



Analysis of 11 endocannabinoids in human plasma using column switching high-performance liquid chromatography–mass spectrometry

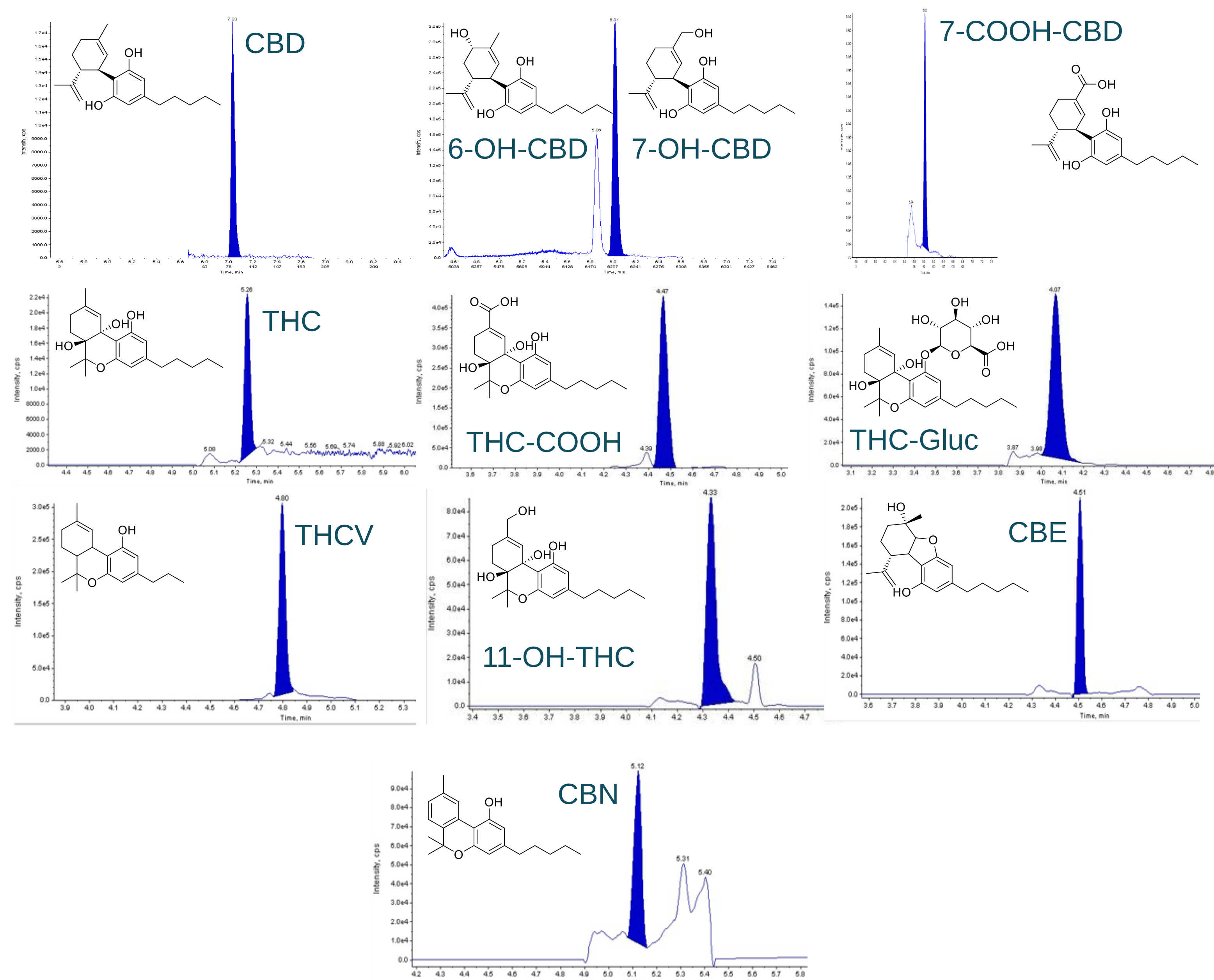
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Introduction

The endocannabinoid system is an intricate cellular signalling network. In response to the increasing demand for analytical methods targeting endocannabinoid levels from different legalized product categories across the globe, we developed and validated an assay for the quantification of 11 endocannabinoids and their metabolites including CBD, 6-OH-CBD, 7-OH-CBD, 7-COOH-CBD, CBN, CBE as well as delta-9-THC, trans-11-OH-THC, THC-COOH, THC-Gluc, THCV. We employed a straightforward extraction method utilizing protein precipitation with ZnSO_4 , followed by online-trapping high-performance liquid chromatography–tandem mass spectrometry (LC/LC-MS/MS) to monitor the levels in plasma. 200 μL of plasma are precipitated with ZnSO_4 in methanol and subsequently injected onto the LC-MS/MS system (Shimadzu Nexera X2 UHPLC coupled to Sciex QTrap6500+ MS/MS). Online extraction is achieved by means of a Zorbax C8 column (Agilent) followed by gradient elution on an Amid UHPLC column (Merck). The method was validated according to ICH M10 guidelines, assessing intra-run and inter-run accuracies and imprecisions, as well as matrix effects, recoveries, and sample stabilities. The LLOQ of 0.39 ng/mL and broad linear range of up to 200 ng/mL in combination with the fast sample preparation demonstrate the suitability of this method for its application in PK studies.

Chromatography



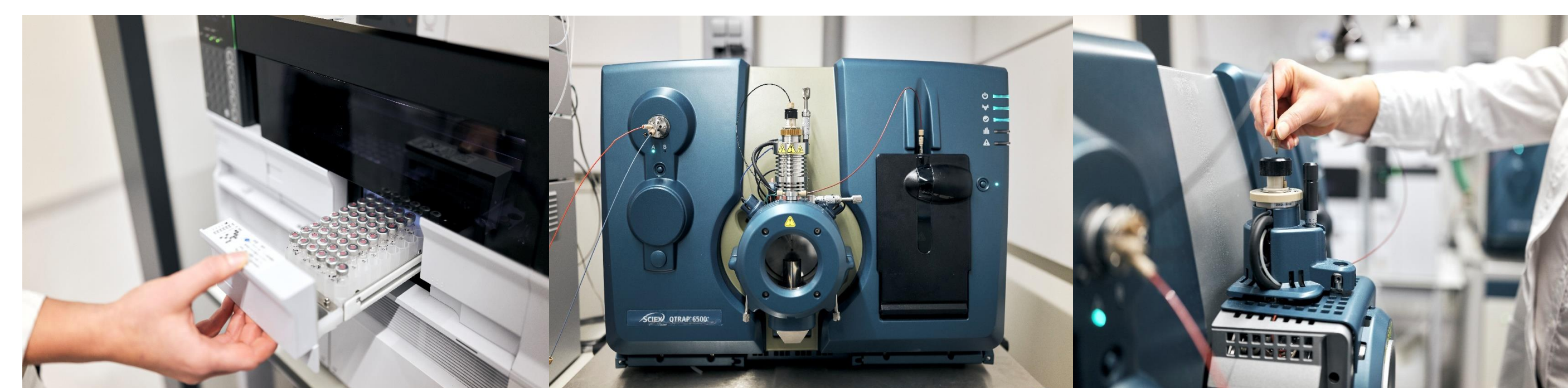
Analytical Method Scheme

50 μL plasma
20 μL Internal Std Mix
0.2M ZnSO_4 (Protein precipitation)

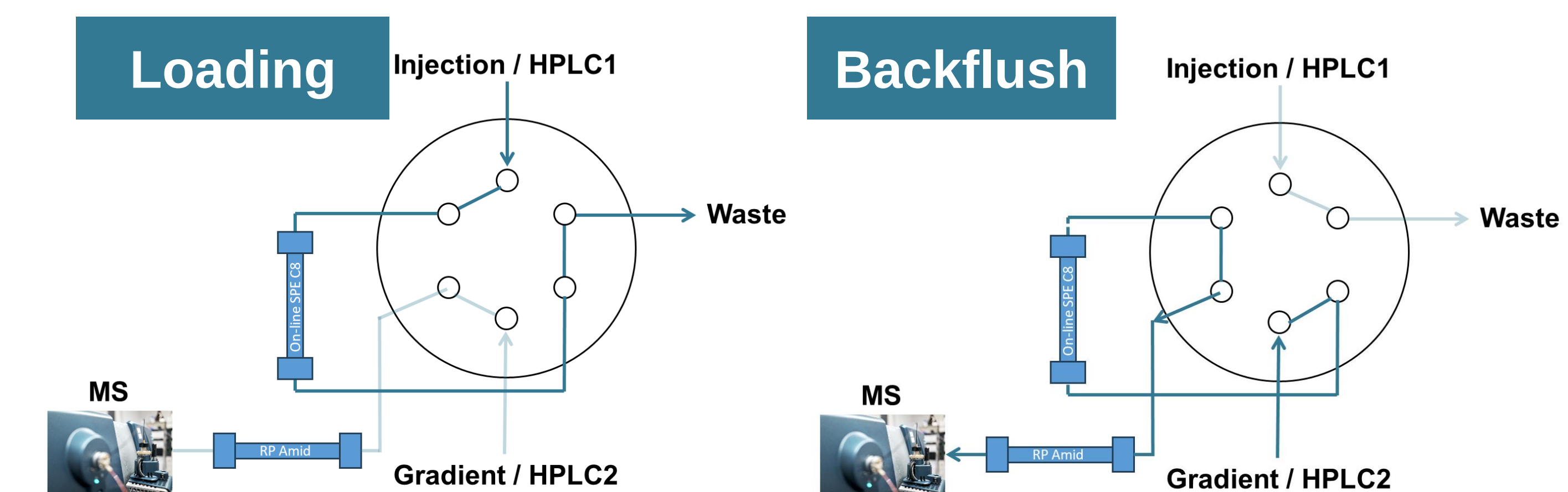
Centrifugation



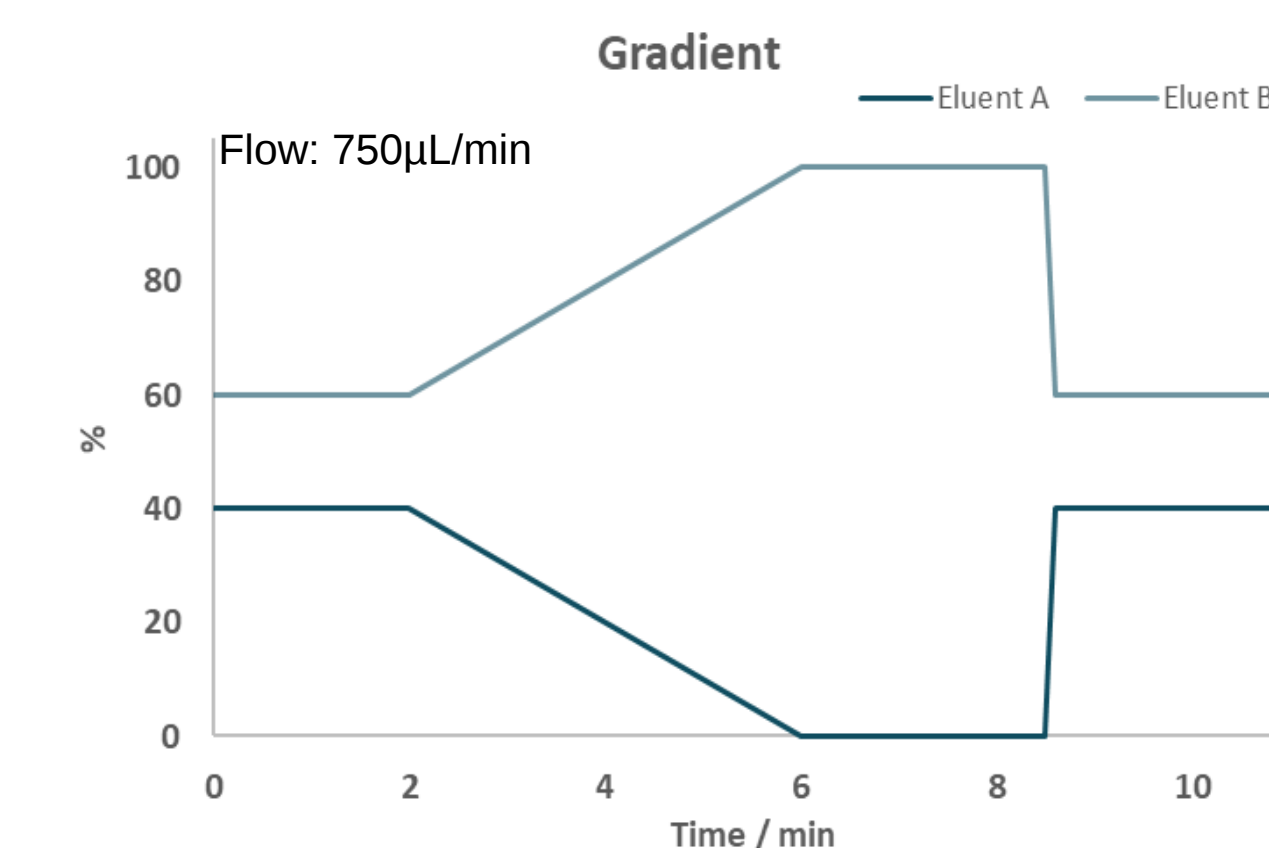
Sample Analysis by LC-MS/MS



Online-SPE-LC-MS/MS Analysis



- Online SPE: Zorbax C8 (4.6x12.5 mm; 5 μm)
- LC column: RP-Amid (3.0x150 mm, 2.7 μm)
- Injection volume: 50 μL
- Column temperature: 60°C
- Gradient flow: 750 $\mu\text{L}/\text{min}$
- 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	5-7	104-110	0.39	0.39-200
6-OH-CBD	4-9	90.5-102	0.39	0.39-200
7-OH-CBD	2-6	104-108	0.39	0.39-200
7-COOH-CBD	4-11	100-104	0.39	0.39-200
THC	1-4	100-107	0.39	0.39-200
11-OH-THC	1-3	91-105	0.39	0.39-200
THC-COOH	1-5	102-107	0.39	0.39-200
THC-COOH-Gluc	1-3	98-111	2.00	2.00-200
THCV	3-10	99-113	0.39	0.39-200
CBN	1-5	92-107	0.39	0.39-200
CBE	3-5	89-102	0.39	0.39-200

Summary & Conclusion

- Method fully validated according to the new, harmonized ICH M10 guideline
- Excellent separation of the isobaric compounds
- Automated 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 analysis and exposure assessment of CBD and THC including major metabolites in plasma