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Implementation of Quantification in Exploratory Metabolomic Analyses

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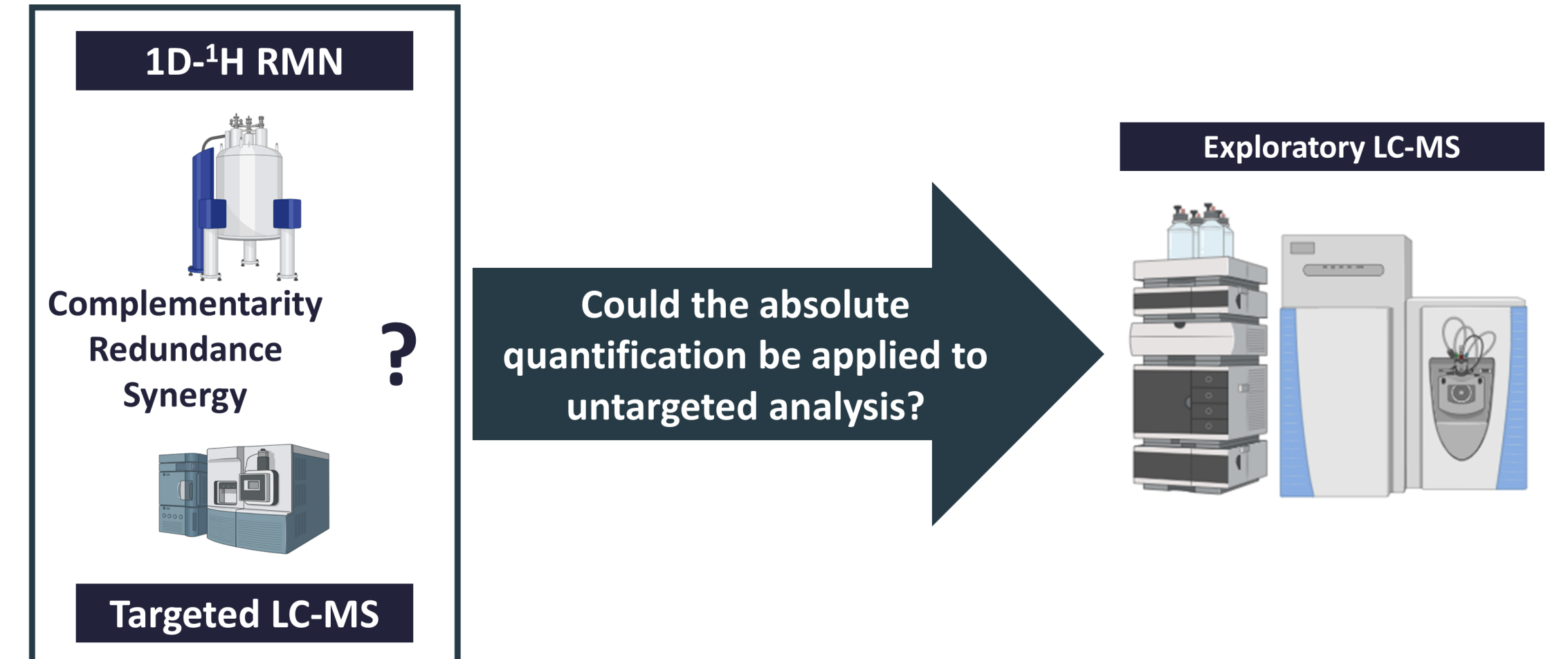
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INTRODUCTION

- Challenges of the personalized medicine are to make the right diagnosis in order to provide the right treatment at the right time to the right patient
- Metabolomics approach allows to enhance patient biological data to describe the individual health status and to enhance the clinical biology parameters
- In order to be able to **integrate metabolomics data into biomedical data, metabolomics should be quantitative**
- Moreover, in order to provide **FAIR temporal information for monitoring the health status over time, metabolomics needs to become quantitative**

How can we overcome this bottleneck in non-targeted analysis applied to biological studies?



MATERIAL & METHODS

SAMPLE : Pool serum ; n = 10 aliquots / analysis

Exploratory Analysis (Q-Orbitrap)

- Extraction: 50 µL serum + 150 µL Cold MeOH
- Vortex – Centrifugation - Evaporation
- Solubilisation : 100 µL H₂O/ACN (C18 +/- & HILIC +)

Data Analysis

- ProteoWizard
- W4M
- Identification: IROA-database ; CV < 30 %



1D-¹H NMR (600MHz, CryoProbe)

- 200 µL serum + Folch extraction
- Vortex – Centrifugation - Evaporation
- Solubilisation: 200 µL D₂O
- 10 µL Internal Reference (3.2 mM)

Quantification Protocol

- TopSpin & NMR.Proc.Flow
- Calibration Curve: 1D-Peak Area (u.a.)
- Linear Dynamic Range : 250 - 100,000 nM



Targeted Analysis (QqQ)

- 25 µL of serum +200 µL MeOH
- Vortex – Centrifugation – Evaporation
- Solubilisation : 100 µL H₂O/MeOH

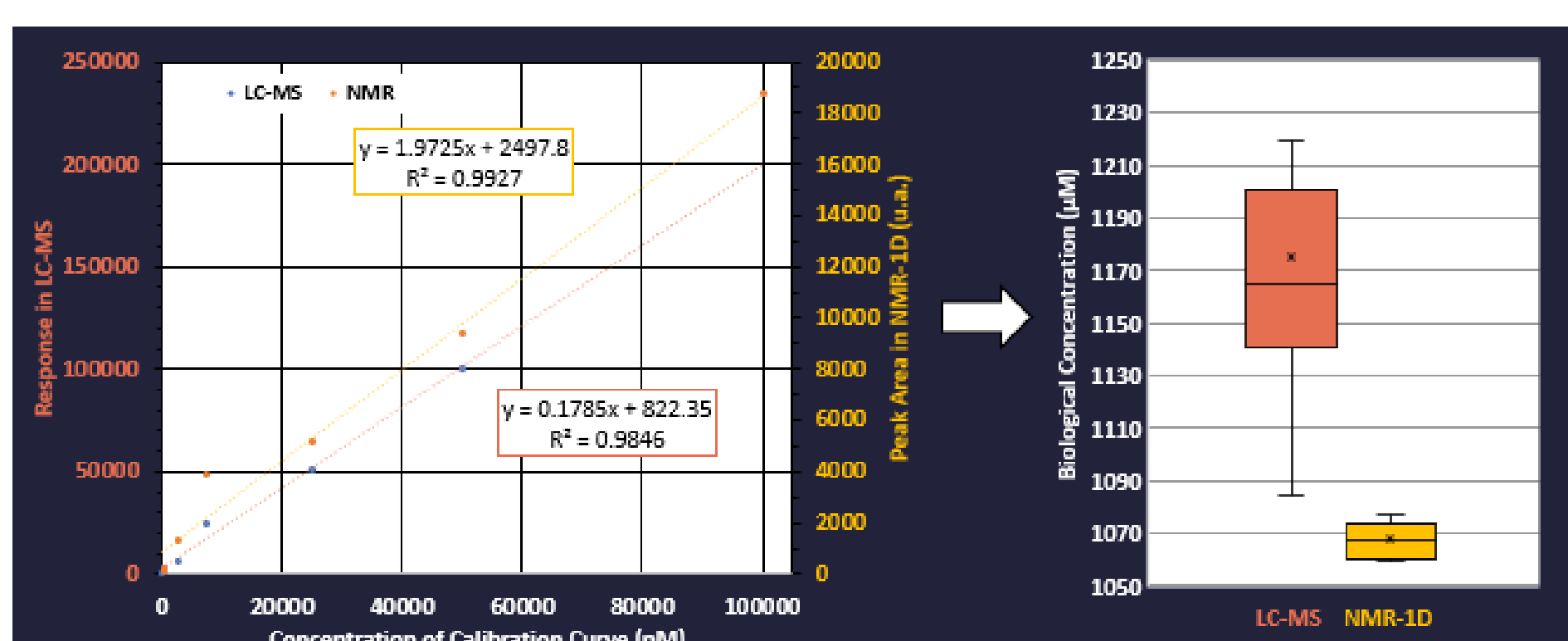
- MassLynx
- Calibration Curve: Response (STD Area / IS Area)
- Linear Dynamic Range : 75 - 50,000 nM



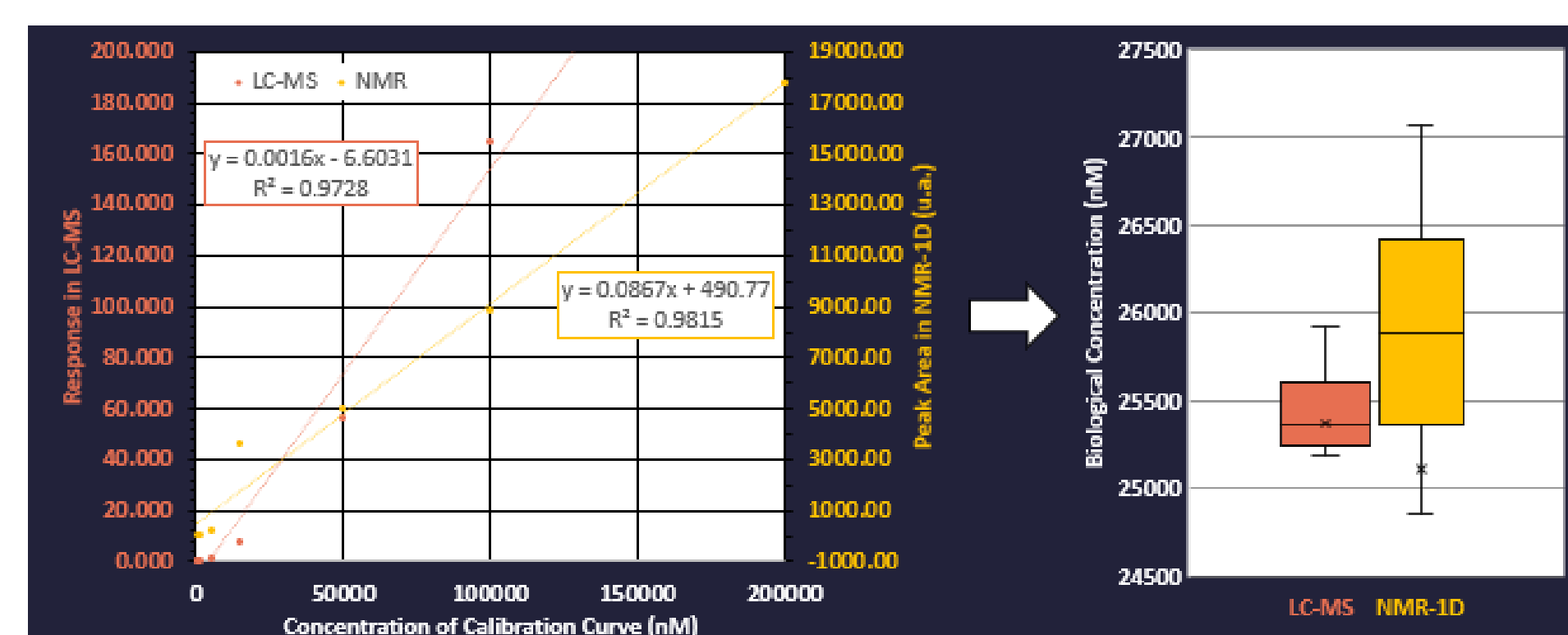
PRELIMINARY RESULTS

Absolute Quantification

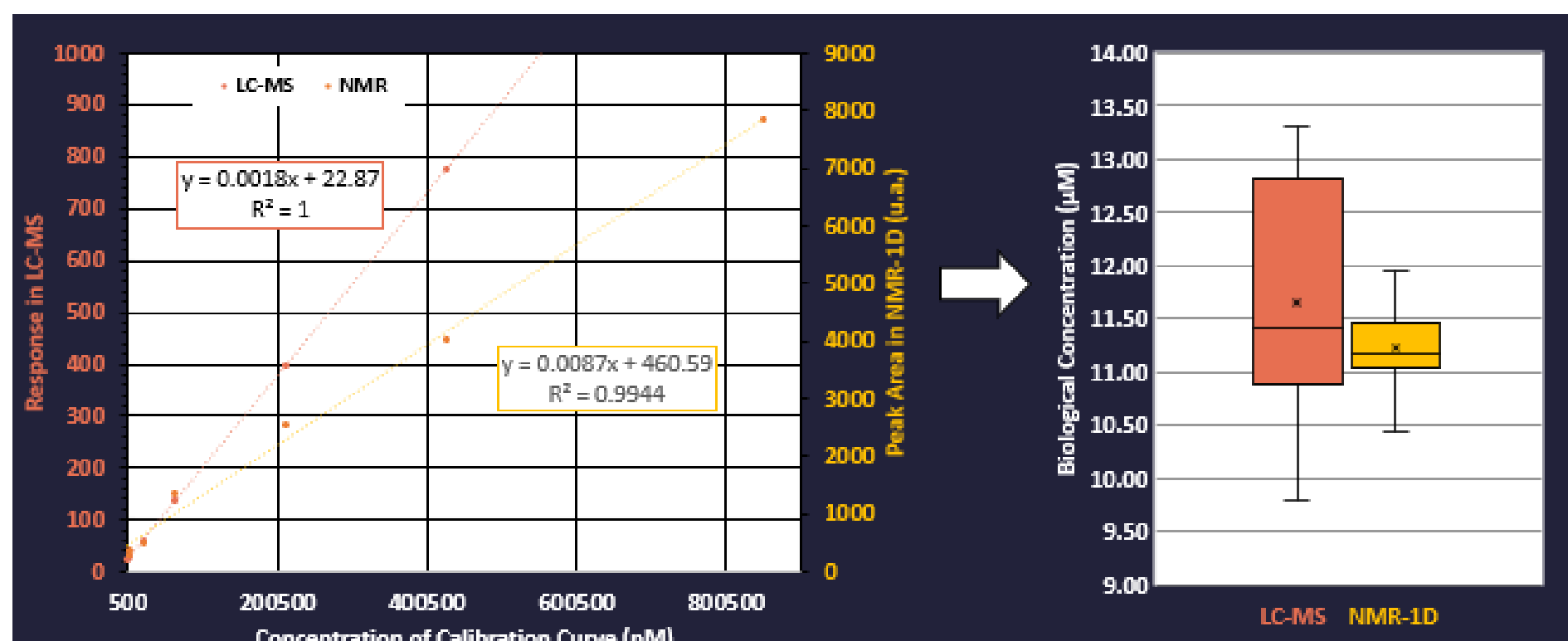
Lactic Acid



Citric Acid



Acetic Acid



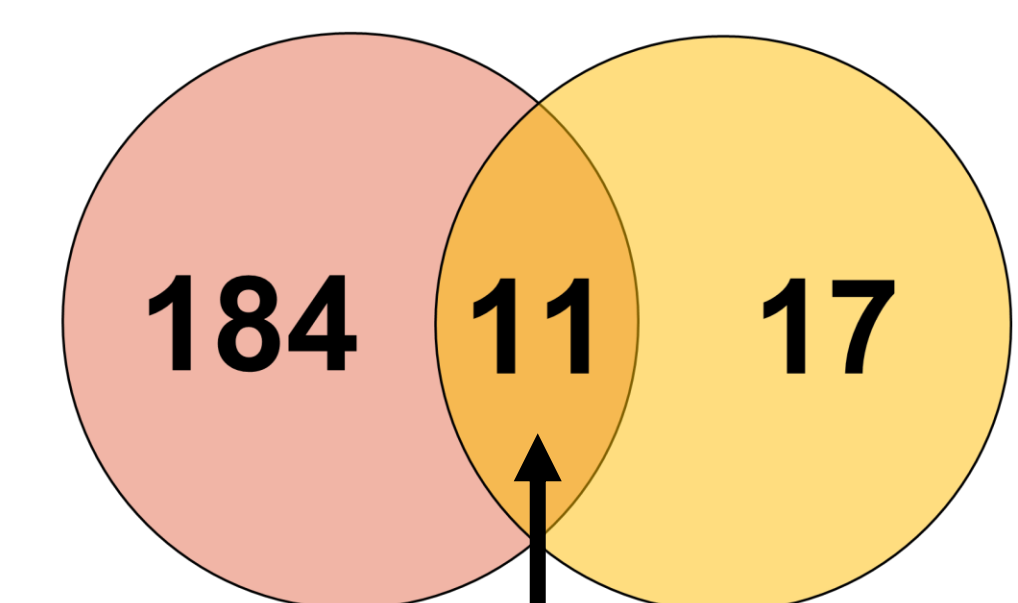
Quantification Table

Modality	LACTIC ACID		ACETIC ACID		CITRIC ACID	
	LC-MS	NMR	LC-MS	NMR	LC-MS	NMR
Biological Concentration (µM)	1 176	1 068	11.64	11.22	25.37	25.11
sd (µM)	69	18	1.17	0.41	0.39	2.84
CV (%)	5.87	1.69	10.01	3.66	1.52	11.33

Exploratory LC-MS vs. 1D-¹H NMR

LC-MS
Nb(identified) = 195
CV_{mean} (%) = 8.26

NMR
Nb(identified) = 28
CV_{mean} (%) = 11.43



Common Compounds

α-Glucose	L-Glutamine	L-Alanine
Citric Acid	L-Phenylalanine	Leucine
Hippuric Acid	Creatine	L-Tyrosine
Glycine	Hipoxanthin	-

- Screening : LC-MS >> 1D-¹H NMR & both are repeatable (CV < 15 %)
- 11 compounds are common:
 - Quantifiable in NMR without specific preparation
 - Quantifiable in Targeted LC-MS but required internal standard in LC-MS & more method development

- On serum samples, absolute quantification are very similar between NMR & LC-MS
- Both are repeatable (CV < 10 %)

CONCLUSION

- Combination of NMR & LC-MS advantages = ↑ quality of untargeted data
- Some compounds quantified in NMR are detected in Exploratory LC-MS
- Identified compounds in Exploratory LC-MS can be quantified in Targeted LC-MS → Cost of IS ; Time Consuming Development
- △ Corrective factors has to be determined

PROSPECTS

- NMR : Comparison of softwares for quantification (Amix, ChenomX, MesTreNova)
- NMR Coverage : ¹H, ¹³C & ³¹P & 2D-NMR
- Investigations of others matrices