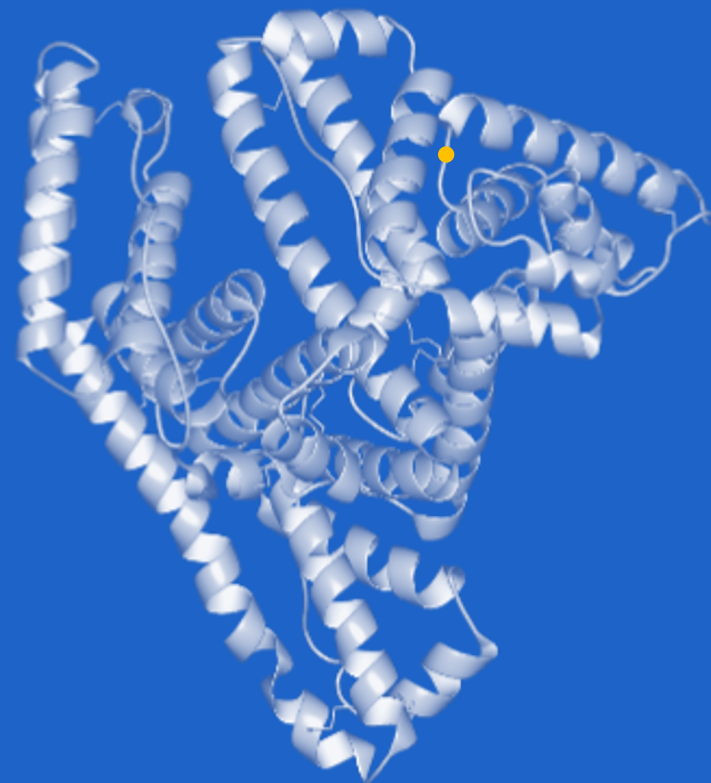


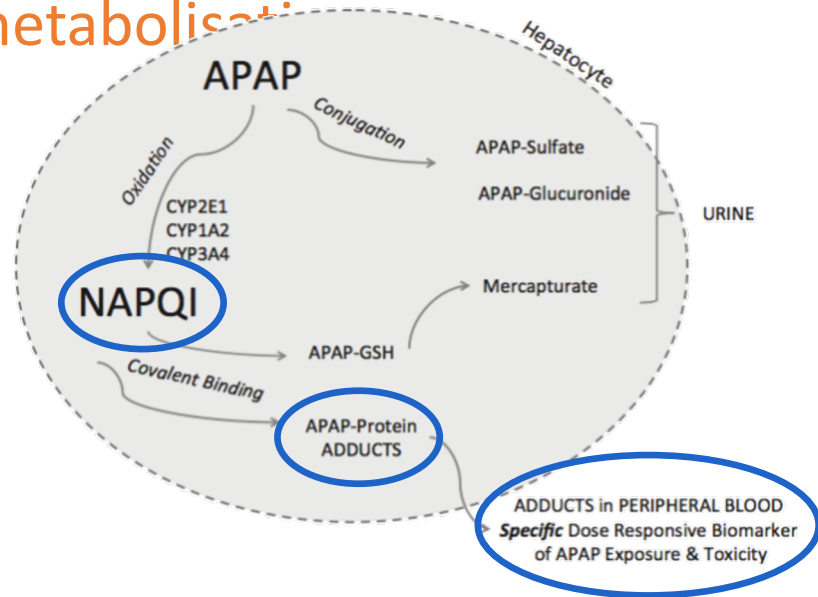
DETERMINATION OF PARACETAMOL-ALBUMIN ADDUCTS VIA LC-(HR)MS

Lisa Delahaye, Christophe Stove



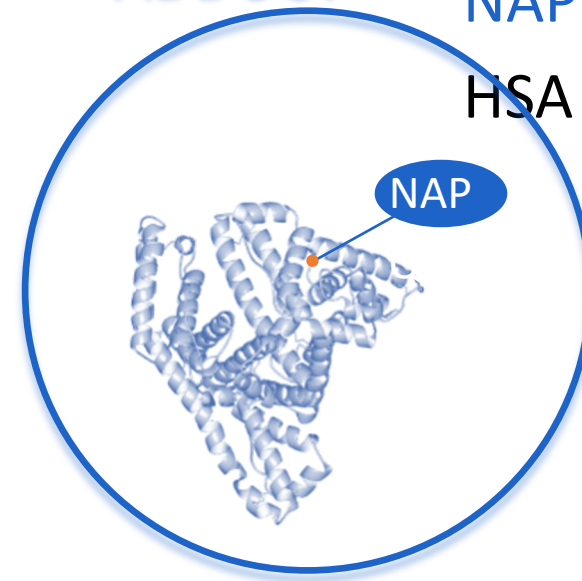
PARACETAMOL-ADDUCTS

Paracetamol (APAP)
metabolism



PROTEIN
ADDUCT

NAPQI bound to Cys³⁴ of
HSA



Biomarker **specific** for paracetamol

intoxication

Suitable for evaluation of **acute and chronic**

intoxication

Study and steer **paracetamol treatment** in patients with

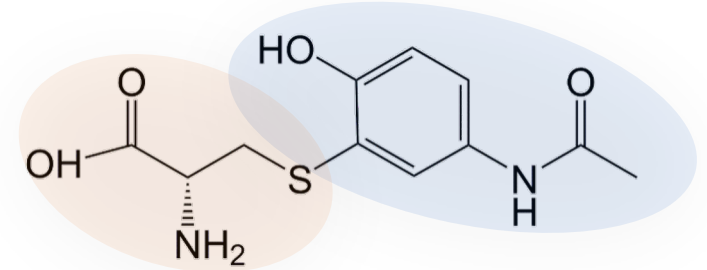
increased risk

BLOOD VS. PLASMA



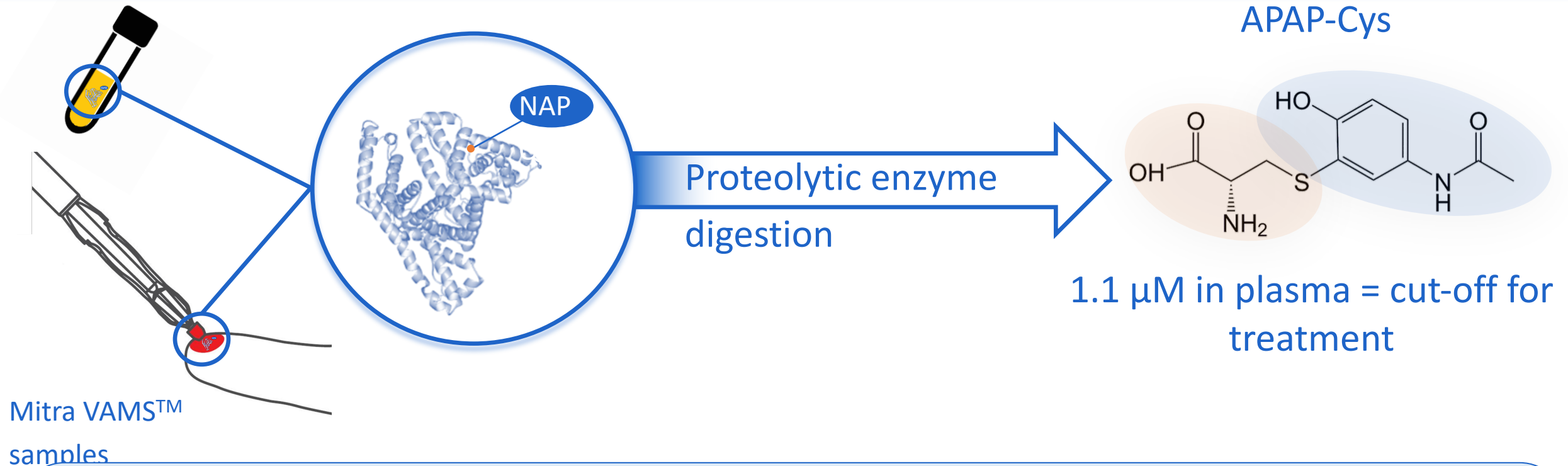
Proteolytic enzyme
digestion

APAP-Cys



1.1 μM in plasma = cut-off for
treatment

BLOOD VS. PLASMA

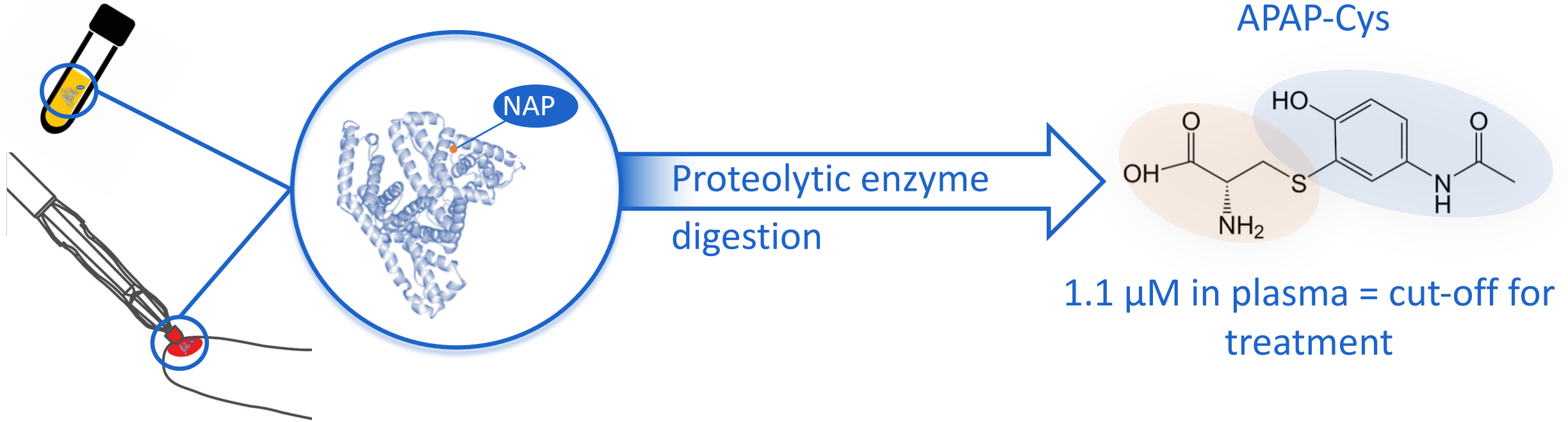


Validated methods: Protein derived APAP-Cys in blood VAMS and plasma VAMS samples

Application: Patient population: Pediatric ICU

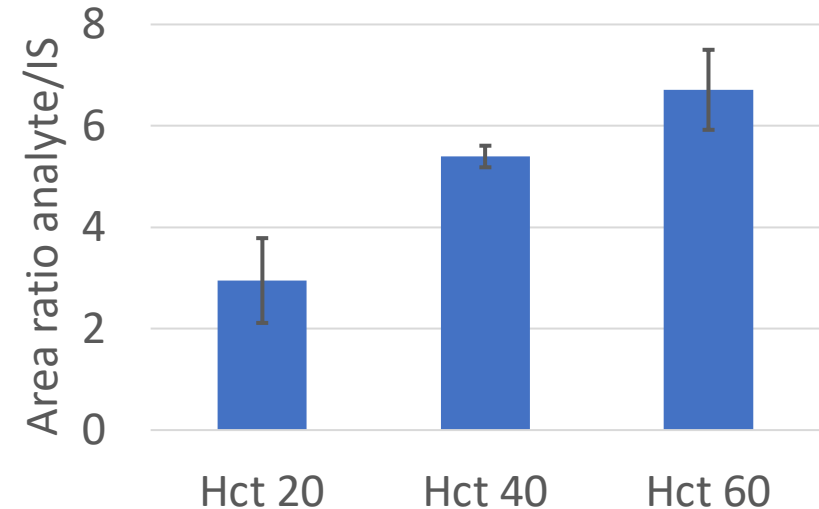
Therapeutic doses of APAP (IV)

BLOOD VS. PLASMA

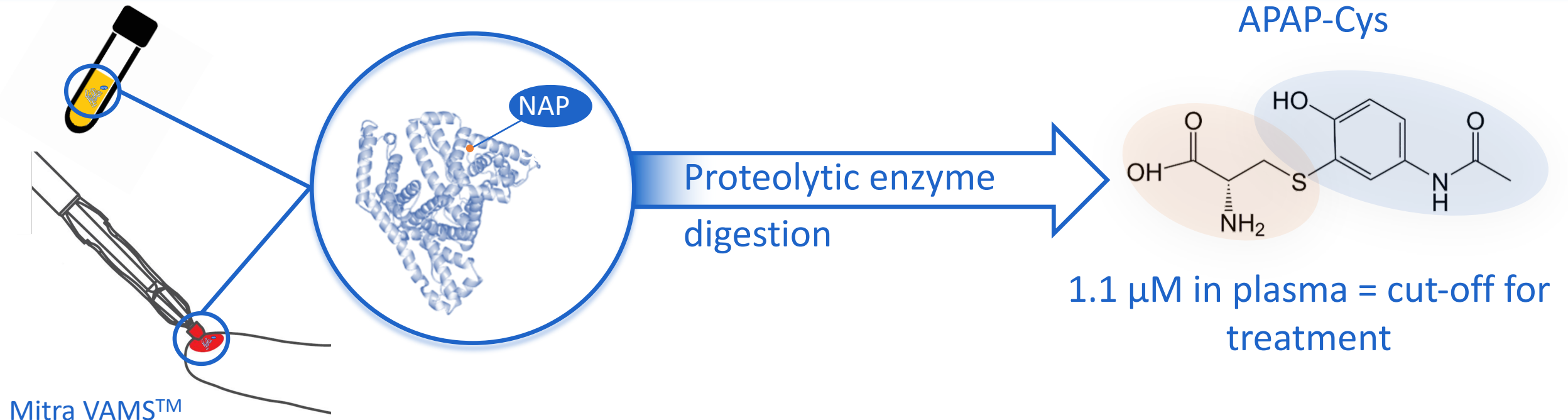


Spike paracetamol (80 $\mu\text{g}/\text{mL}$) to blank blood with different hematocrits (n=3)

Detection of APAP-Cys:
Increasing concentration with increasing Hct



BLOOD VS. PLASMA



Mitra VAMS™
samples

Validated methods: APAP-Cys in blood VAMS and plasma VAMS samples

Application: Patient population: Pediatric ICU

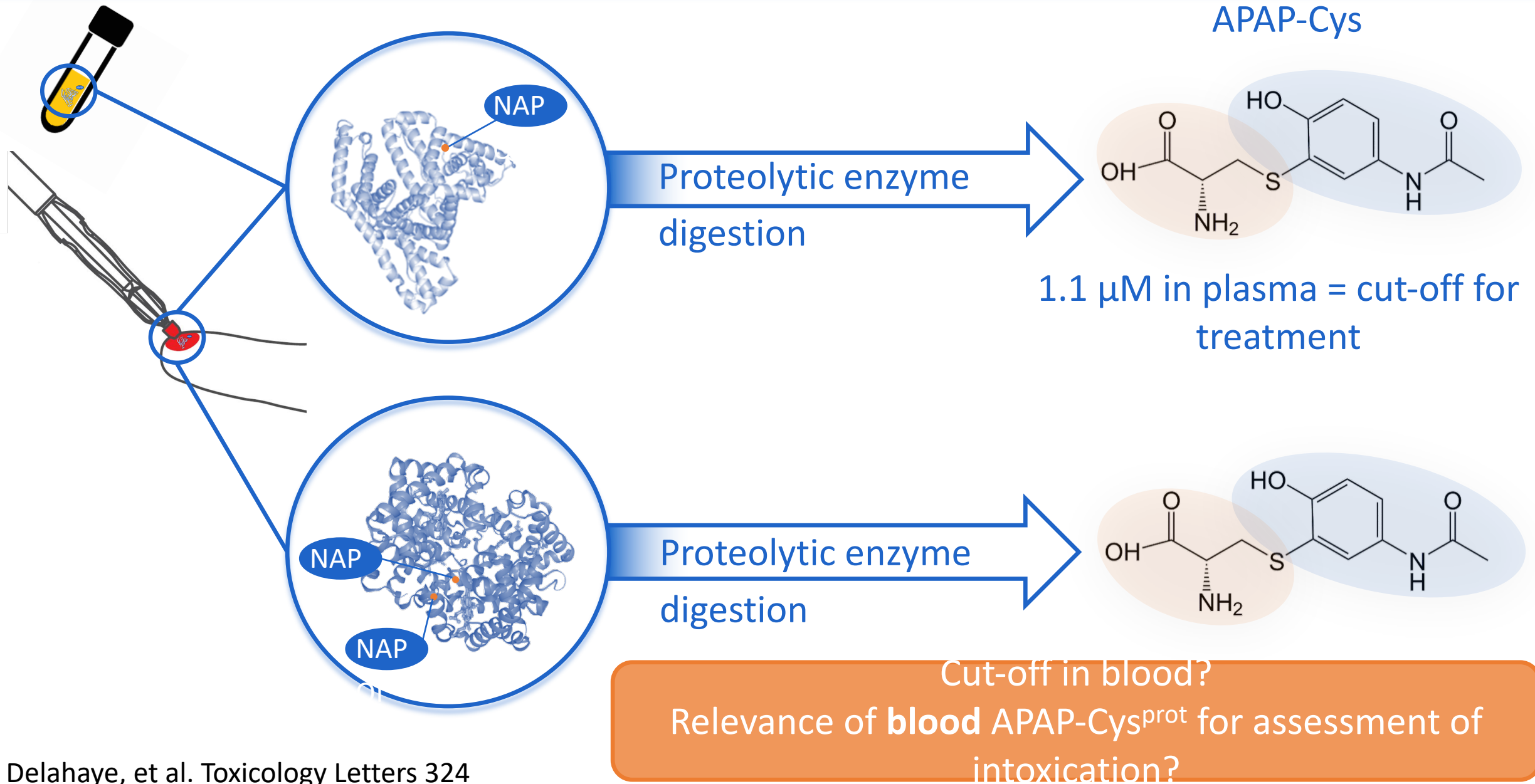
Therapeutic doses of APAP

Presence of APAP-Cys in blood spiked with APAP (no metabolisation)

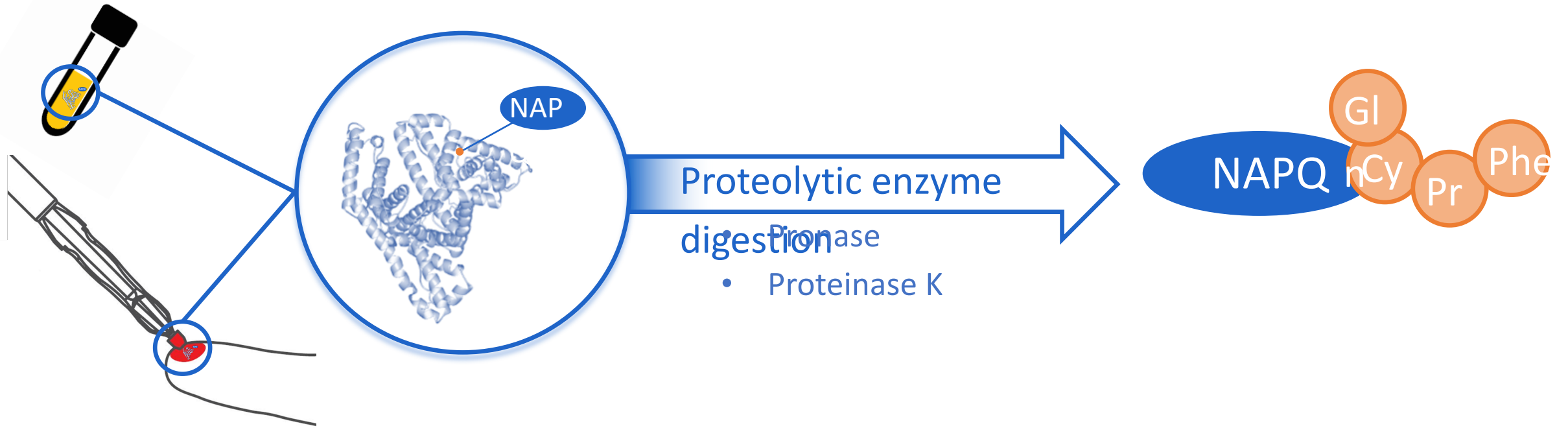
Concentration of some samples >

1.1 μM

BLOOD VS. PLASMA: INTERPRETATION?



ALTERNATIVE APPROACH



No interference from blood-specific NAPQI/APAP-protein-adducts ?

Increased selectivity for APAP-HSA?

ALTERNATIVE APPROACH



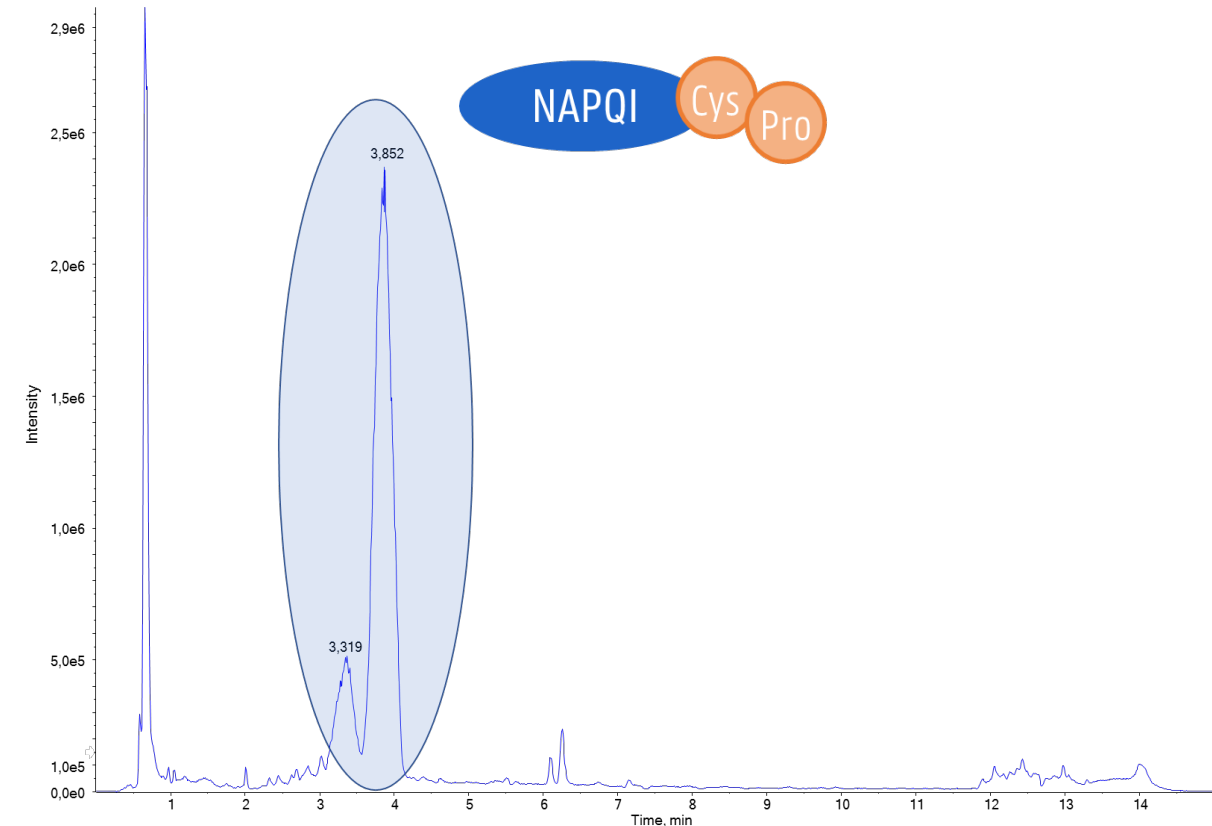
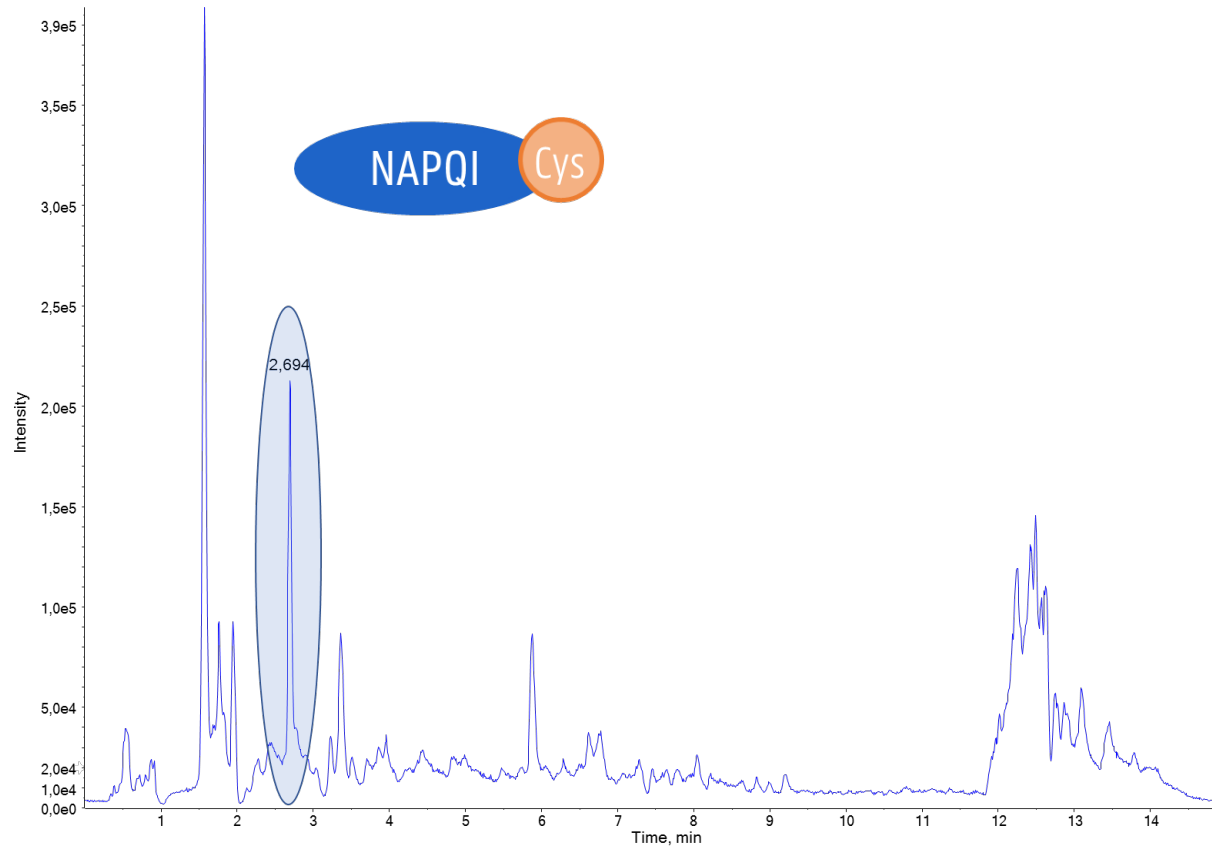
Identification of digestion products
in blood and plasma samples via LC-HRMS (SCIEX
TT 5600+)



IDENTIFICATION OF DIGESTION PRODUCTS

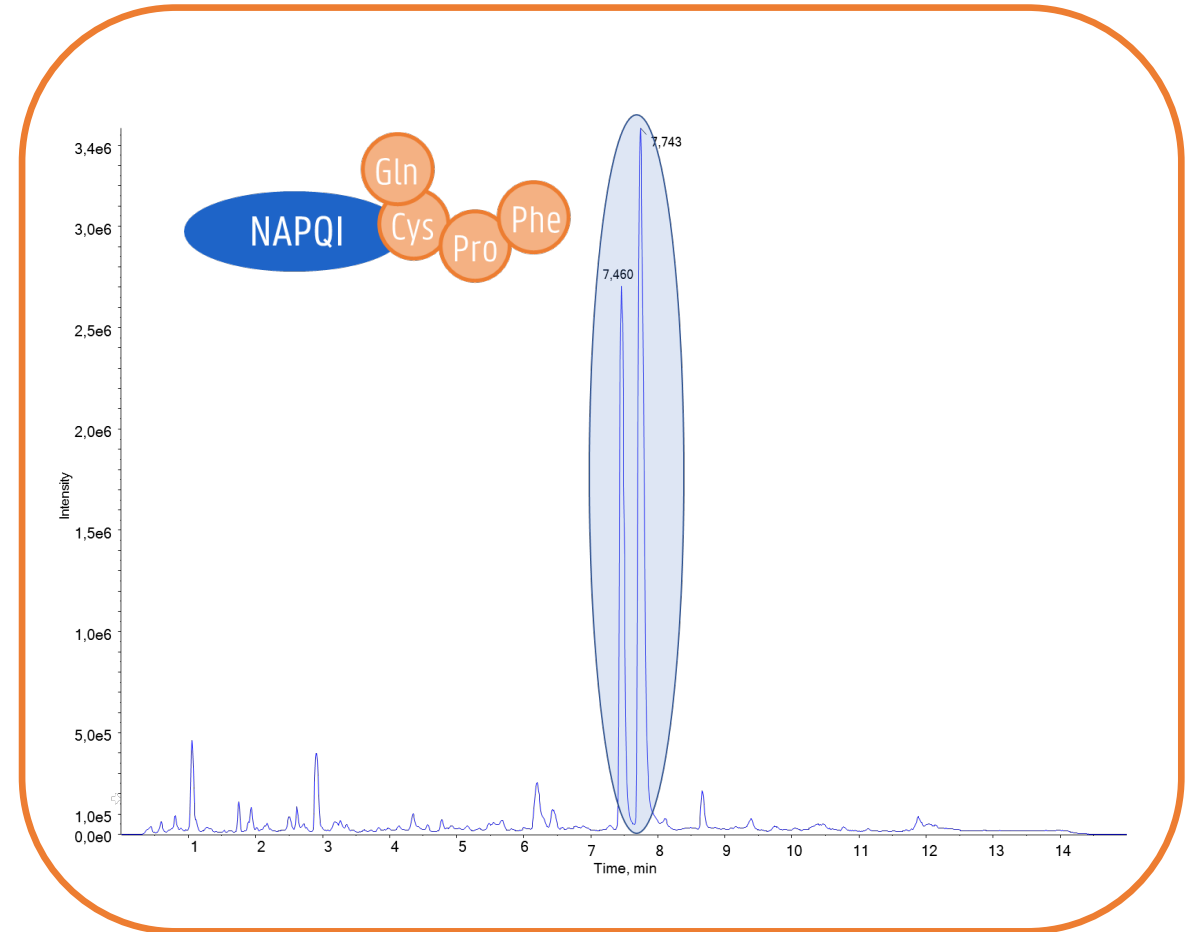
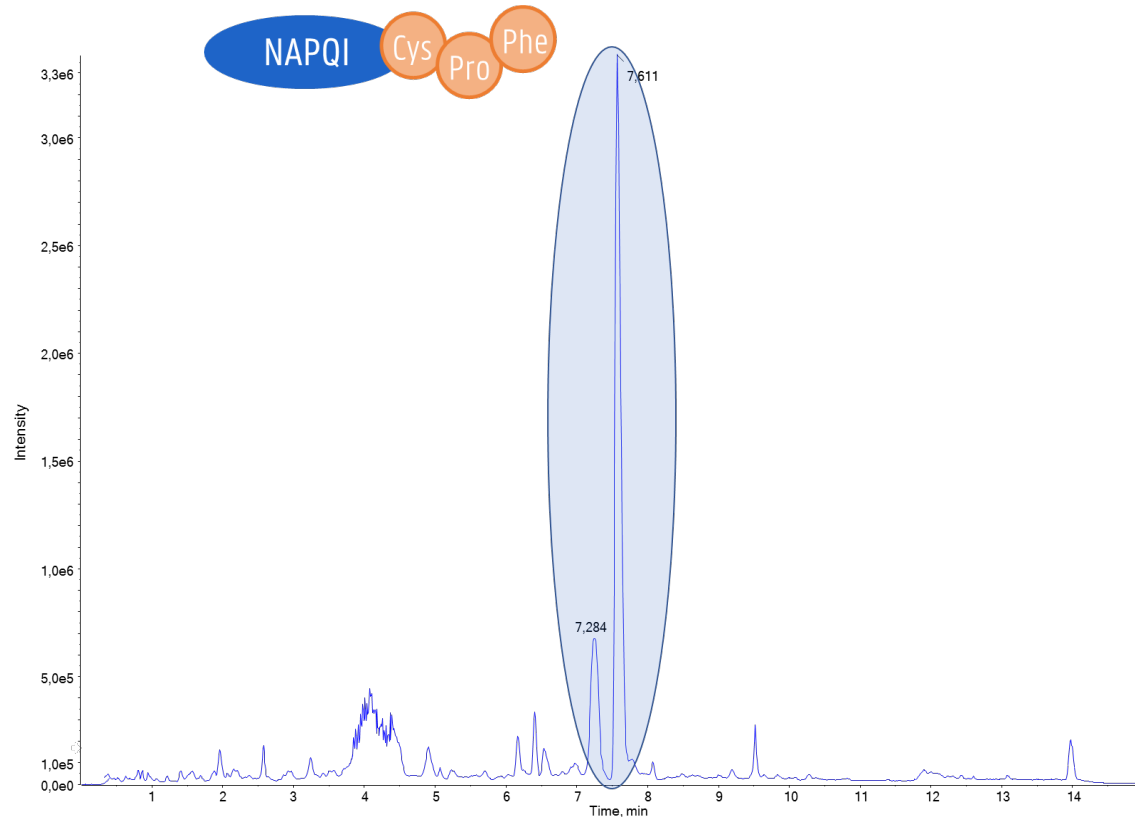
Digestion of native plasma VAMS sample with both enzymes:

1. Pronase: APAP-Cys and APAP-CP



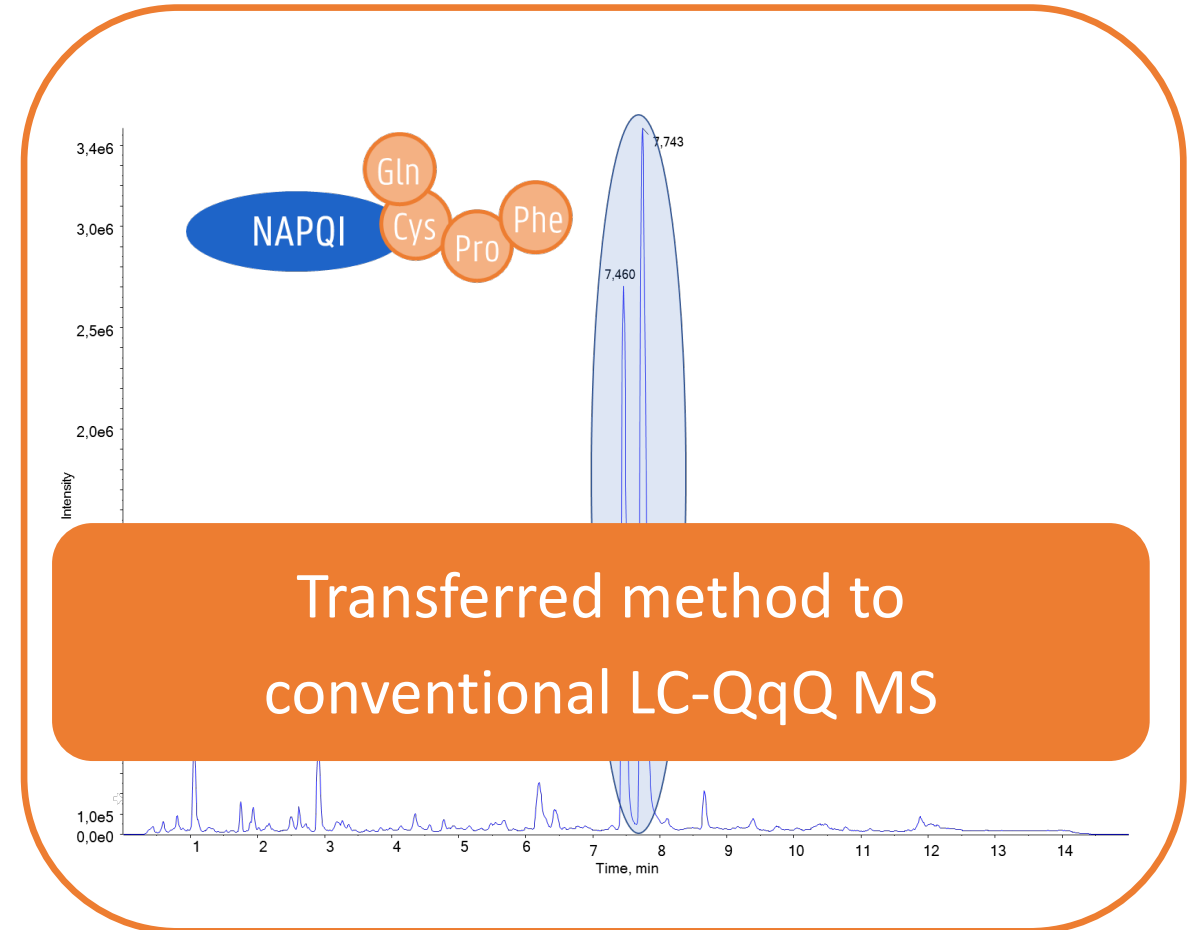
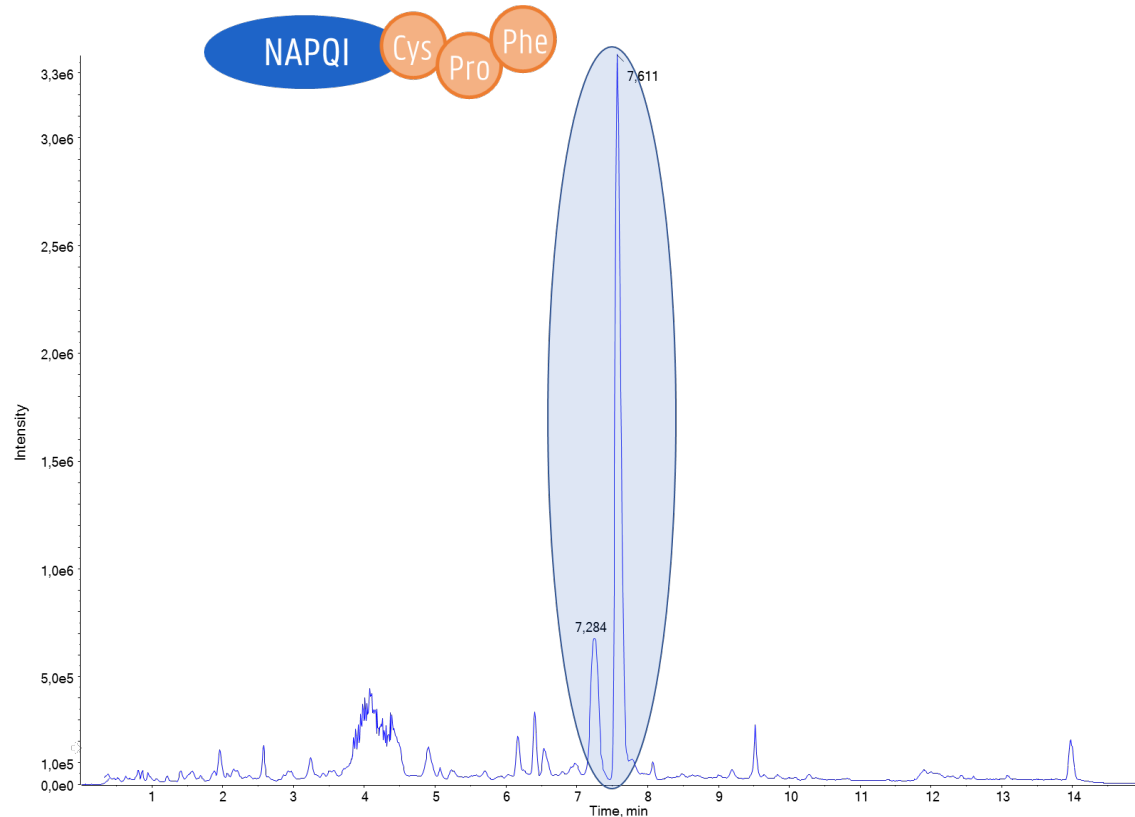
IDENTIFICATION OF DIGESTION PRODUCTS

Digestion of native plasma VAMS sample with both enzymes:
2. Proteinase K: APAP-CPF and APAP-QCPF



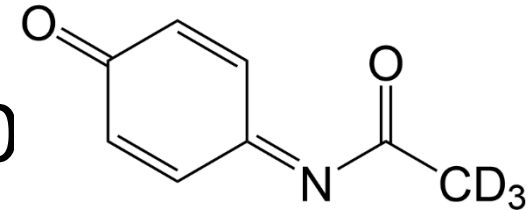
IDENTIFICATION OF DIGESTION PRODUCTS

Digestion of native plasma VAMS sample with both enzymes:
2. Proteinase K: APAP-CPF and APAP-QCPF



Generation of labeled NAPQI-HSA

d3-labeled NAPQI became available in 20



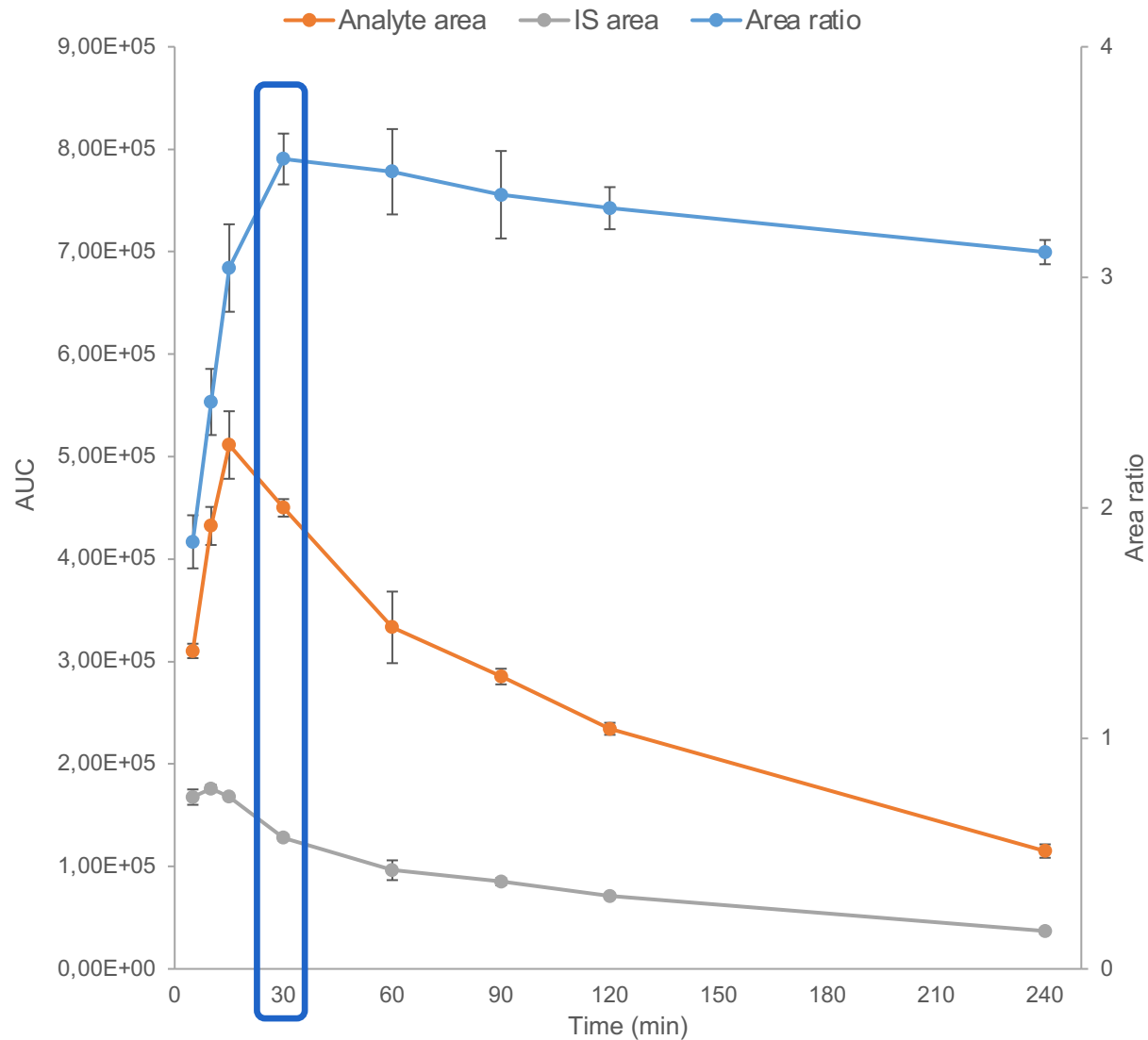
Incubation of d3-NAPQI with HSA → labeled IS

Add IS prior to digestion

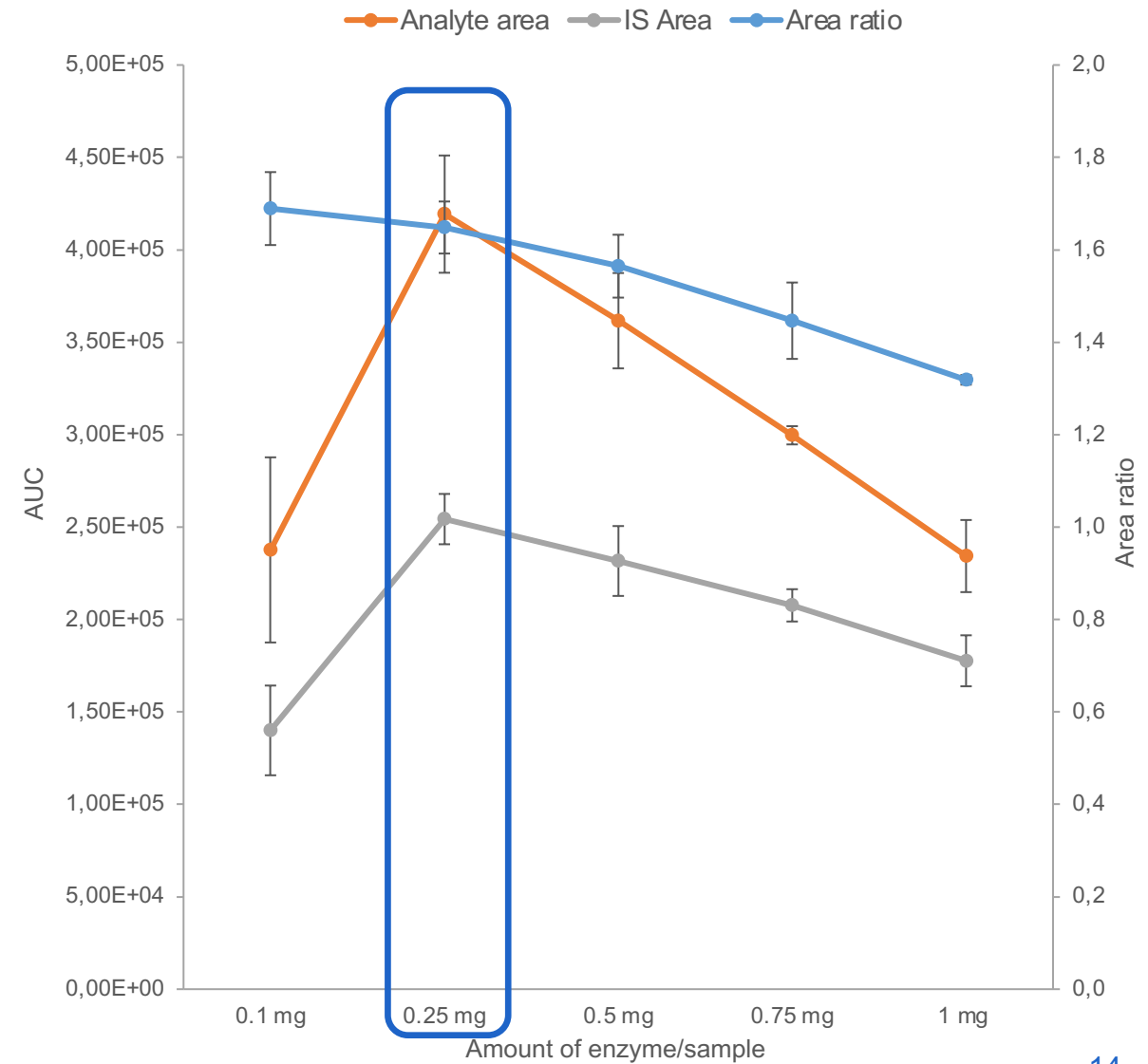
- Compensation for ME and analytical variation
- Compensation for digestion efficiency

OPTMIZATION OF THE SAMPLE PREPARATION PROCEDURE

Digestion time



Amount of enzyme

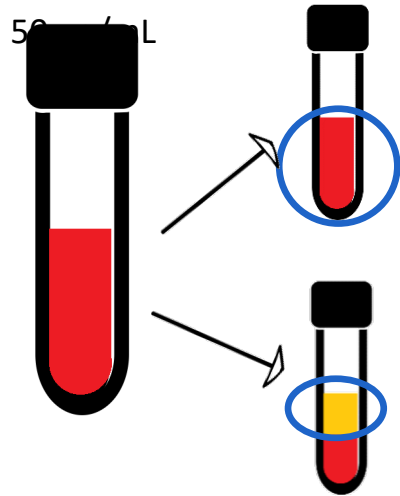


BLOOD VS PLASMA: ISSUE SOLVED?

Spike with

APAP

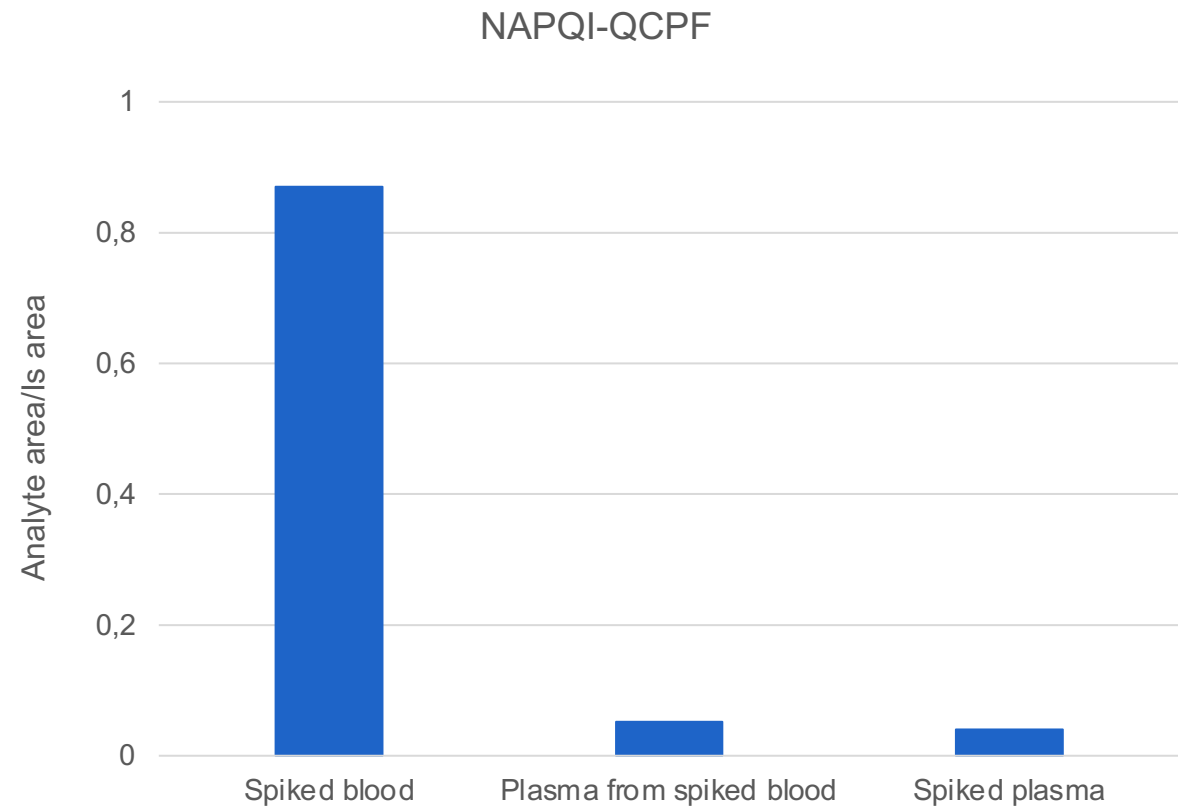
50 µL



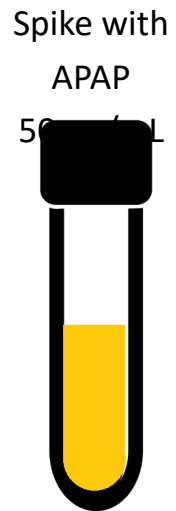
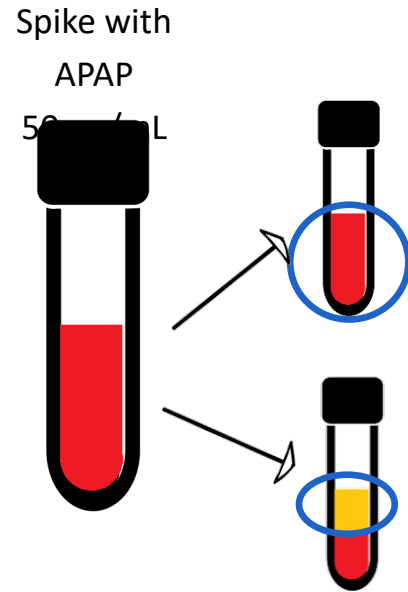
Spike with

APAP

50 µL



BLOOD VS PLASMA: ISSUE SOLVED?



? Still increased signals when spiking APAP to blood

→ Results confirmed with HRMS method

→ Hb does not contain QCPF AA-sequence

? Other protein in RBC fraction of blood which can bind NAPQI/APAP?

? Other cause for increased signal in spiked blood?

CONCLUSION & FUTURE PERSPECTIVES

Validation of the method for the determination of APAP-Cys in blood, using Mitra[®] devices, revealed:

Formation of additional protein adducts, which contribute to the signal of APAP-Cys, due to the presence of paracetamol in blood

Therefore, we:

Identified **alternative digestion products** using HRMS, aiming to develop a more specific method;

Made use of another proteolytic enzyme, **proteinase K**, generating **NAPQI-QCPF**;

Optimized the digestion process, to increase the sensitivity of the method;

Included a **SIL internal standard**. generated via incubation of HSA with labeled

Future perspectives:

Determine concentration in blood samples from patients taking therapeutic doses of APAP

Possibly switch to (capillary) plasma samples for NAPQI-HSA adduct

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