Enantioselective Analysis of Amphetamines in Serum, Urine, Hair and Dried Blood Spots

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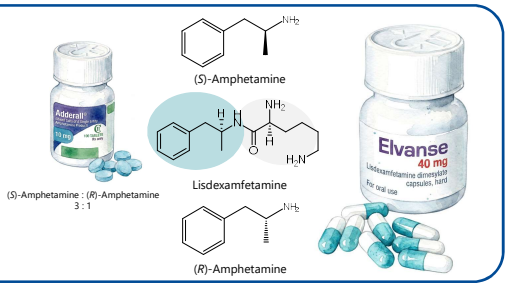
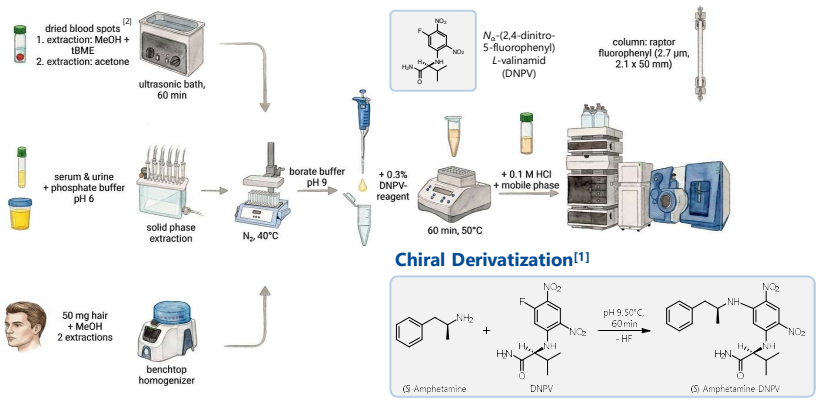
Introduction

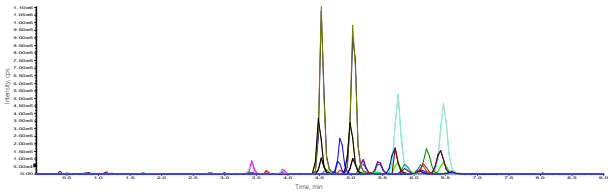
- Amphetamine and amphetamine-type stimulants rank among the most commonly consumed drugs worldwide
- MDMA and methamphetamine are primarily used for their stimulant effects
- Amphetamine or its prodrug (lisdexamfetamine) can also be prescribed as a medication for attention-deficit hyperactivity disorder (ADHD)
- Therapeutic drugs most often contain (*S*)-amphetamine as the active compound, whereas illicit amphetamine is typically traded as the racemate
- Chiral analysis of amphetamine enantiomers enables differentiation between therapeutic use of enantiomerically pure amphetamine and additional use of illicit amphetamine
- Here a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for chiral separation and quantification of amphetamine enantiomers and amphetamine-type stimulants in serum, urine, hair and dried blood spots (DBS) was developed and validated
- Validation was performed according to the guidelines of the German speaking *Society of Toxicological and Forensic Chemistry* (GTFCh)
- **Validation parameters:** selectivity, linearity, accuracy, stability, limit of detection (LOD), limit of quantification (LOQ), recovery and matrix effects
- **Enantiomers:** (*S*)-/(*R*)-amphetamine, (*S*)-/(*R*)-methamphetamine, (*S*)-/(*R*)-MDMA, (*S*)-/(*R*)-MDA, (*S*)-/(*R*)-MDEA



Methods



Chiral Derivatization[1]

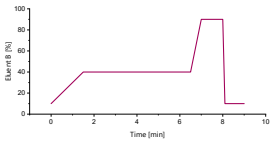


Liquid Chromatography

- Raptor Fluorophenyl Column: 2.7 µm, 50 mm x 2.1 mm
- A = H_2O, 0.1% formic acid, 2 mM ammonium formate
- B = Acetonitrile, 0.1% formic acid, 2 mM ammonium formate
- Flow = 0.45 mL/min
- Injection volume: 5 µL
- Run time: 9 min

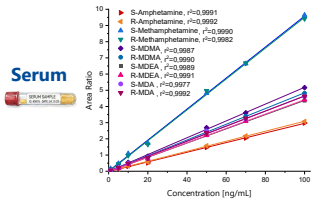
Mass Spectrometry

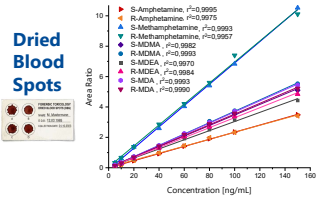
- AB SCIEX Triple Quad™ 6500+
- ESI [H+] & MRM

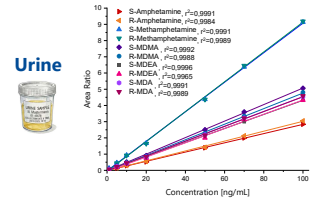


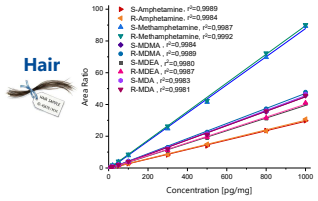
Results

Linearity

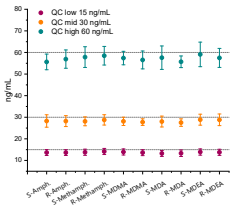


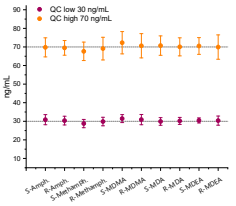


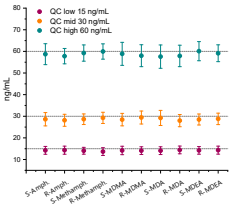


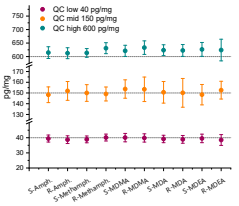


Accuracy

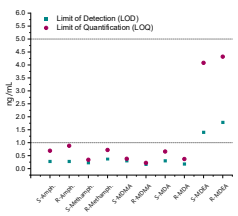


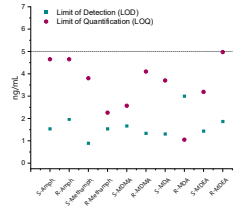


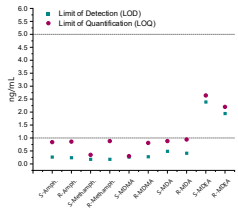


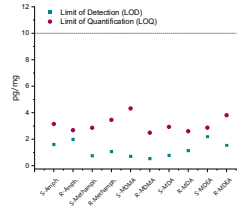


LOD/LOQ





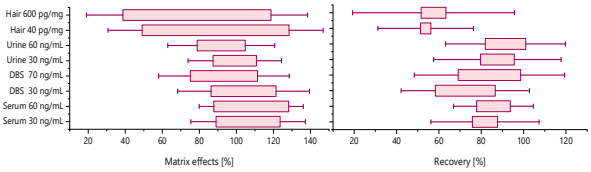




Stability

- All autosampler stability measurements yielded results within a ± 25% range over a timespan of a regular analysis

Matrix Effects and Recovery



- Matrix effects: 75-125% with a standard deviation of max. 25%
- Recovery rates: >50%
- Matrix effects in hair were additionally examined and were compensated for by using a deuterated internal standard

Conclusion

- The method allowed for selective and sensitive detection of amphetamine and amphetamine-type stimulant enantiomers
- Compliance monitoring of therapy in ADHD patients
- Differentiation between therapeutic administration of (*S*)-amphetamine and abuse of illicit street amphetamine
- Dried blood spot samples allow for a less invasive sampling process compared to serum samples and offer advantages for sample transportation and storage
- This method enables future studies on the enantioselective metabolism of amphetamine and amphetamine-type stimulants
- Successful method application to routine samples (n=15) and participation in proficiency tests (n=5)

References

[1] Steuer, A. E., Schmidhauser, C., Liechti, M. E., and Kraemer, T. (2015), Development and validation of an LC-MS/MS method after chiral derivatization for the simultaneous stereoselective determination of methylenedioxy-methamphetamine (MDMA) and its phase I and II metabolites in human blood plasma. *Drug Test. Analysis*, 7, 592–602. doi: 10.1002/dta.1740.

[2] Tretzel, L., Thomas, A., Geyer, H., Pop, V., Schänzer, W., Thevis, M.; Dried blood spots (DBS) in doping controls: a complementary matrix for improved in- and out-of-competition sports drug testing strategies. Anal. Methods 2015; 7 (18): 7596–7605. doi: 10.1039/c5ay01514f

[3] Images were created by Adobe Firefly® and BioRender®

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