



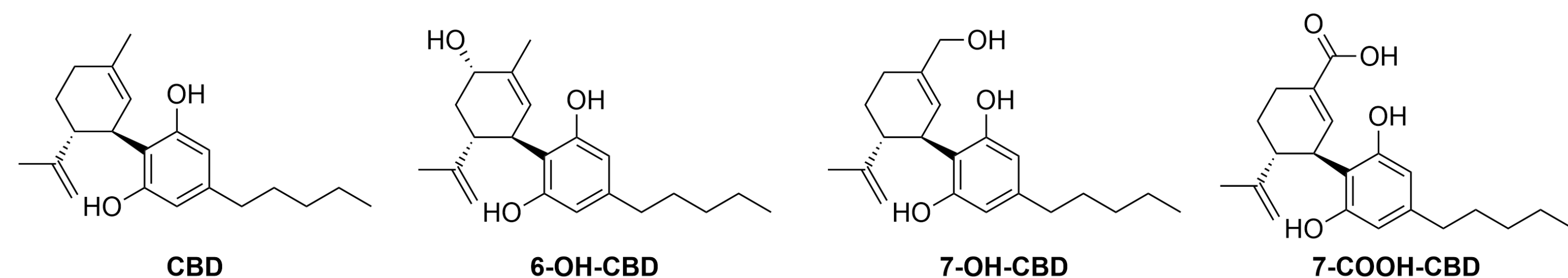
Analysis of cannabidiol and its 3 major metabolites in human plasma by online extraction LC-MS/MS

N. Pluym, R. Kanis, K. Riedel, T. Burkhardt, G. Scherer, M. Scherer
ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany

www.abf-lab.com | @: nikola.pluym@abf-lab.com

Introduction

The main active ingredients of *Cannabis sativa* are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). CBD as the main non-psychoactive constituent has been marketed in a growing number of products for recreational use such as CBD oils and vapes. Pharmacokinetics (PK) studies are needed to determine the absorption, distribution and metabolism of CBD after administration, which is largely affected by the formulation of the product in analogy to nicotine-containing products. This prompted us to develop a quantitative bioanalytical method for the determination of CBD and its three major metabolites 6-hydroxy-CBD (6-OH-CBD), 7-hydroxy-CBD (7-OH-CBD), and 7-carboxy-CBD (7-COOH-CBD) – in plasma by online extraction LC-MS/MS.



Structures of CBD and its 3 major metabolites

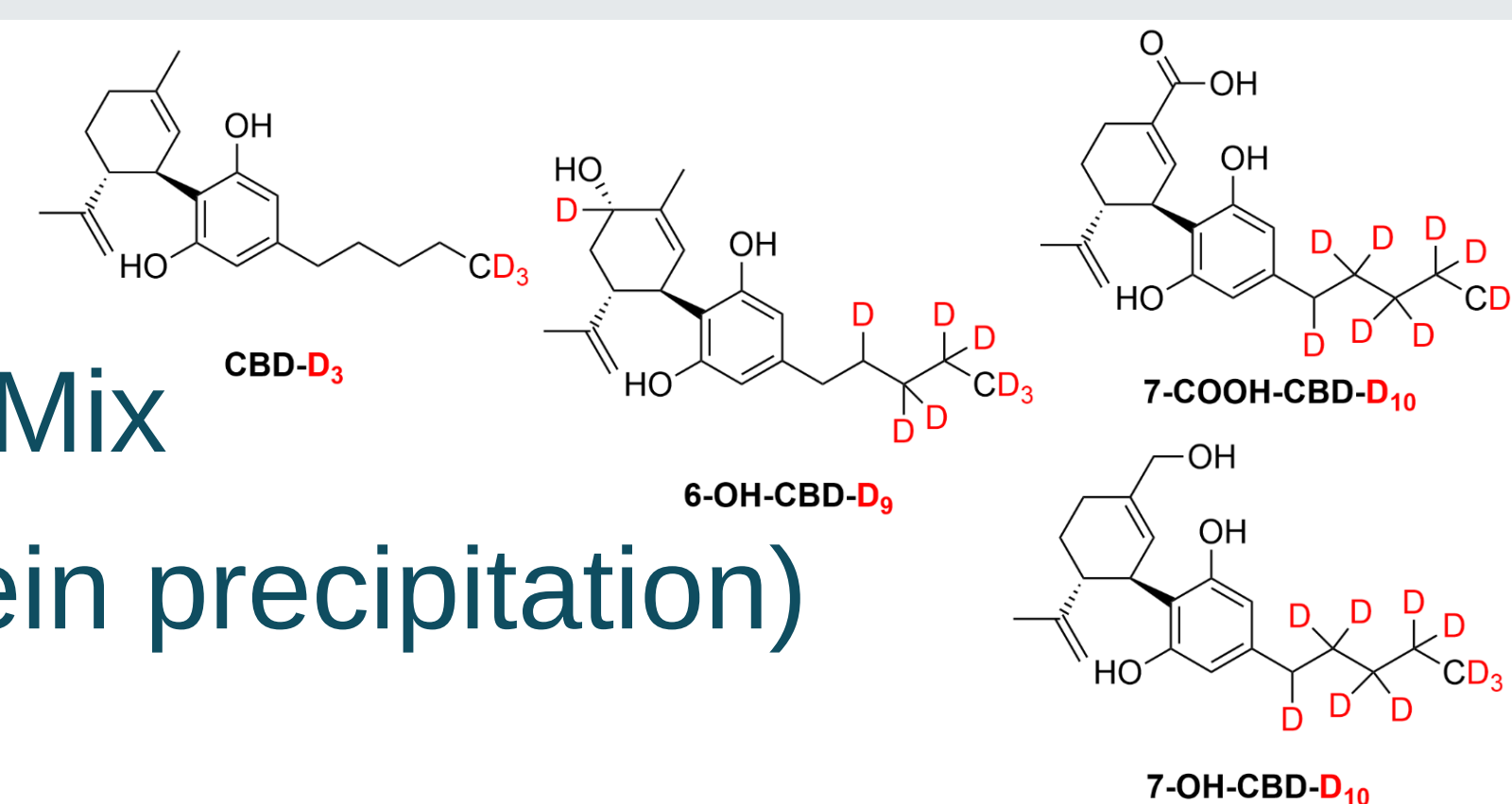
Analytical Method Scheme



200 μ L plasma

20 μ L Internal Std Mix

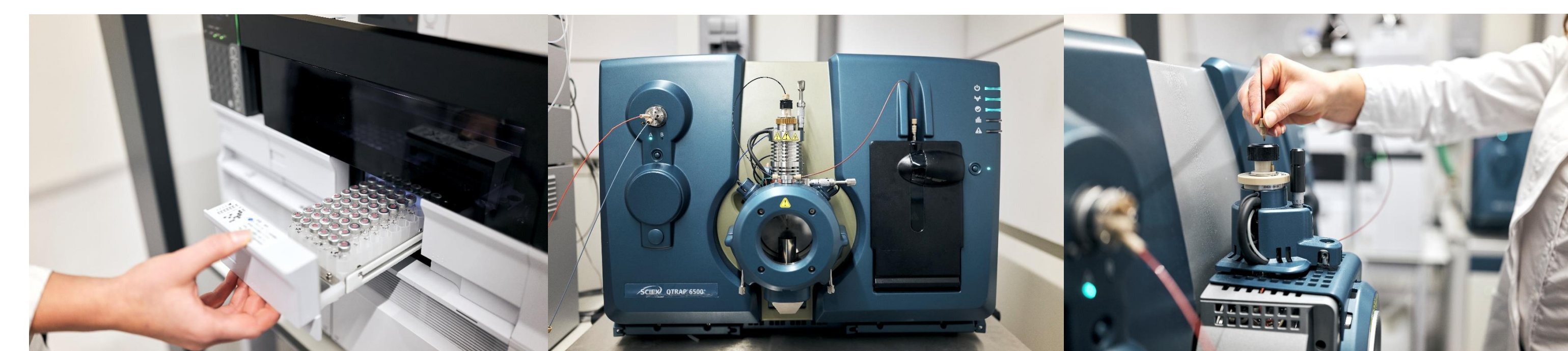
0.2M ZnSO₄ (Protein precipitation)



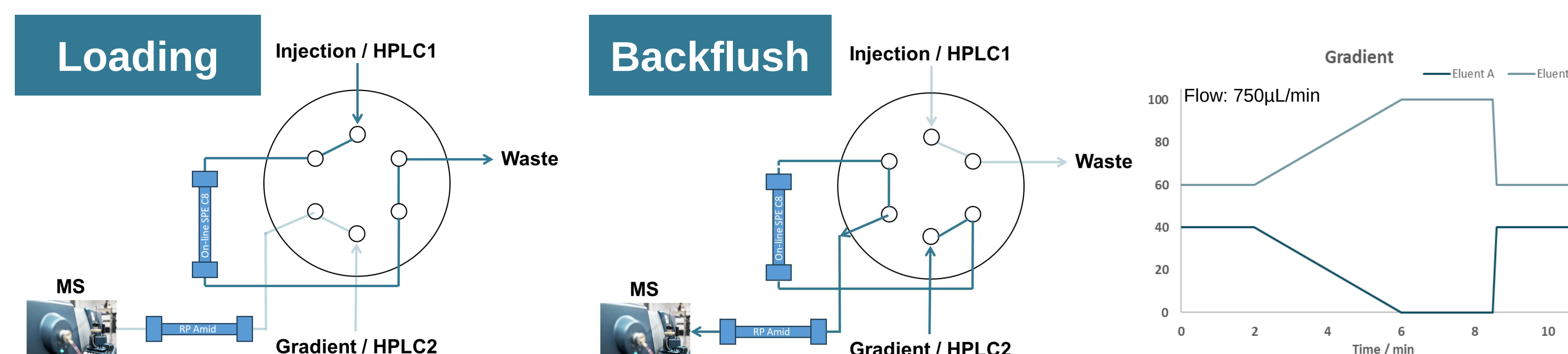
Centrifugation



Sample Analysis by LC-MS/MS



Online-SPE-LC-MS/MS Analysis

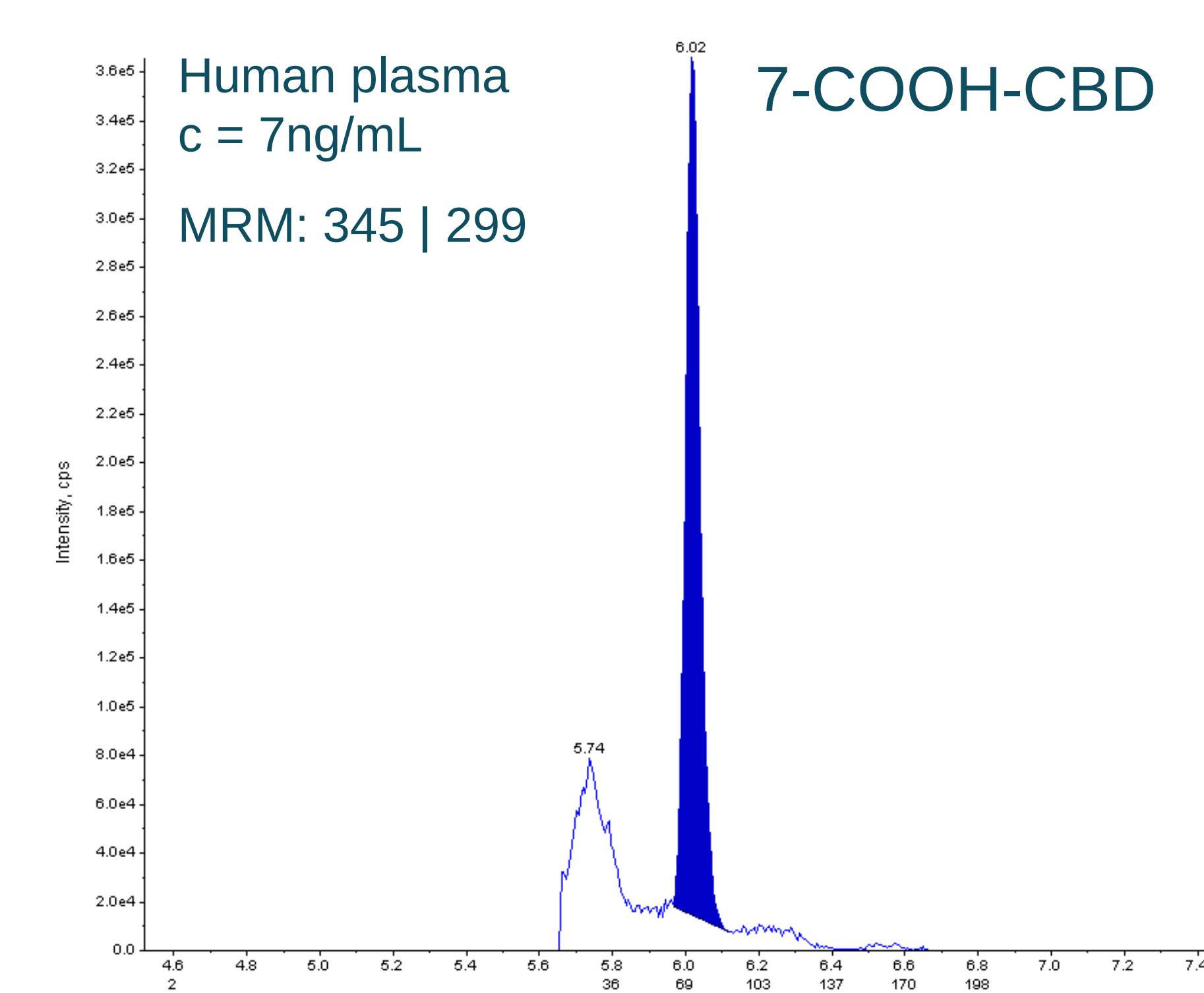
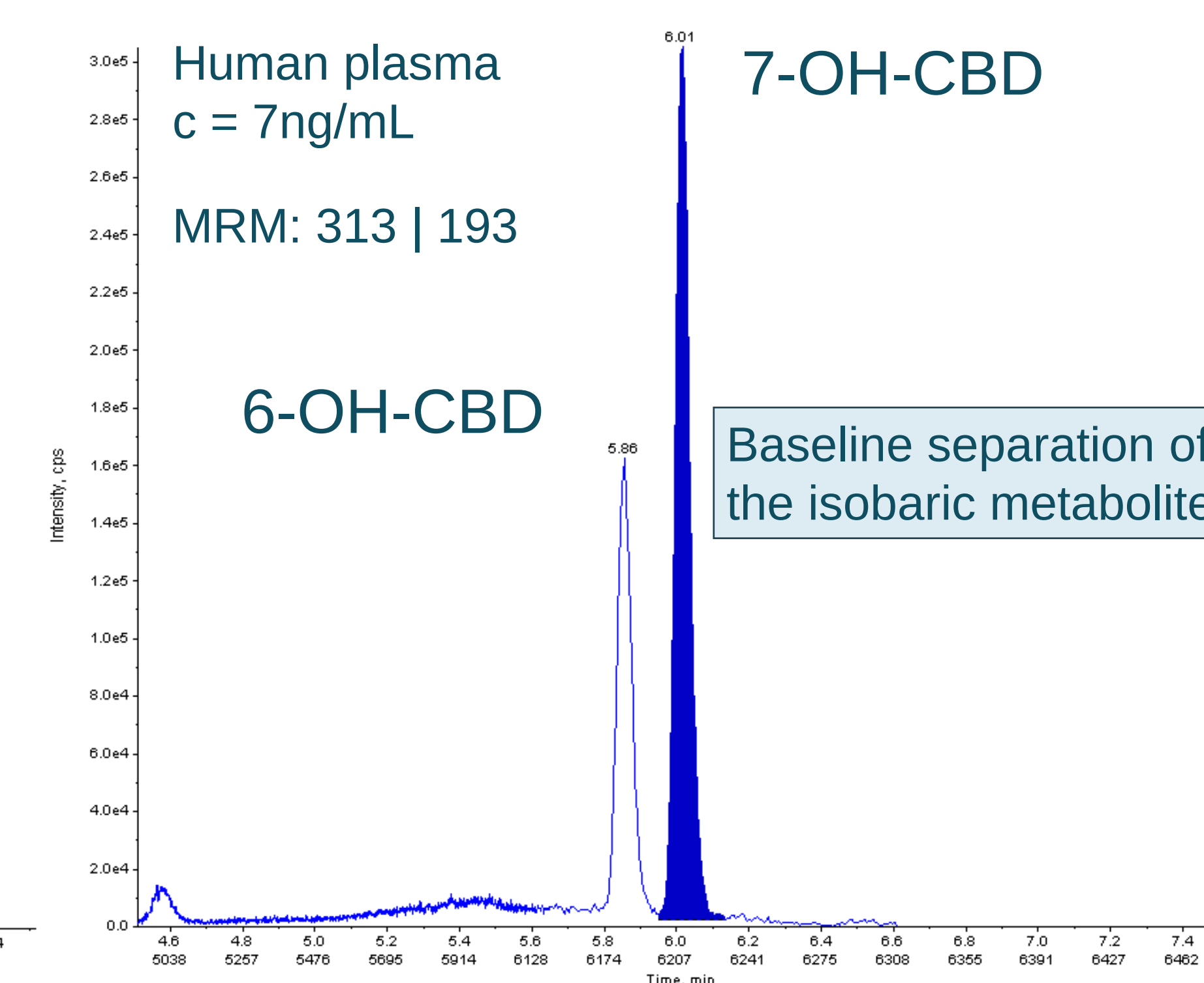
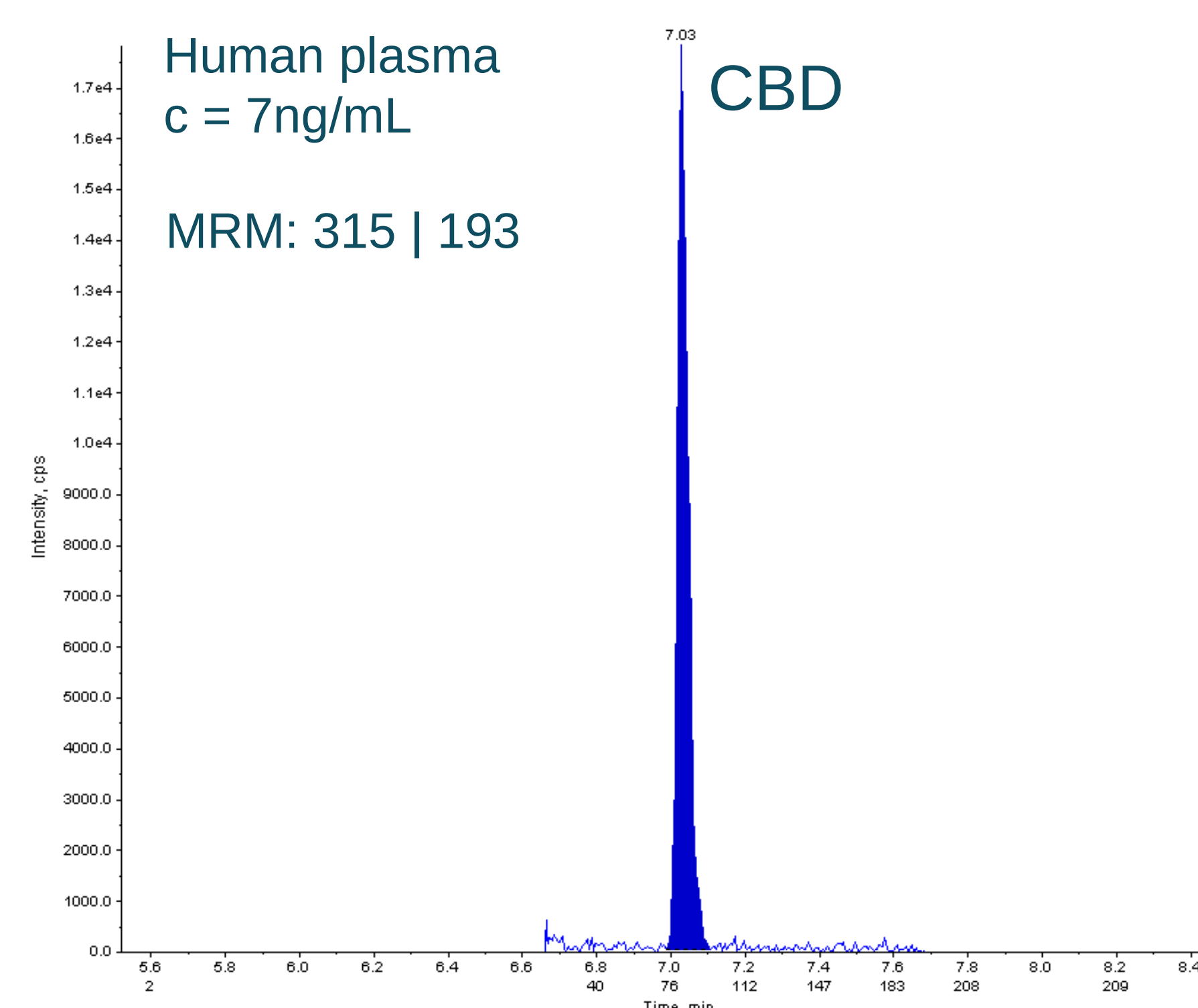


- Online SPE: Zorbax C8 (4.6x12.5 mm; 5 μ m)
- LC column: RP-Amid (3.0x150 mm, 2.7 μ m)
- Injection volume: 50 μ L
- Column temperature: 60°C
- Gradient flow: 750 μ L/min
- Overall runtime: 11min
- Easy sample clean-up by protein precipitation
- Online-SPE purification for high-throughput

Method Validation Results

Analyte	Precision / %CV	Accuracy / %	LLOQ / ng/mL	Range / ng/mL
CBD	4.6-6.8	104-110	0.39	0.39-200
6-OH-CBD	4.2-9.3	90.5-102	0.39	0.39-200
7-OH-CBD	2.3-5.7	104-108	0.39	0.39-200
7-COOH-CBD	4.1-11.4	100-104	0.39	0.39-200

- Full method validation according to ICH M10 proved accuracy, precision and sensitivity of the method
- Linear range: 0.39 – 200 ng/mL for each analyte suited for pharmacokinetic studies



Summary & Conclusion

- Method fully validated according to the new, harmonized ICH M10 guideline
- Excellent separation of the isobaric compounds 6-OH-CBD / 7-OH-CBD
- Minimum sample clean up for high throughput with 1000+ plasma samples per week
- High sensitivity, broad range, fast turnaround
- New LC-MS/MS method well suited for PK / TK analysis and exposure assessment of CBD + 3 major metabolites in plasma

