



Analysis of caffeine in human plasma by LC-MS/MS

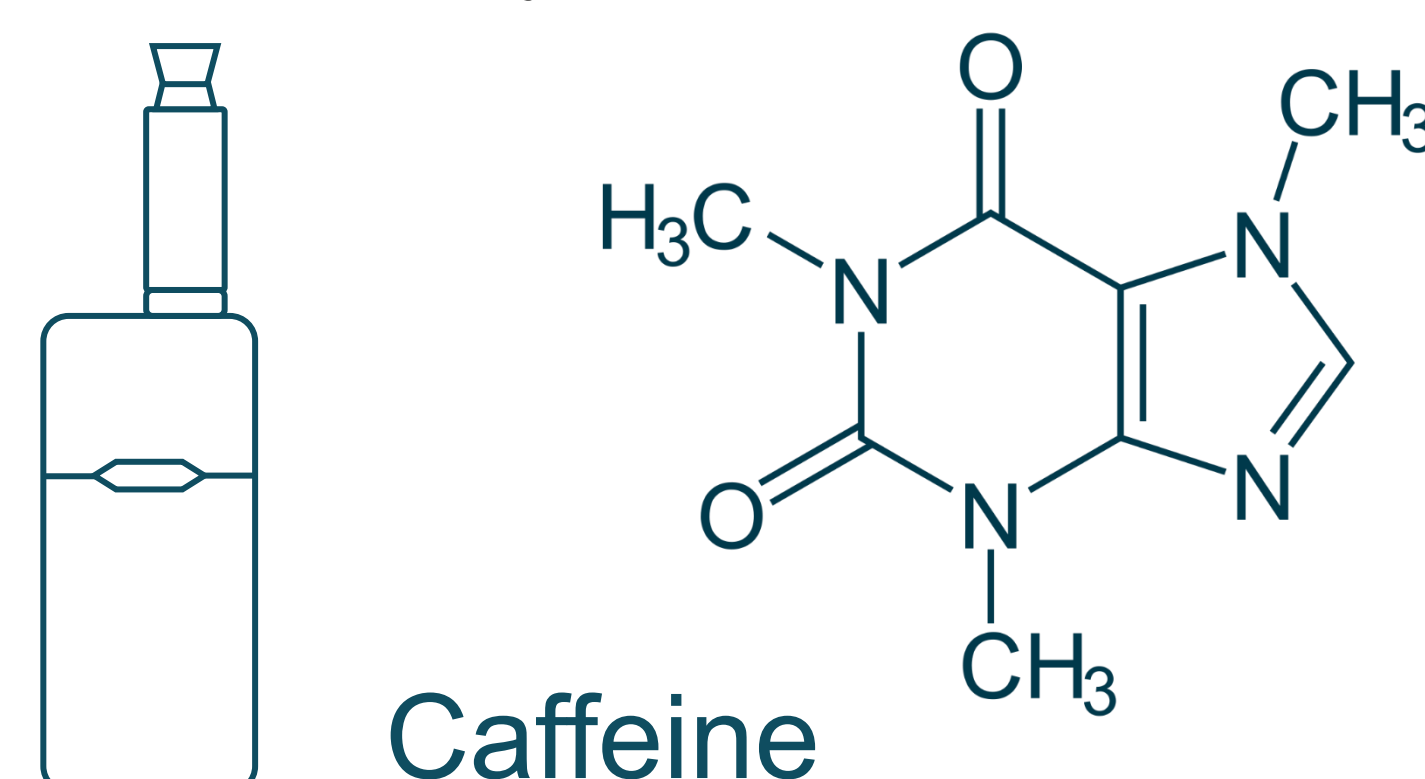
Max Scherer, Johanna Lehocky, Nikola Pluym

ABF Analytisch-Biologisches Forschungslabor GmbH, Semmelweisstr. 5, 82152 Planegg, Germany

www.abf-lab.com | @: max.scherer@abf-lab.com

Introduction

Caffeine is the most widely consumed psychostimulant globally, commonly found in tea, coffee, cocoa, energy drinks, and various food products. More recently, caffeine-containing e-cigarettes have entered the market; however, no comprehensive risk assessment of these products has yet been conducted. A crucial step in evaluating the physiological effects and abuse liability of caffeine, taken in via this new route of administration, is understanding its absorption, which requires a sensitive and reliable analytical method for the quantification in human plasma. We have developed a liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method to enable the sensitive quantification of caffeine in human plasma.



Analytical Method



Centrifugation
Evaporation
Reconstitution

50 µL Plasma
50 µL Internal Standard
50 µL Methanol



Sample Analysis by LC-MS/MS

Method Validation

Complete method validation according to **Bioanalytical Method Validation and Study Sample Analysis M10**



LLOQ

5 ng/mL



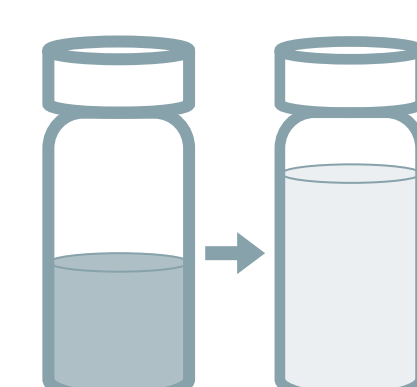
Accuracy

Intra-Day
93-103 %
LLOQ: 90-97 %
Inter-Day
96-99 %
LLOQ: 97 %



Recovery

Low: 104 %
Medium: 104 %
High: 97 %



Dilution Integrity

1:5 dilution
100-107 % (CV 5-9 %)



Stability

Short-term (24 h, 21 °C): Low 113 %, High 98 %
Freeze-Thaw (6 cycles): Low 100 %, High 99 %
Post preparative (10 °C, 11 days): Low 107 %, High 96 %



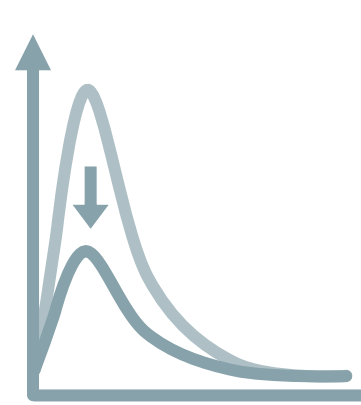
Linear Range

5 – 7500 ng/mL



Precision

Intra-Day
2-10 %
LLOQ: 3-14 %
Inter-Day
3-8 %
LLOQ: 18%



Matrix Effect

Accuracy
Low: 108-119 %
High: 104-107 %
Signal Recovery
Analyte: 98-102 %
IS: 87-93 %

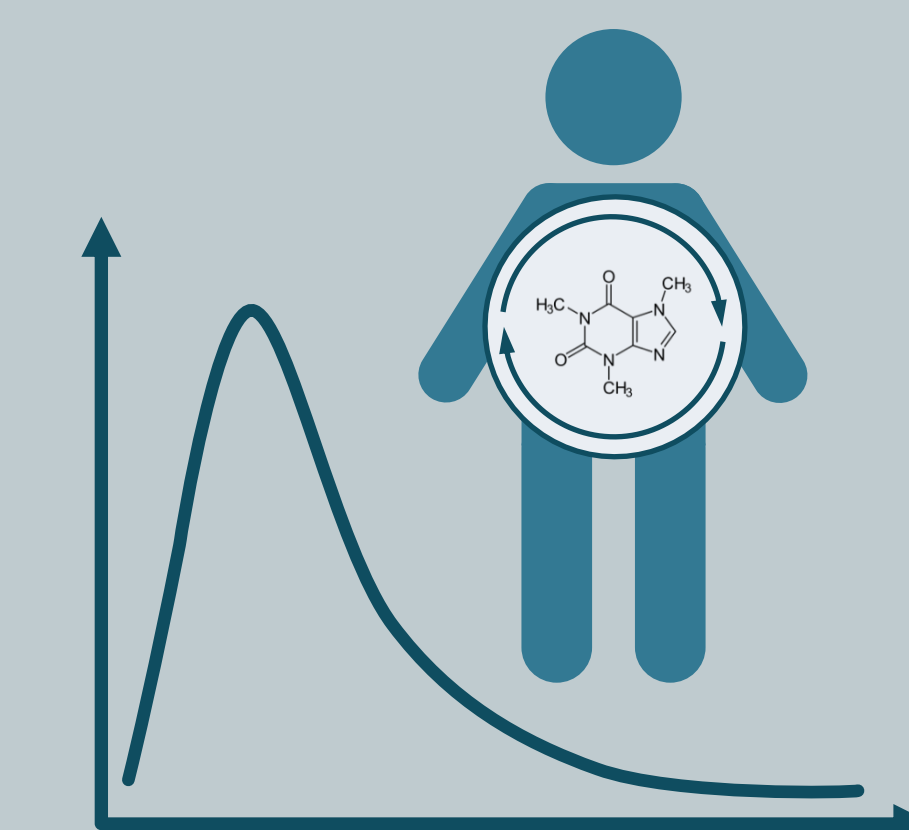


Re-Injection

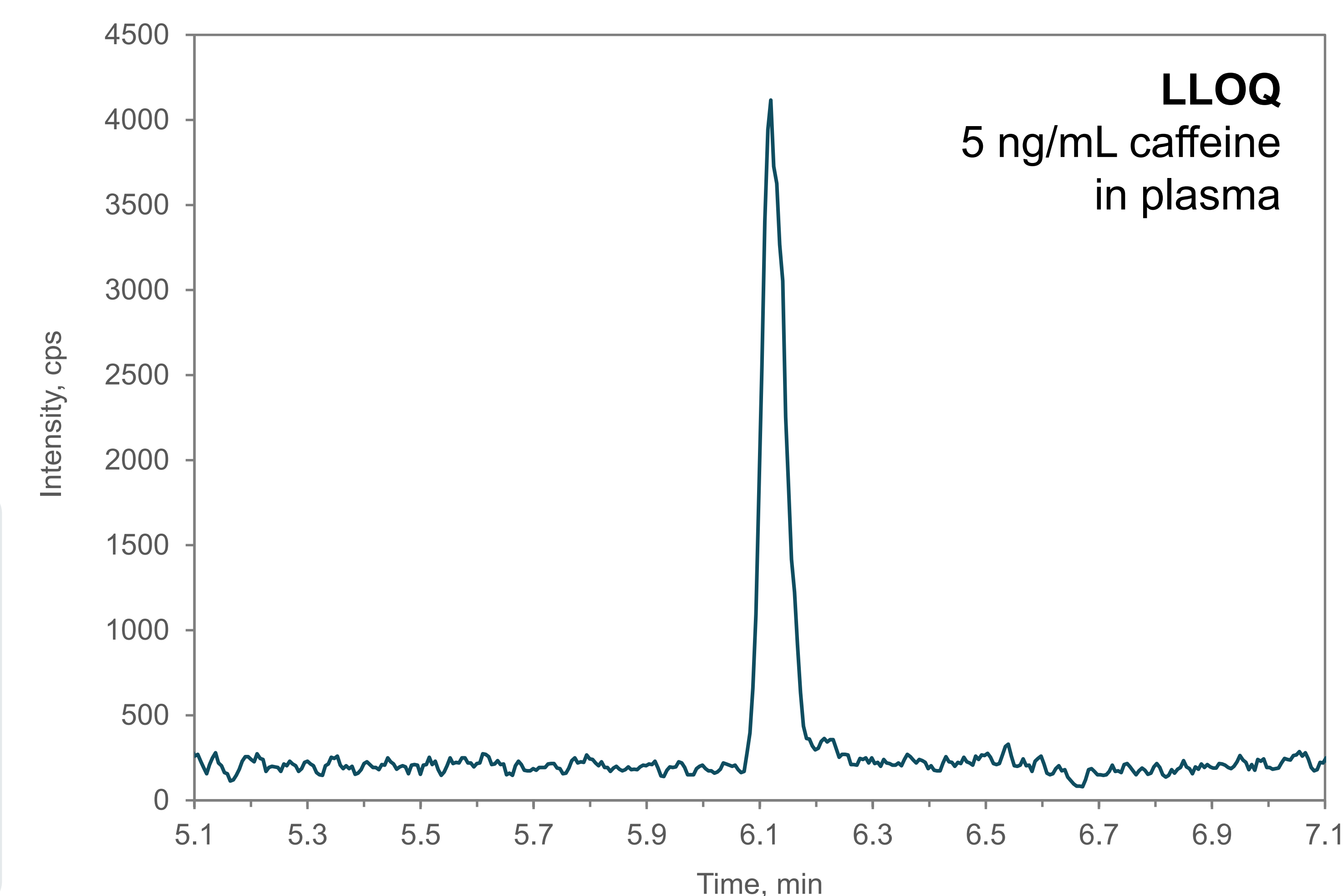
Accuracy: 98-101 %
Precision: 3-6 %

Conclusion

- Method fully validated according to the new, harmonized **ICH M10 guideline**
- Rapid sample clean-up for **high throughput** with 1000+ plasma samples per week
- High sensitivity, broad range, fast turnaround
- Reliable, robust, and suitable for routine plasma analysis of caffeine in **pharmacokinetic** and **clinical studies**



Chromatogram



Method accredited
DIN EN ISO/IEC
17025:2018

