Comprehensive databases available	• Complex sample processing • Derivatization leads to some analyte loss • Derivatization can cause artifacts

- High stability and reproducibility

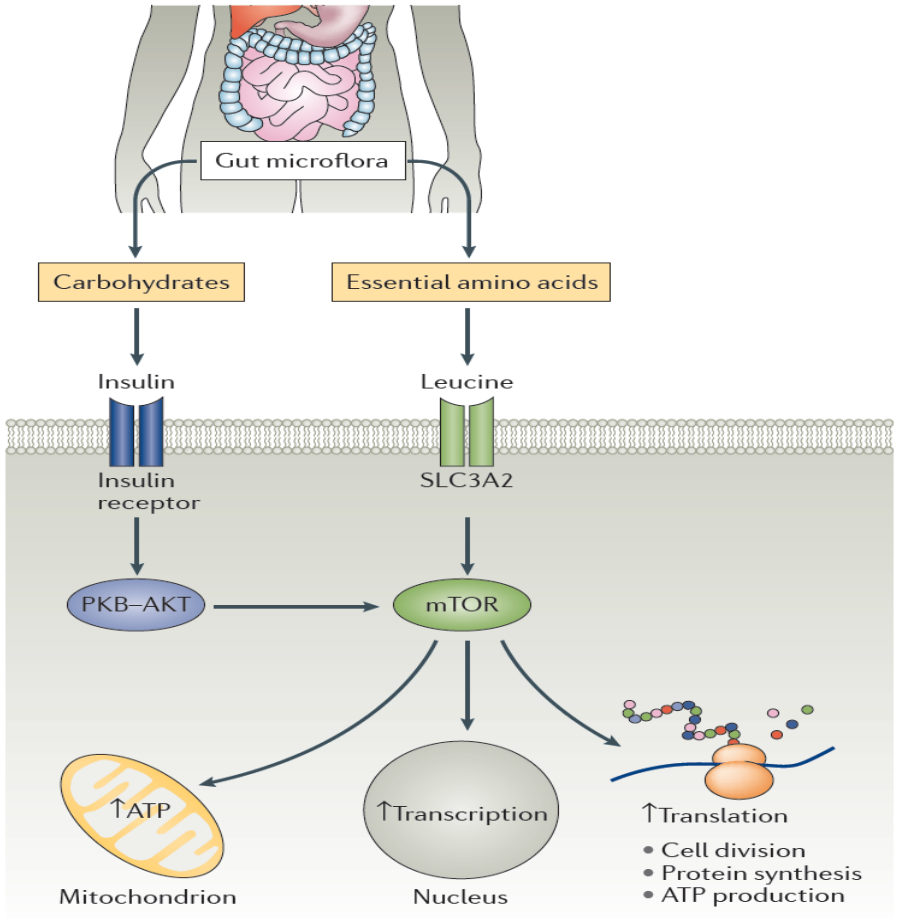


Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease



Nature. 2011 Apr 7;472(7341):57-63 (5060 Citations)

Metabolite Profiles and the Risk of Developing Diabetes



Chronic exposure to high levels of carbohydrates and/or high levels of essential amino acids (Ile, Leu, Val, Phe, Tyr) eventually overwhelm the insulin-signaling process, leading to insulin resistance, which is a hallmark of type 2 diabetes.

BCAAs can act as biomarkers for type 2 Diabetes.

Nat. Med. 2011 Apr 7; 17(4): 448-453 (3027 Citations)

Cancer Metabolomics

Table 2 | **Oncometabolites and their roles in cancer**

Oncometabolite*	Mechanism or role
2-hydroxyglutarate	• Inhibits ATP synthase and mTOR signalling • Inhibits 2-oxoglutarate-dependent oxygenases, which activate oncogenic hypoxia-induced factor pathways and alter DNA methylation patterns • Produced by gain-of-function mutations in the gene encoding isocitrate dehydrogenase • Elevated in gliomas and acute myeloid leukemia
Fumarate	• Inhibits 2-oxoglutarate-dependent oxygenases, which activate oncogenic hypoxia induced factor pathways and alter DNA methylation patterns • Leads to protein succination and disrupted metabolism • Produced by loss-of-function mutations in the gene encoding fumarate hydratase • Elevated in renal carcinoma
Succinate	• Inhibits 2-oxoglutarate-dependent oxygenases which activate oncogenic hypoxia induced factor pathways and alter DNA methylation • Produced by loss-of-function mutations in the genes encoding succinate dehydrogenase • Elevated in paraganglioma and renal and thyroid tumours
Sarcosine	• Activates mTOR signalling pathway • Elevated by mutant glycine *N*-methyl transferase • Elevated in metastatic prostate cancer
Glucose	• Essential source of carbon to support cancer cell anabolism, TCA anaplerosis and aerobic glycolysis • Activates hexokinase II • Activates glucose-regulated proteins that alter signalling, proliferation, invasion and apoptosis • Elevated in most cancers
Glutamine	• Essential source of nitrogen to support cancer cell anabolism and aerobic glycolysis • Essential source of carbon for TCA anaplerosis • Elevated in MYC-dependent cancers
Asparagine	• Essential source of nitrogen to support cancer cell anabolism and aerobic glycolysis • Anti-apoptotic agent • Elevated in acute lymphoblastic leukemia
Choline	• Serves as a methyl donor for DNA methylation which disrupts DNA repair and gene expression • Modifies lipid signalling • Essential source of carbon and nitrogen to support phospholipid synthesis in rapidly dividing cells • Elevated in breast, brain and prostate cancer
Lactate	• Lowers extracellular pH and induces metastasis • Induces local immunosuppression • Elevated in most cancers

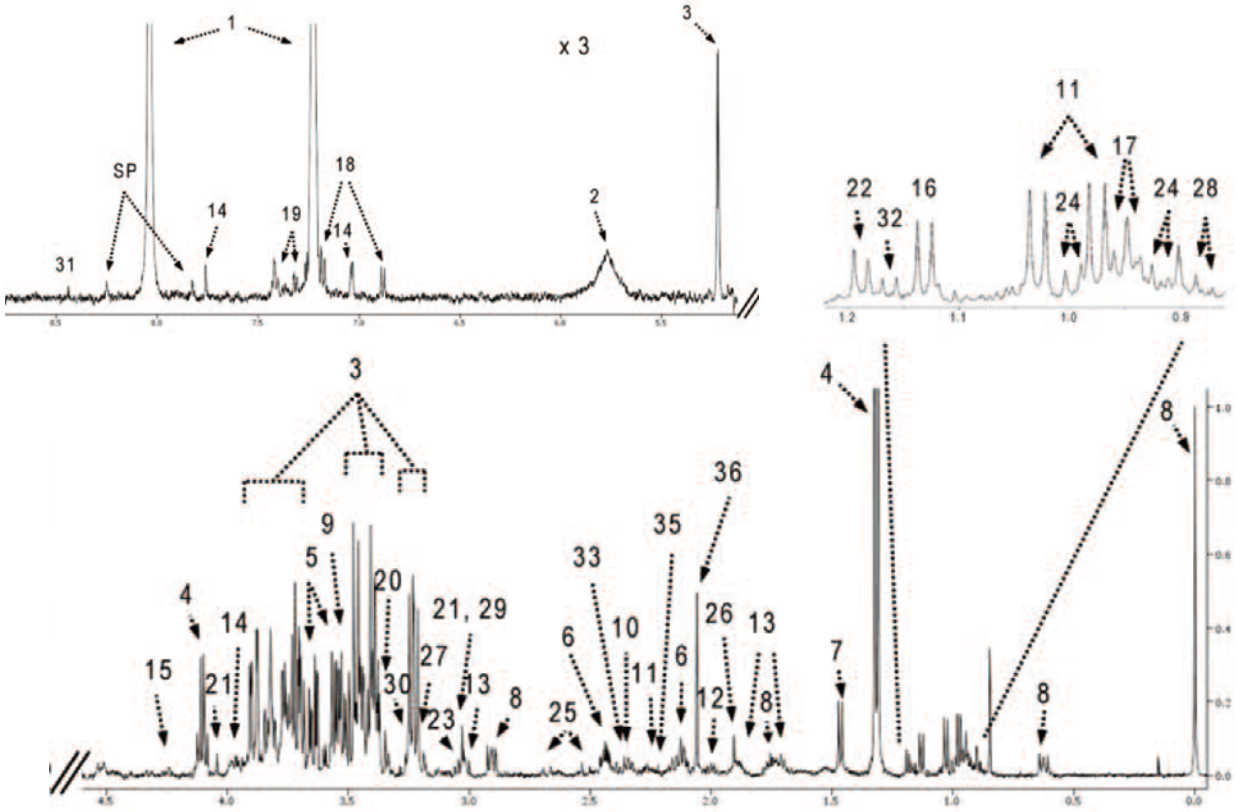
Nat. Rev. Drug Discov. 15, 473–482 (2016).

Why NMR Metabolomics ?

Advantages	Disadvantages
• Quantitative • Non-destructive • Fast (2–3 min per sample) • Requires no derivatization • Requires no separation • Detects most organic classes • Allows identification of novel chemicals • Most spectral features are identifiable • Robust, mature technology • Can be used for metabolite imaging (fMRI or MRS) • Can be fully automated • Compatible with liquids and solids • Long instrument lifetime (over 20 years)	• Not sensitive (LOD = 5 μM) • High start-up cost (>US$1 million) • Large instrument footprint • Cannot detect or identify salts and inorganic ions • Cannot detect non-protonated compounds • Requires larger sample volumes (0.1–0.5 mL)

Nat. Rev. Drug Discov. 15, 473–482 (2016).

The Human Serum Metabolome



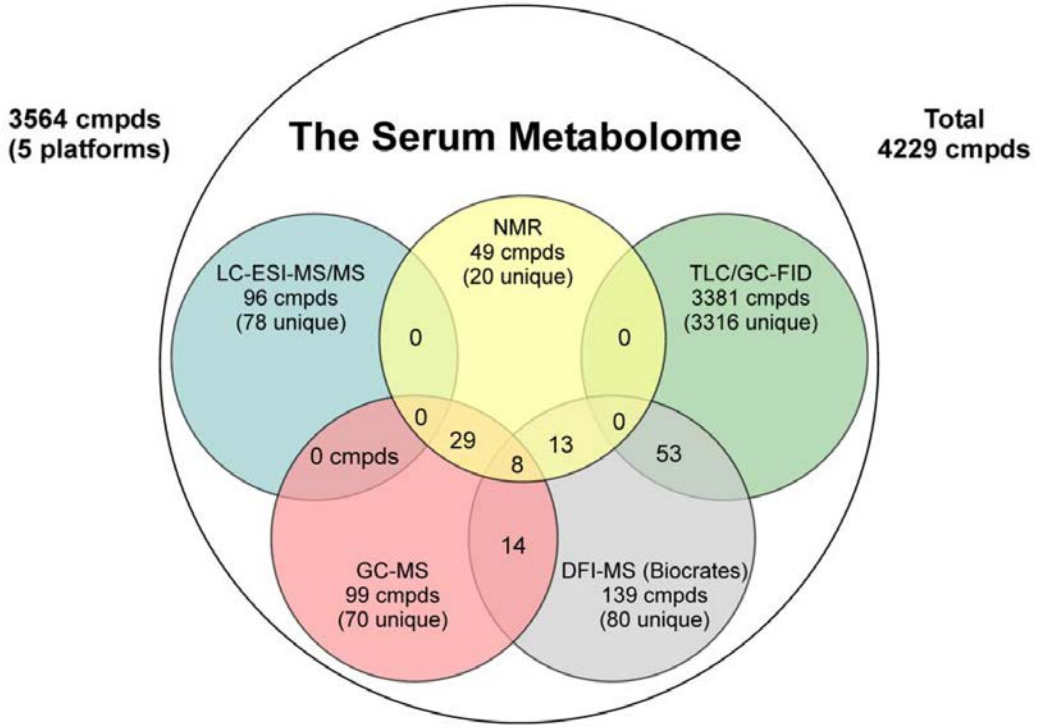


Figure 1. Typical 500 MHz ^{1}H-NMR spectrum of healthy human serum. Numbers indicate the following metabolites: 1, imidazole; 2, urea; 3, D-glucose; 4, L-lactic acid; 5, glycerol; 6, L-glutamine; 7, L-alanine; 8, DSS; 9, glycine; 10, L-glutamic acid; 11, L-valine; 12, L-proline; 13, L-lysine; 14, L-histidine; 15, L-threonine; 16, propylene glycol; 17, L-leucine; 18, L-tyrosine; 19, L-phenylalanine; 20, methanol; 21,creatinine; 22, 3-hydroxybutyric acid; 23, ornithine; 24, L-isoleucine; 25, citric acid; 26, acetic acid; 27, carnitine; 28, 2-hydroxybutyric acid; 29, creatine; 30, betaine; 31, formic acid; 32, isopropyl alcohol; 33, pyruvic acid; 34, choline; 35, acetone; 36, glycerol.

PLoS One. 2011; 6(2): e16957. (1317 Citations)

The Human Urine Metabolome

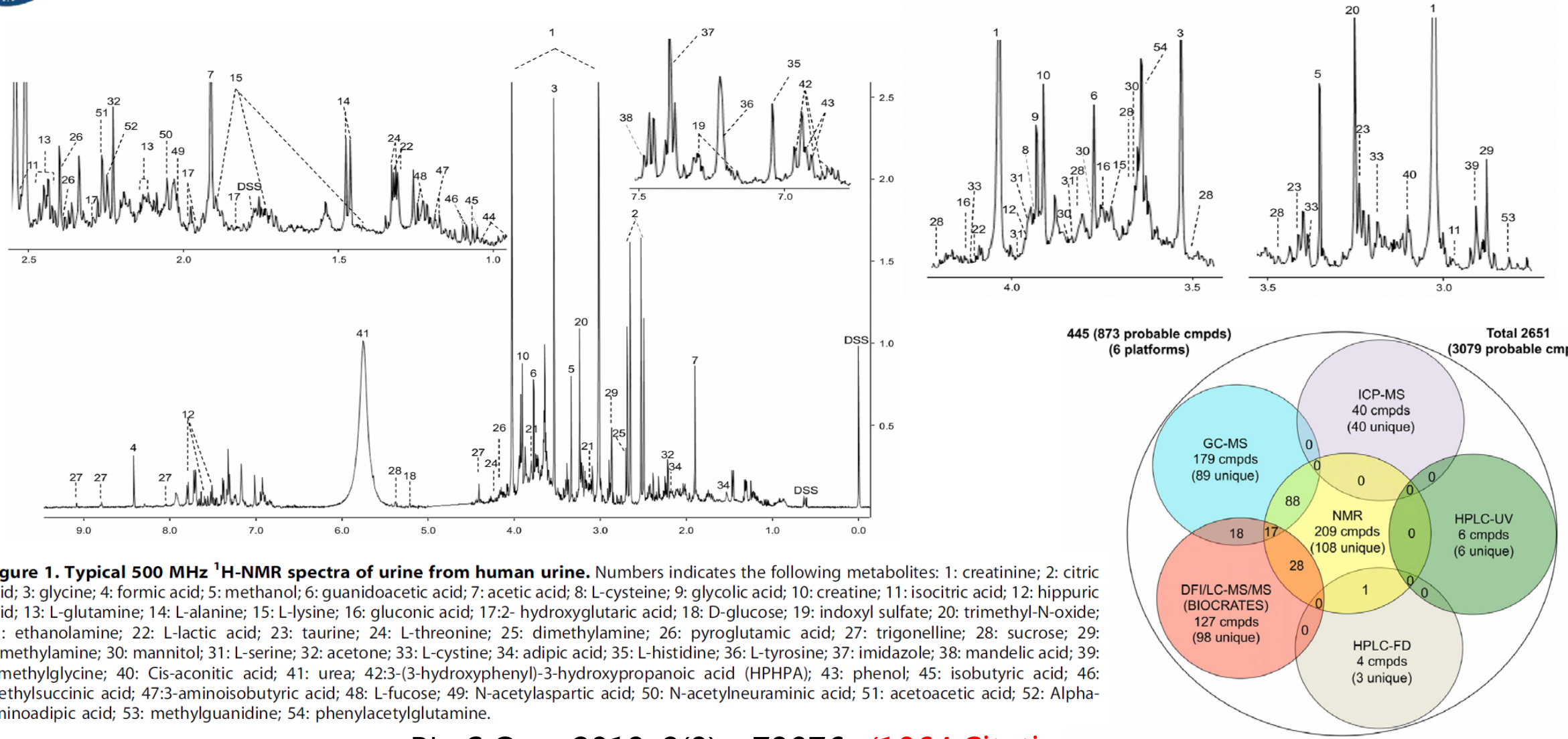


Figure 1. Typical 500 MHz ^{1}H-NMR spectra of urine from human urine. Numbers indicates the following metabolites: 1: creatinine; 2: citric acid; 3: glycine; 4: formic acid; 5: methanol; 6: guanidoacetic acid; 7: acetic acid; 8: L-cysteine; 9: glycolic acid; 10: creatine; 11: isocitric acid; 12: hippuric acid; 13: L-glutamine; 14: L-alanine; 15: L-lysine; 16: gluconic acid; 17:2- hydroxyglutaric acid; 18: D-glucose; 19: indoxyl sulfate; 20: trimethyl-N-oxide; 21: ethanolamine; 22: L-lactic acid; 23: taurine; 24: L-threonine; 25: dimethylamine; 26: pyroglutamic acid; 27: trigonelline; 28: sucrose; 29: trimethylamine; 30: mannitol; 31: L-serine; 32: acetone; 33: L-cystine; 34: adipic acid; 35: L-histidine; 36: L-tyrosine; 37: imidazole; 38: mandelic acid; 39: dimethylglycine; 40: Cis-aconitic acid; 41: urea; 42:3-(3-hydroxyphenyl)-3-hydroxypropanoic acid (HPHPA); 43: phenol; 45: isobutyric acid; 46: methylsuccinic acid; 47:3-aminoisobutyric acid; 48: L-fucose; 49: N-acetylaspartic acid; 50: N-acetylneuraminic acid; 51: acetoacetic acid; 52: Alpha-aminoadipic acid; 53: methylguanidine; 54: phenylacetylglutamine.

PLoS One. 2013; 8(9): e73076. (1064 Citations)

NMR Metabolomics Experiment Design

What do you want to see?

- Identity
  - Databases
- # of metabolites
  - Limit of Detection (LoD)
- Concentration
  - Limit of Quantification (LoQ)

→ Spectra → Data → Results

PCA, PLS-DA, Etc.

- Sample
- Spectrum type
- Instrument
- Processing

Spectral Analysis

Statistical Analysis

Quality Management (SOPs)

Sample Preparation:

ACCURATE

- Sample preparation to be simple → save time, less chance of errors, requires less training, less contaminants
  - Fewer steps per sample
  - Automation – Push-button operation
- Sample concentration to be high → less NS, higher S/N, more accurate quantification
  - Add less buffer
- Duplicate samples → better statistics, error averages out
- Sample viscosity to be low → better spectral resolution
  - Optimization of buffer, temperature, etc.

Spectrum type:

NOESY-PRESATuration (noesypr1d)

- Adequate suppression of solvent
- Easy to implement
- Does not require exotic hardware
- Consistency (everybody is using it)

- Disadvantages
  - Not good for very dilute samples

- Improvements
  - Gradients (noesygppr)

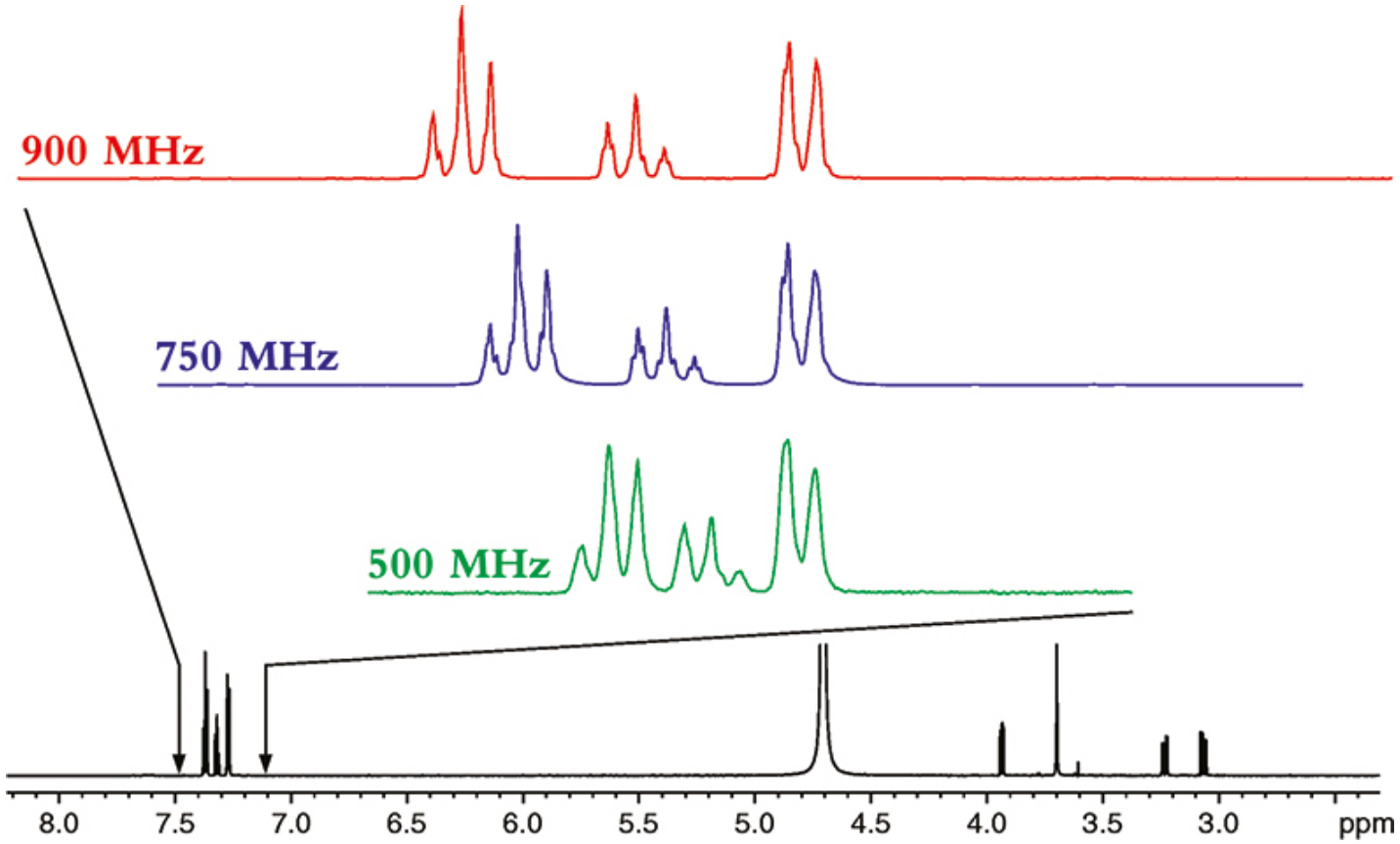
CPMG-PRESAturation (cpmgpr1d)

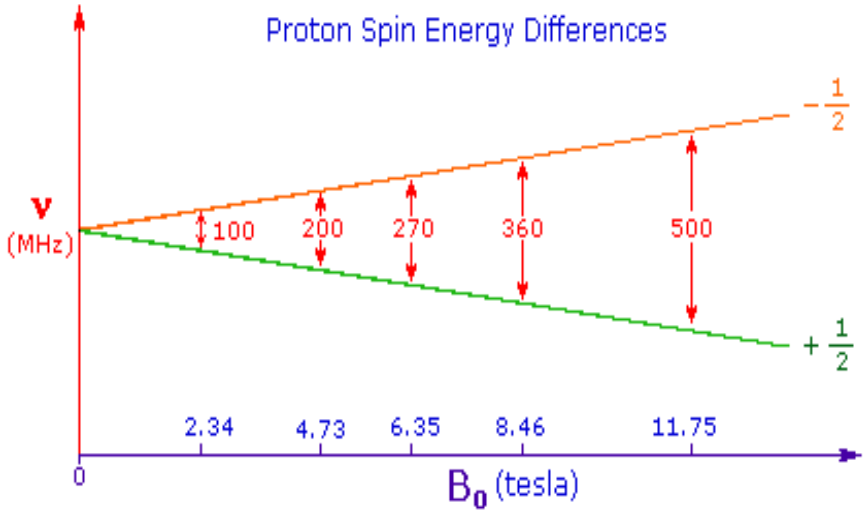
- Carr-Purcell-Meiboom-Gill
- Selects for specific time ranges of motion → molecular weight filter
- Allows detection of small molecules in a mixture of large and small molecules.

- Lower signal intensity
- Tricky quantitation

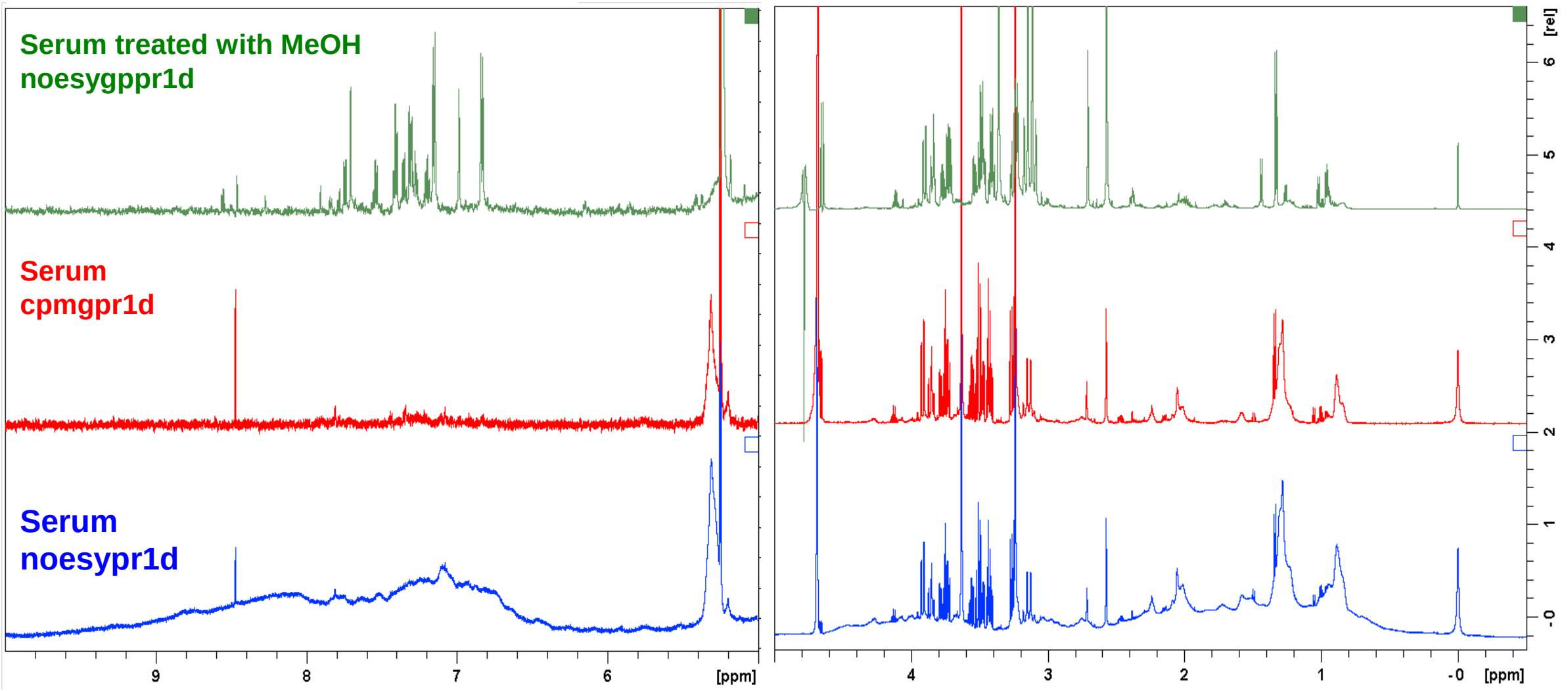
NMR Instrument:

Better dispersion (within same range)



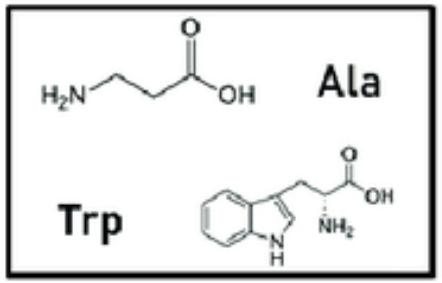


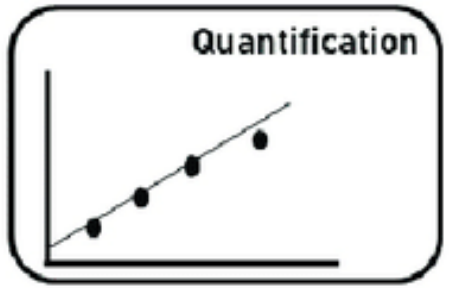
Example: Serum



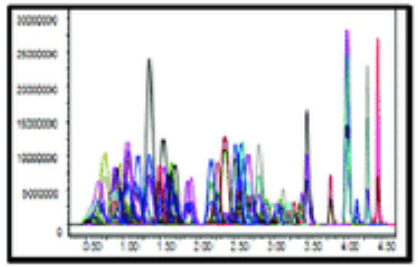
Targeted & Untargeted NMR Metabolomics

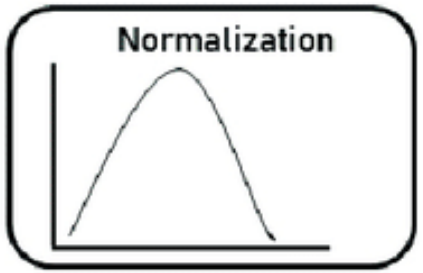
Targeted NMR Metabolomics





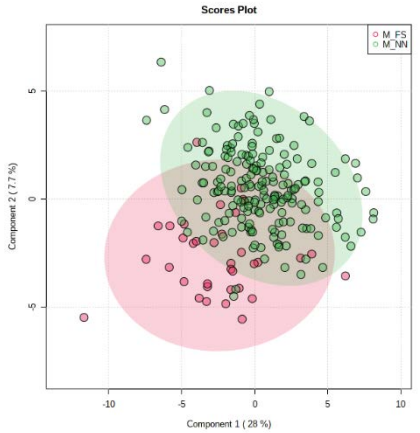
Untargeted NMR Metabolomics



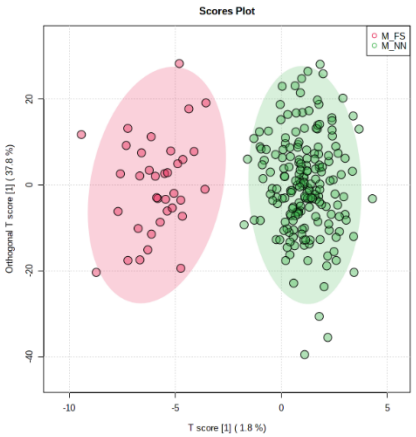


Statistical Analysis

PCA



PLSDA

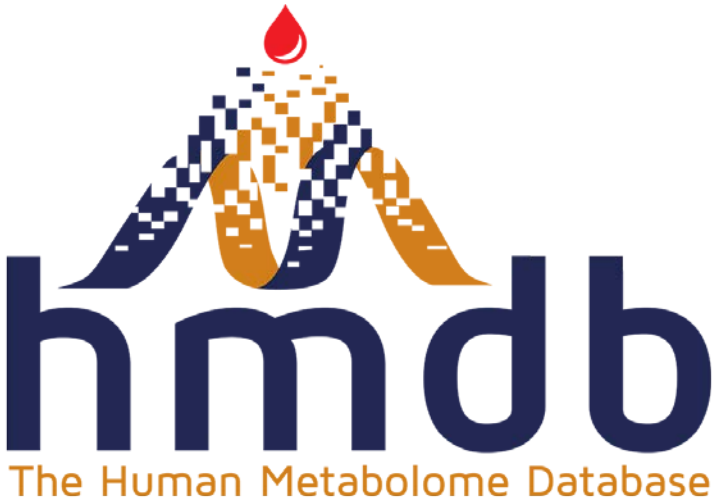


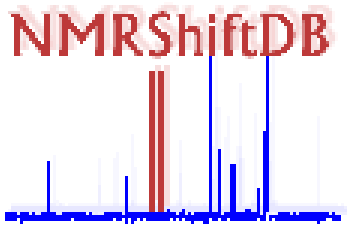
Metabolites Identification

Tips for Metabolites Identification

- Is the metabolite known to exist in the biospecimen (or the species)?
- What is the typical concentration range of the metabolite?
- What are the (other) typical metabolite signals in your observation window?
- What other experiments or techniques could you perform or use to obtain more information?
- If in doubt, leave the signals alone!

NMR Databases



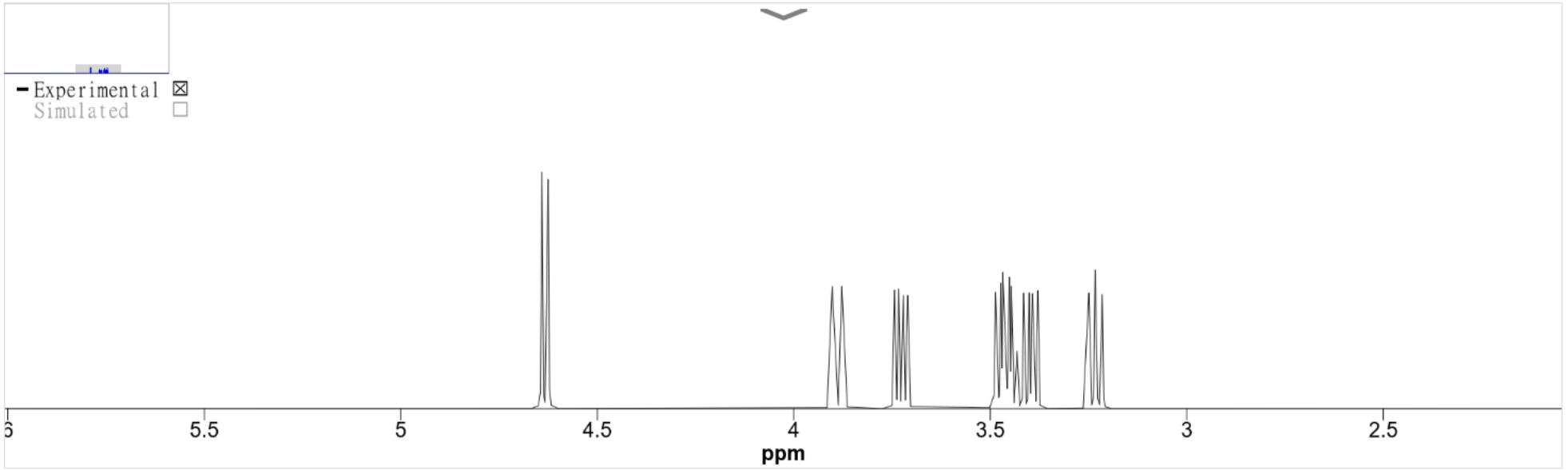


- Chemical shift position of each nucleus
- Splitting patterns
- Sample type and origin

- Extras
  - 2D spectra
  - Other spectroscopy data
  - Metabolite information
  - Etc…

HMDB

D-Glucose (HMDB0000122)



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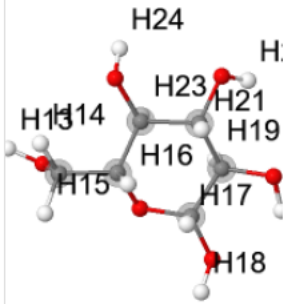
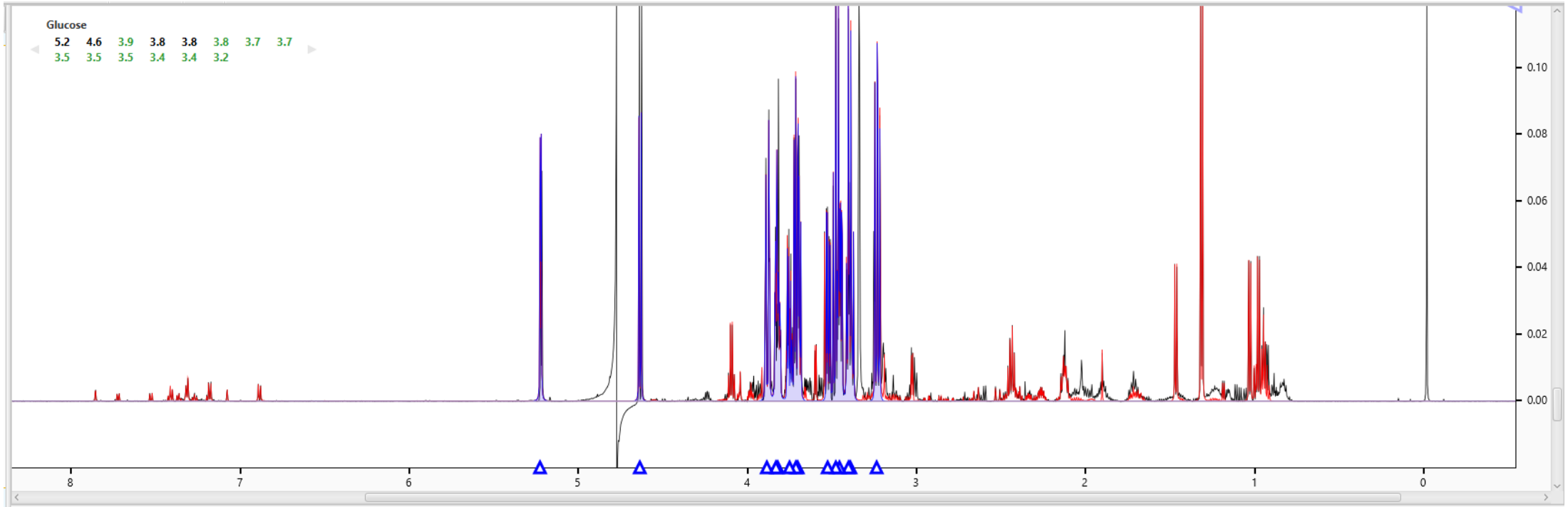


Multiplets 4.63 3.89 3.73 3.47 3.46 3.40 3.23

Assignment Table

Row No.	Cluster Midpoint	No. Peaks	Coupling Type	No. H's	Atom No.	Peak Centers (ppm)
1	4.63	2	d	1	17	4.63 4.64
2	3.89	4	dd	1	15	3.87 3.88 3.90 3.90
3	3.73	4	dd	1	14	3.71 3.72 3.73 3.74
4	3.47	4	dd	1	21	3.45 3.47 3.47 3.49
5	3.46	6	dt	1	16	3.43 3.45 3.45 3.46 3.47 3.48
6	3.40	4	dd	1	23	3.38 3.39 3.40 3.42
7	3.23	4	dd	1	19	3.22 3.23 3.23 3.25

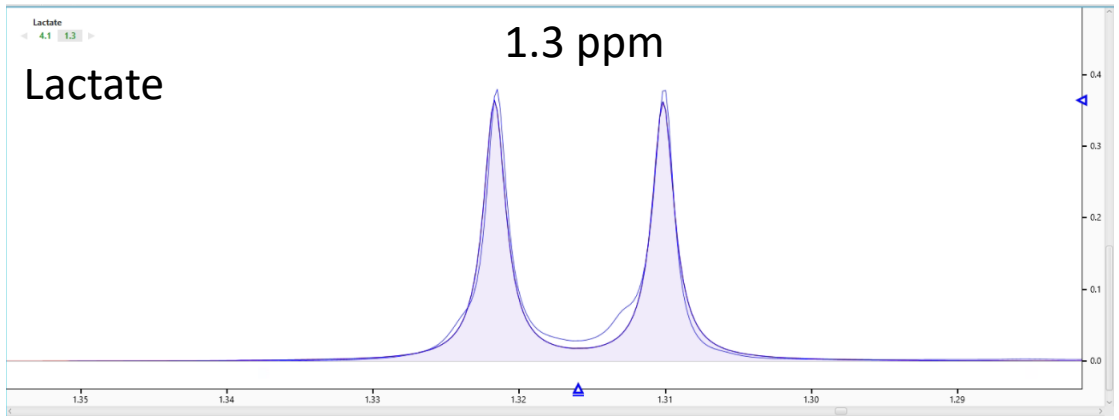
Metabolites Identification using Chenomx

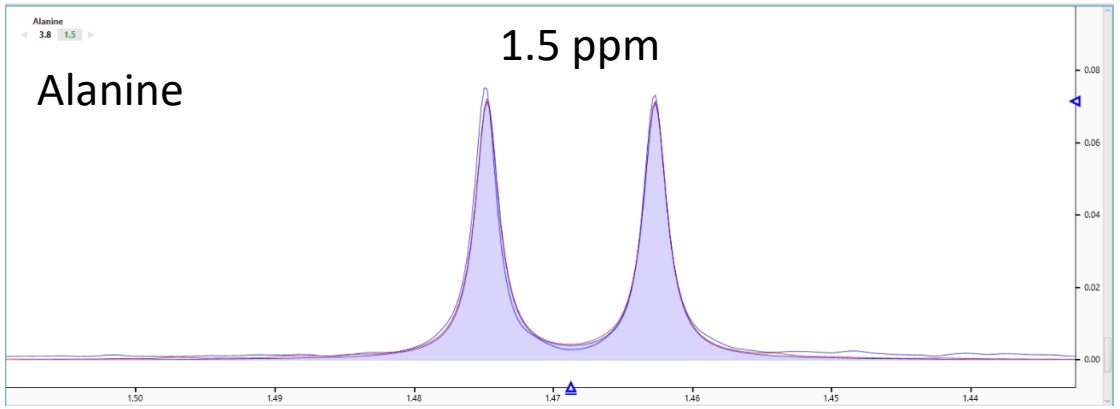


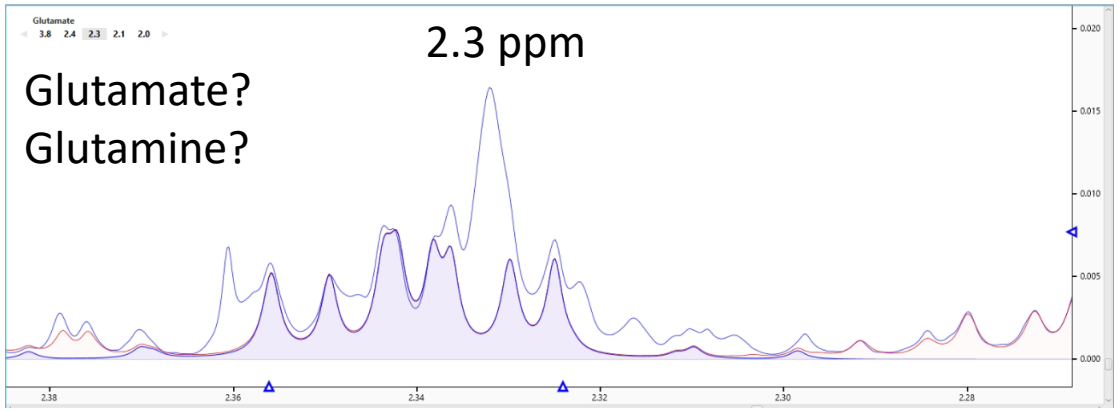
Find in Table (Ctrl+L)

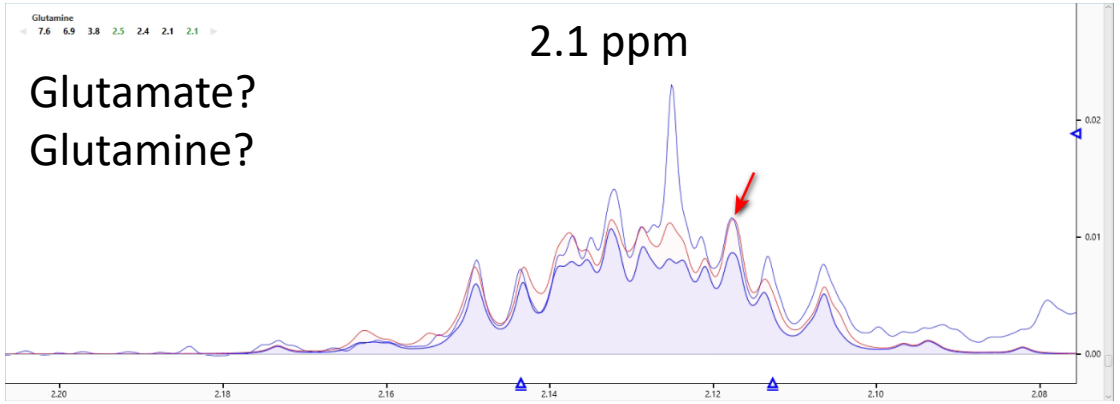
Compound Name	Concentration (mM)	Maximum (mM)	Author	Area (sa)	Match Factor
Glucose	2.2869	0.8830	Chenomx Inc.	4.3516	99.49
Lactate	0.3447	0.3271	Chenomx Inc.	0.3809	97.41
Glutamine	0.2414	0.1921	Chenomx Inc.	0.3419	91.63
Valine	0.1407	0.1333	Chenomx Inc.	0.2943	99.76
Alanine	0.1243	0.1230	Chenomx Inc.	0.1325	99.17
Glycine	0.1042	0.1218	Chenomx Inc.	0.0483	99.58
Leucine	0.0688	0.0589	KF	0.1793	90.57
Phenylalanine	0.0378	0.0268	Chenomx Inc.	0.0807	99.63
Tyrosine	0.0353	0.0310	Chenomx Inc.	0.0543	98.97
Isoleucine	0.0335	0.0577	Chenomx Inc.	0.0867	96.49

Easy Metabolites First



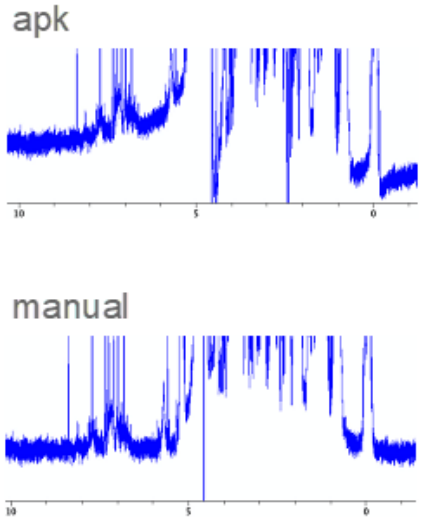


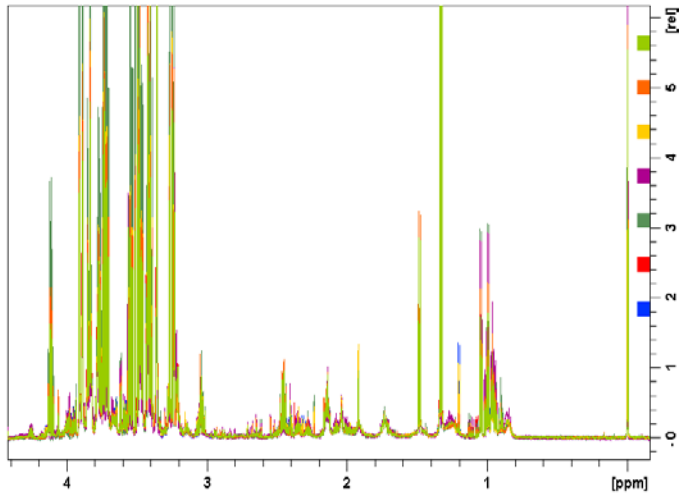


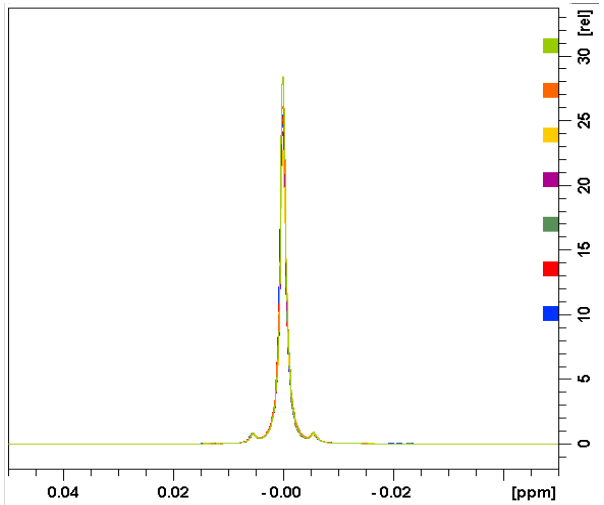


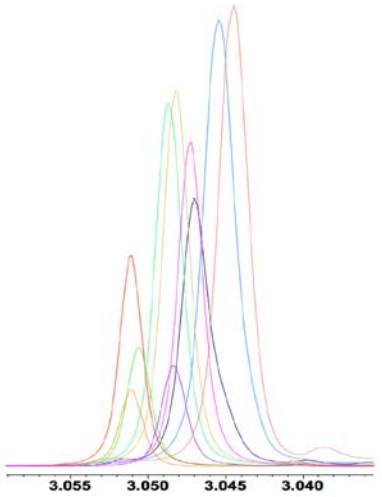
Untargeted NMR Metabolomics using Mnova

Manual Phase → Baseline Correction → Reference → Alignment →









Binning → Normalize → Exclude Regions → Save as CSV file

0.04 ppm ?

What IVDr can Help ?

Statistical Analysis

Identity

Databases

# of metabolites

Limit of Detection (LoD)

Concentration

Limit of Quantification (LoQ)

Spectra

Data

Results

Sample

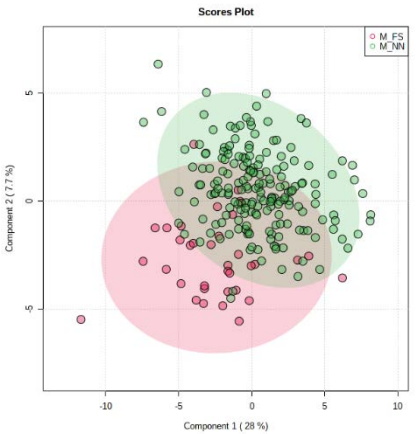
Spectrum type

Instrument

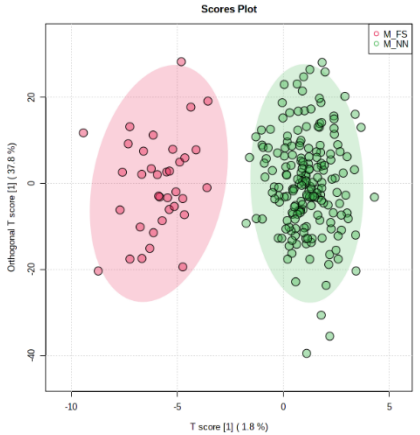
Processing

Spectral Analysis

PCA

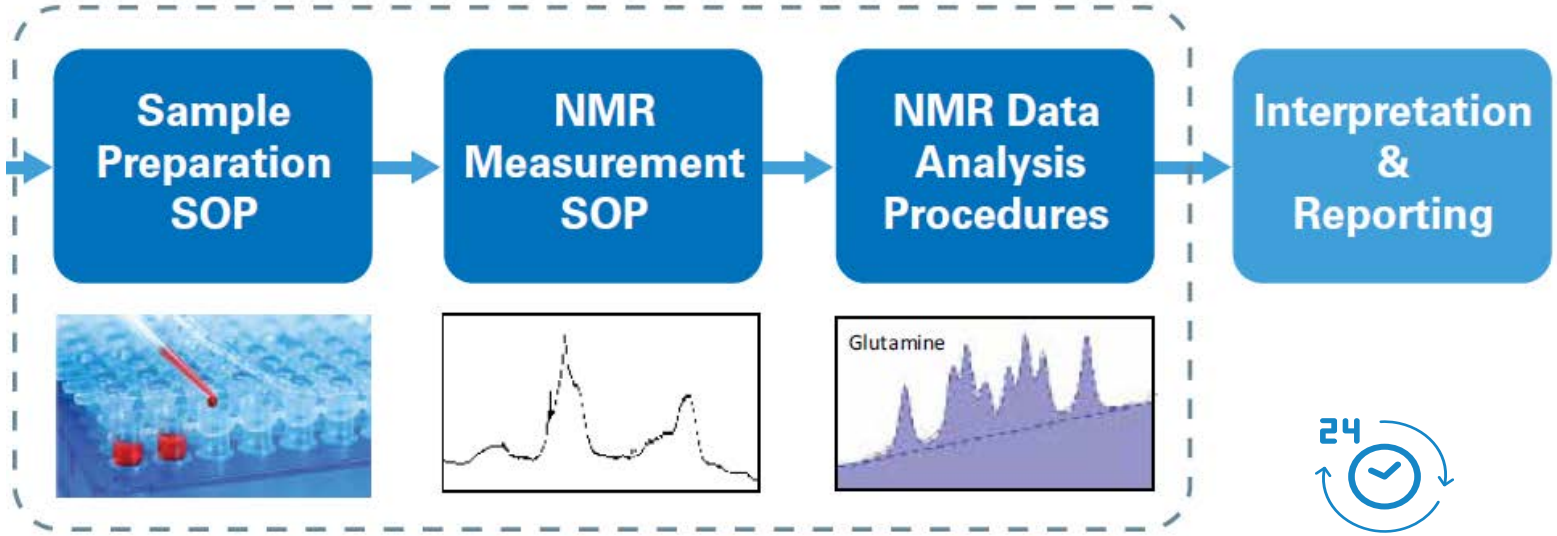


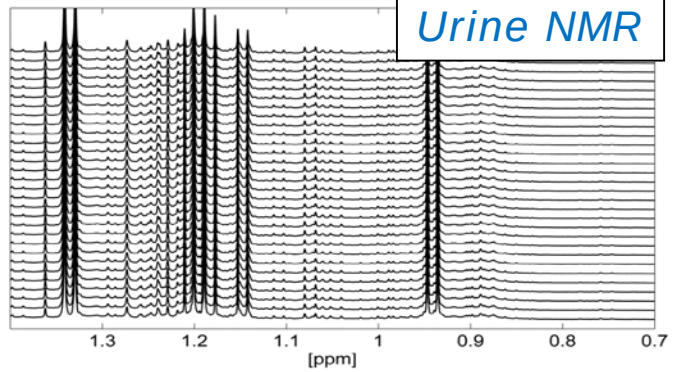
PLSDA

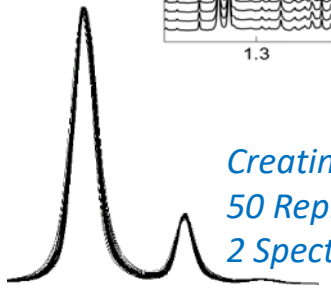


Bruker IVDr Platform for Human Plasma/Serum and Urine

IVDr Platform: Fully Automated NMR Metabolic Profiling for Human Bodyfluids Samples



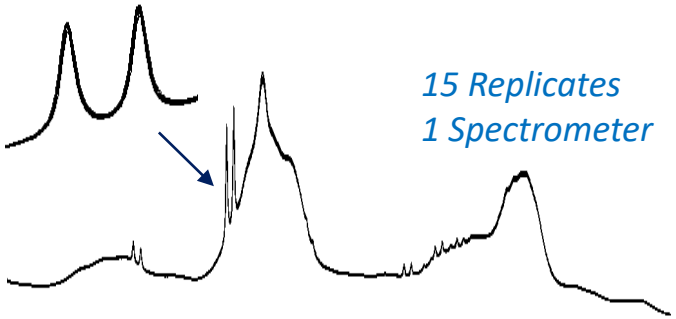




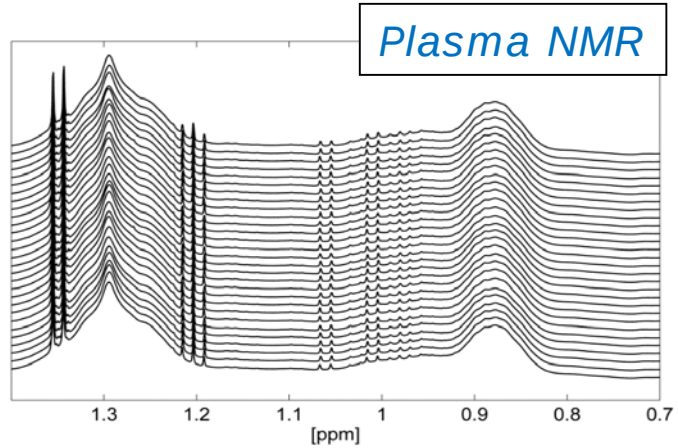
Creatinine - Creatine
50 Replicates
2 Spectrometers

Anywhere & anytime!

- *Standardisation*
- *Automation*
- *Scalability*
- *Sustainability*



15 Replicates
1 Spectrometer



Bruker IVDr platform is for Research Use Only and not for Use in Clinical Diagnostic Procedures

Steps in Bruker IVDr Platform

Follow SOPs

1. 解凍

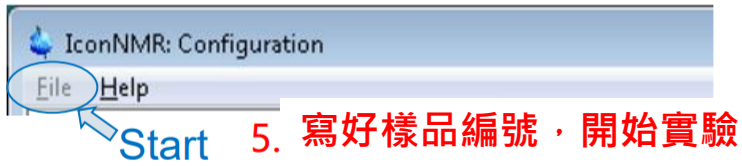
2. 加緩衝液

3. 移入核磁管

* 可使用扫码器
* 可使用配样机器人

4. 放入SampleJet

5*96+99
低温



5. 寫好樣品編號，開始實驗

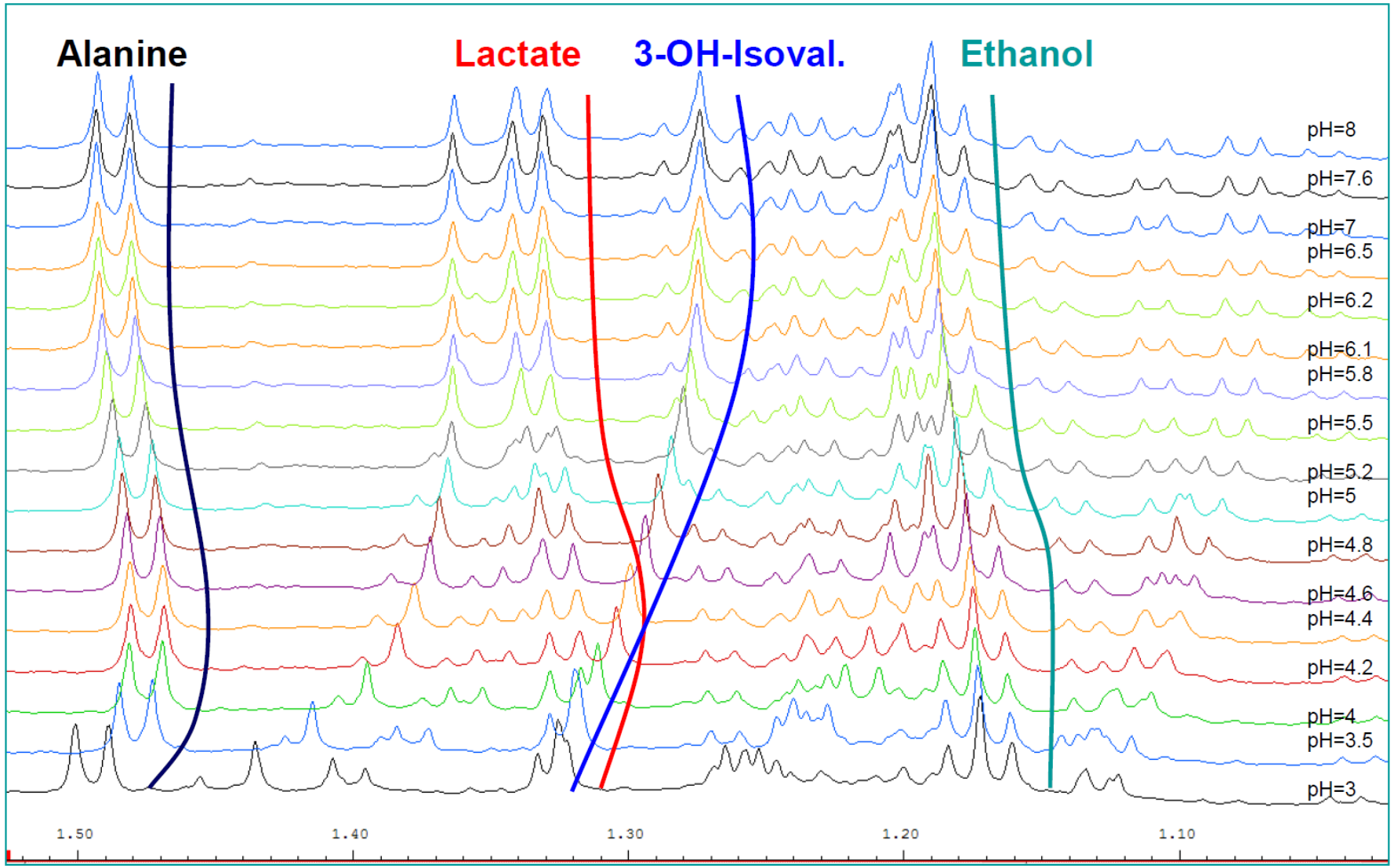
DISK	SAMPLE NAME	SOLVENT	EXPERIMENT	HOLDER	TITLE
C:\IVDrData\data\UrineDemo\nmr	Urine1	Urine	N PROF_URINE_NOESY	1 A1	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine1	Urine	N PROF_URINE_JRES	1 A1	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine1	Urine	PROF_URINE_DAS_E	1 A1	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine2	Urine	N PROF_URINE_NOESY	1 A2	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine2	Urine	N PROF_URINE_JRES	1 A2	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine2	Urine	PROF_URINE_DAS_E	1 A2	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine3	Urine	N PROF_URINE_NOESY	1 A3	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine3	Urine	N PROF_URINE_JRES	1 A3	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine3	Urine	PROF_URINE_DAS_E	1 A3	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine4	Urine	N PROF_URINE_NOESY	1 A4	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine4	Urine	N PROF_URINE_JRES	1 A4	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1
C:\IVDrData\data\UrineDemo\nmr	Urine4	Urine	PROF_URINE_DAS_E	1 A4	Sample: 900 ul PseudoUrine + 100 ul Urine Buffer - sample No.1

~20 mins for 1 sample

IVDr Sample Preparation:

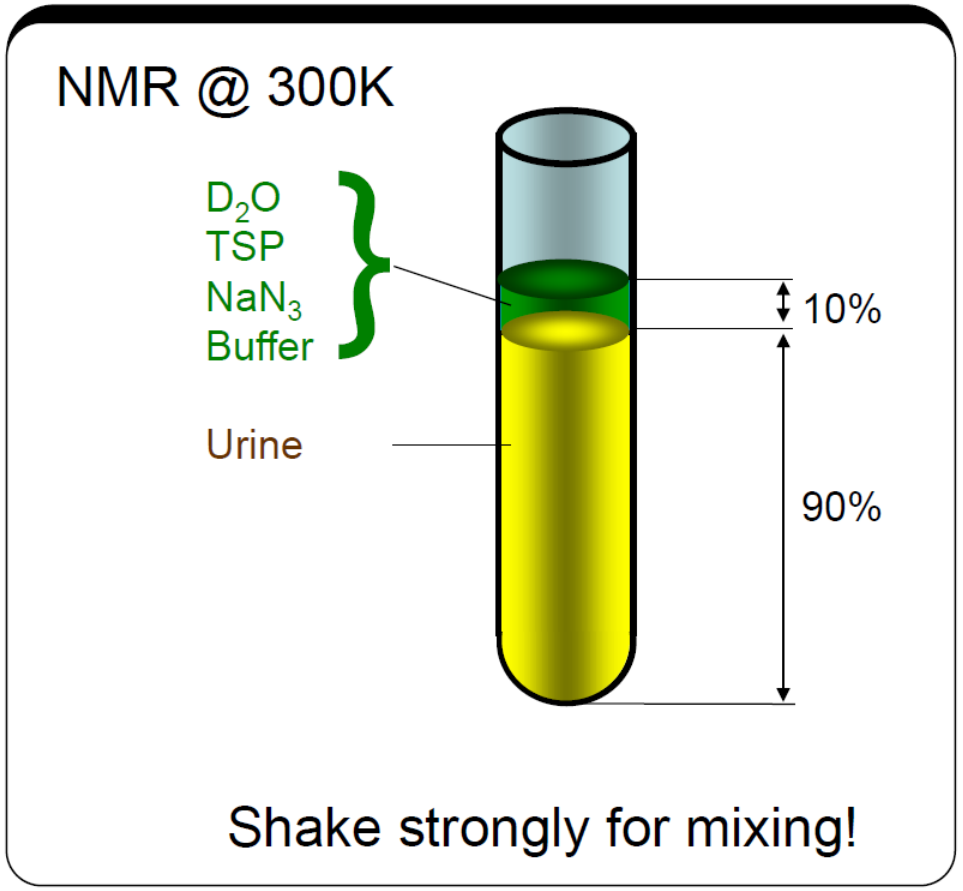
- Lock solvent needed (e.g. 10% of D_2O)
- Stabilizing line positions of pH-dependent signals
  - *buffer addition*
  - *pH – adjustment*
- Biological stabilization (e.g. addtion of NaN_3)
- Dilution
  - *lower initial concentration (e.g. juice concentrate)*
  - *lower the viscosity*
  - *decrease risk of foam and bubbles in flow*
  - *lower the salt concentration*
  - *adjust sample to target volume (e.g. 200µL to 600µL final volume)*
- Concentrating up and / or change solvent to NMR “friendly” solvent
  - *lyophilization and redilution in D_2O*
  - *partial evaporation of solvent*

pH -Effect on urinary NMR signal positions



Preparation Procedure - Urine

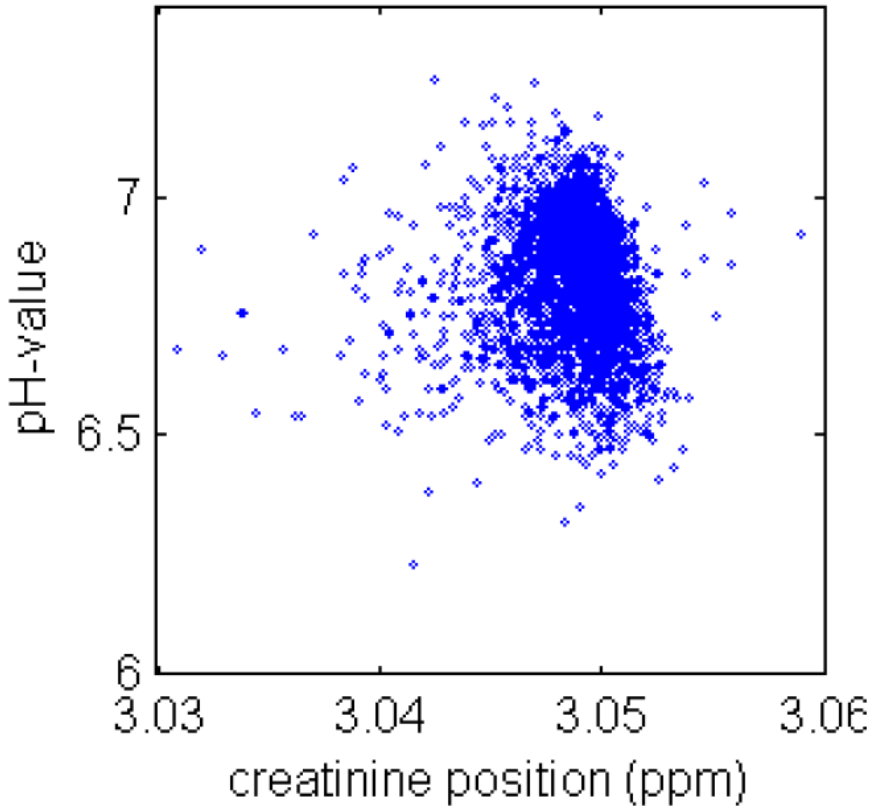
- Carefully thaw the samples at room temperature for ca.30 min.
- (Centrifuge the samples for 5 to 10min at ~2000 RCF).
- Add 100µL of Bruker urine buffer.
- Add 900µL of urine into a Cryovial / Eppendorf
- Mix the buffered urine for 30 seconds on the Vortex mixer.
- Transfer 600uL of well mixed sample into a 5mm 7” NMR tube or a 5mm SampleJet rack tube.



In urine, pH -adjustment does not reduce positional variability.

pH Estimation for Urine

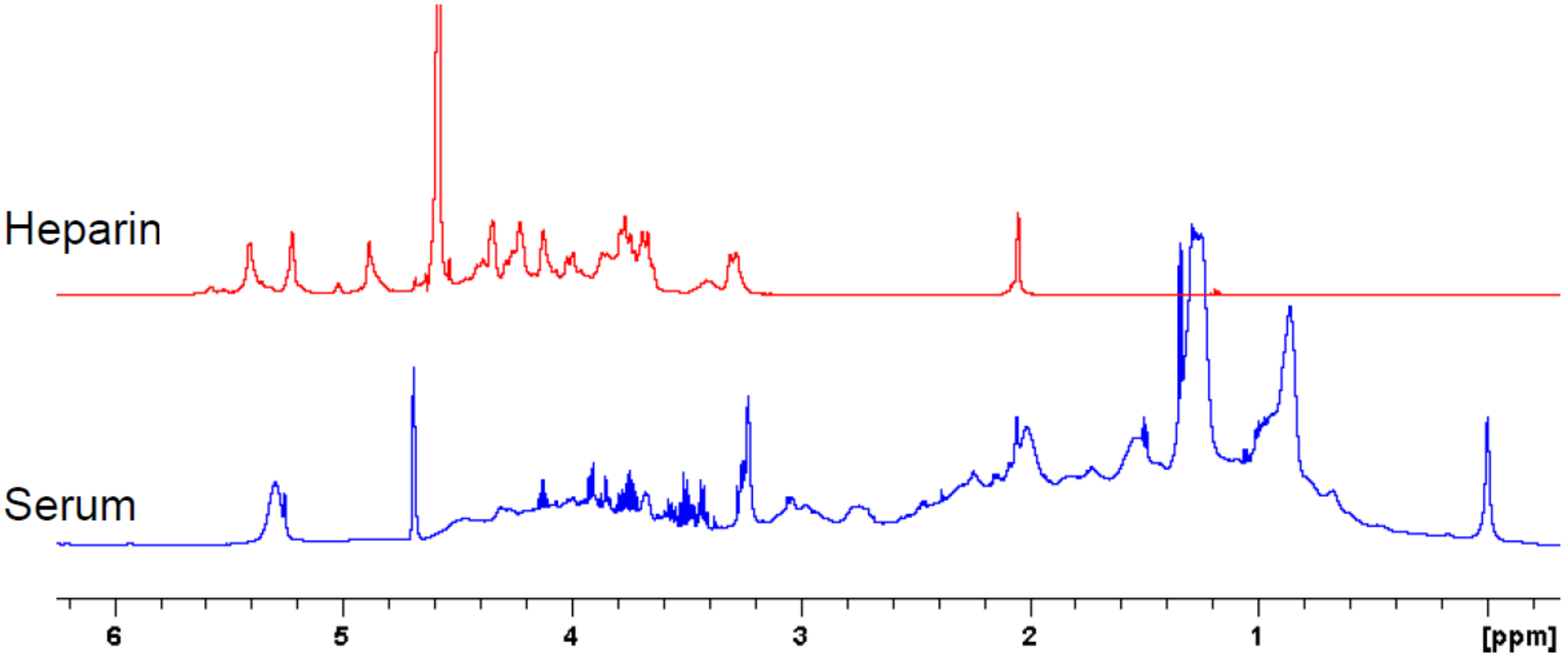
Correlation = 0.00



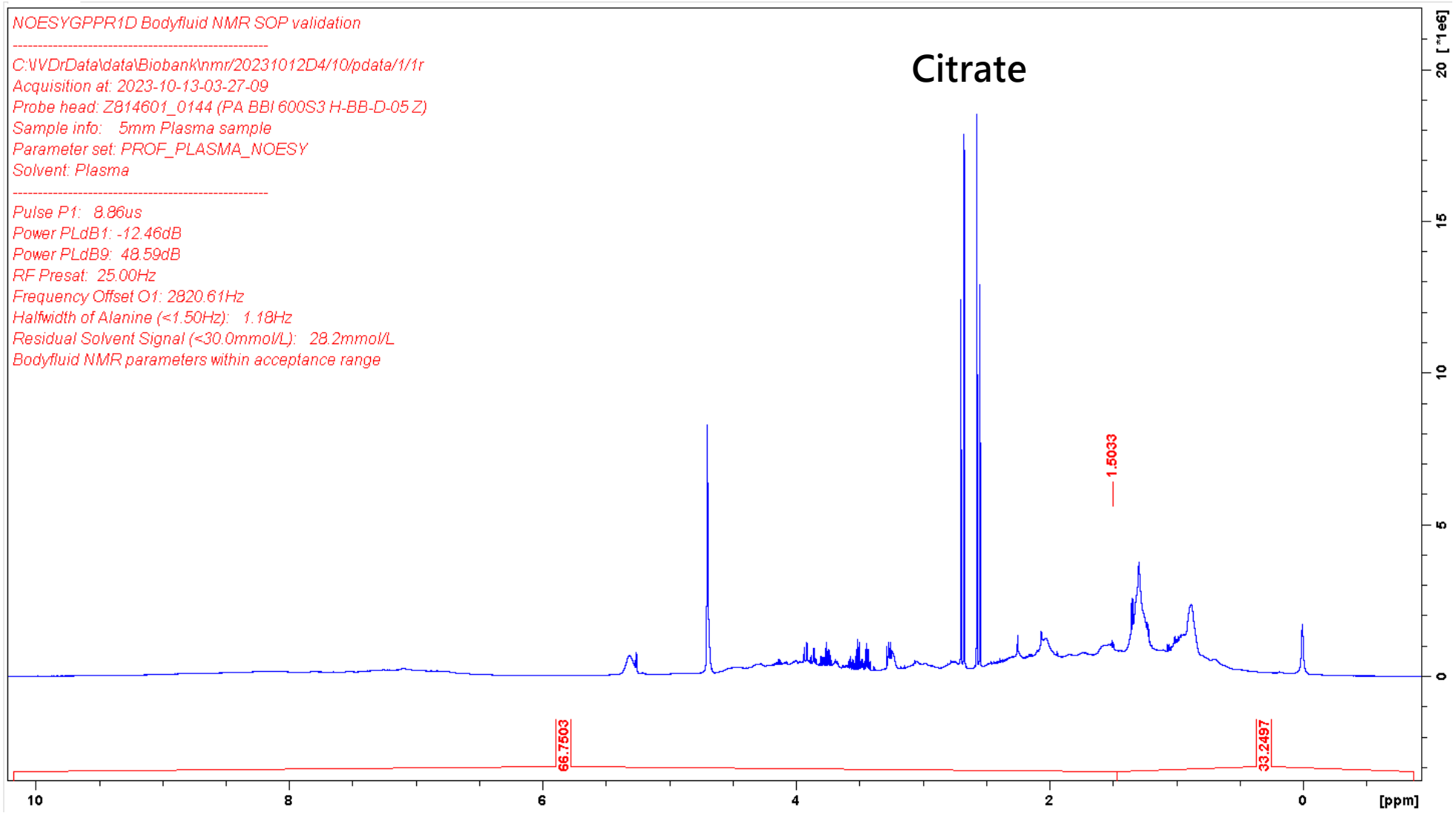
Data from > 1000 urine samples (20 – 80 years)

In urine, NMR signal positions are affected by pH, ion composition and concentration. pH -adjustment does not reduce positional variability.

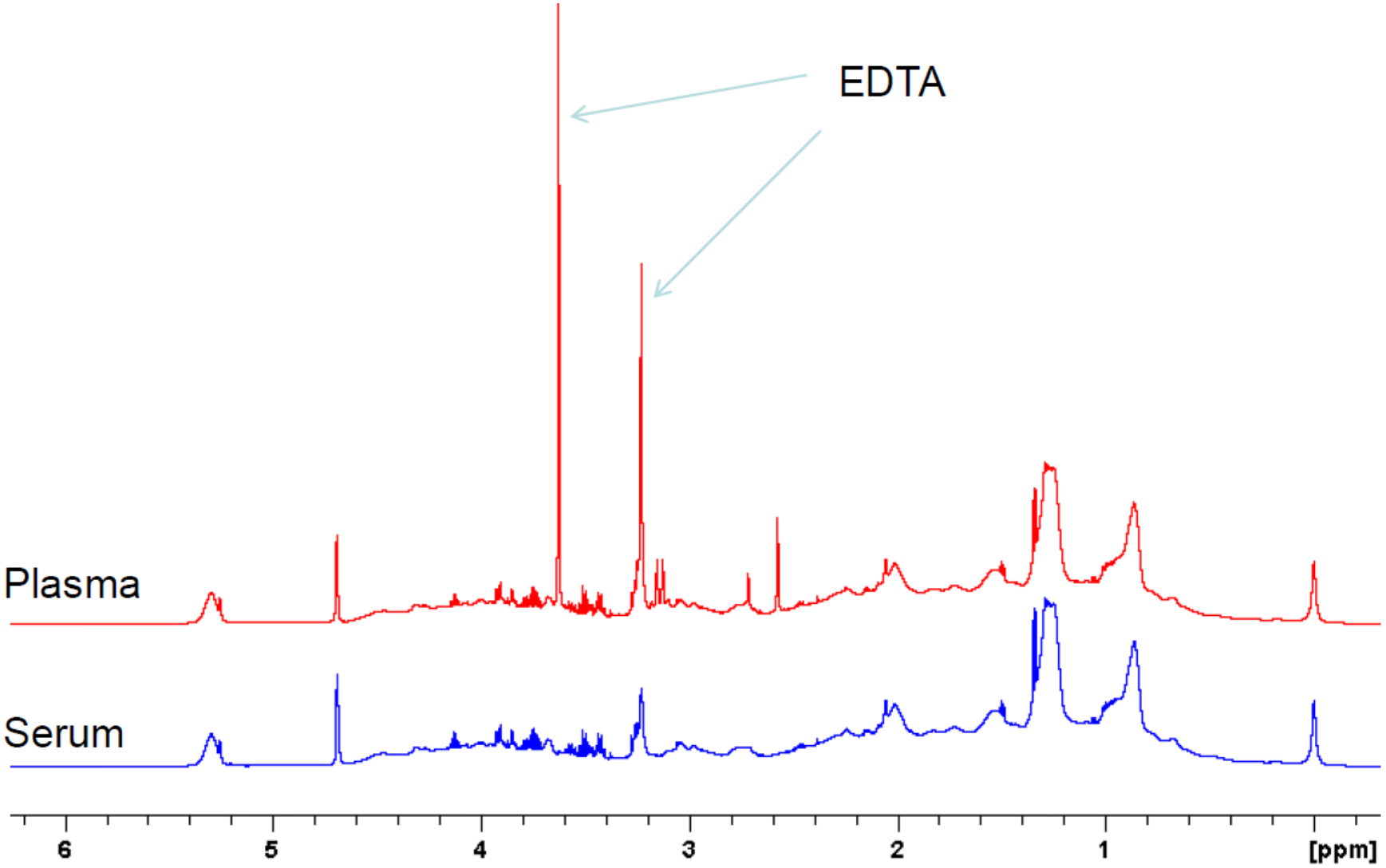
Plasma with Heparin (NO !!!)



Plasma with Sodium Citrate (OK)



Plasma with K2-EDTA (Recommended)

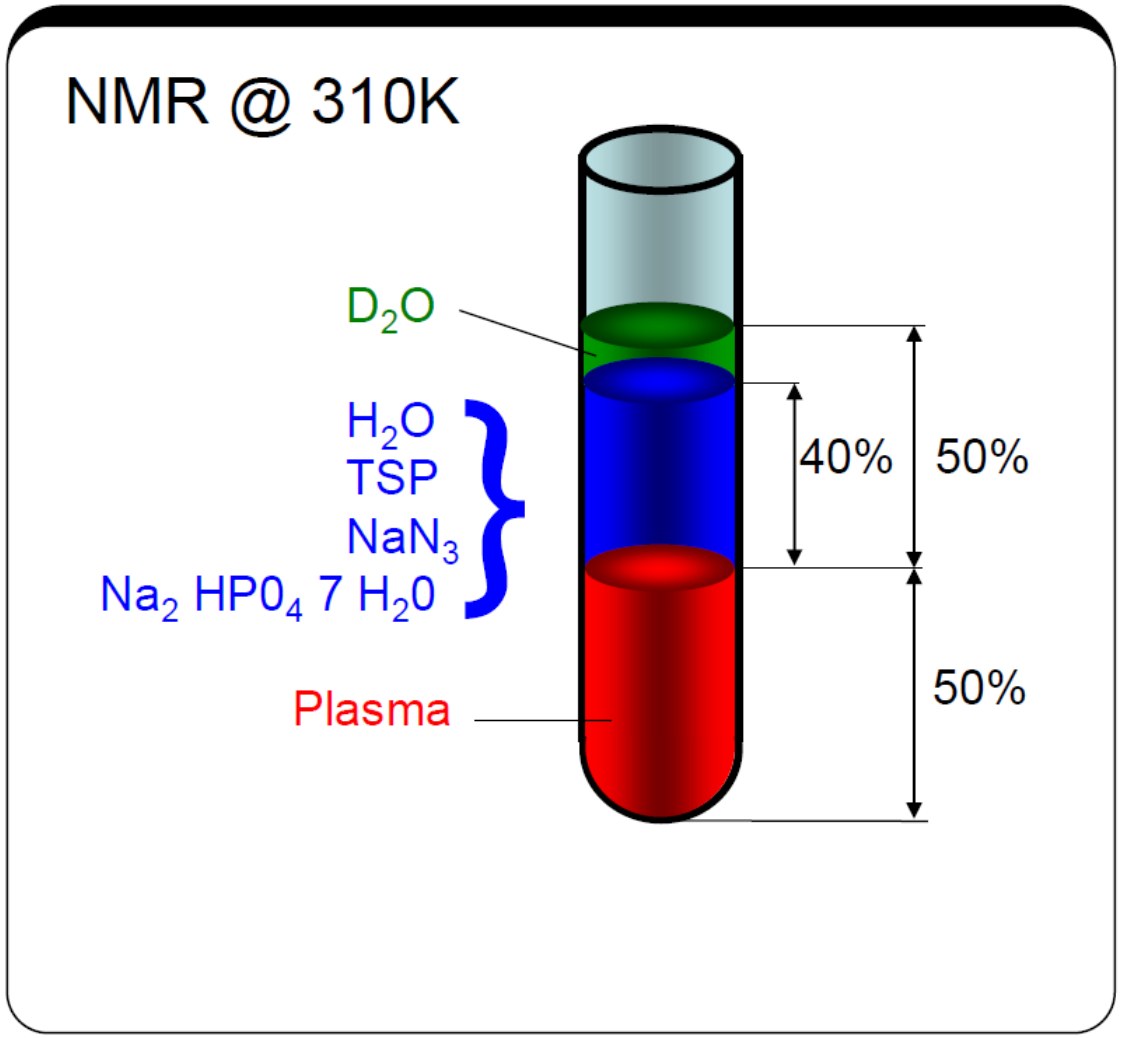


SOP for Blood Collection

- Blood collection in fasted state (e.g in the morning, 12 h fasted).
- Fresh EDTA-plasma into 6ml-tubes EDTA
  (BD Ref. 367864, Vacutainer K2E 10.8 mg),
- Needle diameter 0.8mm.
- Gentle blood withdrawal, avoiding manipulation of the vein or
  a long time of tourniquet, which might result in hemolysis.
- Centrifugation at (6° C, 13 min at 1700 G).
- 1mL plasma per aliquot into Biozym-containers
  (Biozyme Ref. tube: 710020, cap: 710030).
- Immediate deep freezing of plasma at -80° C.

Preparation Procedure – Plasma/Serum

- Carefully thaw the samples at room temperature for ca.30min.
- Add 400µL of Bruker plasma buffer.
- Add 400µL of plasma/serum into a Cryovial / Eppendorf.
- Shake the mixture gently for 1min (do NOT use the Vortex mixer).
- Transfer 600µL of well mixed sample into a 5 mm 7” NMR tube or a 5 mm SampleJet rack tube.



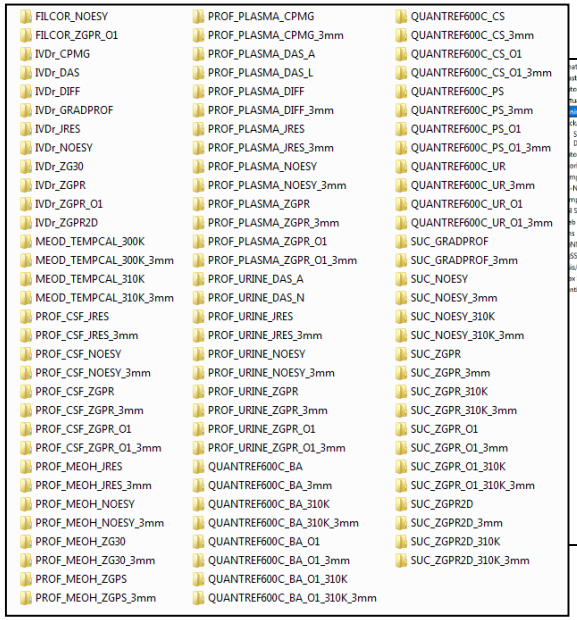
SOPs in Calibration and Measurement

- **Software**
  - Automation software scripts
  - Experimental parametersets
  - ICON NMR configuration
  - SampleTrack configuration

- **Reference Samples and Buffers**
  - Calibration samples for daily Quality Control and Optimization
  - Pseudo Bodyfluid test samples
  - Buffer solutions (starter kit)

- **Sample preparation SOPs / Sample NMR Measurements SOPs**

- **Quality Control SOPs**



IVDr Sample Kit

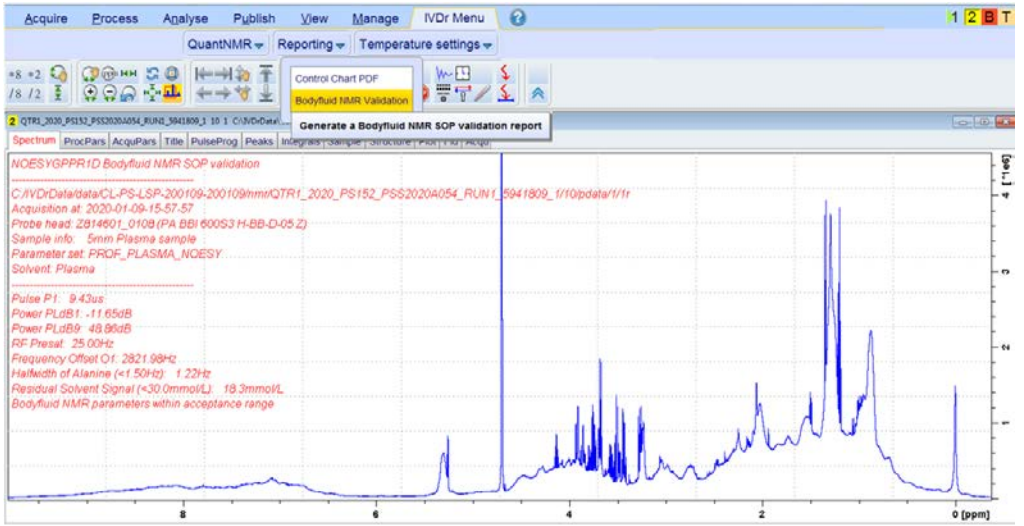
IVDr Buffers

Quality Control in NMR

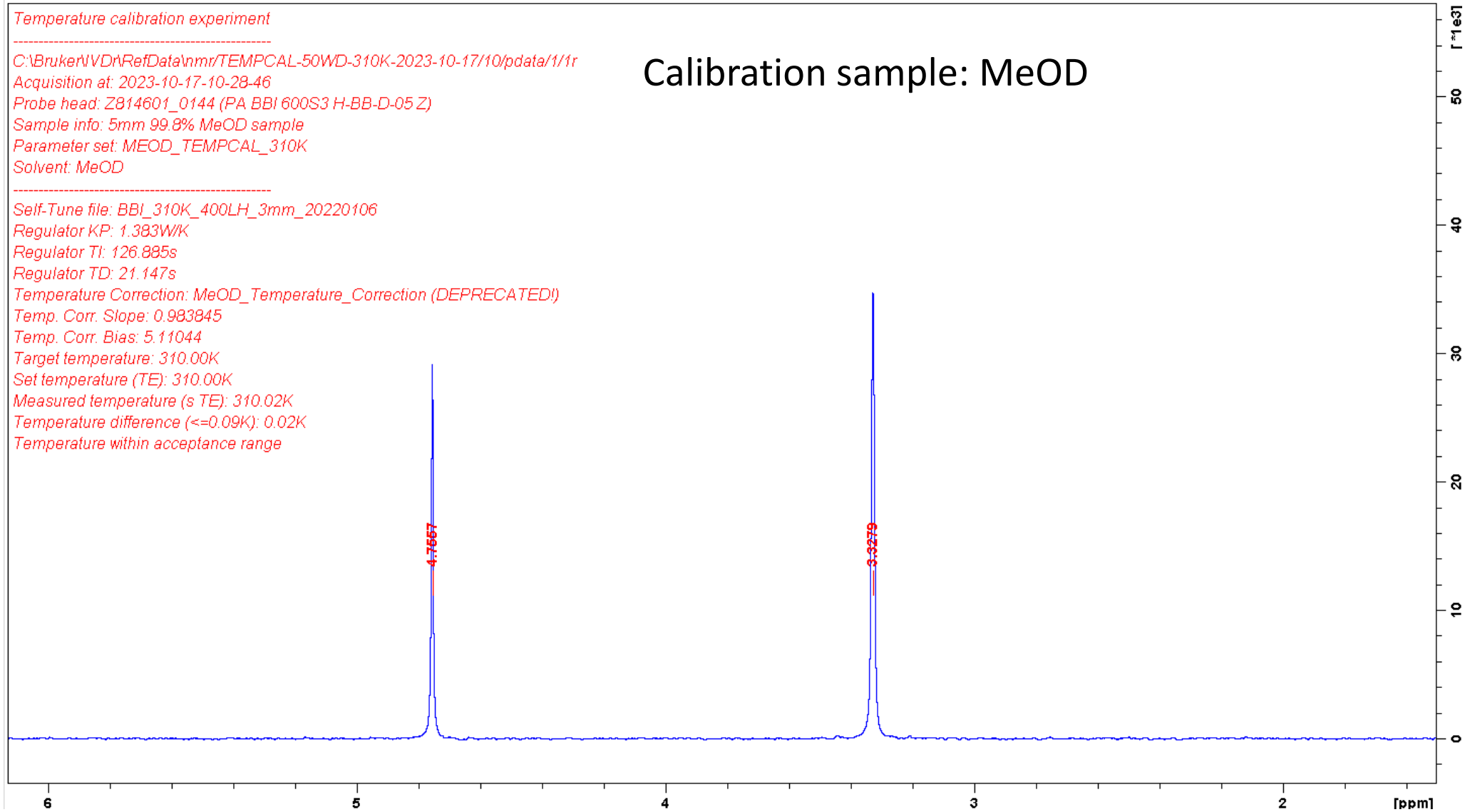
- Advanced **DAILY quality control of the system** by calibration, validation and reporting methods: temperature <0.09 °C , shim (TSP < 0.8 Hz), water suppression (water hump -50% < 30Hz), quantification (98-102%)
- **system suitability functionality** i.e. temperature management and operation mode (5mm/3mm) push buttom
- Advanced **monitoring of NMR parameters** (p1, residual water, shim)
- Supports reporting needs for laboratory accreditation.

Quality Control	QC Report	Control Charts
(Filcor Calibration)	√	√
Temperature Calibration	√	√
Shim & Watersuppression Optimization	√	√
QuantRef Calibration & Validation	√	√
Bodyfluid screening NMR quality validation	√	√

- *QC Report:*
  - *spectrum title*
  - *XML*
  - *PDF*
- *Control Chart:*
  - *CSV table*
  - *PDF*

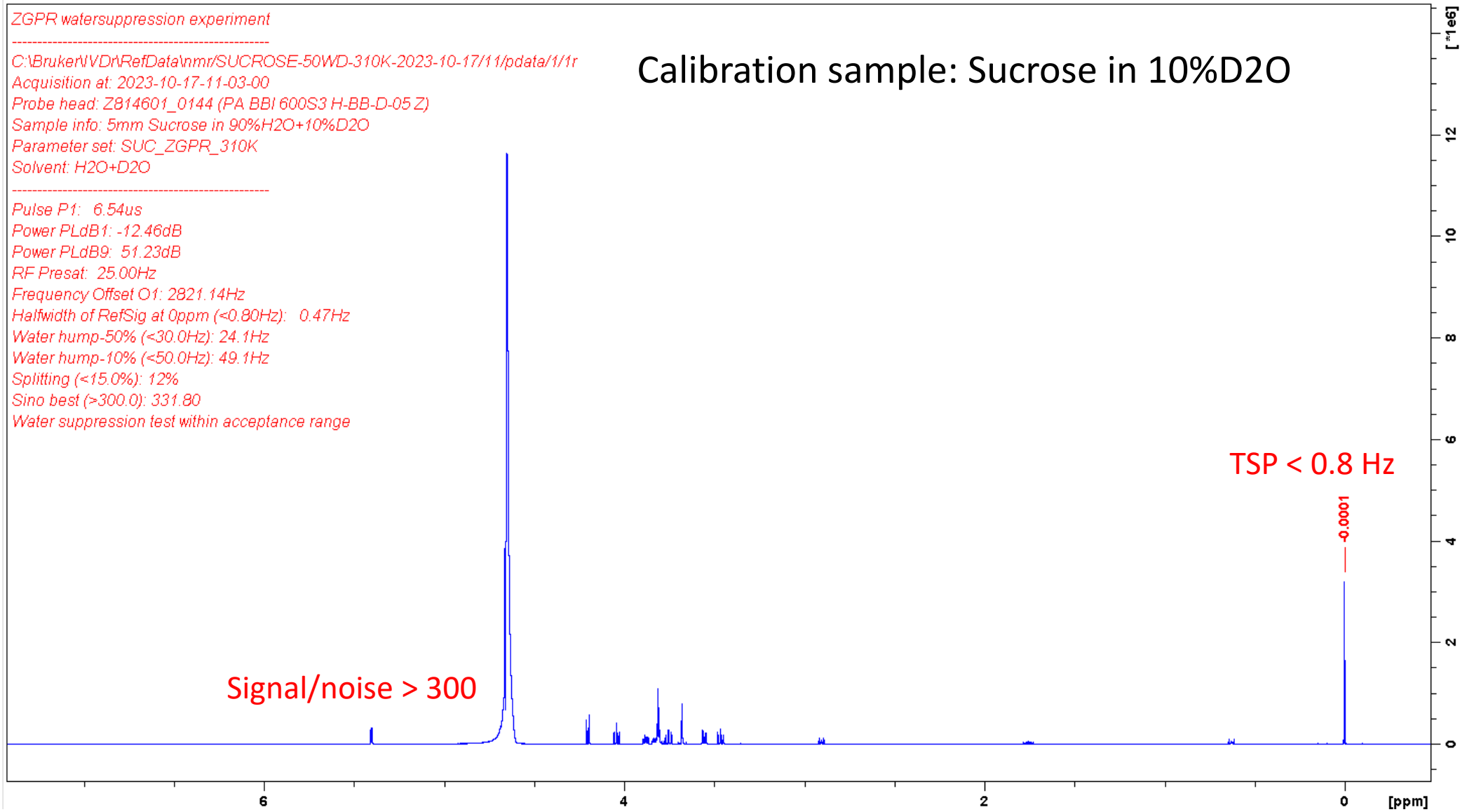


Temperature Calibration <0.09 °C

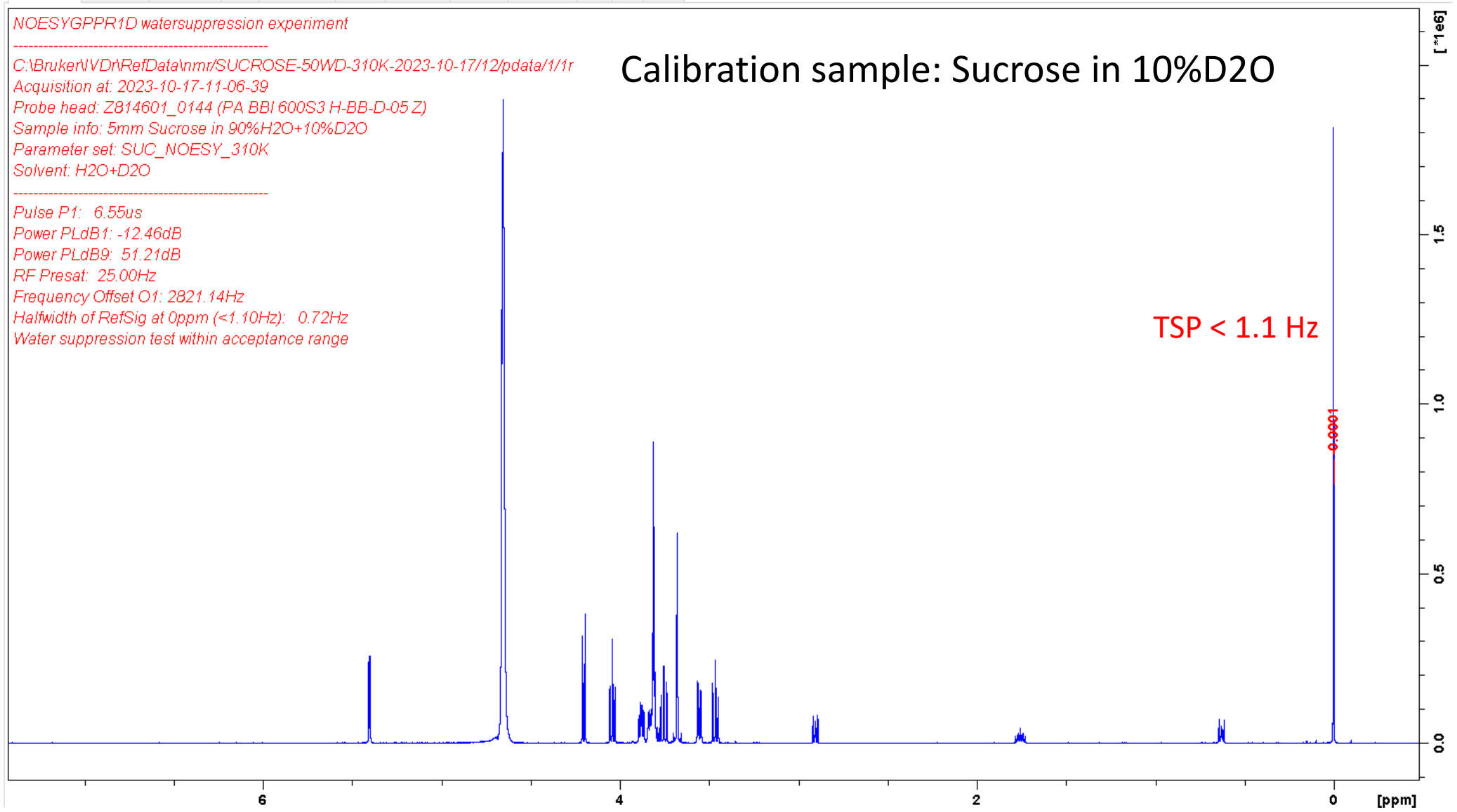


Calibration sample: MeOD

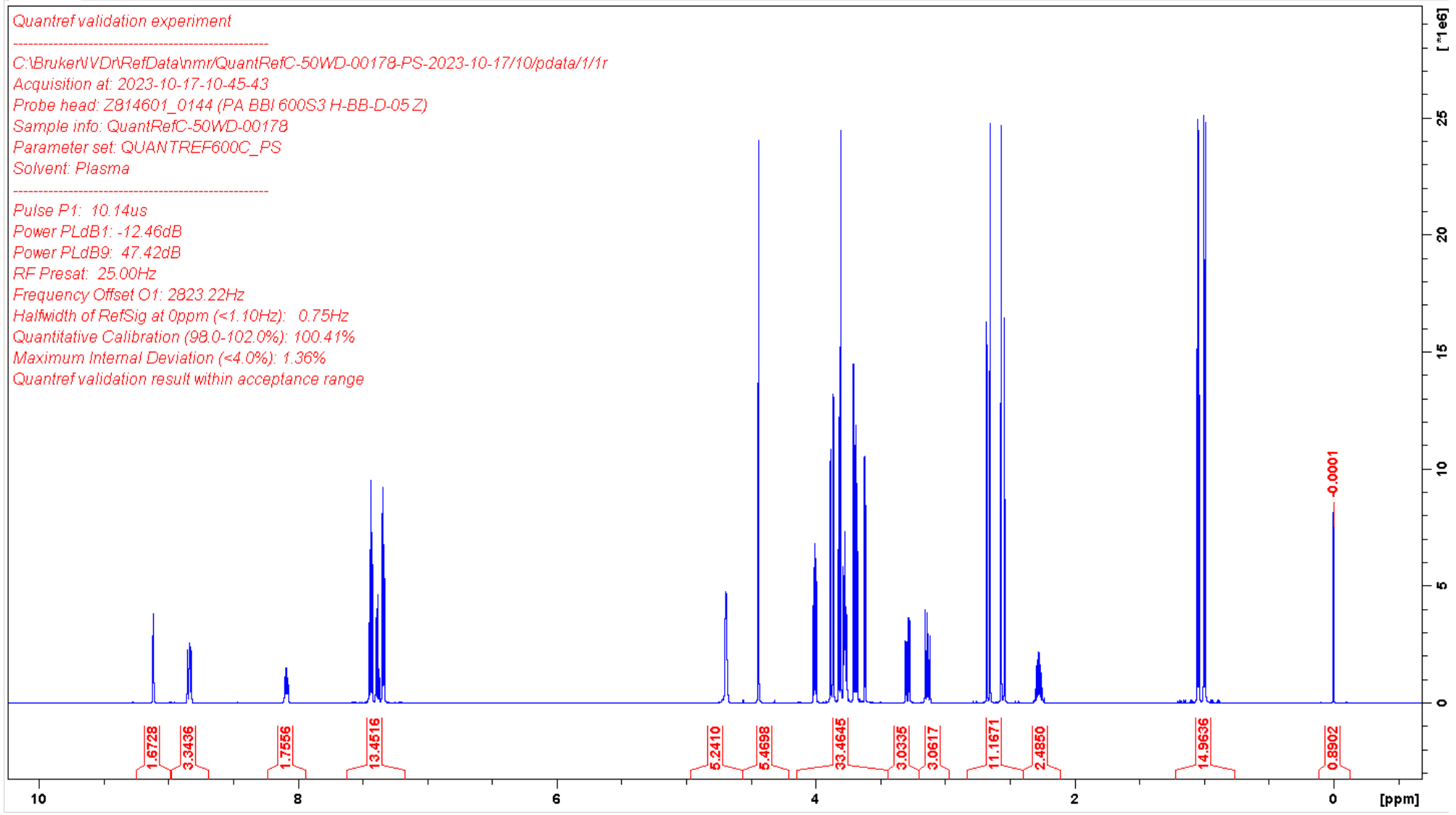
Shim and Water Suppression (zgpr)



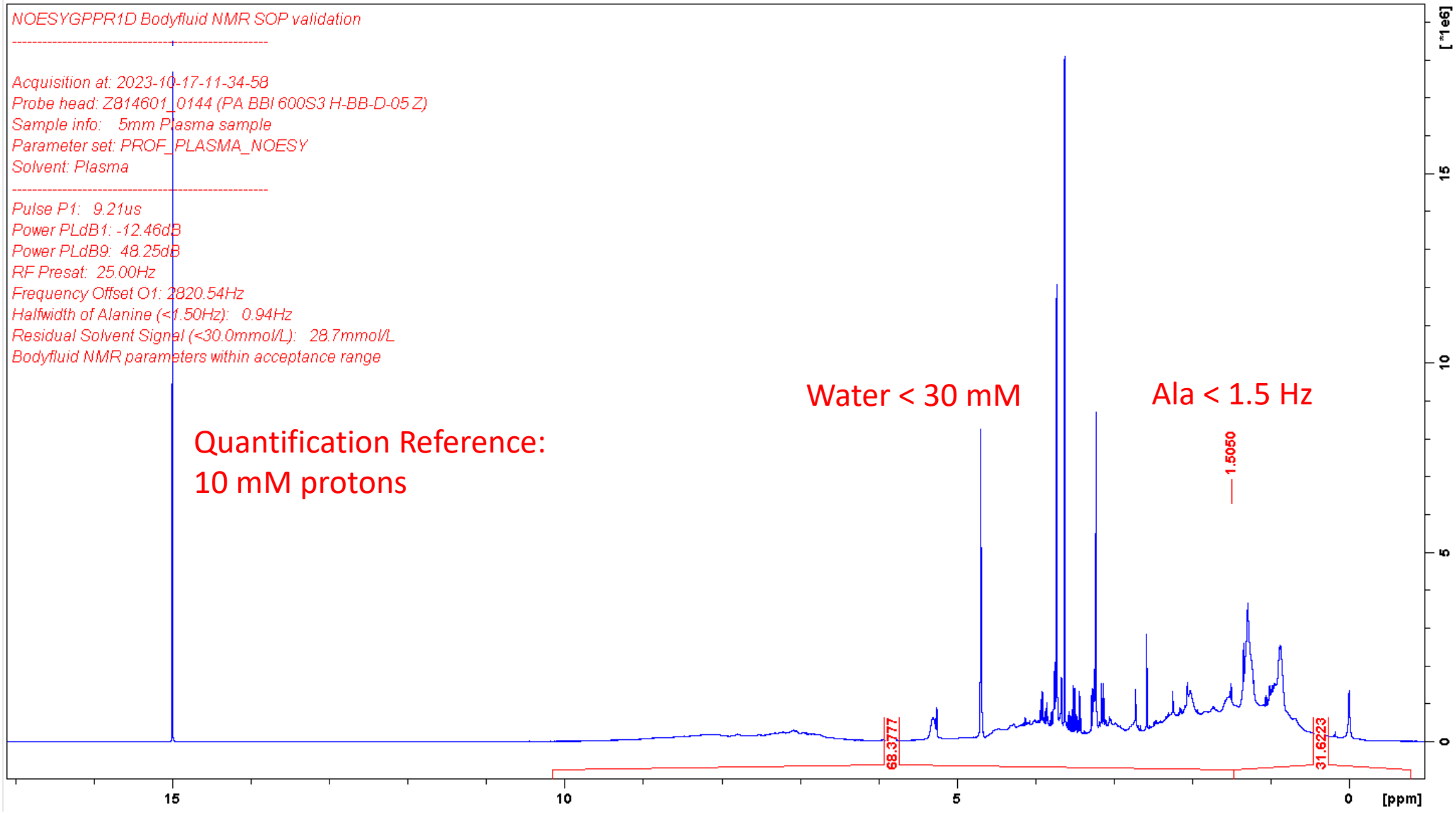
Shim and Water Suppression (noesygppr1d)



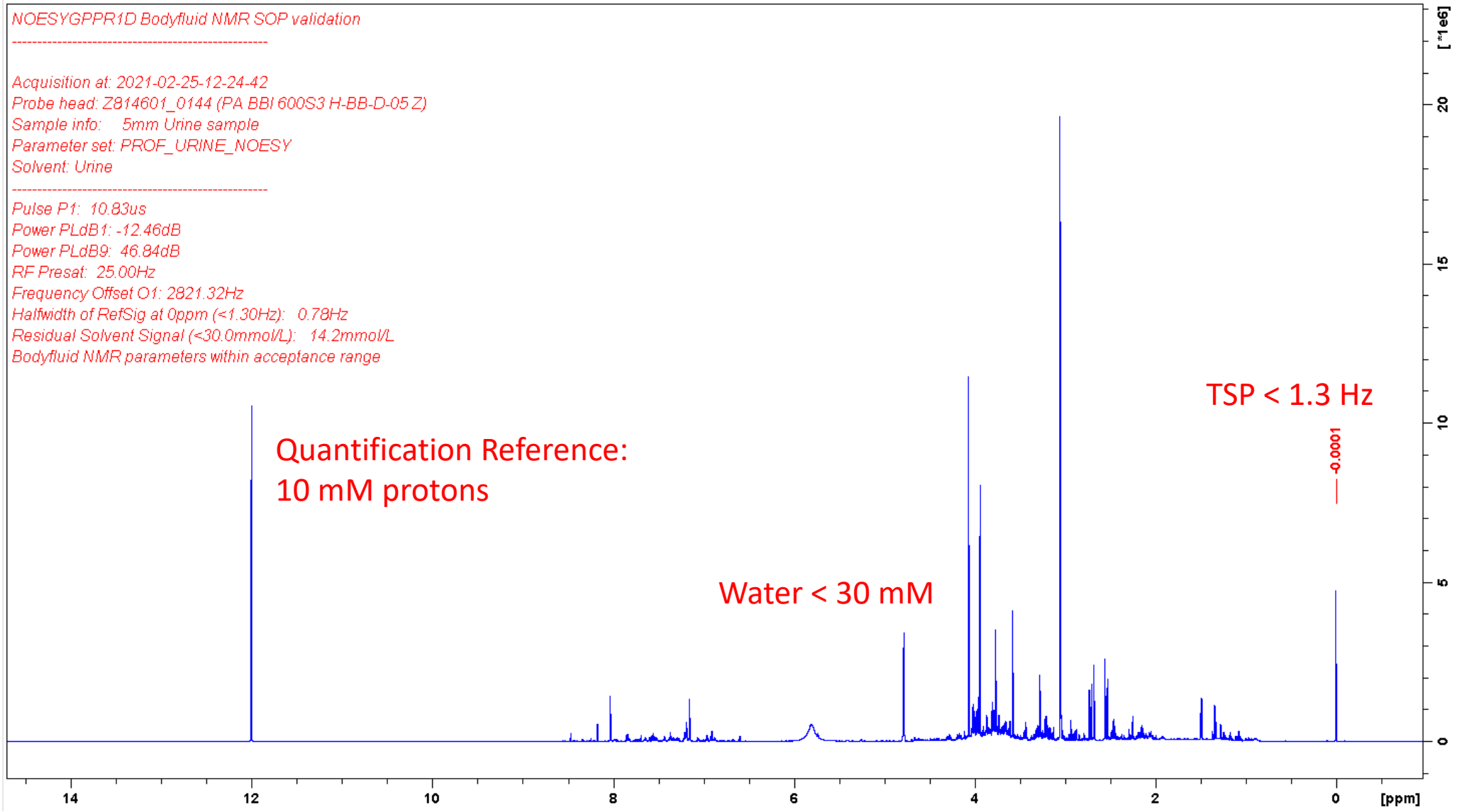
Quantification Validation (98-102%)



Validation for Plasma



Validation for Urine



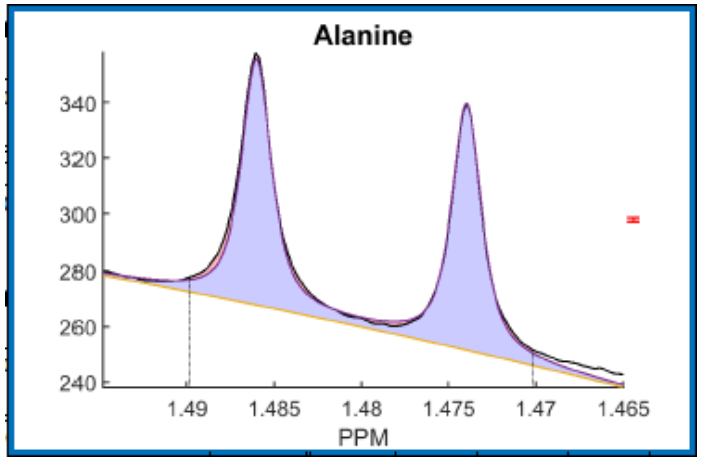
Automatic Matabolite Identification & Quantification

Bruker IVDr provides automatic identification on:

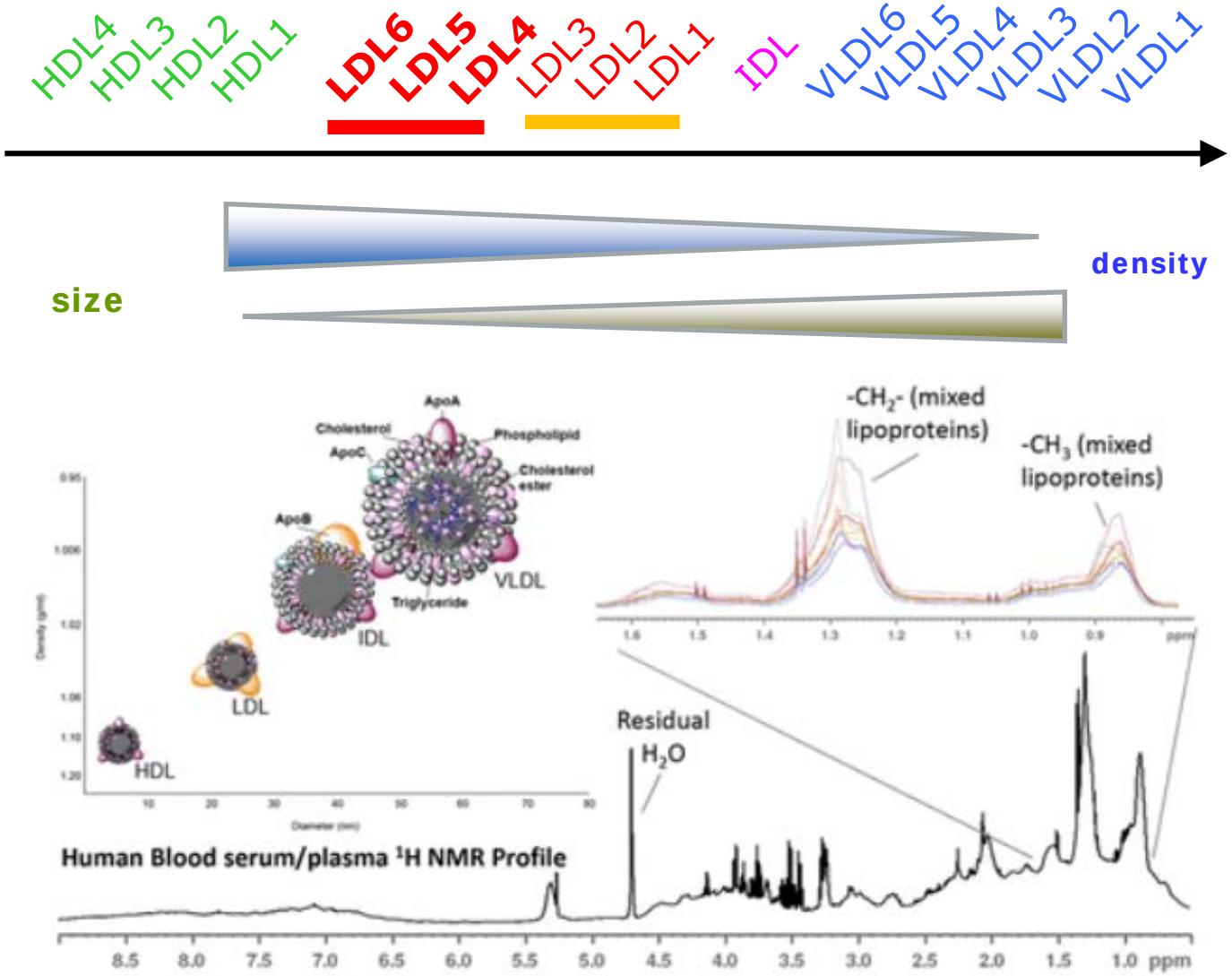
- 41 metabolites in Plasma/Serum
- 150 metabolites in Urine
- 115 Lipoprotein parameters in Plasma/Serum

3 Amino acids and derivatives

Compound	Conc. mmol/L	LOD mmol/L	r mmol/L	ρ %	Δ mmol/L	95% Range mmol/L	Graphics (*)
2-Aminobutyric acid	< 0.05	0.05	0.000	0 ○	0.827	⩽ 0.10	
Alanine	0.42	0.02	0.423	100 ●	0.011	0.29 - 0.64	
Asparagine	< 0.05	0.05	0.000	0 ○	4.734	⩽ 0.08	
Creatine	0.02	0.01	0.024	100 ●	0.002	⩽ 0.07	
Creatinine	0.06	0.01	0.059	100 ●	0.002	0.06 - 0.14	
Glutamic acid	0.11	0.05	0.106	25 ○	0.083	⩽ 0.24	
Glutamine	0.69	0.02	0.687	99 ●	0.018	0.30 - 0.83	
Glycine	0.30	0.01	0.298	100 ●	0.007	0.17 - 0.44	

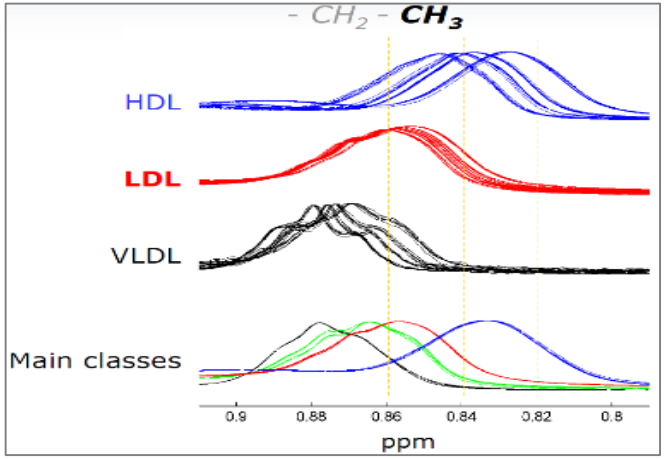


Lipoprotein Analysis



115 Parameters:
CH, FC, TG, PL,
ApoA1,A2,B100

Main and subfractions
VLDL, LDL, HDL



Lipoprotein Report

Particle numbers

Total Concentration of ApoB carrying Particles

Key	Parameter	Value	Unit	95% Range of Model	Graphics (*)
TBPN	Total Particle Number	1777	nmol/L	876 - 2908	

(*) Gray horizontal boxes represent 95% range of model, black vertical lines represent sample value.

Lipoprotein Main Fractions

Key	Parameter	Value	Unit	95% Range of Model	Graphics (*)
VLPN	VLDL Particle Number	138	nmol/L	50 - 473	
IDPN	IDL Particle Number	98	nmol/L	36 - 316	
LDPN	LDL Particle Number	1452	nmol/L	760 - 2560	

(*) Gray horizontal boxes represent 95% range of model, black vertical lines represent sample value.

LDL Subfractions

Key	Parameter	Value	Unit	95% Range of Model	Graphics (*)
L1PN	LDL-1 Particle Number	232	nmol/L	98 - 567	
L2PN	LDL-2 Particle Number	268	nmol/L	47 - 427	
L3PN	LDL-3 Particle Number	282	nmol/L	51 - 499	
L4PN	LDL-4 Particle Number	300	nmol/L	77 - 577	
L5PN	LDL-5 Particle Number	248	nmol/L	86 - 615	
L6PN	LDL-6 Particle Number	182	nmol/L	91 - 815	

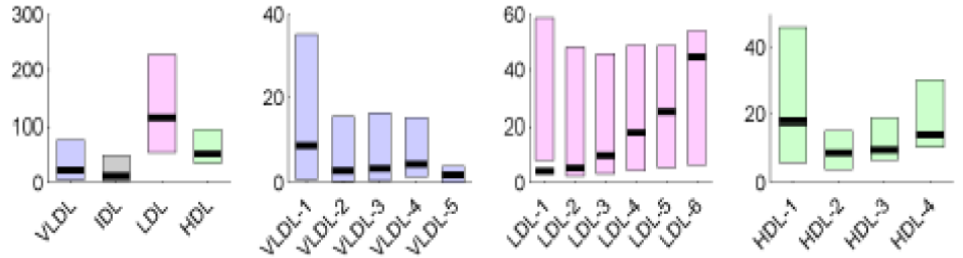
(*) Gray horizontal boxes represent 95% range of model, black vertical lines represent sample value.

Main class

Key	Parameter	Value	Unit	95% Range of Model	Graphics (*)
TPTG	TG	115	mg/dL	53 - 490	
TPCH	Chol	204	mg/dL	140 - 341	
LDCH	LDL-Chol	134	mg/dL	55 - 227	
HDCH	HDL-Chol	50	mg/dL	35 - 96	
TPA1	Apo-A1	146	mg/dL	112 - 217	
TPA2	Apo-A2	32	mg/dL	24 - 48	
TPAB	Apo-B100	98	mg/dL	48 - 160	

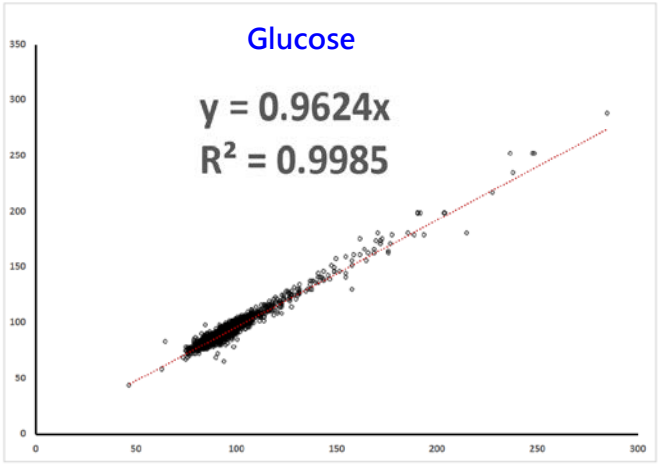
Subclass

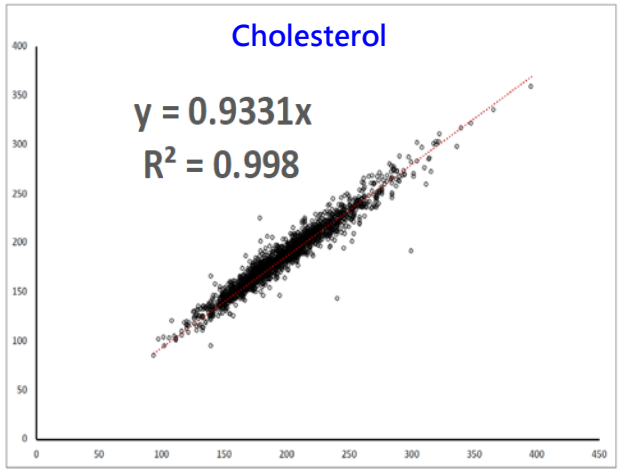
Cholesterol distribution (concentrations in mg/dL together with 95% range of model)

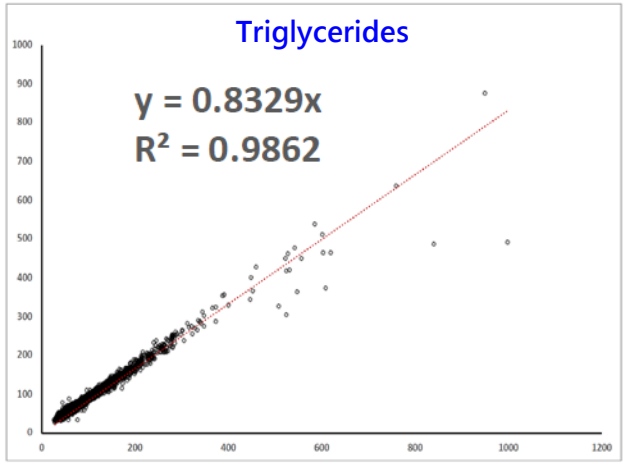


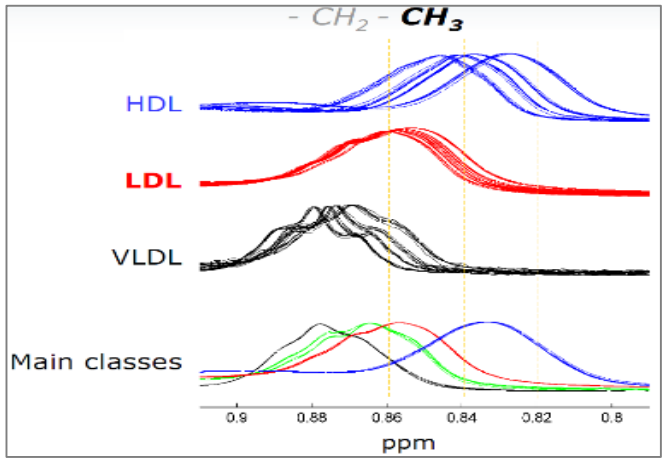
GOOD Correlation between NMR and Biochemical Values

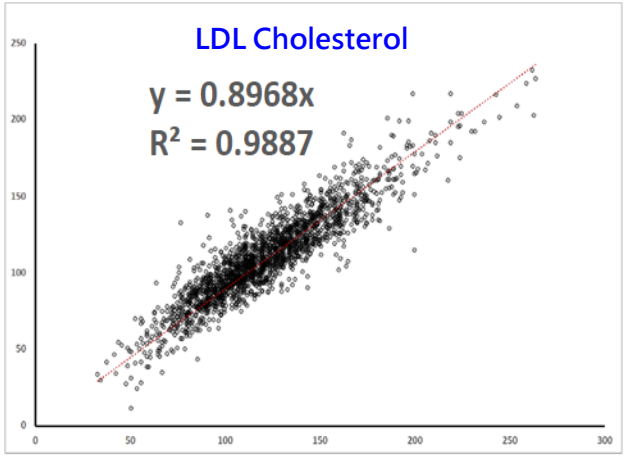
Provided by Taiwan BioBank

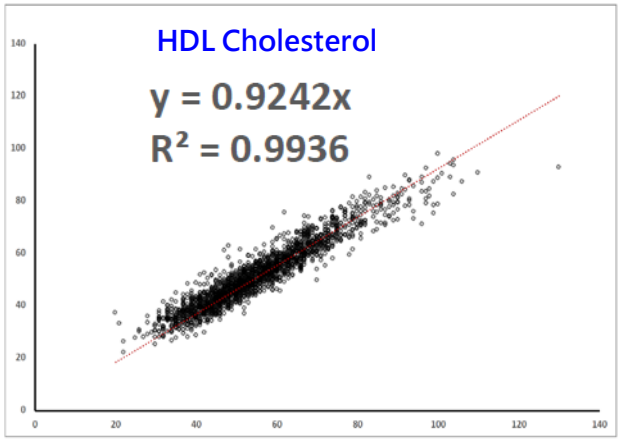












The super BAD LDL: sLDL

Atherosclerosis & Lipoproteins

Low-Density Lipoprotein Subfractions and the Long-Term Risk of Ischemic Heart Disease in Men

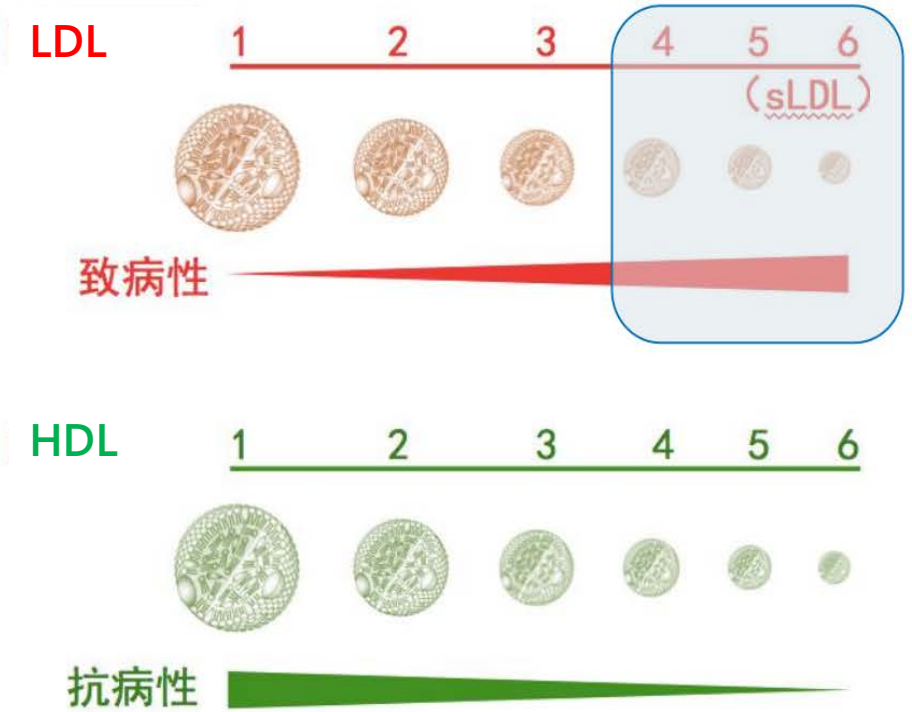
13-Year Follow-Up Data From the Québec Cardiovascular Study

Annie C. St-Pierre, Bernard Cantin, Gilles R. Dagenais, Pascale Mauriège, Paul-Marie Bernard, Jean-Pierre Després, Benoît Lamarche

Objective—The objective of the present study was to investigate the association between large and small low-density lipoprotein (LDL) and long-term ischemic heart disease (IHD) risk in men of the Québec Cardiovascular Study.

Methods and Results—Cholesterol levels in the large and small LDL subfractions (termed LDL-$C_{\geq 260Å}$ and LDL-$C_{<255Å}$, respectively) were estimated from polyacrylamide gradient gel electrophoresis of whole plasma in the cohort of 2072 men of the population-based Québec Cardiovascular Study. All men were free of IHD at the baseline examination and followed-up for a period of 13 years, during which 262 first IHD events (coronary death, nonfatal myocardial infarction, and unstable angina pectoris) were recorded. Our study confirmed the strong and independent association between LDL-$C_{<255Å}$ levels as a proxy of the small dense LDL phenotype and the risk of IHD in men, particularly over the first 7 years of follow-up. However, elevated LDL-$C_{\geq 260Å}$ levels (third versus first tertile) were not associated with an increased risk of IHD over the 13-year follow-up (RR=0.76; P=0.07).

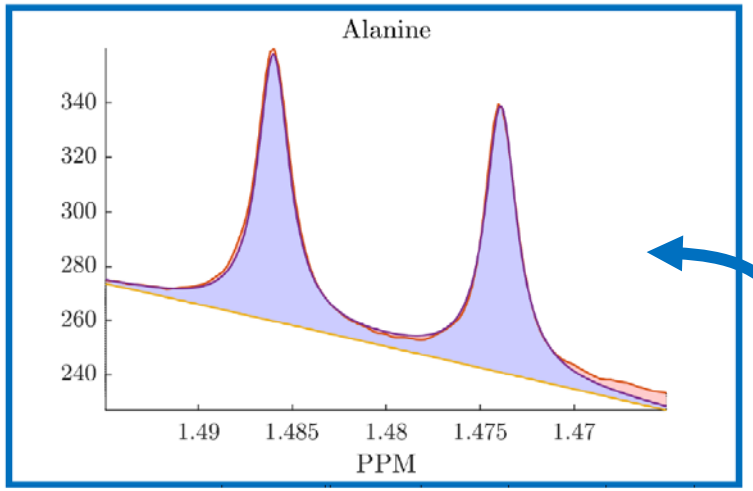
Conclusions—These results indicated that estimated cholesterol levels in the large LDL subfraction were not associated with an increased risk of IHD in men and that the cardiovascular risk attributable to variations in the LDL size phenotype was largely related to markers of a preferential accumulation of small dense LDL particles. (*Arterioscler Thromb Vasc Biol*. 2005;25:553-559.)

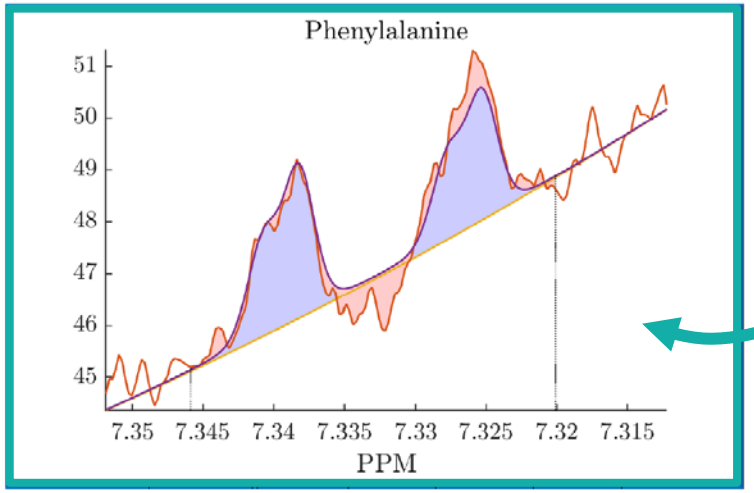


The classical lipid risk factors (Total Chol./HDL) CANNOT perfectly predict cardiovascular disease in patients, sLDL subfraction has the potential to improve risk prediction.

小顆低密度脂蛋白(sLDL)容易黏附至血管，且容易被氧化，導致心血管的疾病。因此，sLDL的顆粒數可做為預測心血管疾病的重要風險因子

41 Plasma Metabolites:





by positing the mouse cursor on a metabolite name, the corresponding graphical fit display pops up in PDF report

4 Amino acids and derivatives

Compound	Conc. mmol/L	LOD mmol/L	r mmol/L	ρ %	Δ mmol/L	95% Range mmol/L	Graphics (*)
Alanine	0.43	0.02	0.433	100 ●	0.016	0.29 - 0.63	
Creatine	0.02	0.01	0.024	99 ●	0.004	$\leq$ 0.07	
Glutamic acid	0.07	0.05	0.075	38 ○	0.106	$\leq$ 0.25	
Glutamine	0.58	0.02	0.579	99 ●	0.023	0.29 - 0.82	
Glycine	0.22	0.10	0.224	99 ●	0.013	0.15 - 0.42	
Histidine	0.10	0.02	0.100	99 ●	0.002	0.07 - 0.17	
Isoleucine	0.05	0.03	0.054	95 ●	0.006	0.03 - 0.11	
Leucine	0.07	0.01	0.072	96 ●	0.010	0.06 - 0.20	
Phenylalanine	$<$ 0.05	0.05	0.028	95 ●	0.004	$\leq$ 0.06	
Tyrosine	0.05	0.03	0.052	97 ●	0.005	$\leq$ 0.08	
Valine	0.22	0.03	0.216	100 ●	0.007	0.15 - 0.36	

(*) Gray horizontal boxes represent 95% concentration range, black vertical lines represent sample value.

150 Urine Metabolites:

Bruker BioSpin GmbH

Analysis Report

Bruker IVDr Quantification in URine B.I.Quant-UR e™

1 Creatinine

2 Alcohols and derivatives

3 Amines and derivatives

4 Amino acids and derivatives

5 Benzene and substituted derivatives

6 Carboxylic acids

7 Cosmetics, vitamines, drugs and drug metabolites

8 Fatty acids and derivatives

9 Hydroxy acids and derivatives

10 Keto acids and derivatives

11 Purine, Pyridine and Pyrimidine derivatives

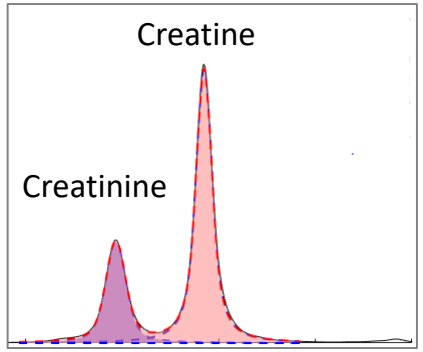
12 Sugars and derivatives

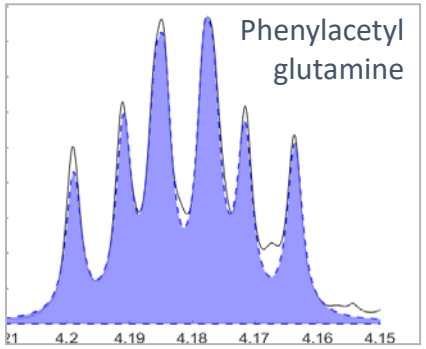
13 Explanations

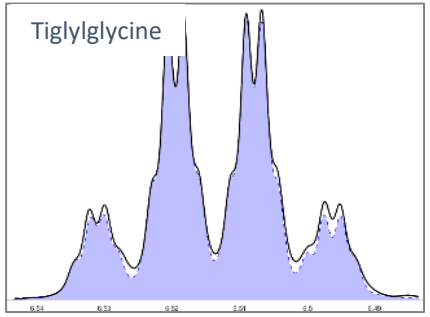
4 Amino acids and derivatives

Compound	Conc. mmol/L	Conc. mmol / mol Crea	LOD mmol / mol Crea	r mmol/L	ρ %	Δ mmol/L	95% Range(*) mmol / mol Crea
1-Methylhistidine	< 0.12	< 15	15	0.000	0 ○	0.085	≤ 15
2-Furoylglycine	< 0.30	< 39	39	0.000	0 ○	0.038	≤ 40
3-Aminoisobutyric acid	< 0.66	< 85	85	0.000	0 ○	0.489	≤ 85
3-Methylcrotonylglycine	< 0.06	< 8	8	0.000	0 ○	0.007	≤ 8
4-Aminobutyric acid	< 0.15	< 20	20	0.000	0 ○	0.306	≤ 20
5-Aminopentanoic acid	< 0.73	< 94	94	0.000	0 ○	0.972	≤ 94
Alanine	0.18	23	10	0.180	100 ●	0.012	11 - 72
Arginine	< 5.8	< 750	750	0.433	0 ○	1.526	≤ 750
Argininosuccinic acid	< 0.22	< 29	29	0.000	0 ○	0.387	≤ 29
Betaine	0.10	13	7	0.097	100 ●	0.031	9 - 78
Citrulline	< 5.3	< 690	690	0.299	1 ○	2.260	≤ 690
Creatine	< 0.39	< 50	50	0.032	100 ●	0.250	≤ 280
Cystine	< 3.8	< 490	490	0.000	0 ○	2.862	≤ 490
DL-Alloisoleucine	< 0.37	< 48	48	0.000	0 ○	0.184	≤ 48
DL-Tyrosine	< 0.34	< 44	44	0.000	0 ○	0.295	≤ 44
Glutamic acid	< 3.5	< 460	460	0.000	0 ○	1.590	≤ 460
Glutamine	< 3.4	< 440	440	0.000	0 ○	9.383	≤ 440
Glycine	0.66	85	34	0.658	100 ●	0.036	38 - 440
Guanidinoacetic acid	< 0.80	< 100	100	0.277	35 ○	0.264	≤ 140
Isobutyrylglycine	< 0.05	< 7	7	0.000	0 ○	0.071	≤ 7
L-Carnosine	< 0.99	< 130	130	0.000	0 ○	0.157	≤ 130
L-Homocystine	< 7.1	< 910	910	0.000	0 ○	0.343	≤ 910
L-Isoleucine	< 0.12	< 16	16	0.010	67 ○	0.007	≤ 16
L-Pyroglutamic acid	< 0.25	< 32	32	0.000	0 ○	1.457	≤ 67
L-Tryptophan	< 0.75	< 97	97	0.079	73 ○	0.121	≤ 97
Leucine	< 0.17	< 22	22	0.029	74 ○	0.011	≤ 22
Methionine	< 0.14	< 18	18	0.010	95 ●	0.032	≤ 18
N,N-Dimethylglycine	< 0.04	< 5	5	0.029	91 ●	0.015	≤ 15
N-Acetylaspartic acid	< 0.77	< 99	99	0.083	16 ○	0.269	≤ 99
N-Acetylglutamate	< 0.33	< 42	42	0.000	0 ○	0.209	≤ 42
N Acetylphenylalanine	< 1.00	< 130	130	0.000	0 ○	0.559	≤ 130
N-Acetyltyrosine	< 2.9	< 380	380	0.000	0 ○	1.382	≤ 380
N-Isovaleroylglycine	< 0.01	< 2	2	0.012	64 ○	0.010	≤ 5
Phenylalanine	< 1.5	< 200	200	0.125	0 ○	0.661	≤ 200
Proline betaine	< 0.19	< 25	25	0.100	96 ●	0.033	≤ 280
Propionylglycine	< 0.10	< 12	12	0.000	0 ○	0.166	≤ 12
Sarcosine	< 0.01	< 2	2	0.005	0 ○	0.009	≤ 7
Taurine	< 1.1	< 140	140	0.202	46 ○	0.191	≤ 170
Tiglylglycine	< 0.15	< 19	19	0.000	0 ○	0.069	≤ 19
Valine	0.03	4	2	0.034	80 ○	0.014	≤ 7

(*) Gray horizontal boxes represent 95% concentration range, black vertical lines represent sample value.







Conclusions for IVDr

- NMR is a robust and highly reproducible spectroscopic method, allowing to integrate spectral data from all over the globe into post processing like statistical analysis and quantification for urine, plasma, CSF and Methanol extract of Bodyfluids, cell cultures or tissues.
- On Avance IVDr platform there are efficient enabling tools to generate large number of parameters out of 1 measurement, including targeted and non-targeted results
- Urine quantification is a tool generating up to 150 metabolites and the results can be linked to associated external knowledgebases (Metagene) to maximize information content
- Lipoprotein subclass analysis is a tool generating 115 lipoprotein related parameters in 1 measurement under full automation, clinical relevance in cardiovascular risks. With the same measurement quantification of 41 small molecules can be done allowing to have 2 different angles on a disease
- NMR can support biobanks offering input QC of samples and at the same time creating spectra that offer a substantial value add for the biobank