# Zeiteinschätzung des Konsums von Stimulantien in Blutserumproben über enantioselektive Analytik

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# Publikationen

Die der vorliegenden kumulativen Dissertationsschrift zugrundeliegenden Arbeiten, die am Institut für Rechtsmedizin der Universitätsmedizin Mainz entstanden sind, wurden in den folgenden Publikationen veröffentlicht:

Losacker M., Zörntlein S., Schwarze B., Staudt S., Röhrich J., Hess C. Determination of the enantiomeric composition of amphetamine, methamphetamine and 3,4-methylendioxy-*N*-methylamphetamine (MDMA) in seized street drug samples from southern Germany. *Drug Testing and Analysis* 2021, doi:10.1002/dta.3118.

Losacker M., Tönnes S., de Sousa Fernandes Perna E., Ramaekers J., Röhrich J., Hess C. Chiral Serum Pharmacokinetics of 4-Fluoroamphetamine after Controlled Oral Administration: Can (R)/(S)-Concentration Ratios Help in Interpreting Forensic Cases? *Journal of Analytical Toxicology* 2020, https://doi.org/10.1093/jat/bkaa156.

Losacker, M., Krämer, M., Dücker, K., Philipsen, A., Dreimüller, N., Engelmann, J., Röhrich, J., Hess, C. Enantioselective quantification of amphetamine and metabolites in serum samples: Forensic evaluation and estimation of consumption time. *Metabolites* 2021, 11, 521, https://doi.org/10.3390/metabo11080521.

Losacker, M., Röhrich, J., Hess, C. Enantioselective determination of plasma protein binding of common amphetamine-type stimulants. *Journal of Pharmaceutical and Biomedical Analysis* 2021, 205, 114317, https://doi.org/10.1016/j.jpba.2021.114317.

Die notwendigen Lizenzen zum Nachdruck der Publikationen in dieser Dissertation wurden von den entsprechenden Verlagen erteilt.

**Weitere Publikationen, die nicht in dieser Arbeit enthalten sind:**

Krämer, M.; Schäper, M.; Dücker, K.; Philipsen, A.; Losacker, M.; Dreimüller, N.; Engelmann, J.; Madea, B.; Hess, C. Detectability of cannabinoids in the serum samples of cannabis users: Indicators of recent cannabis use? A follow-up study. *Drug Testing and Analysis* 2021, 13, 1614–1626, doi:10.1002/dta.3110.

Hess, C.; Losacker, M.; Maas, A. Chromatographic separation of R-(−)/S-(+)-enantiomers of amphetamine and methamphetamine: differentiation between single methamphetamine consumption and co-consumption with amphetamine using enantioselective quantitative LC-MS/MS analysis. *International Journal of Legal Medicine* 2019, 133, 467–473, doi:10.1007/s00414-018-1979-1.

Maas, A.; Losacker, M.; Hess, C. Chromatographic separation of R/S-enantiomers of amphetamine and methamphetamine: Pathways of methamphetamine synthesis and detection in blood samples by qualitative enantioselective LC–MS/MS analysis. *Forensic Science International* 2018, 291, 138–143, doi:10.1016/j.forsciint.2018.08.013.

**Zudem wurden Teile der Arbeit auf folgenden Kongressen präsentiert:**

Losacker M., Tönnes S., de Sousa Fernandes Perna E., Ramaekers J., Röhrich J., Hess C. Chiral serum pharmacokinetics of 4-fluoroamphetamine after controlled oral administration - can (R)/(S)-concentrations ratios help in Interpreting forensic cases? *XXII. Symposium der GTFCh*, Mosbach-online, 15. - 17.04.2021

Losacker M. Möglichkeiten der chiralen Stimulantienanalytik. *6. Symposium zur chiralen Chromatographie*, Phenomenex LTD, Aschaffenburg, 23.10.2019

**Weitere Präsentationen zu Inhalten, die nicht in dieser Arbeit enthalten sind:**

Losacker M., Madea B., Hess C., Maas A. Enantioselective detection of S-/R-amphetamine and S-/R-methamphetamine and differentiation between pure methamphetamine consumption and amphetamine co-consumption. *XXI. Symposium der GTFCh*, Mosbach, 11. - 13.04.2019 (Posterpräsentation)

# Abkürzungsverzeichnis

| | |
|---|---|
| 4-FA | 4-Fluoramphetamin |
| ADHS | Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung |
| AGP | Saures Alpha1-Glykoprotein |
| ATS | Stimulantien des Amphetamin -Typs (engl. *amphetamine-type stimulants*) |
| AUC | Fläche unter der Kurve (engl. *Area Under the Curve*) |
| BtMG | Betäubungsmittelgesetz |
| CEM | Channeltron (engl. *channel electron multiplier)* |
| CID | kollisionsinduzierte Dissoziation (engl. *collision induced dissociation*) |
| $c_{max}$ | maximale Serumkonzentration |
| DAD | Diodenarray-Detektor |
| EMCDDA | Europäische Beobachtungsstelle für Drogen und Drogensucht (engl. *European Monitoring Centre for Drugs and Drug Addiction*) |
| engl. | englisch |
| ESI | Elektrosprayionisation |
| EU | Europäische Union |
| g | Gramm |
| griech. | griechisch |
| GTFCh | Gesellschaft für Toxikologische und Forensische Chemie |
| GÜG | Grundstoffüberwachungsgesetz |
| h | Stunde(n) |
| HHA | 3,4-Dihydroxyamphetamin |
| HHMA | 3,4-Dihydroxymethamphetamin |
| HMA | 4-Hydroxy-3-methoxyamphetamin |
| HMMA | 4-Hydroxy-3-methoxymethamphetamin |
| HPLC | Hochleistungsflüssigkeitschromatographie (engl. high performance liquid chromatography) |
| IUPAC | Internationale Union für reine und angewandte Chemie (engl. *International Union of Pure and Applied Chemistry*) |
| KKWT/ED | Kommission Kriminalwissenschaft und -technik/Erkennungsdienst |
| L | Liter |
| lat. | lateinisch |
| LKA | Landeskriminalamt |

| | |
|---|---|
| LOD | Nachweisgrenze (engl. *limit of detection*) |
| LOQ | Bestimmungsgrenze (engl. *limit of quantification*) |
| m/z | Masse-zu-Ladungsverhältnis |
| MDA | 3,4-Methylendioxyamphetamin |
| MDMA | 3,4-Methylendioxy-*N*-methylamphetamin |
| MDP2P | 3,4-Methylenedioxyphenylpropan-2-on (Piperonylmethylketon, PMK) |
| MRM | *engl. multiple reaction monitoring* |
| MS/MS | Tandem-Massenspektrometrie |
| NPS | Neue psychoaktive Stoffe |
| NRF | Neues Rezeptur-Formularium |
| p | Signifikanzwert (lat. *probabilitas*) |
| P2P | Phenylaceton (IUPAC: 1-Phenyl-2-propanon) |
| pH-Wert | Potential des Wasserstoffs (lat. *potentia hydrogenii*) |
| PMK | Piperonylmethylketon |
| PNS | Peripheres Nervensystem |
| PPB | Plasmaproteinbindung |
| PTBS | Posttraumatische Belastungsstörung |
| QqQ | Triple-Quadrupol-Massenspektrometer |
| $R^2$ | Bestimmtheitsmaß |
| SEV | Sekundärelektronenvervielfacher |
| SPE | Festphasenextraktion (engl. *solid phase extraction*) |
| StGB | Strafgesetzbuch |
| StVG | Straßenverkehrsgesetz |
| $t_{1/2}$ | Serumhalbwertszeit |
| $t_{max}$ | Zeit zum Erreichen der maximalen Serumkonzentration |
| UF | Ultrafiltration |
| ZNS | Zentrales Nervensystem |

# Abstract

Amphetamine-type stimulants (ATS) are a globally abused type of narcotic drugs. Effects range from stimulant effects such as increased concentration ability and psychomotor performance to restlessness, confusion and aggression. The acute phase is followed by symptoms of exhaustion such as fatigue and lack of concentration, which can last for days. Due to their psychomimetic properties and the widespread use of ATS, forensic toxicologists are frequently confronted with the assessment of offenses related to the consumption of these stimulants. These primarily include questions related to driving ability, but also the assessment of criminal responsibility. In this context, experts are often challenged by the fact that it is hardly possible to make statements regarding the influence at the moment of the crime, in particular due to the unknown quantity consumed. The verification of the defendant's information on the time of the last consumption is therefore of crucial forensic interest. While time estimation based on blood serum concentrations as determined routinely in forensic laboratories is not possible, enantioselective analysis offers an approach that potentially expands the forensic interpretation possibilities. Since ATS are mostly consumed as racemates, but their enantiomers are metabolized at different rates in the human body, conclusions can be drawn about the time of consumption if the time course of the enantiomeric ratios is known. The studies summarized in this dissertation describe the investigation of the enantioselective pharmacokinetics of 4-fluoroamphetamine (4-FA) and amphetamine with respect to forensic issues. In addition, the plasma protein binding (PPB) of various ATSs was determined enantioselectively and seized substance samples of illicit ATS were analyzed for their chiral composition.

For this purpose, two liquid chromatographic-tandem mass spectrometric (LC-MS/MS) methods for the detection of the enantiomers of amphetamine, methamphetamine, 4-FA, 3,4-methylenedioxy-N-methylamphetamine (MDMA), and the amphetamine metabolites norephedrine and 4-hydroxyamphetamine were developed and successfully validated for application in serum samples. Sample preparation was performed by solid phase extraction (SPE) and enantiomer separation was performed using a chiral column.

The analysis of police seizures showed exclusively racemic composition for amphetamine and MDMA. On the one hand, this allows the interpretation of enantiomeric ratios in serum for the purpose of time estimation and, on the other hand, the differentiation from a therapeutic intake of approved (S)-amphetamine-containing drugs. While the majority of the seized methamphetamine samples exclusively contained the more potent (S)-enantiomer, in some cases the pure

(R)-enantiomer as well as racemic and non-racemic mixtures were also detected. These results reflect the current changes in the drug market and may also have legal implications for the interpretation of the so-called ‘nicht geringe Menge’ (non-small amount) according to the German Narcotics Act (BtMG).

Analysis of serum samples obtained from 12 subjects in a clinical study with oral ingestion of 4-FA showed significantly shorter half-lives for the (S)-enantiomer. The increase in (R)/(S)-concentration ratios was linear for all subjects and was independent of the given dose. However, the slope of the (R)/(S)-ratios showed considerable interindividual differences, resulting in enantiomeric ratios between 1.08 and 2.77 after 12 hours. Nevertheless, for forensic interpretation, a cut-off at a (R)/(S)-ratio of 1.60 could be suggested, indicating the absence of acute effects.

For time estimation in amphetamine use, samples were collected from psychiatric inpatients who voluntarily provided information on their last consumption. Here, a good correlation of the (R)/(S)-concentration ratios with the reported time difference to the last consumption was found, although significant outliers were also observed. Analogous to the 4-FA study, this study indicated a cut-off at a (R)/(S)-ratio of 1.09 for the assumption that acute effects have passed. Limitations include the relatively small sample size of 30 samples, the reliability of the self-reported data and the frequent presence of binge consumption. Examination of a total of 425 amphetamine-positive forensic serum samples yielded (R)/(S) concentration ratios between 0.88 and 4.04. Furthermore, the enantiomers of the metabolites norephedrine and 4-hydroxyamphetamine could be detected in many of these samples.

The determination of the PPB of amphetamine, methamphetamine, 4-FA and MDMA by ultrafiltration (UF) revealed no or only insignificant differences in the binding of the enantiomers. A significant influence of the PPB on the stereospecific differences in pharmacokinetics and pharmacodynamics could therefore be excluded.

## Zusammenfassung

Stimulantien des Amphetamin-Typs (*amphetamine-type stimulants,* ATS) sind eine weltweit missbrauchte Gruppe von Betäubungsmitteln. Ihre Wirkung reicht von stimulierenden Effekten wie Konzentrations- und Leistungssteigerung bis hin zu Unruhe, Verwirrtheit und Aggressivität. Im Anschluss an die akute Wirkungsphase treten Erschöpfungssymptome wie Müdigkeit und Konzentrationslosigkeit auf, die mitunter über Tage andauern können. Aufgrund ihrer psychomimetischen Eigenschaften und der weiten Verbreitung von ATS sind Forensische Toxikologen häufig mit der Beurteilung von Delikten im Zusammenhang mit dem Konsum dieser Stimulantien konfrontiert. Dazu gehören vor allem Fragestellungen bezüglich der Fahrtüchtigkeit aber auch der Einschätzung von Schuldfähigkeit. Hierbei stehen Gutachter oft vor dem Problem, dass sich insbesondere aufgrund der unbekannten Konsummenge kaum Aussagen zur Beeinflussung zum Tatzeitpunkt treffen lassen. Die Überprüfung der Angaben des Beschuldigten zum Zeitpunkt des letzten Konsums ist somit von zentralem forensischen Interesse. Während eine Zeiteinschätzung auf Basis der in forensischen Laboren routinemäßig ermittelten Blutserumkonzentrationen nicht möglich ist, bietet die enantioselektive Analytik einen Ansatz, der potentiell die forensischen Interpretationsmöglichkeiten erweitert. Da ATS meist in Form des Racemates konsumiert werden, die Enantiomere im menschlichen Körper jedoch unterschiedlich schnell metabolisiert werden, können unter Kenntnis des zeitlichen Verlaufs der Enantiomerenverhältnisse Rückschlüsse auf den Konsumzeitpunkt gezogen werden. Die in dieser Arbeit zusammengefassten Studien beschreiben die Untersuchung der enantioselektiven Pharmakokinetik von 4-Fluoramphetamin (4-FA) und Amphetamin in Hinblick auf forensische Fragestellungen. Darüber hinaus wurde die Plasmaproteinbindung (PPB) von verschiedenen ATS enantioselektiv ermittelt und beschlagnahmte Stoffproben illegaler ATS auf ihre chirale Zusammensetzung untersucht.

Zu diesem Zweck wurden zwei flüssigkeitschromatographisch-tandem-massenspektrometrische (LC-MS/MS) Methoden zur Detektion der Enantiomere von Amphetamin, Methamphetamin, 4-FA, 3,4-Methylendioxy-*N*-methylamphetamin (MDMA), sowie der Amphetamin-Metabolite Norephedrin und 4-Hydroxyamphetamin entwickelt und erfolgreich für die Anwendung in Serumproben validiert. Die Probenaufarbeitung erfolgte hierbei mittels Festphasenextraktion (*solid phase extraction*, SPE) und die Enantiomerentrennung mithilfe einer chiralen Säule.

Bei der Analyse von polizeilich beschlagnahmten Stoffproben zeigten sich für Amphetamin und MDMA ausnahmslos racemische Zusammensetzungen. Dies erlaubt zum einen die Interpretation

von Enantiomerenverhältnissen im Serum im Sinne einer Zeiteinschätzung und zum anderen die Differenzierung von einer therapeutischen Aufnahme zugelassener (S)-amphetaminhaltiger Medikamente. Während der Großteil der beschlagnahmten Methamphetaminproben ausschließlich das potentere (S)-Enantiomer enthielt, konnte in einigen Fällen auch das reine (R)-Enantiomer sowie racemische und nicht-racemische Mischungen detektiert werden. Diese Ergebnisse spiegeln den aktuell stattfindenden Wandel auf dem Drogenmarkt wider und können darüber hinaus rechtliche Auswirkungen auf die Auslegung der sogenannten „nicht geringen Menge" im Sinne des deutschen Betäubungsmittelgesetzes (BtMG) haben.

Bei der Analyse von Serumproben, die von 12 Probanden im Rahmen einer klinischen Studie mit oraler Einnahme von 4-FA gewonnen wurden, zeigten sich deutlich kürzere Halbwertszeiten für das (S)-Enantiomer. Der Anstieg der (R)/(S)-Konzentrationsverhältnisse verlief für alle Probanden linear und war unabhängig von der konsumierten Dosis. Bei der Steigung der (R)/(S)-Verhältnisse zeigten sich jedoch erhebliche interindividuelle Unterschiede, sodass sich nach 12 Stunden Enantiomerenverhältnisse zwischen 1,08 und 2,77 ergaben. Für die forensische Interpretation konnte dennoch ein Cut-off bei einem (R)/(S)-Verhältnis von 1,60 vorgeschlagen werden, der die Abwesenheit von Akuteffekten nahelegt.

Zur Zeiteinschätzung bei Konsum von Amphetamin wurden Proben von psychiatrischen Patienten gesammelt, die freiwillig Angaben zu ihrem letzten Konsum machten. Hierbei zeigte sich eine gute Korrelation der (R)/(S)-Konzentrationsverhältnisse mit der angegebenen Zeitdifferenz zum letzten Konsum, wobei auch hier deutliche Ausreißer zu beobachten waren. Analog zur 4-FA-Studie ergab sich aus dieser Studie für die Annahme des Nichtvorliegens einer akuten Beeinflussung ein Cut-off bei einem (R)/(S)-Verhältnis von 1,09. Hierbei stellen neben dem relativ kleinen Probenumfang von 30 Proben sowohl die Verlässlichkeit der Eigenangaben als auch der oftmals vorliegende Mehrfachkonsum Limitationen dar. Bei der Untersuchung von insgesamt 425 Amphetamin-positiver forensischer Serumproben ergaben sich (R)/(S)-Konzentrationsverhältnisse zwischen 0,88 und 4,04. In vielen dieser Proben konnten darüber hinaus die Enantiomere der Metabolite Norephedrin und 4-Hydroxyamphetamin nachgewiesen werden.

Bei der Bestimmung der PPB von Amphetamin, Methamphetamin, 4-FA und MDMA mittels Ultrafiltration (UF) konnten keine oder nur unerhebliche Unterschiede in der Bindung der Enantiomere festgestellt werden. Ein nennenswerter Einfluss der PPB auf die stereospezifischen Unterschiede in Pharmakokinetik und Pharmakodynamik konnte somit ausgeschlossen werden.

# 1 Einleitung

## 1.1 Stimulantien des Amphetamin-Typs

Amphetamin und seine strukturverwandten Analoga werden in der Gruppe der sogenannten *amphetamine-type stimulants* (ATS, Stimulantien des Amphetamin-Typs) zusammengefasst [1]. In der Literatur wird bisweilen der Oberbegriff „*Amphetamine*“ generisch verwendet [2–4], historisch fand der etwas weiter gefasste Begriff der „*Weckamine*“ Gebrauch [5,6]. ATS sind Derivate des 2-Phenethylamins und gehören daher zur Stoffgruppe der Phenethylamine [2,7]. Sie besitzen somit die selbe Grundstruktur wie die endogenen Neurotransmitter der Katecholamine [2]. Allgemein bewirken ATS eine Konzentrationserhöhung der Neurotransmitter Dopamin, Noradrenalin und Serotonin im synaptischen Spalt der Nervenzellen (Neuronen) des Gehirns [3,8,9]. Zu den dadurch bedingten stimulierenden Effekten zählen Leistungssteigerung, Euphorie und Enthemmung. Gleichzeitig werden Müdigkeit und Hungergefühl unterdrückt [2,9,10].

Zu den wichtigsten Vertretern der ATS gehören neben dem namensgebenden Amphetamin das deutlich potentere Methamphetamin und das meist als sogenannte „*Partydroge*“ verwendete 3,4-Methylendioxy-*N*-methylamphetamin (MDMA) [9,11–13]. Daneben existieren unzählige weitere Substanzen der ATS-Gruppe, von denen einige der Gruppe der *Neuen psychoaktiven Stoffe* (NPS, auch „Designerdrogen“) zugeordnet werden [7,13]. Durch gezielte Modifizierung der chemischen Struktur wird versucht, betäubungsmittelrechtliche Beschränkungen zu umgehen. Dies führt mitunter dazu, dass die Substanzen nur kurzzeitig auf dem Markt erscheinen und dann wieder an Bedeutung verlieren [7,14,15]. Während einige Vertreter der ATS auch natürlich vorliegen und seit Jahrtausenden genutzt werden, wie zum Beispiel das im Kathstrauch enthaltene Cathinon [3,10,16], werden die heute in der westlichen Welt relevanten Vertreter voll- oder halbsynthetisch hergestellt [17,18].

Wurden Amphetamin und Methamphetamin nach ihrer Entdeckung zunächst therapeutisch für vielfältige Indikationen angewendet, offenbarte sich schnell das große Missbrauchs- und Abhängigkeitspotential der Stimulantien [2,19]. So stehen die am häufigsten missbrauchten Vertreter der ATS seit 1971 unter internationaler Kontrolle [20]. Dennoch sind ATS nach Cannabis und Opioiden die weltweit am dritthäufigsten missbrauchte Gruppe von Betäubungsmitteln [11]. In Deutschland konsumieren etwa ein Prozent aller Erwachsenen mindestens einmal im Jahr Amphetamine, während die Prävalenz unter jungen Erwachsenen in etwa bei zwei Prozent liegt [21,22]. Einzelne Vertreter der ATS und weitere Derivate der Phenethylamine finden jedoch auch

heute noch therapeutische Anwendung, insbesondere zur Behandlung der Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung (ADHS) [23,24].

Die folgenden Kapitel beinhalten kurze Substanzmonographien der in dieser Arbeit untersuchten ATS.

### 1.1.1 Amphetamin

Der Name Amphetamin (vgl. Abbildung 1) ist von der veralteten systematischen Bezeichnung alpha-Methylphenethylamin abgeleitet [25]. Die systematische deutsche Bezeichnung nach IUPAC-Nomenklatur (International Union of Pure and Applied Chemistry) lautet 1-Phenylpropan-2-amin. Amphetamin wurde erstmals 1887 in Berlin von dem rumänischen Chemiker Lazăr Edeleanu synthetisiert [3,5,26]. Im Jahr 1927 wurde es von Gordon Alles resynthetisiert, der nach einer kostengünstigen Alternative zum natürlich vorkommenden Phenethylamin Ephedrin suchte und den stimulierenden Effekt des Amphetamins zuerst beschrieb [27–30]. Erstmals zur medizinischen Anwendung zugelassen wurde es im Jahr 1932 unter dem Namen *Benzedrine*® in Tablettenform und zur nasalen Applikation [29,30]. Mit der Zulassung für Indikationen wie Narkolepsie und Depression stiegen die Verschreibungen von amphetaminhaltigen Arzneimitteln [5,31]. In Folge des zweiten Weltkrieges, wo Amphetamin breite Anwendung unter alliierten Truppen fand, wurde es auch in der zivilen Bevölkerung immer häufiger missbraucht [2,31]. Heute wird Amphetamin weltweit konsumiert und stellt in Deutschland sowie vielen anderen europäischen Ländern das am häufigsten missbrauchte ATS dar [11,12,22]. Das in Deutschland sichergestellte Amphetamin stammt überwiegend aus den Niederlanden [32,33]. Amphetamin, genannt „*Speed*“ oder „*Pep*“, wird meist als Pulver, seltener in Tablettenform, angeboten [13,34]. Der mittlere Reinheitsgrad betrug im Jahr 2019 etwa 13 Prozent [35]. Der Konsum erfolgt in der Regel nasal, jedoch werden auch oraler, inhalativer und intravenöser Konsum gelegentlich praktiziert [12].

### 1.1.2 Methamphetamin

Methamphetamin (IUPAC: *N*-Methyl-1-phenylpropan-2-amin) ist das *N*-methylierte Derivat des Amphetamins (vgl. Abbildung 1) [25]. Die Erstsynthese gelang dem Japaner Nagayoshi Nagai im Jahr 1893 [36–38]. Der Durchbruch hin zu einer industriellen Herstellung erfolgte 1919, als Akira Ogata erstmals kristallines Methamphetamin synthetisierte [36,38]. Die Temmler-Werke brachten Methamphetamin 1938 unter dem Handelsnamen Pervitin® als Fertigpräparat in Deutschland auf den Markt [30,39]. Das im Zweiten Weltkrieg vor allem von deutschen und japanischen Truppen

zur Leistungssteigerung genutzte Stimulanz [30,38] wurde aufgrund seines Missbrauchspotentials bereits 1941 dem Reichsopiumgesetz unterstellt [40,41]. Über die darauf folgenden Jahrzehnte entwickelte sich der Methamphetaminmissbrauch zu einem weltweiten Problem [11,42].

Heute ist Methamphetamin im asiatischen Raum das am häufigsten missbrauchte Stimulanz, jedoch gewinnt es auch in Zentraleuropa an Relevanz [11,43]. Während Methamphetamin in den letzten Jahrzehnten vor allem in der Tschechischen Republik hergestellt und missbraucht wurde, unterliegt der europäische Markt momentan einem Wandel [33,43]. So stammte im Jahr 2019 der Großteil des in Deutschland sichergestellten Methamphetamins aus den Niederlanden [21,32]. Die 12-Monats-Prävalenz unter den Erwachsenen liegt bei 0,2 Prozent [21], wobei der Konsum vor allem in den an Tschechien angrenzenden Bundesländern verbreitet ist [44,45].

Die Wirkung von Methamphetamin ist qualitativ mit der von Amphetamin vergleichbar [2,34,46]. Ob die Wirkung von Methamphetamin etwa aufgrund höheren Lipophilie und somit leichterer Passage der Blut-Hirnschranke oder aufgrund der allgemein höheren Reinheit zu einer intensiveren Wirkung und damit verbundenem höherem Abhängigkeitspotential führt, wird kontrovers diskutiert [2,3,34,46–48]. Während Methamphetamin in Europa meist als Pulver („*Crank*“) oder in kristalliner Form („*Crystal Meth*“) gehandelt wird, tritt es vor allem in Asien auch in Tablettenform („*Yaba*“) auf [12,30,49–51]. Der Konsum erfolgt in der Regel nasal (Pulver) oder oral (Tabletten), seltener intravenös [2,12,43]. Die hochreine kristalline Form, auch “*Ice*” genannt, kann geraucht werden [30,49].

### 1.1.3 3,4-Methylendioxy-*N*-methylamphetamin (MDMA)

3,4-Methylendioxy-*N*-methylamphetamin (IUPAC: 1-(1,3-Benzodioxol-5-yl)-*N*-methylpropan-2-amin), kurz MDMA, ist ein ringsubstituiertes Phenethylamin (vgl. Abbildung 1) [52]. Es wurde erstmals 1912 von der Darmstädter Firma E. Merck als Syntheseintermediat hergestellt (Patenschrift Nr. 274350). Erste pharmakologische Untersuchungen fanden jedoch erst 1927 statt. [53,54]. 1978 führte der US-Amerikanische Halluzinogenforscher Alexander Shulgin erste Studien am Menschen durch [55,56]. Erste Belege für den Missbrauch im amerikanischen Mittleren Westen finden sich jedoch schon in den frühen Siebzigern [54,57,58]. In den Achtzigerjahren verbreitete sich der MDMA Konsum im Rest der USA und auch in Europa, insbesondere in der Technokultur („*Raveszene*“) [2,58–60]. Nach einem Tiefpunkt im Jahr 2010 ist in Deutschland zuletzt wieder ein deutlich ansteigender Trend bei den Konsumdelikten von MDMA zu beobachten [35,61].

MDMA unterscheidet sich in seiner psychotropen Wirkung grundsätzlich von Amphetamin und Methamphetamin. Während bei letzteren Stimulantien die Wirkung von Dopamin und Noradrenalin im Vordergrund steht, bewirkt MDMA einen deutlich höheren Anstieg von Serotonin im synaptischen Spalt [62–65]. Der US-amerikanische Pharmakologe David E. Nichols prägte zur Beschreibung der emotional öffnenden und empathiesteigernden Wirkung den Begriff „*entactogen*“, was so viel bedeutet wie „das Innere berührend“ [13,63].

Im Jahr 2019 betrug die 12-Monats-Prävalenz in Deutschland unter den Erwachsenen 1,1 Prozent, während der Konsum unter jungen Erwachsenen (18 bis 25 Jahre) mit 3,6 Prozent deutlich verbreiteter war [21]. MDMA wird typischerweise in Form von bunten Tabletten („*Ecstasy*“, kurz „*E*“ oder „*XTC*“) vertrieben, die mit Symbolen oder Markenlogos versehen sind [55,60,66–68]. Während in der Vergangenheit mitunter auch andere psychotrope Substanzen in *Ecstasy*-Tabletten enthalten waren [69,70], beinhalten in Deutschland sichergestellte Tabletten heute fast immer MDMA [61], sodass der Begriff „*Ecstasy*“ mitunter auch Synonym verwendet wird [69,71]. Der mittlere Wirkstoffgehalt von in Deutschland sichergestellten *Ecstasy*-Tabletten hat sich in den letzten zehn Jahren annähernd verdreifacht und betrug 2019 im Schnitt 146 Milligramm pro Tablette [35,61]. Seit 2010 ist MDMA darüber hinaus auch in unverschnittener kristalliner Form auf dem Markt [11,68], dessen Wirkstoffgehalt meist über 70 Prozent beträgt [35]. Der Großteil des in Deutschland sichergestellten MDMA stammt aus den Niederlanden, in denen eine besonders hohe Konsumprävalenz herrscht [11,22]. *Ecstasy*-Tabletten werden in der Regel geschluckt, die kristalline Form kann auch geschnupft werden [22]. Seltener wird es intravenös injiziert oder geraucht [72].

### 1.1.4 4-Fluoramphetamin

4-Fluoramphetamin (IUPAC: 1-(4-Fluorphenyl)propan-2-amin), kurz 4-FA, ist das para-fluorierte Analogon des Amphetamins (vgl. Abbildung 1) [73]. Als halogenierte Variante einer bereits gebräuchlichen Droge ist 4-FA der Gruppe der NPS zuzuordnen [74–76]. 4-FA wurde erstmals im Januar 2003 in Deutschland sichergestellt [76]. Seitdem wurde es in einigen europäischen Ländern wie Dänemark [77,78], Deutschland [73,79], Belgien [80], Schweden [81,82], Finnland [83] und der Schweiz [84] detektiert. Darüber hinaus gibt es Berichte von schweren Intoxikationen aus den USA [85–87]. Besonders populär war 4-FA in den Niederlanden, wo es 2013 das am häufigsten konsumierte NPS war [88–93]. Dort wurde es zunächst als Bestandteil von Amphetamin- und Ecstasyzubereitungen festgestellt, entwickelte sich aber im Laufe der Zeit zu einer Droge der Wahl, die eigenständig vermarktet wurde [88,90]. Nach der Aufnahme in das

niederländische Opiumgesetz (nl.: *Opiumwet*) und einer öffentlichen Informationskampagne verlor es jedoch deutlich an Popularität [94]. Bereits 2012 wurde 4-FA in Anlage I (nicht verkehrsfähige Betäubungsmittel) des deutschen Betäubungsmittelgesetzes (BtMG) aufgenommen [95].

4-FA ist auch bekannt unter Szenenamen wie „*PFA*“, „*4-FMP*“, „*flava*“, „*4floor*“, „*4-fluor*“, und wurde darüber hinaus als „*Flux CD cleaner*“ vermarktet [92]. Die Substanz wird hauptsächlich in Form von Tabletten oder Kapseln und seltener als Pulver angeboten [89,90]. In der Regel erfolgt der Konsum oral, der nasale Konsum wird seltener praktiziert und ist mit starken Schmerzen in der Nase verbunden [91]. Die Wirkung lässt sich zwischen den stimulierenden Effekten des Amphetamins und den entaktogenen Effekten von MDMA einordnen [91,96] und kann als „mild psychedelisch“ bezeichnet werden [96].

Amphetamin | 4-Fluoramphetamin | Methamphetamin | MDMA

**Abbildung 1** Strukturformeln der in dieser Arbeit untersuchten Phenethylamine Amphetamin, 4-Fluoramphetamin, Methamphetamin und 3,4-Methylendioxy-*N*-methylamphetamin (MDMA)

### 1.1.5 Epidemiologie

Während Konsum von ATS in der Regel nicht zu körperlicher Abhängigkeit führt, bieten sie ein erhebliches psychisches Abhängigkeitspotential [97,98]. Die Konsummuster reichen von gelegenheitsmäßigem Gebrauch bis hin zu chronischem, mehrfach täglichem Missbrauch [10,99,100]. Bei weitem am häufigsten werden ATS in der Gruppe der jungen Erwachsenen (15 bis 24 Jahre) konsumiert, dabei etwas häufiger von Männern [12,22,101]. In den freiwilligen Entzug begeben sich jedoch in etwa dreimal so viele Männer wie Frauen [12,22,101]. Laut dem Report der Europäischen Beobachtungsstelle für Drogen und Drogensucht (EMCDDA) liegt die 12-Monats-Prävalenz für MDMA-Konsum unter jungen Erwachsenen (15 bis 34 Jahre) in Deutschland mit etwa 1,3 Prozent im EU-Mittel, Spitzenreiter ist die Niederlande mit 7,1 Prozent. Beim Amphetamin-Konsum befindet sich Deutschland mit 1,9 Prozent in der EU-Spitze, während auch hier die Niederlande mit 3,5 Prozent deutlich heraussticht [22]. Vor allem MDMA wird vermehrt in der Techno- und Partyszene konsumiert [2,58–60], häufig gemeinsam mit anderen Substanzen, insbesondere Alkohol, Cannabis und anderen ATS [22,101]. Auch unter

Gelegenheitskonsumenten von ATS ist der *Bingekonsum*, also der Konsum von mehreren Dosen in relativ kurzen Zeitabständen, ein verbreitetes Phänomen [99,100,102]. Der regelmäßige Gebrauch von ATS führt zu einer raschen Toleranzentwicklung, sodass Dosen gesteigert und Konsumintervalle verkürzt werden [10,100]. Chronisch Abhängige wählen zudem vermehrt den Applikationsweg der Injektion, um einen stärkeren Effekt zu erzielen [10,103].

## 1.2 Chiralität

Chiralität ist eine Form der chemischen Isomerie (vgl. Abbildung 2). In erster Linie tritt Chiralität bei Molekülen auf, die vier unterschiedliche Substituenten an einem Kohlenstoffatom tragen [104,105]. Dieses Atom wird als „*asymmetrisches C-Atom*", „*Asymmetriezentrum*", „*stereogenes Zentrum*" oder kurz „*Stereozentrum*" bezeichnet [104–106]. Durch die dreidimensionale Anordnung der vier Substituenten als Tetraeder (*absolute Konfiguration*) existieren zwei mögliche Konfigurationsisomere, die sich wie Bild und Spiegelbild verhalten und somit nicht zur Deckung gebracht werden können (vgl. Abbildung 3) [104–108]. Die beiden Verbindungen werden als Enantiomere (*enantios*, griech.: entgegengesetzt) bezeichnet [106,109]. Die Eigenschaft der Enantiomere wird *Chiralität* genannt (*cheir*, griech.: Hand), da sich die beiden Verbindungen zueinander verhalten wie rechte und linke Hand [104–107]. Eine äquimolare (1:1) Mischung von zwei Enantiomeren wird als „*racemische Mischung*" oder „*Racemat*" (*racemus*, lat. etwa: Weinrebe) bezeichnet [104–107].

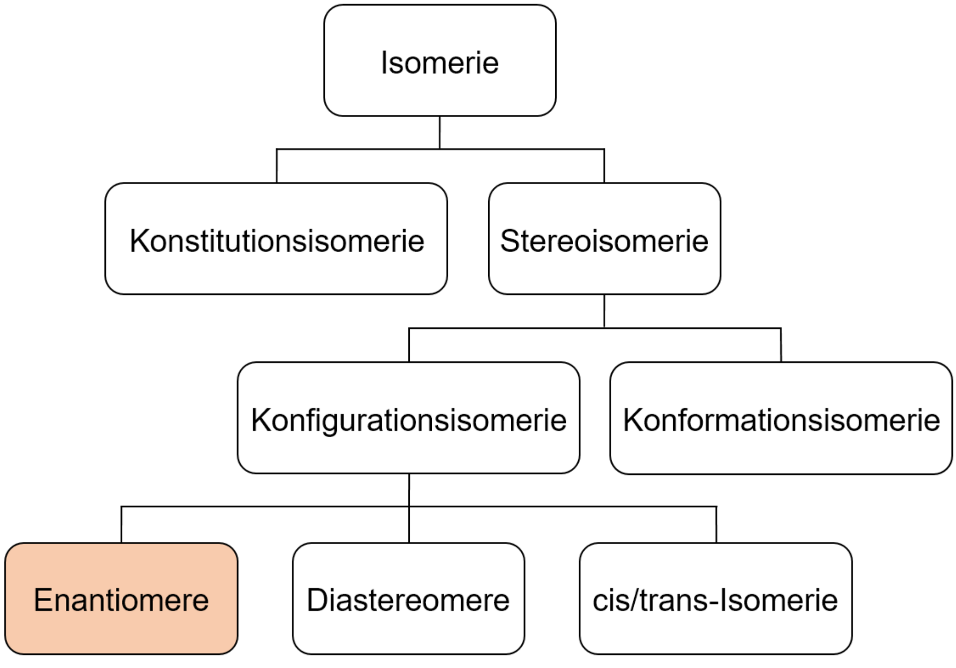


**Abbildung 2** Übersicht der unterschiedlichen Formen der Isomerie (modifiziert nach [110]).

Enantiomere können sich unter bestimmten Umständen ineinander umwandeln. Die Einstellung auf ein äquimolares Gleichgewicht wird „*Racemisierung*“ genannt [104–106]. Besitzt ein Molekül mehrere (n) Stereozentren, existieren in der Regel $2^n$ Stereoisomere, wobei jeweils n Enantiomerenpaare vorliegen [111]. Alle Stereoisomere, deren absolute Konfiguration sich nicht an sämtlichen (n) Stereozentren voneinander unterscheiden und sich somit nicht wie Spiegelbilder verhalten, werden *Diastereomere* genannt [104,107]. Während Diastereomere sich in ihren grundlegenden physikalischen und chemischen Eigenschaften unterscheiden, sind diese bei Enantiomeren identisch [104,107,112]. Während achirale Substanzen und Racemate in Lösung die Richtung von linear polarisiertem Licht in der Regel nicht ändern, können Enantiomere anhand ihrer spezifischen Drehrichtung unterschieden werden (optische Aktivität) [104,106].

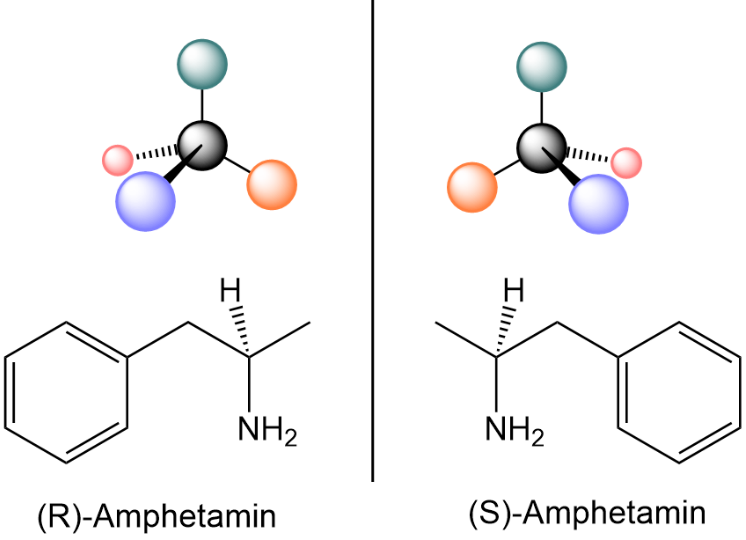


**Abbildung 3** Veranschaulichung der Chiralität am Beispiel des Amphetaminmoleküls im Tetraedermodell

Um die absolute Konfiguration in der zweidimensionalen Ebene sichtbar zu machen, hat sich die sogenannte Keilstrichformel etabliert [106,108]. Hierbei werden Bindungen in der Zeichenebene als einfache Linie dargestellt, Bindungen vor der Zeichenebene als Keil und hinter der Zeichenebene als gestichelter Keil [108,113]. Bei unbekannter Konfiguration oder Gemischen (Racematen) wird eine wellenförmige Linie genutzt (vgl. Abbildung 1) [108]. Zur Beschreibung der absoluten Konfiguration im Substanznamen existieren mehrere Deskriptoren. Historisch wurden die Enantiomere anhand ihrer optischen Aktivität in rechtsdrehend (+) und linksdrehend (-) unterschieden. Ebenso können synonym die Minuskel d für rechtsrotierend (lat.: *dexter*) und l für linksrotierend (lat. *laevus*) genutzt werden [104,112]. Diese Deskriptoren korrelieren jedoch nicht mit der tatsächlichen Lage der Atome im Raum [106,112]. Die von Emil Fischer 1891 eigeführten Stereodeskriptoren D und L (geschrieben als sogenannte Kapitälchen)

beruhen auf der absoluten Konfiguration und sind heute noch für Aminosäuren und Kohlenhydrate gebräuchlich [107,108]. Sowohl die Fischer Nomenklatur als auch die Beschreibung der optischen Drehrichtung lassen jedoch nur die Unterscheidung von zwei Enantiomeren zu. Diastereomeren Verbindungen müssen daher zwingend unterschiedliche Substanznamen zugeordnet werden [108]. Aus diesem Grund wird heute meist die von der IUPAC empfohlene Cahn-Ingold-Prelog-Nomenklatur (CIP) genutzt [104,107,112]. Dazu werden die vier Substituenten beginnend mit der höchsten Ordnungszahl priorisiert. Besitzen Atome die gleiche Ordnungszahl, wird die Kette weiterverfolgt, bis eine eindeutige Priorisierung möglich ist. Anschließend wird die Bindung mit der geringsten Priorität (oftmals Wasserstoff) nach hinten gedreht. Erscheinen nun die restlichen Substituenten mit aufsteigender Priorität (1, 2, 3) im Uhrzeigersinn, liegt die (R)-Konfiguration (*rectus*, lat.: rechts) vor. Andernfalls liegt die (S)-Konfiguration (*sinister*, lat.: links) vor [104–106]. Die hier vorgestellten Stereodeskriptoren (optische Drehrichtung, Fischer- oder IUPAC-Nomenklatur) stehen in keinem direkten Zusammenhang und werden daher mitunter nebeneinander angegeben [105,106,112].

Alle in dieser Arbeit untersuchten Substanzen besitzen ein Stereozentrum. Eine Ausnahme bildet Norephedrin, da es zusätzlich an der Seitenkette hydroxyliert ist und somit zwei Stereozentren besitzt. Die (1R,2S) bzw. (1S,2R) konfigurierten Enantiomere werden Norephedrin genannt, die (1S,2S) bzw. (1R,2R) konfigurierten Enantiomere dagegen Norpseudoephedrin [114].

### 1.2.1 Chirale Arzneimittel

Neben ihrer optischen Aktivität unterscheiden sich Enantiomere lediglich in ihrer Wechselwirkung mit anderen chiralen Systemen [104,107]. Da alle proteinogenen Aminosäuren außer Glycin chirale Moleküle sind, bilden die daraus aufgebauten Zielstrukturen wie Rezeptoren, Enzyme, Transporter und Ionenkanäle, mit denen Arzneistoffe und Drogen im menschlichen Körper interagieren, eine chirale Umgebung [104,112,115]. Dementsprechend unterscheidet sich die Pharmakologie von Enantiomeren grundsätzlich [115–117]. Enantiomere können unter anderem unterschiedlich stark an Rezeptoren [118,119] oder Plasmaproteine [120] binden, unterschiedliche Potenz oder Toxizität aufweisen [121,122], agonistisch oder antagonistisch wirken [123,124] und unterschiedlich schnell zu unterschiedlichen Metaboliten verstoffwechselt werden [125,126]. Dabei ist zu beachten, dass unter Umständen auch enantiomerenreine Stoffe im Körper invertieren oder racemisieren können, wie es zum Beispiel für Thalidomid (Contergan®) [127] oder Ibuprofen [125] der Fall ist. Das Enantiomer, welches die gewünschte pharmakologische Wirkung hervorruft oder eine höhere pharmakologische Potenz aufweist, wird

„*Eutomer*“ genannt. Das andere Enantiomer, welches weniger aktiv bzw. inaktiv ist oder sogar unerwünschte bis toxische Effekte hervorruft, wird als „*Distomer*“ bezeichnet [104,108]. Da durch die Gabe des reinen Eutomers die Dosis halbiert und Nebenwirkungen reduziert werden können, werden immer weniger racemische Arzneimittel in Deutschland zugelassen [115,128]. Zur Kennzeichnung von enantiomerenreinen Arzneimitteln werden abgewandelte Präfixe verwendet. Die Zusätze „*Dex*-“ und „*Lev*-“ beziehen sich auf die Fischer-Nomenklatur, „*Ar*-“ oder „*Er*-“ sowie „*Es*-“ auf die CIP-Nomenklatur. Auch der Zusatz „*rac*-“ wird mitunter für racemische Arzneimittel verwendet [129,130].

### 1.2.2 Enantiomerenzusammensetzung illegaler ATS

ATS können über diverse Synthesewege hergestellt werden. Abhängig von den zur Verfügung stehenden Vorläuferstoffen und den entsprechend angewandten Methoden können entweder racemische oder enantiomerenreine Produkte sowie nichtracemische Enantiomerengemische entstehen [18,131–134]. Im Zuge der Umsetzung des *Übereinkommens der Vereinten Nationen gegen den unerlaubten Verkehr mit Suchtstoffen und psychotropen Stoffen* von 1988 [135] kontrollieren die Europäische Union (EU) sowie nationale Gesetzgeber den Verkehr von möglichen Synthesevorläuferstoffen [136–138]. Da viele Edukte und Reagenzien auch von pharmazeutischer oder industrieller Bedeutung sind, sind den Gesetzgebern allerdings Grenzen gesetzt [139,140]. In Deutschland regelt das Grundstoffüberwachungsgesetz (GÜG) den Verkehr von „Grundstoffen, die für die unerlaubte Herstellung von Betäubungsmitteln missbraucht werden können“ [141]. Laut § 2 GÜG liegen ihr die EU-Verordnungen (EG) Nr. 111/2005, (EG) Nr. 1277/2005 und (EG) Nr. 273/2004 zu Grunde. Die kontrollierten Stoffe selbst sind lediglich in den EU-Verordnungen, nicht aber im GÜG gelistet und werden in drei Kategorien eingeteilt. Kategorie 1 umfasst Grundstoffe (Edukte), Kategorie 2 Reagenzien zur Umsetzung und Kategorie 3 Lösemittel und Säuren [138,141].

**Abbildung 4** Schematische Darstellung der stereoselektiven und nicht stereoselektiven Synthesewege für Amphetamin und Methamphetamin (modifiziert nach [142])

Das Syntheseschema in Abbildung 4 zeigt die grundlegenden stereoselektiven und nicht stereoselektiven Synthesewege für Amphetamin und Methamphetamin. Amphetamin wird üblicherweise aus Phenylaceton (IUPAC: 1-Phenyl-2-propanon, kurz P2P) [138,143,144] hergestellt. Die am häufigsten angewandten Methoden sind die reduktive Aminierung und die sogenannte Leuckart-Methode (Leuckart-Wallach-Reaktion) [144,145]. Bei allen gängigen Synthesemethoden ausgehend von P2P entsteht racemisches Amphetamin [18,146,147]. Analog zum Amphetamin kann racemisches Methamphetamin aus P2P hergestellt werden [131,139,148]. Vor allem in Osteuropa und insbesondere in der Tschechischen Republik [138] wird Methamphetamin aus den pharmazeutisch genutzten Vorläuferstoffen (1S,2S)-Pseudoephedrin oder seltener (1R,2S)-Ephedrin hergestellt [43,68,131,148–150]. Da die jeweils anderen Enantiomere in der Natur nicht vorkommen und auch nicht pharmazeutisch

genutzt werden [151,152], entsteht aus diesen Vorläufern unter Retention der absoluten Konfiguration an der 2-Position enantiomerenreines (S)-Methamphetamin [151,153]. Hierfür am häufigsten angewandt werden die Reduktionswege nach Birch, Emde und Nagai sowie die „Moscow“-Route [131,148,149]. Da P2P sowie Ephedrin und Pseudoephedrin kontrollierte Substanzen der Kategorie 1 sind, werden immer häufiger nicht-kontrollierte Vorläufer von P2P - sogenannte *„pre-precursor“*, auch *„Designer-Grundstoffe“* - als Ausgangsstoff für die Synthese verwendet [33,138]. Aufgrund der Nutzung von Chlorephedrin als Ephedrin-Alternative, wurde auch dieses zusammen mit Chlorpseudoephedrin in Kategorie 1 aufgenommen [138,154]. Die Restriktion von Vorläuferstoffen führen zu kontinuierlichen Veränderungen in der Herstellung [18,43,138]. So wurde in den letzten Jahren der Trend beobachtet, das als minderwertig angesehene racemische Methamphetamin mittels Racematspaltung (engl. *chiral resolution*) aufzureinigen [43,131,155]. Bei der sogenannten „Pope-Peachy“-Methode bilden sich durch die Zugabe von Weinsäure diastereomere Salze, welche mittels fraktionierter Kristallisation getrennt werden können [43,155]. Es ist möglich, die unerwünschte Fraktion zu racemisieren [156], um durch erneute Racematspaltung höhere Ausbeuten zu erzielen [157].

MDMA wird analog zu Amphetamin und Methamphetamin über reduktive Aminierung oder die Leuckart-Reaktion aus Piperonylmethylketon (PMK, auch MDP2P) synthetisiert [158–160]. PMK selbst wird oft über die ebenfalls kontrollierten Vorläuferstoffe Safrol, Isosafrol oder Piperonal hergestellt [161,162]. Eine andere Synthesemöglichkeit bildet die Bromierung und anschließende Substituierung von Safrol („Bromopropan-Route“) [158,159]. Die angewandten Synthesemethoden sind nicht stereoselektiv, weshalb illegales MDMA in racemischer Form vorliegt [134,163]. Bis heute gibt es keine Belege für das Auftreten nicht-racemischer Beschlagnahmungen.

Wie für Amphetamin kommen auch für 4-FA diverse (nicht stereoselektive) Syntheserouten ausgehend von entsprechenden 4-fluorierten Analoga in Frage [90]. Dennoch existieren zumindest theoretisch stereoselektive Synthesemethoden oder Methoden zur optischen Aufreinigung [164–166].

### 1.2.3 Enantiomerenzusammensetzung zugelassener Arzneimittel

Das deutsche Betäubungsmittelgesetz (BtMG) unterteilt Substanzen in drei Gruppen (Anlagen). Die in Anlage 1 genannten Substanzen sind generell nicht verkehrsfähig. Stoffe, die in Anlage 2 genannt sind, sind verkehrs- jedoch nicht verschreibungsfähig. In Anlage 3 sind schließlich Betäubungsmittel aufgeführt, die sowohl verkehrs- als auch verschreibungsfähig sind. Mitunter

werden einzelne Enantiomere in unterschiedlichen Anlagen geführt. Dies ist auch für Amphetamin der Fall. Während (R)-Amphetamin (Levamphetamin oder Levamfetamin) in Anlage 2 geführt wird, stehen sowohl racemisches Amphetamin als auch (S)-Amphetamin (Dexamphetamin oder Dexamfetamin) in Anlage 3 und sind somit verschreibungsfähig [167]. In Deutschland sind Amphetaminpräparate ausschließlich für die Behandlung von ADHS zugelassen. Attentin® (Dexamfetaminhemisulfat Tabletten, 5 - 20 mg) ist für Kinder und Jugendliche von 6 bis 17 Jahren zugelassen [168]. Das Retardpräparat Elvanse® (Lisdexamfetamindimesilat) wird im Gastrointestinaltrakt enzymatisch zu L-Lysin und (S)-Amphetamin gespalten (vgl. Abbildung 5) [169]. Es ist für Kinder und Jugendliche ab 6 Jahren (Dosierung 20 - 70 mg) und seit 2019 unter dem Namen Elvanse Adult® (Dosierung 30 - 70 mg) auch für Erwachsene zugelassen [168,170]. Darüber hinaus können amphetaminhaltige Präparate als Individualrezeptur nach dem Neuen Rezeptur-Formularium (NRF) angefertigt werden. Neben enantiomerenreinen Dexamfetaminsulfat-Tropfen (25 mg/mL, NRF 22.9) sind dort auch die racemischen Präparate Amfetaminsulfat-Saft (2 mg/mL, NRF 22.4) und Amfetaminsulfat-Kapseln (5 mg / 10mg, NRF 22.5) geführt [171].

Methamphetamin („Metamfetamin“) wird in Anlage 2 des BtMG geführt, wobei laut der dort angegebenen IUPAC-Bezeichnung lediglich das (S)-Enantiomer gemeint ist. Im Gegensatz zum Amphetamin finden sich für das Racemat oder das (R)-Enantiomer keine separaten Einträge [167]. Diese Diskrepanz lässt den betäubungsmittelrechtlichen Status von reinem (R)-Methamphetamin fraglich erscheinen. Da für (R)-Methamphetamin unter anderem eine sogenannte *nicht geringe Menge* im Sinne des BtMG vorgeschlagen wird [172], legt dies nahe, dass der Eintrag in Anlage 2 des BtMG für alle Enantiomere gleichermaßen Geltung findet. Seit dem Produktionsende von Pervitin® im Jahr 1988 [173,174] ist kein methamphetaminhaltiges Medikament in Deutschland zugelassen. Das auch in Deutschland zugelassene Parkinson Medikament Selegilin wird jedoch zu (R)-Methamphetamin (sowie (R)-Amphetamin) metabolisiert (vgl. Abbildung 5) [175,176]. Etwa in den USA ist (R)-Methamphetamin zur nasalen Vasokonstriktion als Vicks Vapor Inhaler® oder in racemischer Form zur Behandlung von ADHS als Desoxyn® zugelassen [177,178].

**Abbildung 5** Metabolismus von Lisdexamfetamin zu (S)-Amphetamin (oben, modifiziert nach [169]) und von Selegilin zu (R)-Methamphetamin und (R)-Amphetamin (unten, modifiziert nach [179])

In Deutschland sind MDMA sowie 4-FA als nicht verkehrsfähig eingestuft, eine Unterscheidung zwischen den Enantiomeren wird im BtMG nicht vorgenommen [167]. MDMA wird seit den Achtzigerjahren experimentell in der Psychotherapie eingesetzt und aktuelle klinische Studien ergaben vielversprechende Ergebnisse [180]. So zeigte eine placebokontrollierte Phase-III-Studie aus 2021 eine deutliche Verbesserung des Therapieerfolgs bei posttraumatischen Belastungsstörungen (PTBS), wenn den Patienten vor und während der Sitzungen MDMA verabreicht wurde [181].

## 1.3 Pharmakologie

### 1.3.1 Pharmakodynamik

Die Wirkung von ATS beruht auf einer Konzentrationserhöhung von Dopamin, Noradrenalin und Serotonin im synaptischen Spalt der Neuronen des Gehirns [3,8,9]. In diesem Zusammenhang spielen vor allem zwei Wirkmechanismen eine Rolle. Zum einen hemmen ATS die Wiederaufnahme in die Präsynapse und führen zum anderen gleichzeitig zu einer erhöhten Ausschüttung der genannten Katecholamine (vgl. Abbildung 6) [63,64,182]. Die Wirkung von Amphetamin und Methamphetamin beruht hauptsächlich auf der Wirkung von Dopamin und Noradrenalin [3,46,63,64,183]. Die akute Wirkphase dieser Stimulantien ist allgemein geprägt durch Euphorie, Antriebs- und Leistungssteigerung, sowie durch reduziertes Schlafbedürfnis und Hungergefühl [2,10,184,185]. Zu den unerwünschten akuten Nebenwirkungen gehören

beispielsweise Aggressivität, Schlaflosigkeit und zwanghaftes Verhalten [10,186,187]. Die akute Wirkung nach Konsum des Entaktogens MDMA unterscheidet sich von der rein stimulierenden Wirkung von Amphetamin und Methamphetamin. Verbunden mit dem Konzentrationsanstieg von Serotonin im synaptischen Spalt [62–65] wird die Sinneswahrnehmung verändert und Gefühle des Glücks, der Empathie, Nähe und Verbundenheit verstärkt [13,63,188]. Typische Nebenwirkungen bei MDMA-Konsum sind etwa Hyperthermie (Überwärmung), Tachykardie (Herzrasen), Bruxismus (Zähneknirschen), Trismus (Kieferklemme) und Mundtrockenheit [2,189]. Nach Konsum von 4-FA treten sowohl stimulierende Effekte wie nach Amphetamin-Konsum als auch entaktogene Effekte wie nach MDMA-Konsum auf [91,96]. Auch die von 4-FA-Konsumenten am häufigsten berichteten Nebenwirkungen sind entsprechend vergleichbar [91]. Bei Überdosierung von ATS kann es neben psychischen Symptomen wie Agitation (Unruhe) und Verwirrtheit zu schwerwiegenden physischen Akutkomplikationen wie Kreislaufdysregulation, Hyperthermie, Krampfanfällen und kardialen Arrhythmien (Herzrhythmusstörungen) kommen [2,10,34,190,191]. Amphetamin- und Methamphetaminkonsum können zu Hirnblutungen und insbesondere bei vorerkrankten Personen zu tödlich verlaufenden kardialen Arrhythmien oder Infarkten führen [192–195]. MDMA-assoziierte Todesfälle werden oft mit Hyperthermie [196,197] oder dem Serotoninsyndrom [192,198–201] in Verbindung gebracht. Der Konsum von 4-FA kann unter anderem Schlaganfälle, Hirnblutungen oder eine schwerwiegende Takotsubo-Kardiomyoparthie („Stress-Kardiomyopathie“) verursachen [85,89,202,203].

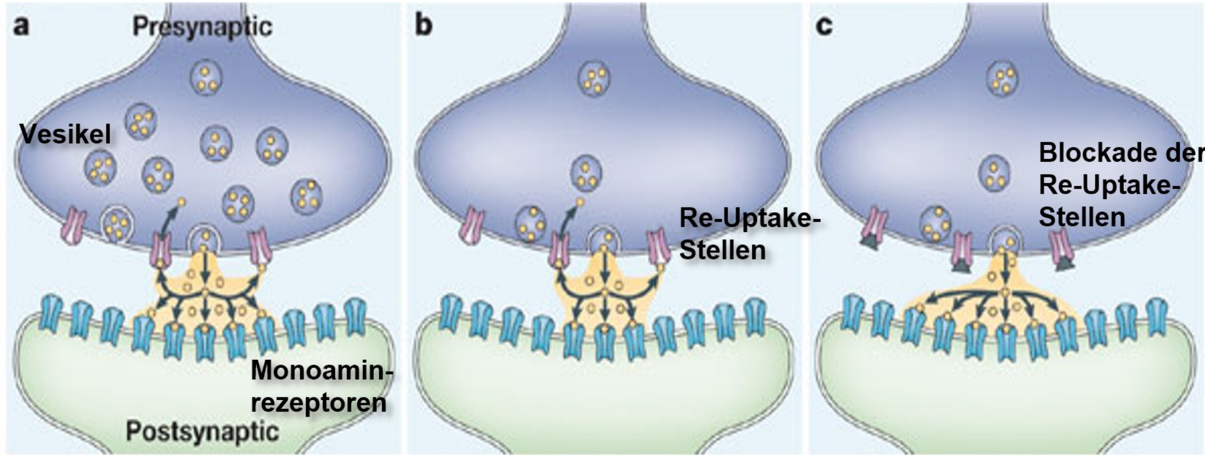


**Abbildung 6** Schematische Darstellung der ATS-induzierten Wiederaufnahmehemmung von Monoaminen im synaptischen Spalt. [13] Mit freundlicher Genehmigung der W. Kohlhammer GmbH.

Die Dauer der akuten Phase nach ATS-Konsum wurde nur in wenigen Studien untersucht und beträgt in der Regel nicht mehr als 6 Stunden. In einer Studie von Holze et al. näherten sich 6 Stunden nach Konsum von (S)-Amphetamin sowohl Vitalparameter als auch subjektive

Wirkungen der Basislinie, wobei das Abklingen jeglicher subjektiver Effekte im Mittel erst nach 8,5 Stunden erfolgte. In der gleichen Studie zeigte sich für MDMA eine etwas kürzere akute Wirkdauer. Im Mittel konnten nach etwa 6 Stunden keinerlei subjektive Effekte mehr wahrgenommen werden, während sich die Vitalparameter in ähnlicher Geschwindigkeit wie nach Amphetaminkonsum der Basislinie näherten [204]. Mendelson et al. beobachteten nach separater Injektion der Methamphetamin-Enantiomere eine Wirkdauer der kardiovaskulären Effekte von maximal 6 Stunden für (S)-Metamphetamin und 4 Stunden für (R)-Methamphetamin. Auch die subjektive Wirkung hielt für das (S)-Enantiomer mit 6 Stunden länger an als für das (R)-Enantiomer mit 3 Stunden [139]. In einer Umfrage unter 4-FA-Konsumenten gaben zwei Drittel der Befragten eine Wirkdauer von maximal 6 Stunden an, etwa 11 Prozent berichteten jedoch von einer Wirkdauer von über 8 Stunden [91]. Auch in einer klinischen Studie zeigte sich 6 Stunden nach Konsum von 4-FA ein erheblicher Rückgang der subjektiven Effekte [96].

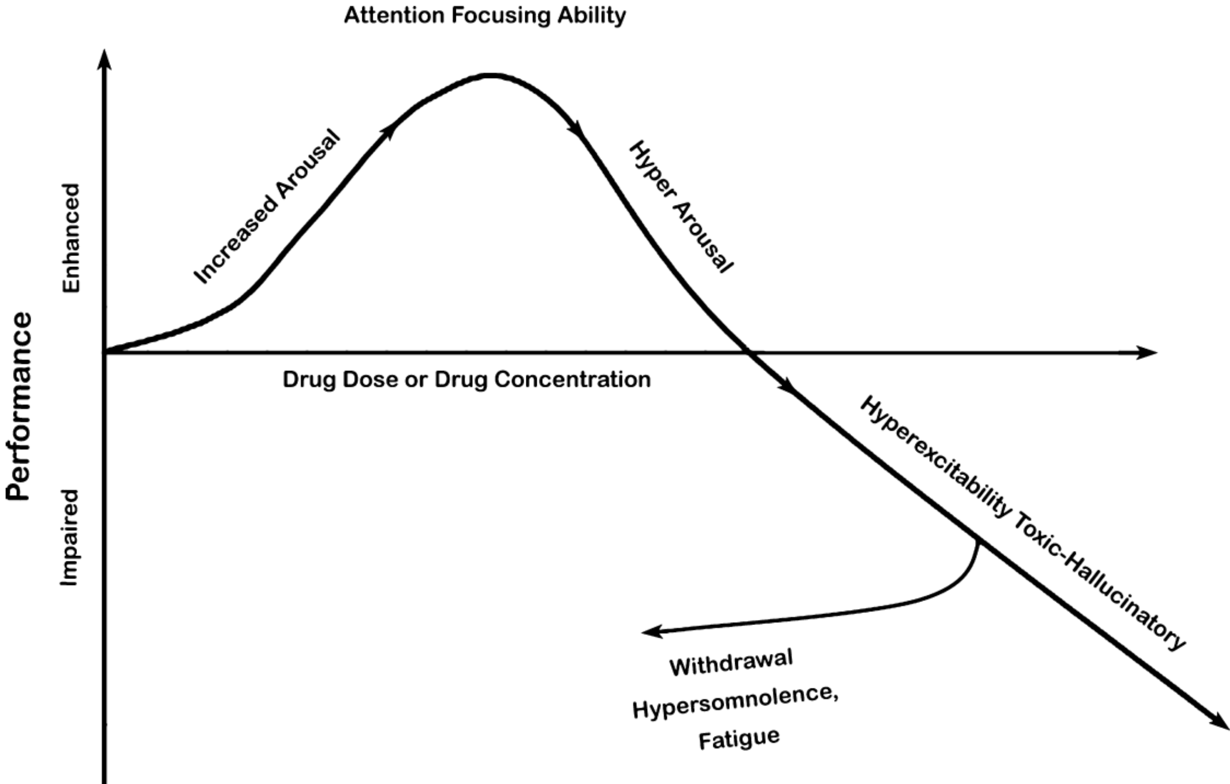


**Abbildung 7** Dosis- und Zeitabhängigkeit der Wirkung von Stimulantien: Zu hohe Dosen wirken sich negativ auf die Leistungsfähigkeit aus. Auf die akute Phase folgen Erschöpfungsreaktionen. [187]

An die akute Wirkungsphase schließt sich die durch Erschöpfungsreaktionen geprägte Subakutphase an. Sie ist typischerweise geprägt durch Symptome wie Müdigkeit, Schlafstörungen, Konzentrations- und Motivationslosigkeit, verzögerte Reaktion sowie

depressive Verstimmungen aber auch Aggression [2,186,187,205–208]. Derartige subakute Effekte können mitunter über Tage andauern [205,207–211]. Abbildung 7 zeigt den dosis- bzw. zeitabhängigen Verlauf der pharmakodynamischen Effekte nach Stimulantienkonsum. Dort ist veranschaulicht, dass sowohl bei Überdosierung als auch in der Subakutphase die geistige Leistungsfähigkeit durch Agitation bzw. durch Erschöpfungssymptome eingeschränkt wird.

Sowohl für Amphetamin und Methamphetamin als auch für MDMA belegen unterschiedliche Studien eine höhere pharmakologische Potenz der jeweiligen (S)-Enantiomere. Smith und Davis fanden in einer Studie mit Einnahme der Amphetamin-Enantiomere eine doppelt so hohe anregende und Euphorie steigernde Potenz des (S)-Enantiomers im Vergleich zum (R)-Enantiomer [212]. Prinzmetal und Alles geben basierend auf einer Studie mit psychosomatischen Patienten für (S)-Amphetamin die drei- bis vierfache zentral stimulierende Potenz an [213]. Anderson et al. fanden in einer klinischen Studie für (S)-MDMA eine im Vergleich zum (R)-Enantiomer etwa dreifach höhere Potenz subjektive Intoxikationssymptome auszulösen. Hierbei ergab sich für das Racemat eine höhere Potenz als sich aus der Summe der beiden Enantiomere ergeben würde [55]. Nishimura et al. schlossen auf Grundlage der an Mäusen beobachteten Auswirkungen auf psychomotorische Effekte auf eine mindestens 10-fach höhere Potenz von (S)-Methamphetamin [150]. In-vitro Studien von Rothmann et al. zeigten für (S)-Methamphetamin vielfach höhere Aktivitäten in der Freisetzung und Wiederaufnahmehemmung von Dopamin, Noradrenalin und Serotonin [64]. Kuczenski et al. fanden bei Experimenten mit Ratten ähnliche Ergebnisse für den Konzentrationsanstieg dieser Neurotransmitter [183]. Darüber hinaus beobachteten Mendelson et al. in Humanexperimenten ähnliche subjektive und kardiovaskuläre Effekte für (S)-Methamphetamin und das Racemat [139].

### 1.3.2 Pharmakokinetik

Die grundlegenden Reaktionen im Metabolismus von Amphetamin und Methamphetamin sind die aromatische Hydroxylierung, die N-Dealkylierung (nur für Methamphetamin), die β-Hydroxylierung, die oxidative Deaminierung und die Oxidation der Seitenkette (vgl. Abbildung 8). Die aromatische Hydroxylierung (1) führt zur Bildung von 4-Hydroxymethamphetamin, 4-Hydroxyamphetamin und 4-Hydroxynorephedrin. Durch N-Dealkylierung (2) von Methamphetamin entsteht Amphetamin und durch β-Hydroxylierung (3) entstehen Norephedrin bzw. 4-Hydroxynorephedrin. Durch Oxidation der Seitenkette (4) entsteht zunächst Phenylaceton, welches zu Benzoesäure oxidiert wird (5) und nach Konjugation mit Glycin (6) als Hippursäure ausgeschieden wird. [126,176,214,215]

**Abbildung 8** Metabolismus von Methamphetamin und Amphetamin (modifiziert nach [126])

Die p-Hydroxylierung und die N-Demethylierung sind Monooxygenierungen und werden von CYP2D6, einem Leberenzym der Cytochrom-P450-Familie, katalysiert [216]. CYP2D6 weist einen genetischen Polymorphismus auf und der Genotyp der Konsumenten kann somit einen Einfluss auf Pharmakokinetik und Pharmakodynamik der ATS haben [217,218]. Etwa 71 Prozent der auch als *Kaukasier* bezeichneten europäischstämmigen Bevölkerung besitzen eine „normale“

Aktivität von CYP2D6 (*extensive metabolizer*), während etwa 1 – 2 Prozent durch Duplikation oder Mutation der Allele eine gesteigerte Aktivität besitzen (*ultrarapid metabolizer*) [219]. Dagegen besitzen etwa 5 – 10 Prozent zwei nicht funktionierende Allele und weisen somit eine deutlich verringerte Aktivität von CYP2D6 auf (*poor metabolizer*) [219,220]. CYP2D6 zeichnet sich darüber hinaus durch eine Selektivität bezüglich der absoluten Konfiguration seiner Substrate aus [221–223]. So kann nach Konsum von racemischem Methamphetamin oder Amphetamin in der Regel eine höhere Konzentration des (R)-Enantiomers im Blut nachgewiesen werden [139,224]. Die Serumhalbwertszeit von (R)-Amphetamin ist im Vergleich zum (S)-Enantiomer je nach pH-Wert des Urins um 13 – 238 Prozent höher [39,225]. Mendelson et al. fanden bei Aufnahme von racemischem Methamphetamin eine 40 Prozent längere Serumhalbwertszeit für das (R)-Enantiomer [139].

Die β-Hydroxylierung wird von der Dopamin-β-Hydroxylase katalysiert, welche im zentralen Nervensystem (ZNS) und peripheren Nervensystem (PNS) lokalisiert ist [126,224,226]. Die β-Hydroxylierung ist auf primäre Amine beschränkt und findet darüber hinaus selektiv nur für die (S)-Enantiomere von Amphetamin und der entsprechenden Metabolite statt [126,224]. Die Hydroxygruppe wird in der (R)-Konfiguration eingeführt [126], sodass entsprechend lediglich die (1R,2S)-Formen von Norephedrin und p-Hydroxynorephedrin gebildet werden.

In der einzigen pharmakokinetischen Studie mit Aufnahme von 4-FA konnten zwei Diastereomere von 4-Fluornor(pseudo)ephedrin und ein ringhydroxylierter Metabolit, bei dem es sich wahrscheinlich um 3-Hydroxy-4-fluoramphetamin handelte, im Urin nachgewiesen werden [227]. Im Serum konnten diese Metabolite nicht nachgewiesen werden [228].

Abbildung 9 zeigt schematisch den Metabolismus von MDMA. Der primäre Metabolismusweg von MDMA erfolgt über Demethylenierung (1) zum 3,4-Dihydroxymethamphetamin (HHMA) und anschließende Methylierung (2) der Hydroxygruppe in 3-Position zum 4-Hydroxy-3-methoxymethamphetamin (HMMA). Darüber hinaus wird MDMA *N*-demethyliert (3) zum 3,4-Methylendioxyamphetamin (MDA). Durch anschließende Demethylenierung entsteht 3,4-Dihydroxyamphetamin (HHA) und durch weitere Methylierung 4-Hydroxy-3-methoxyamphetamin (HMA). [221,229,230] Die Demethylenierung wird hauptsächlich durch CYP2D6 katalysiert [221,231–233]. Wie auch für Amphetamin und Methamphetamin beobachtet, zeigt das (R)-Enantiomer von MDMA signifikant höhere (61 – 208 Prozent) Serumhalbwertszeiten [134,163,230,233].

MDMA
3,4-Methylendioxymethamphetamin

MDA
3,4-Methylendioxyamphetamin

HHMA
3,4-Dihydroxymethamphetamin

HHA
3,4-Dihydroxyamphetamin

HMMA
4-Hydroxy-3-methoxymethamphetamin

HMA
4-Hydroxy-3-methoxyamphetamin

**Abbildung 9** Metabolismus von MDMA (modifiziert nach [229])

### 1.3.3 Plasmaproteinbindung

Neben Zielstrukturen im ZNS binden chemische Stoffe im Körper auch unspezifisch an Plasmaproteine [234–237]. Basische Substanzen wie ATS werden vor allem durch das *saure Alpha1-Glykoprotein* (AGP, auch *Orosomucoid*) gebunden. Dieses ist im menschlichen Blut mit einer Konzentration von etwa 12 – 30 µmol/L vorhanden und ist somit in der Lage, die freie (ungebundene) Plasmakonzentration erheblich zu beeinflussen [234,236–239]. Der *free drug hypothesis* zufolge können nur ungebundene Stoffe mit Zielstrukturen wie Rezeptoren,

Transportern und Enzymen interagieren und das Ausmaß der Plasmaproteinbindung (PPB) hat somit einen wesentlichen Einfluss auf Pharmakodynamik und Pharmakokinetik [234,235,237,239,240]. Das Ausmaß der PPB kann je nach Substrat erheblich variieren und beträgt für Amphetamin und Methamphetamin etwa 20 Prozent [241–243]. Die Stereoselektivität der Bindung zu AGP wurde bereits für andere Substanzen gezeigt [120,244], ist jedoch für ATS größtenteils unerforscht. Einzig Wan et al. (1978) untersuchten die PPB von (S)- und (R)-Amphetamin und fanden keine Unterschiede [225].

Die Ultrafiltration (UF) bietet eine einfache und kostengünstige Möglichkeit, die PPB zu ermitteln. Hierbei werden Plasmaproteine wie AGP zusammen mit denen an sie gebundenen Substanzen mittels einer Cellulosemembran durch Zentrifugation abgetrennt. Während die Proteine aufgrund ihrer Molekülgröße im Probenreservoir verbleiben, kann das Filtrat zusammen mit dem ungebundenen Anteil der Substanzen durch die Membran dringen. Durch Quantifizierung des ungebundenen Anteils im Ultrafiltrat kann so die PPB errechnet werden. [245–247]

## 1.4 Forensische Toxikologie

Die Forensische Toxikologie dient dem Zweck des Nachweises oder des Ausschlusses von Vergiftungen in rechtlich relevanten Fällen. Neben dem Nachweis absichtlicher Vergiftungen durch fremde Hand und akzidentieller Vergiftungen stehen hierbei vor allem absichtliche Vergiftungen durch eigene Hand im Vordergrund. Diese geschehen abgesehen von der suizidalen Absicht vor allem mit dem Ziel sich zu berauschen. Der Einfluss von Drogen und Arzneimitteln spielt etwa bei der Beurteilung der Schuldfähigkeit oder Fahrtüchtigkeit eine Rolle. [248–250]

Als Untersuchungsmatrices werden meist Blut, Urin oder Haare verwendet, wobei auch Matrices wie Speichel, Schweiß oder Mageninhalt bei Lebenden in Frage kommen. Bei der Postmortem Toxikologie kommen neben Femoralvenenblut, Herzblut und Urin auch Organe oder Körperflüssigkeiten wie Liquor oder Glaskörperflüssigkeit als Untersuchungsmaterial in Betracht. Auch die Untersuchung verdächtiger Stoffproben wie Tabletten, Flüssigkeiten und Pulver fällt in das Aufgabengebiet der Forensischen Toxikologie. [249]

Für die Begutachtung von Intoxikationen durch Drogen oder Medikamente ist Blut bzw. Blutserum oder Blutplasma die bevorzugte Matrix, da die Wirkstoffkonzentration im Blut Rückschlüsse auf eine Beeinflussung zulässt [249,251]. Für die Beurteilung der Fahrtüchtigkeit oder Schuldfähigkeit ist eine Blutabnahme somit zwingend erforderlich. Die Nachweisbarkeitsdauer ist dagegen im

Blut beschränkt und beträgt bei ATS in der Regel nur wenige Tage [252,253]. Die Untersuchung von Urin und insbesondere von Haaren ermöglicht den Nachweis eines Konsums über einen deutlich längeren Zeitraum, lässt jedoch keine Rückschlüsse auf eine akute Beeinflussung zu [249,252]. Neben den Muttersubstanzen selbst können im Blut oftmals auch Metabolite nachgewiesen werden, deren Nachweis zusätzliche Schlüsse bezüglich Beeinflussung oder Konsumhäufigkeit erlaubt [254–256].

### 1.4.1 Rechtlicher Hintergrund

Der Umgang und der Konsum von Betäubungsmitteln wie Stimulantien kann weitreichende straf- und zivilrechtliche Konsequenzen nach sich ziehen, auf die im Folgenden eingegangen werden soll. Forensisch-toxikologische Analysenbefunde und Gutachten geben der Rechtsprechung in diesen Fällen Anhaltspunkte für die Beurteilung des Tatbestandes.

Das BtMG regelt in Deutschland den Verkehr mit Betäubungsmitteln und definiert entsprechende Straftaten und Ordnungswidrigkeiten. § 29 BtMG verbietet unter anderem unerlaubten Anbau, Herstellung, Handel, Ein- und Ausfuhr, Veräußerung, Abgabe und Inverkehrbringung von Betäubungsmitteln. In bestimmten Fällen kann jedoch von einer Strafe (§ 29 Abs. 5 BtMG) oder Verfolgung (§ 31a Abs. 1 BtMG) absehen werden, wenn es sich um eine sogenannte *geringe Menge* zum Eigenverbrauch handelt. Die geringe Menge soll sich dabei nach dem Augenblicks- oder Tagesbedarf eines nicht abhängigen Konsumenten richten und umfasst maximal drei Konsumeinheiten. Darüber hinaus definiert das BtMG den Begriff der nicht geringen Menge (§§ 29 Abs. 1, 30 Abs. 1, 30a Abs. 1 und 2 BtMG) als solche, bei deren Überschreitung unabhängig vom Eigenverbrauch stets ein Verbrechen vorliegt, das mit einer Mindestfreiheitsstrafe von einem Jahr bedroht ist. Sowohl geringe als auch nicht geringe Menge sind nicht rechtsverbindlich definiert, jedoch wurden von der Rechtsprechung entsprechende Grenzwerte ausgearbeitet. Die Feststellung der Menge bedarf im Einzelfall einer Bestimmung des vorliegenden Wirkstoffgehaltes. [257]

Laut § 316 StGB (Strafgesetzbuch) wird mit Geldstrafe oder Freiheitsstrafe bis zu einem Jahr bestraft, wer ein Fahrzeug führt obwohl er „infolge des Genusses alkoholischer Getränke oder anderer berauschender Mittel [...] nicht in der Lage ist, das Fahrzeug sicher zu führen". Wenn darüber hinaus „Leib oder Leben eines anderen Menschen oder fremde Sachen von bedeutendem Wert gefährdet" werden, drohen dem Fahrer nach § 315c StGB Freiheitsstrafen bis zu fünf Jahren.

Gemäß § 24a StVG Abs. 2 (Straßenverkehrsgesetz) handelt ordnungswidrig, „wer unter der Wirkung eines in der Anlage zu dieser Vorschrift genannten berauschenden Mittels im Straßenverkehr ein Kraftfahrzeug führt“. Der Nachweis einer Wirkung gilt oberhalb eines „analytischen Grenzwertes“, der von der *Gemeinsamen Arbeitsgruppe für Grenzwertfragen und Qualitätskontrolle* (Grenzwertkommission) für die in der Anlage genannten Stimulantien Amphetamin, Methamphetamin und MDMA auf jeweils 25 ng/mL festgelegt wurde [258]. Die Empfehlungen der Grenzwertkommission können dem Gericht als Anhaltspunkt für eine Beurteilung dienen, allerdings muss eine Mindestgrenze nicht zwingend überschritten sein. Laut Urteil des Bundesverfassungsgerichtes [259], welches in seiner Geltung auf alle Substanzen des § 24a StVG ausgeweitet wurde, muss vielmehr eine Konzentration vorliegen, die es geeignet erscheinen lässt, die Fahrtüchtigkeit einzuschränken. Somit steht ähnlich der §§ 315c und 316 StGB auch bei der Auslegung des § 24a StVG die momentane Wirkung der Betäubungsmittel zum Tatzeitpunkt im Vordergrund [260]. Entscheidend für einen möglichen Entzug der Fahrerlaubnis ist darüber hinaus das vom Konsumenten geforderte Trennungsvermögen und somit auch der zeitliche Zusammenhang von Konsum und Führen eines Kraftfahrzeuges [261,262]. Keine Ordnungswidrigkeit nach § 24a StVG Abs. 2 liegt vor, „wenn die Substanz aus der bestimmungsgemäßen Einnahme eines für einen konkreten Krankheitsfall verschriebenen Arzneimittels herrührt“. Dieses sogenannte *Medikamentenprivileg* ist somit insbesondere bei der forensisch-toxikologischen Begutachtung von Blutproben von ADHS-Patienten zu beachten [23].

Nach § 20 StGB besteht Schuldunfähigkeit, wenn der Beschuldigte „wegen einer tiefgreifenden Bewusstseinsstörung [...] unfähig ist, das Unrecht der Tat einzusehen oder nach dieser Einsicht zu handeln“. Verminderte Schuldfähigkeit besteht nach § 21 StGB, wenn die Fähigkeit zur Einsicht erheblich vermindert ist. Wird das Vorliegen eines akuten drogeninduzierten Rauschzustandes belegt, kann entsprechend die Schuldfähigkeit zugunsten des Beschuldigten gemindert werden [263]. Für die Beurteilung der Schuldfähigkeit sowie von Verstößen gegen §§ 315c und 316 StGB existieren keine Grenzwerte, da aufgrund der interindividuellen Wirkungsunterschiede eine individuelle und differenzierte Betrachtung notwendig ist.

### 1.4.2 Analytik

#### 1.4.2.1 Hochleistungsflüssigkeitschromatographie

Die Hochleistungsflüssigkeitschromatographie (engl.: *high performance liquid chromatography*, HPLC) ist eine der wichtigsten analytischen Methoden in der Forensischen Toxikologie [248]. Hierbei wird ein Aliquot der zuvor aufgearbeiteten Probe mittels Autosampler in das chromatographische System injiziert. Die sogenannte mobile Phase ist bei der HPLC flüssig und besteht in der Regel aus einer wässrigen Komponente (A) und einer organischen Komponente (B). Das Substanzgemisch wird mittels leistungsstarker Pumpen durch dünne Kapillaren zu einer chromatographischen Trennsäule gepumpt. Die Säule ist auf der Innenseite mit Kieselgel besetzt, welches wiederum mit speziellen chemischen Gruppen modifiziert ist und die sogenannte stationäre Phase bildet. Durch unterschiedlich starke Wechselwirkung der Analyten mit der stationären Phase werden bestimmte Substanzen länger retardiert (zurückgehalten) als andere. Durch Anpassung der Zusammensetzung der mobilen Phase mit der Zeit (Gradientenelution) kann die chromatographische Trennung so optimiert werden, dass die Analytmoleküle ausreichend voneinander und von störenden Matrixbestandteilen getrennt werden. [248,264,265]

Auch Enantiomere lassen sich flüssigkeitschromatographisch voneinander trennen. Neben der Möglichkeit der Derivatisierung von chiralen Analytmolekülen zu Diastereomeren, die auf herkömmlichen Säulen trennbar sind, existiert heute eine Vielzahl chiraler Säulen. Die chromatographische Trennung basiert hierbei auf der Bildung von intermediären diastereomeren Assoziationskomplexen der Enantiomere mit der chiralen stationären Phase. Bedingt durch unterschiedliche Dissoziationskonstanten dieser Komplexe wird ein Enantiomer länger retardiert als das andere (vgl. Abbildung 10). [264,266]

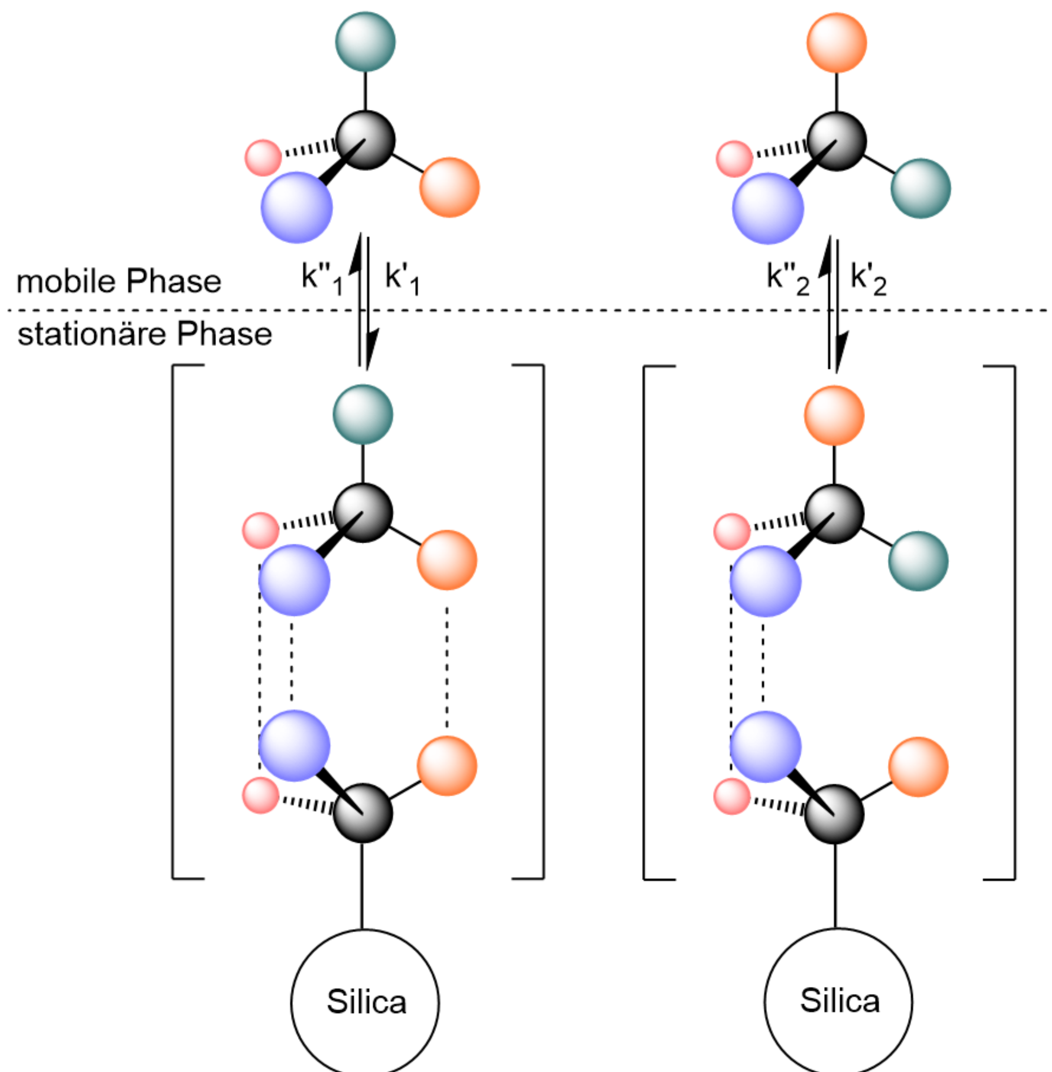


**Abbildung 10** Trennungsprinzip einer chiralen stationären Phase (modifiziert nach [266] und [267]). Das linke Enantiomer hat eine größere attraktive Wechselwirkung mit dem chiralen Selektor der stationären Phase. Der diastereomere Komplex hat somit eine höhere Komplexbildungskonstante und ist stabiler. Die chromatographische Trennung basiert auf den unterschiedlichen Assoziationskonstanten (k'1, k'2) und Dissoziationskonstanten (k"1, k"2).

#### 1.4.2.2 Tandem-Massenspektrometrie

In der Forensischen Toxikologie wird die HPLC zur Detektion der chromatographisch aufgetrennten Analyte meist mit Massenspektrometern gekoppelt. Als sogenannte Ionenquelle wird häufig die Elektrosprayionisation (ESI) verwendet. Hierbei wird die Flüssigkeit kontinuierlich mittels einer Kapillare versprüht und gleichzeitig elektrisch geladen. Durch Verdampfen des Lösungsmittels überträgt sich die Ladung auf die Analytmoleküle, die anschließend in das

Hochvakuum des Massenspektrometers gelangen. Für basische Analyten wie ATS ist die positive Ionisierung geeignet. Jedes ATS-Molekül wird hierbei einfach protoniert und die Atommasse des $[M+H]^+$-Ions wird somit um 1 erhöht. [248,268,269]

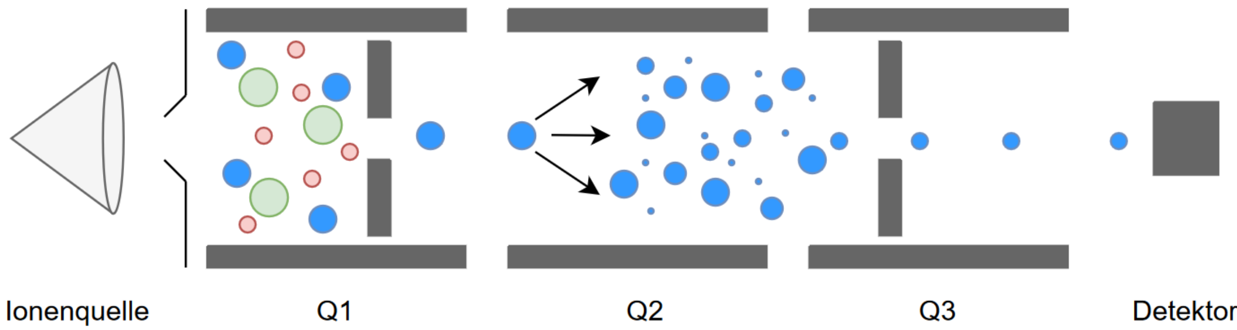


**Abbildung 11** Schematische Darstellung eines Triple-Quadrupol-Massenspektrometers (modifiziert nach [270])

Bei der sogenannten Tandem-Massenspektrometrie (MS/MS), bei der eine besonders hohe Selektivität und Sensitivität erreicht werden kann, werden zwei Massenspektrometer miteinander gekoppelt. Am häufigsten werden hierfür die kostengünstigen und kompakten Triple-Quadrupol-Massenspektrometer (QqQ) eingesetzt (vgl. Abbildung 11). Im ersten Quadrupol (Q1) werden die Vorläuferionen (engl. *precursor*) nach ihrem Masse-zu-Ladungsverhältnis (m/z) ausgewählt. Aufgrund der elektrischen Spannung, die an die Stäbe des Quadrupols angelegt wird, können sich nur Ionen mit einem bestimmten m/z auf einer stabilen, oszillierenden Flugbahn bewegen und anschließend in die sogenannte Kollisionszelle (Q2) gelangen. Dort treffen die Ionen auf das sogenannte Kollisionsgas (z.B. Stickstoff) und zerfallen durch kollisionsinduzierte Dissoziation (engl. *collision induced dissociation*, CID) in spezifische Molekülfragmente. Diese werden wiederum im dritten Quadrupol (Q3) herausgefiltert. Das sogenannte *multiple reaction monitoring* (MRM) erlaubt durch schnelle Variation der Spannungen an den Quadrupolen die nahezu gleichzeitige Detektion mehrerer Analyten. Die Tochterionen treffen letztendlich auf einen Detektor, der bei Auftreffen eines Ions ein elektrisches Signal wiedergibt [268,269,271]. Hierzu werden zum Beispiel Sekundärelektronenvervielfacher (SEV) oder sogenannte Channeltrons (engl. *channel electron multiplier*, CEM) verwendet [271].

#### 1.4.2.3 Validierung

Um verlässliche und gerichtsfeste Ergebnisse zu gewährleisten, ist die Validierung einer analytischen Methode in der forensischen Toxikologie von entscheidender Bedeutung. Die Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) hat zu diesem Zweck einen Validierungsleitfaden erarbeitet [272], deren Parameter im Folgenden dargelegt werden sollen.

**Selektivität und Spezifität** Die Selektivität beschreibt die Fähigkeit einer Methode, verschiedene Analyten eindeutig, also ohne gegenseitige Störung oder Störungen von anderen Probenbestandteilen wie Metaboliten oder Matrix, zu identifizieren. Die Spezifität beschreibt dagegen die Fähigkeit einen einzigen Analyten oder eine Substanzklasse ohne Verfälschung durch andere in der Probe vorhandene Komponenten zu identifizieren.

**Linearität** Für den gewählten Kalibrationsbereich einer Methode muss ein geeignetes Maß an Linearität gezeigt werden. Dies ist der Fall, wenn Peakflächenverhältnisse und Sollkonzentrationen der Kalibratoren direkt proportional zueinander sind.

**Genauigkeit, Richtigkeit und Präzision** Die Genauigkeit beschreibt den Abstand eines einzelnen Messwertes vom Sollwert. Die Richtigkeit einer Methode beschreibt den mittleren Abstand einer ausreichend großen Anzahl an Messwerten vom Sollwert, welcher durch systematische Fehler (Bias) bedingt ist. Die Präzision ist Ausdruck für zufällige Fehler und beschreibt das Maß der Streuung von Messwerten um den Mittelwert.

**Stabilität** Die chemische Stabilität von Analyten in einer gegebenen Matrix wird unterschieden in Langzeitstabilität, Einfrier-/Auftaustabilität und Stabilität der aufgearbeiteten Probe. Letztere ist methodenabhängig und muss zwingend ermittelt werden.

**Analytische Grenzen** Die Nachweisgrenze (engl. *limit of detection*, LOD) beschreibt die geringste Konzentration eines Analyten in der Probe, die nötig ist, um die Substanz eindeutig zu identifizieren. Die Bestimmungsgrenze (engl. *limit of quantification*, LOQ) beschreibt die geringste Konzentration, die eine Quantifizierung mit akzeptabler Genauigkeit und Präzision zulässt.

**Wiederfindung und Matrixeffekte** Die Wiederfindung beschreibt das in der aufgearbeiteten Probe (Matrixprobe) wiedergefundene absolute Messsignal im Vergleich zu einer nachträglich mit Originallösung versetzten aufgearbeiteten Probe (Extrakt). Matrixbestandteile können das Messsignal direkt oder indirekt verstärken (engl. *Ion enhancement*) oder unterdrücken (engl. *Ion suppression*). Die Matrixeffekte werden durch Vergleich der Peakfläche des Extraktes mit der Peakfläche von Reinsubstanzlösungen (Kontrollen) ermittelt.

## 1.5 Fragestellungen und Zielsetzung

Der Konsum von *amphetamine-type stimulants* (ATS, Stimulantien des Amphetamin-Typs) ist weltweit verbreitet und kann bei Konsumenten erhebliche psychische und physische Beeinträchtigungen hervorrufen. Diese können sowohl durch die stimulierende Akutwirkung als auch durch subakute Erschöpfungssymptome bedingt sein. In der Forensischen Toxikologie spielen Beeinträchtigungen durch ATS-Konsum vor allem im Zusammenhang mit Verkehrsdelikten eine große Rolle, wobei hier die Evaluation der Fahrtüchtigkeit von zentraler Bedeutung ist. Sowohl bei der Bewertung von Delikten zu §§ 315c und 316 StGB als auch zu § 24a StVG gilt es daher, die Konsumangaben des Beschuldigten zu verifizieren oder zu widerlegen. Insbesondere die Einlassung, dass der Konsum bereits sehr weit zurückliege und daher kein Betäubungsmitteleinfluss mehr vorliege, wird in diesem Zusammenhang häufig abgegeben. Ferner erklären Beschuldigte einen positiven Amphetaminbefund gelegentlich mit der Einnahme der (S)-amphetaminhaltigen Medikamente Elvanse® oder Attentin® und berufen sich auf das sogenannte Medikamentenprivileg nach § 24a StVG.

Auch bei Fragestellungen zur Schuldfähigkeit gilt es, eine Einschätzung der Beeinflussung zum Tatzeitpunkt zu geben. Durch eine vorgebliche Bewusstseinsstörung aufgrund einer Beeinflussung durch ATS-Konsum kann es im Sinne des Beschuldigten sein, eine Strafmilderung nach §§ 20 oder 21 StGB zu erwirken. Auch zur Beurteilung der Schuldfähigkeit ist somit zu prüfen, ob die Konsumangaben mit den toxikologischen Befunden zu vereinbaren sind.

Die forensisch-toxikologische Beurteilung der Beeinflussung durch ATS-Konsum ist jedoch mit der herkömmlichen (achiralen) Laboranalytik, also durch die Feststellung absoluter Serumkonzentrationen kaum möglich. Eine Vielzahl unbekannter Faktoren macht eine genauere Einschätzung nahezu unmöglich. So ist vor allem die Konsummenge, die insbesondere bei chronisch abhängigen Konsumenten deutlich erhöht sein kann, in der Regel unbekannt. Serumkonzentrationen sind zudem abhängig von der Konsumform und können durch wiederholten Konsum (Bingekonsum) kumulieren. So können auch längere Zeit nach Konsumende bei anhaltend hohen Serumspiegeln bereits Symptome der Subakutphase auftreten.

Da illegale ATS mit Ausnahme des Methamphetamins in der Regel als Racemat konsumiert werden, der Metabolismus jedoch enantioselektiv ist, bieten sich durch stereoselektive Analytik weitere forensische Interpretationsmöglichkeiten. Obwohl die Stereoselektivität schon seit Jahrzehnten bekannt ist und verschiedene Studien vielversprechende Resultate ergeben haben,

bleibt das Potential der stereoselektiven Analytik im forensischen Anwendungsbereich noch weitgehend unausgeschöpft.

Voraussetzung für die forensische Forschung ist die Entwicklung geeigneter analytischer Methoden. Ziel war also zunächst die Trennung der Enantiomere von Amphetamin, Methamphetamin, 4-FA, 4-Hydroxyamphetamin, Norephedrin, MDMA. Im Anschluss sollte eine Validierung für die Analyse humaner Serumproben nach der Richtlinie der GTFCh erfolgen.

### 1.5.1 Bestimmung der Enantiomerenzusammensetzung von beschlagnahmten Stoffproben

Die Kenntnis der Enantiomerenzusammensetzung der konsumierten Drogen ist Voraussetzung für weitere forensische Evaluationen. In den letzten Jahren häufen sich Berichte, die auf eine Veränderung der chiralen Zusammensetzung von illegalem Methamphetamin hindeuten. Dennoch wird die Zusammensetzung von beschlagnahmten Methamphetaminproben nur selten bestimmt. Eine Kontrolle der chiralen Zusammensetzung von Amphetamin- und MDMA-Proben, für die eine racemische Zusammensetzung angenommen wird, findet de facto nicht statt. Auch für diese Substanzen kommen jedoch enantioselektive Synthesemethoden oder Racematspaltung in Frage. Insbesondere die pharmakologische Potenz der Produkte könnte durch eine veränderte Enantiomerenzusammensetzung beeinflusst werden. Des Weiteren kann die nicht geringe Menge abhängig von der chiralen Zusammensetzung ausgelegt werden. Ferner ist die Kenntnis der Zusammensetzung von Straßenamphetamin für die Differenzierung von einer therapeutischen Einnahme (S)-amphetaminhaltiger Medikamente von grundlegender Bedeutung.

Ziel dieser Studie ist die chirale Charakterisierung von beschlagnahmten Stoffproben von Amphetamin, Methamphetamin und MDMA. Die Ergebnisse sollen im Hinblick auf die mögliche Anwendung alternativer Synthesetechniken sowie von Racematspaltung diskutiert werden. Es soll überprüft werden, ob und, wenn ja, für welche ATS eine chirale Untersuchung routinemäßig stattfinden sollte. Darüber hinaus soll abhängig von der Enantiomerenzusammensetzung der Drogen die Zulässigkeit einer forensischen Interpretation von Enantiomerenverhältnissen im Blut geklärt werden. Zu diesem Zweck sollen in Kooperation mit dem Landeskriminalamt (LKA) Rheinland-Pfalz Stoffproben von Amphetamin und MDMA gesammelt werden. Da dort nur sehr selten Methamphetaminproben asserviert werden, sollen entsprechende Daten vom Institut für Rechtsmedizin der Universität Erlangen in die Studie aufgenommen und diskutiert werden. Die Ergebnisse dieser Studie finden sich in Kapitel 2.

### 1.5.2 Chirale Serum Pharmakokinetik von 4-Fluoramphetamin nach kontrollierter oraler Aufnahme

Am besten geeignet für die Erforschung des Metabolismus von Substanzen sind Serumproben, die aus klinischen Studien nach kontrollierter Aufnahme stammen. Solche Studien sind jedoch für Betäubungsmittel aufgrund der heutigen forschungsethischen Richtlinien sowie des damit verbundenen hohen Kostenaufwandes eine Seltenheit. Solche Serumproben konnten aus einer Kooperation mit der Universität Maastricht gewonnen werden. Die Proben aus dieser Studie mit kontrollierter oraler Einnahme von zwei verschiedenen Dosen 4-FA sollen auf den zeitlichen Verlauf der Konzentrationen von (R)- und (S)-4-FA untersucht werden.

Ziel dieser Studie ist die Untersuchung des enantioselektiven Metabolismus von 4-FA, welcher bis dato noch nicht belegt wurde. Hierzu sollen Unterschiede in Absorption und Elimination anhand pharmakokinetischer Parameter wie maximale Serumkonzentration ($c_{max}$), Zeit zum Erreichen der maximalen Serumkonzentration ($t_{max}$) und Serumhalbwertszeit ($t_{1/2}$) untersucht werden. Darüber hinaus soll der zeitliche Verlauf der (R)/(S)-Konzentrationsverhältnisse evaluiert werden. Hierbei soll ein besonderes Augenmerk auf die interindividuellen Unterschiede sowie eine mögliche Dosisabhängigkeit des Verlaufs gelegt werden. Die Ergebnisse sollen anschließend im Hinblick auf forensische Fragestellungen diskutiert werden, insbesondere bezüglich der Möglichkeit einer Einschätzung des Konsumzeitpunktes und der Etablierung von Entscheidungsgrenzen (*Cut-offs*) für die forensische Begutachtung. Die Ergebnisse dieser Studie finden sich in Kapitel 3.

### 1.5.3 Enantioselektive Bestimmung von Amphetamin und Metaboliten in Serumproben

Besonders relevant ist eine Zeiteinschätzung für das in Deutschland meistkonsumierte ATS Amphetamin. Da hierfür keine Proben aus einer klinischen Studie verfügbar sind, soll auf Serumproben von psychiatrischen Patienten zurückgegriffen werden. Bei Stationsaufnahme für den freiwilligen Entzug können Patienten Angaben zu ihrem letzten Konsumzeitpunkt machen. Darüber hinaus sollen Serumproben aus dem Probengut der Forensischen Toxikologie Mainz untersucht werden, welche im Rahmen polizeilicher Ermittlungsverfahren entnommen wurden. Neben (R)- und (S)-Amphetamin sollen in allen Proben auch die Enantiomere der Metabolite 4-Hydroxyamphetamin und Norephedrin quantifiziert werden, deren Vorkommen in Serumproben nur spärlich belegt ist.

Ziel dieser Studie ist die Beschreibung der Konzentrationen von (R)- und (S)-Amphetamin in einem großen Kollektiv an Polizeiproben, insbesondere im Hinblick auf die Verteilung und Streuung der Enantiomerenverhältnisse. Das Vorhandensein, die Konzentrationen und die Verhältnisse der Enantiomere von 4-Hydroxyamphetamin und Norephedrin sollen ebenfalls beschrieben werden. Durch erneute Untersuchung von Teilen dieser Proben nach zehn Monaten soll darüber hinaus die überprüft werden, ob die Verhältnisse von (R)- und (S)-Amphetamin bei Lagerung stabil sind und somit eine forensische Interpretation legitimieren. Analog zu den Untersuchungen zu 4-FA soll durch Untersuchung der Psychiatrieproben der zeitliche Verlauf der (R)/(S)-Konzentrationsverhältnisse für Amphetamin evaluiert werden. Dazu soll das von den Patienten angegebene Zeitintervall zum letzten Konsum sowie möglicher Bingekonsum berücksichtigt werden. Der Verlauf und die Korrelation der (R)/(S)-Verhältnisse mit der Zeit soll denen von 4-FA und MDMA gegenübergestellt werden. Darüber hinaus soll die ermittelte Steigung mit denen aus Literaturdaten für Amphetamin errechneten theoretischen Steigungen verglichen werden. Die Ergebnisse sollen letztendlich hinsichtlich einer forensischen Anwendung und der Möglichkeit einer Zeiteinschätzung evaluiert werden. Die Ergebnisse dieser Studie finden sich in Kapitel 4.

### 1.5.4 Enantioselektive Bestimmung der Plasmaproteinbindung von gebräuchlichen ATS

Die Plasmaproteinbindung (PPB) von ATS ist nur sehr unzureichend erforscht und die teilweise aus Tierversuchen gewonnenen Daten werden in der Literatur mitunter irreführend oder falsch zitiert. Die Unterschiede in der Pharmakodynamik und Pharmakokinetik der Enantiomere von ATS könnten unter anderem gemäß der *free drug hypothesis* durch unterschiedliche Proteinbindung erklärt werden.

Ziel dieser Studie ist die enantioselektive Bestimmung der PPB von Amphetamin, Methamphetamin, MDMA und 4-FA. Hierzu soll die freie Fraktion von mit diesen Substanzen gespikten Leerserumproben mittels Ultrafiltration (UF) abgetrennt werden. Durch Quantifizierung des ungebundenen Anteils im Ultrafiltrat soll so die PPB errechnet werden. Auf diese Weise sollen Unterschiede in der PPB der Substanzen im Allgemeinen sowie mögliche Unterschiede in der PPB der Enantiomere ermittelt werden. Ein möglicher Einfluss der PPB auf die pharmakodynamischen und pharmakokinetischen Eigenschaften der Enantiomere soll diskutiert werden. Die Ergebnisse dieser Studie finden sich in Kapitel 5.

# 2 Bestimmung der Enantiomerenzusammensetzung von in Süddeutschland beschlagnahmten Amphetamin-, MDMA- und Methamphetaminproben

## 2.1 Zusammenfassung

Zur Detektion der Enantiomere von Amphetamin und MDMA wurden zwei LC-MS/MS-Methoden entwickelt, deren Messgenauigkeit in methanolischer Lösung durch mehrfache Analyse von zertifizierten racemischen Standardlösungen validiert wurde. Insgesamt wurden 143 Asservate von Amphetamin und 94 von MDMA untersucht. Die Analyse von insgesamt 528 beschlagnahmten Methamphetaminproben, welche vorwiegend aus Franken stammten, erfolgte im Institut für Rechtsmedizin in Erlangen mittels chiraler HPLC-DAD (Diodenarray-Detektor). Sämtliche im Rahmen dieser Studie untersuchten Proben stammten aus den Jahren 2019 und 2020.

Alle untersuchten Asservate von Amphetamin (139 Pulver, 4 Flüssigkeiten) lagen als Racemat vor. Ebenso waren alle untersuchten Asservate von MDMA (63 Tabletten, 30 kristalline Proben, 1 Pulver) racemisch. Die einzige flüssige Methamphetaminprobe sowie 28 von 29 pulverförmigen Proben enthielten enantiomerenreines (S)-Methamphetamin. Die verbleibende Pulverprobe enthielt dagegen reines (R)-Methamphetamin. 95 Prozent der kristallinen Methamphetaminproben enthielten ausschließlich (S)-Methamphetamin. Neben 7 kristallinen Proben (1,4 %), die enantiomerenreines (R)-Methamphetamin enthielten, und 10 racemischen Mischungen (2,0 %), lagen weitere 10 Proben als nicht-racemische Mischungen vor. Hiervon enthielten 8 Proben einen Überschuss an (S)-Methamphetamin. 7 der 10 nicht-racemischen Mischungen stammten aus einer einzigen Beschlagnahmung.

Die Detektion von racemischem Amphetamin und MDMA ist im Einklang mit den für diese Stimulantien üblichen Synthesewegen. Dies deckt sich auch mit den wenigen vorhandenen publizierten Studien. Die vorwiegende Detektion von enantiomerenreinem (S)-Methamphetamin wäre zum einen mit der Versorgung aus der an Franken grenzenden Tschechischen Republik erklärbar, wo die Synthese stereoselektiv aus pharmazeutischen Vorläufern erfolgt. Da mittlerweile der Großteil des deutschen Methamphetamins aus vollsynthetischer Herstellung in den Niederlanden stammt, könnte zum anderen auch eine im großen Stil angewandte

Racematspaltung für die Dominanz des enantiomerenreinen (S)-Methamphetamins verantwortlich sein. Vereinzelt scheint auch racemisches Methamphetamin vertrieben zu werden.

Die vorgefundenen nicht-racemischen Proben lassen sich durch eine unvollständige Racematspaltung erklären. Die sichergestellten Proben von (R)-Methamphetamin können als minderwertiges Nebenprodukt der Racematspaltung angesehen werden. Allgemein sind die Beobachtungen für Methamphetamin im Einklang mit internationalen Studien, die einen rückläufigen Trend in den Beschlagnahmungen von reinem (S)-Methamphetamin beobachten.

Obwohl dies theoretisch möglich wäre, konnten in dieser Studie keine Hinweise auf eine Anwendung der Racematspaltung für Amphetamin und MDMA gefunden werden. Ein Grund hierfür könnte sein, dass während für (S)-Methamphetamin im Vergleich zum (R)-Enantiomer eine bis zu zehnfach höhere pharmakologische Potenz angegeben wird, der Unterschied für Amphetamin und MDMA nur etwa das dreifache betragen soll. Demnach wäre das Kosten-Nutzen-Verhältnis einer Racematspaltung bei diesen Substanzen, insbesondere unter Berücksichtigung der allgemein geringeren Marktpreise, ein anderes.

In Anbetracht der durch die KKWT/ED vorgeschlagenen deutlich niedrigeren nicht geringen Mengen für reines (R)-Methamphetamin und das Racemat legen die Ergebnisse dieser Studie nahe, dass jede beschlagnahmte Methamphetaminprobe enantioselektiv untersucht werden sollte. Falls sich der Trend weg von enantiomerenreinem (S)-Methamphetamin fortsetzen sollte, wäre von einer erheblich geringeren pharmakologischen Potenz der Straßendrogen auszugehen. Für Amphetamin und MDMA kann weiterhin von einer racemischen Zusammensetzung ausgegangen werden. Hierdurch wird auch die forensische Interpretation von (R)/(S)-Enantiomerenverhältnissen in Serumproben ermöglicht. Des Weiteren kann aufgrund der racemischen Zusammensetzung von illegalem Amphetamin weiterhin der illegale Konsum von einer therapeutischen Einnahme der (S)-amphetaminhaltigen Medikamente Elvanse® oder Attentin® unterschieden werden.

**Eigenleistung an dieser Publikation:** Konzept, Methodenentwicklung, Validierung, Probenmessung, Datenanalyse, Datenaufbereitung, graphische Aufbereitung, Literaturrecherche, Niederschrift und Einreichung

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RESEARCH ARTICLE

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# Determination of the enantiomeric composition of amphetamine, methamphetamine and 3,4-methylendioxy-*N*-methylamphetamine in seized street drug samples from southern Germany

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## Abstract

Amphetamine (*speed*), methamphetamine (*crystal meth*), and 3,4-methylenedioxy-N-methylamphetamine (MDMA, *ecstasy*) represent the most frequently abused amphetamine-type stimulants (ATS). Differences in pharmacological potency and metabolism have been shown for the enantiomers of all three stimulants. Legal consequences in cases of drug possession may also differ according to the German law depending on the enantiomeric composition of the seized drug. Therefore, enantioselective monitoring of seized specimens is crucial for legal and forensic casework.

Various kinds of samples of amphetamine ($n = 143$), MDMA ($n = 94$), and methamphetamine ($n = 528$) that were seized in southern Germany in 2019 and 2020 were analyzed for their chiral composition using different chromatographic methods.

Whereas all samples of amphetamine and MDMA were racemic mixtures, the chiral composition of the methamphetamine specimens was diverse. Although the vast majority ($n = 502$) was present as (S)-methamphetamine, also specimens containing pure (R)-methamphetamine ($n = 7$) were found. Furthermore, few samples ($n = 8$) were of racemic nature or contained non-racemic mixtures of both enantiomers ($n = 10$).

Because methamphetamine appears in varying enantiomeric compositions, any seizure should be analyzed using an enantioselective method. Amphetamine and MDMA, on the other hand, currently appear to be synthesized exclusively via racemic pathways and are not chirally purified. Nevertheless, regular monitoring of the chiral composition should be ensured.

**KEYWORDS**
amphetamines, chiral chromatography, enantiomers, forensic toxicology, seizures

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## 1 | INTRODUCTION

Over the past decade, global seizures of amphetamine-type stimulants (ATS) have continuously increased.[1] In addition to amphetamine (*speed*) and methamphetamine (*crystal meth*), the two major representatives of the ATS group, MDMA (3,4-methylenedioxy-*N*-methylamphetamine), commonly known as ecstasy, poses another frequently abused member of this group.[2] Use of ATS is on the rise in Europe and especially in Germany, where amphetamine is the most commonly abused ATS.[1,3] At the same time, an increasing trend in the use of *ecstasy* can be observed, whereas the MDMA content of tablets is rising and other high-purity forms such as powder and crystals have also become available.[1,4] The use of methamphetamine is also becoming more widespread in many European countries.[1,3,4]

Amphetamine, methamphetamine, and MDMA each possess one stereogenic center and therefore exist in two enantiomeric forms. For all three substances, studies suggest a higher stimulant activity of the (S)-(+)-enantiomer.[5–9] Furthermore, pharmacokinetic studies showed metabolic preferences for the respective (S)-enantiomers.[7,10–13] Due to the different potencies, for amphetamine and methamphetamine, the threshold of the *non-small amount* in the sense of the German narcotics act (*Betäubungsmittelgesetz*, BtMG) differs depending on the enantiomeric composition of the seized drugs. Consequently, the *non-small amount* for (R)-amphetamine is twice and for (R)-methamphetamine 10 times as high as for the more potent (S)-enantiomers.[14] According to § 31a BtMG, in the case of production, possession, acquisition, etc. of drugs for personal use in amounts below this threshold (*small amount*), the public prosecutor may refrain from prosecution.[15]

The methods used for the synthesis of the stimulants differ both in their precursors and in the chiral composition of the products and are subject to dynamic change due to limitations of the precursor compounds.[1,16,17]

In order to enable assessment of legal and forensic cases, it is therefore necessary to investigate and monitor the enantiomeric composition of seized ATS and to consider possible variations in routine casework. In the present study, two methods of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and one method of liquid chromatography coupled with diode array detector (HPLC-DAD) were used to enantioselectively analyze seized ATS samples from southern Germany. Specimens of confiscated amphetamine, methamphetamine, and MDMA from the years 2019 and 2020 were collected and analyzed. The aim of this study is the assessment of the enantiomeric composition of the seized samples, and the evaluation regarding legal and forensic implications.

## 2 | MATERIAL AND METHODS

### 2.1 | Amphetamine and MDMA

#### 2.1.1 | Material

(RS)-Amphetamine and (RS)-MDMA-$d_5$ were obtained from LGC (Wesel, Germany). (RS)-MDMA was purchased from Merck (Darmstadt, Germany). (RS)-Amphetamine-$d_{11}$, (S)-amphetamine, and (R)-amphetamine were obtained from Lipomed (Weil am Rhein, Germany). All analytical standards were provided as methanolic solutions with concentrations of 0.1 mg/mL (internal standards) and 1 mg/mL (all other solutions). The standards labeled with "RS" were racemic according to the manufacturer's certificates. Methanol (HPLC grade) was acquired from Thermo Fisher Scientific (Dreieich, Germany). Water (LC-MS grade) and acetonitrile were obtained from Carl Roth (Karlsruhe, Germany). LC-MS-grade ammonia solution (25%) and ammonium bicarbonate were purchased from Merck (Darmstadt, Germany).

#### 2.1.2 | Seized drug samples

143 amphetamine samples from a total of 79 seizures and 94 MDMA samples from a total of 60 seizures were available for investigation. All samples were seized by the state police on the territory of Rhineland-Palatinate in the years 2019–2020. Amphetamine was almost exclusively present as powder, with four samples being present in liquid form. MDMA specimens were seized either as tablets of various colors ($n = 63$), in crystalline form ($n = 30$), or as powder ($n = 1$). Some seizures contained both tablets and crystalline samples. Quantitative determination of the sample purity was conducted by achiral HPLC-DAD. Ecstasy pills contained a median of 37.0% (19.3%–50.0%, mean [SD] 36.3 ± 6.0%) MDMA base, whereas the crystalline form exhibited significantly higher purity (median 79.8%, 76.5%–82.7%, mean [SD] 79.6 ± 1.6%), which corresponds to a purity of at least 90.9%, assuming the hydrochloride form. One specimen that was seized as purple powder, was of significantly lower purity (7.1%), and contained traces of amphetamine. Amphetamine powder contained a median of 16.2% (0.4%–73.1%, mean [SD] 21.6 ± 14.9%) amphetamine base, with two samples representing almost pure (>99%) amphetamine, assuming the sulfate form. One powder sample (24.6%) also contained traces of MDMA. Liquid amphetamine samples contained 62.7%, 60.6%, 47.1%, and 2.6% amphetamine base, respectively. The latter one was of blue color and also contained MDMA, ethanol, and gamma-butyrolactone (GBL). Boxplots in Figure 1 illustrate the distribution of purity for the various types of samples seized.

#### 2.1.3 | Sample preparation

Sample preparation was carried out according to a forensic guideline.[18] In brief, the preparation for the different sample types was conducted as follows: At first, all samples were dried to a constant weight. For seizures of tablets in a single packaging unit, at least 30% of the samples were homogenized and diluted in water. For seizures of tablets in multiple packaging units, at least 30% of each unit were homogenized. Subsequently, at least one mixture of equal proportions of the homogenisates was prepared and diluted in water. For powder and crystalline samples in a single packaging unit, a composite sample containing at least 30% of the material was taken from at least three

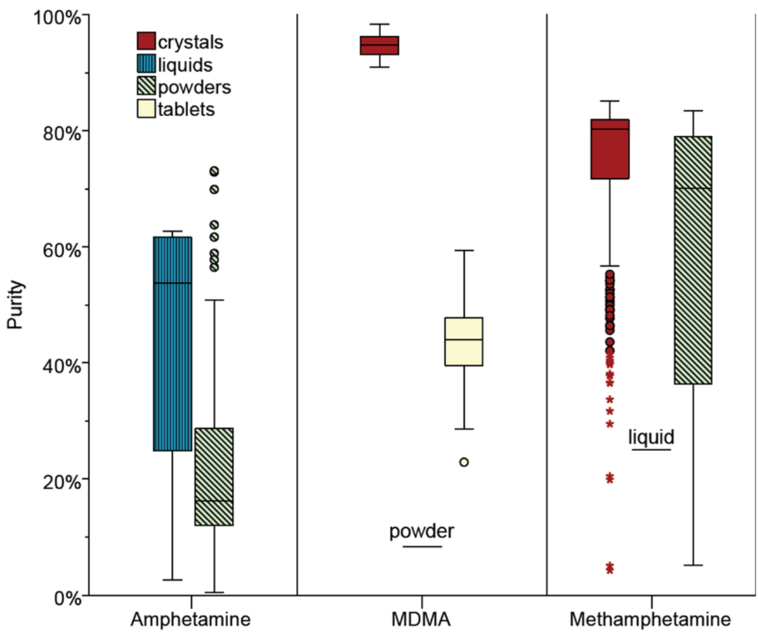


**FIGURE 1** Boxplots for purity of the different sample types of amphetamine, MDMA, and methamphetamine. Horizontal lines represent the median, and boxes represent the range between lower and upper quartile of the subgroups (inter quartile range). Whiskers represent all samples within ±1.5 times the interquartile range. Outliers > median ±1.5 times the interquartile range are shown as circles. Extreme values with >median ±3 times the interquartile range are shown as asterisk. For each MDMA powder and methamphetamine liquid, only one sample was available, which is presented as single line [Colour figure can be viewed at wileyonlinelibrary.com]

spots of the specimen and subsequently diluted in water. For powder and crystalline samples in multiple packaging units, at least one mixture of equal proportions of all units was prepared and diluted in water. All liquid samples were stored sealed until the day of analysis. For analysis, all aqueous solutions were diluted with methanol to reach a final racemic concentration of 100–500 ng/mL. The solutions were transferred into sampling vials, and racemic internal standard solution was added (racemic amphetamine-$d_{11}$ and MDMA-$d_5$, respectively; final concentration 500 ng/mL).

### 2.1.4 | Chiral LC-MS/MS instrumentation and analytical parameters

Enantiomers of amphetamine and MDMA were analyzed using a LC-MS/MS system from Agilent (Waldbronn, Germany). Chromatography was conducted using a 1290 Infinity II LC system, coupled via Jet Stream interface (ESI) to a 6495C triple quadrupole mass spectrometer. Chromatographic separation of both enantiomeric pairs was achieved using a polysaccharide-based chiral Lux® 3-μm AMP 150 × 3.0 mm analytical column, guarded with a Lux AMP® 4 × 2.0 mm security guard cartridge (both Phenomenex; Aschaffenburg, Germany). Injection volume was 1 μL. Mobile Phase A consisted of 5 mM ammonium bicarbonate solution adjusted to pH 11 by addition of ammonia solution. Composition of mobile Phase B and gradient elution differed between the determination of amphetamine and MDMA. For enantiomeric separation of amphetamine, methanol was used as organic mobile phase (B), and for enantiomeric separation of MDMA, a mixture of methanol/acetonitrile (1/1, v/v) was used. Holding a temperature of 30°C and a flow rate of 0.35 mL/min, the following gradients were used for separation of amphetamine (if applicable different percentages for MDMA in parentheses): holding B at 60% (50%) for 13 min, increasing it to 95% within 0.5 min, holding for 3 min, and decreasing to 60% (50%) within 0.5 min. With an additional post-time of 2 min, total run time for both gradients was 19 min. Baseline separation was achieved for all analytes and ISTD. Electrospray parameters were as follows: gas flow 11 L/min at 200°C; nebulizer 15 psi, sheath gas flow 12 L/min at 400°C; capillary voltage +3500 V. Analytes were detected using multiple reaction monitoring (MRM) mode using the following transitions (*m/z*, collision energy in parentheses, target ion underlined): amphetamine 136.1 → <u>91.0</u> (17 eV), 119.0 (5 eV), 65.0 (45 eV); amphetamine-$d_{11}$ 147.2 → <u>98.0</u> (21 eV), 130.1 (5 eV), 70.1 (45 eV); MDMA 194.1 → <u>163.0</u> (9 eV), 105.0 (25 eV), 51.1 (80 eV); MDMA-$d_5$ 199.2 → <u>165.0</u> (13 eV), 107.0 (29 eV), 135.0 (21 eV). The two LC-MS/MS methods used for this study also allowed simultaneous enantioselective detection of 3,4-methylenedioxyamphetamine (MDA) and methamphetamine. Data evaluation was done using Agilent MassHunter Workstation Software (Version B.09.00). Identification of the elution order for amphetamine enantiomers was conducted by injection of enantiopure standard solutions. For all other experiments, a racemic amphetamine solution was used as analytical standard, because the monoenantiomeric solutions (especially for (R)-amphetamine, which eluted first) were not completely pure. Due to the lack of enantiopure standards for MDMA, elution order could not be determined but was deduced. The present chromatographic method was also validated for quantitative detection of MDMA enantiomers in serum samples. Studies show that (S)-MDMA is metabolized more rapidly than (R)-MDMA in humans, and therefore, (R)/(S)-concentration ratios greater than 1 can be found in serum samples.[10,12,13] Because the first eluting enantiomer consistently showed a larger area under the curve (AUC) when serum samples were

measured, it was identified as (R)-MDMA. For each sample, (R)/(S)-concentration ratio was calculated as the quotient of the AUC of the analyte relative to the AUC of the respective ISTD according to Equation 1. To assess the precision of the method, two methanolic solutions of certified racemic amphetamine and MDMA were prepared to reach a final racemic concentration of 250 and 500 ng/mL, respectively. Analogous to the procedure for the seized samples, the solutions were transferred into sampling vials, and racemic internal standard solution was added (final concentration 500 ng/mL). Both low and high quality control (QC) samples were injected 10 times each, and the (R)/(S) ratio was calculated.

$$(\mathrm{R})/(\mathrm{S})-\mathrm{ratio}=\frac{\mathrm{AUC}_{(\mathrm{R})-\mathrm{enantiomer}}}{\mathrm{AUC}_{(\mathrm{R})-\mathrm{enantiomer\,of\,ISTD}}}\Big/\frac{\mathrm{AUC}_{(\mathrm{S})-\mathrm{enantiomer}}}{\mathrm{AUC}_{(\mathrm{S})-\mathrm{enantiomer\,of\,ISTD}}} \quad (1)$$

## 2.2 | Methamphetamine

### 2.2.1 | Material

Water was obtained from Merck (Darmstadt, Germany). Methanol, phosphoric acid (85%), potassium dihydrogen phosphate, and hydrochloric acid (37%) were purchased from VWR (Darmstadt, Germany). (RS)-Methamphetamine, (S)-methamphetamine, and (R)-methamphetamine were obtained from Lipomed (Weil am Rhein, Germany). All analytical standards were provided as methanolic solutions with concentrations of 1 mg/mL. (RS)-Methamphetamine was racemic according to the manufacturer's certificate. All chemicals and solvents were of analytical grade.

### 2.2.2 | Seized drug samples

528 methamphetamine (MA) samples from a total of 237 seizures from the years 2019 and 2020 were collected. All samples were seized by the state police in the Free State of Bavaria. The samples originated from the following governmental districts: Upper Franconia ($n = 125$), Middle Franconia ($n = 298$), Lower Franconia ($n = 1$), Upper Palatinate ($n = 94$), Upper Bavaria ($n = 1$), and Lower Bavaria ($n = 9$). Methamphetamine was mostly present in crystalline form ($n = 498$, 94.3%), less frequently as powder ($n = 29$, 5.5%), and in one case, a liquid was seized. Some seizures contained both crystalline and powder samples. Quantitative determination of the sample purity was conducted by achiral HPLC-DAD. Crystals contained a median of 80.3% (4.3%–85.1%, mean [SD] 74.5 ± 12.5%) MA base, whereas MA powder contained a median of 70.1%, (5.2%–83.4%, mean [SD] 56.3 ± 28.1%), and the liquid had a purity of 25% and a total volume of only 250 μL. Boxplots in Figure 1 illustrate the distribution of purity for the various types of methamphetamine samples seized. Traces of amphetamine (nine samples) and MDMA (one sample) were found in both crystalline and powder samples. The relative amphetamine content in these samples was between 3.9% and 1474% (median 37.1%). The sample with MDMA contained more MDMA than methamphetamine (258%).

### 2.2.3 | Sample preparation

Sample preparation was based on a forensic guideline.[18] All samples were dried at a temperature of 70°C to a constant weight and subsequently homogenized. Up to a sample weight of 500 g, 2 × 25 mg of the homogenized material were taken. Above a weight of 500 g, 3 × 25 g were prepared, and above 1000 g, 4 × 25 mg were used. For analysis, homogenisates were diluted in 25 mL diluted hydrochloric acid (1/99, v/v) to reach a final racemic below 1 mg/mL.

### 2.2.4 | Chiral LC-DAD instrumentation and analytical parameters

Chromatography was conducted using a 1100 series HPLC-DAD system from Agilent (Waldbronn, Germany). Methamphetamine enantiomers were separated on a cyclodextrin-based ChiraDex® 5 μm (250 × 4.0 mm Merck, Darmstadt, Germany) chiral column. Injection volume was 2 μL and column temperature was 40°C. The mobile phase was prepared by adding 49.75 g phosphate buffer solution (pH 6.8) and 2.5 g methanol to 447.75 g water. Separation was achieved by isocratic elution at a flow rate of 0.8 mL/min. The total run time was 10 min. Spectra were detected at 210, 220, 230, 240, and 280 nm, whereas quantification was done at a wavelength of 210 nm. Data evaluation was done using Agilent ChemStation software. Elution order of methamphetamine enantiomers was conducted by injection of enantiopure standard solutions. When enantiomerically pure methamphetamine or racemate was detected by the chiral method, quantitative results of the achiral HPLC-DAD were applied. When non-racemic mixtures were detected, the enantiomers were quantified separately using the chiral method. For both enantiomers, linearity of calibration was tested within a range of 0.001–1 mg/mL. The method's specificity was examined with respect to the also detected analytes amphetamine, MDMA, MDA, MDE, creatine, paracetamol, caffeine, piracetam, and ephedrine. Traces of other phenethylamines were detected up to an absolute content of 0.5%.

# 3 | RESULTS

## 3.1 | Enantiomeric composition of amphetamine and MDMA

Baseline separation was achieved for both analytes and internal standards (cf. Figure 2). Validation experiments showed satisfactory precision for the determination of (R)/(S)-ratios. Low and high amphetamine QC samples showed ratios between 0.96 and 1.06

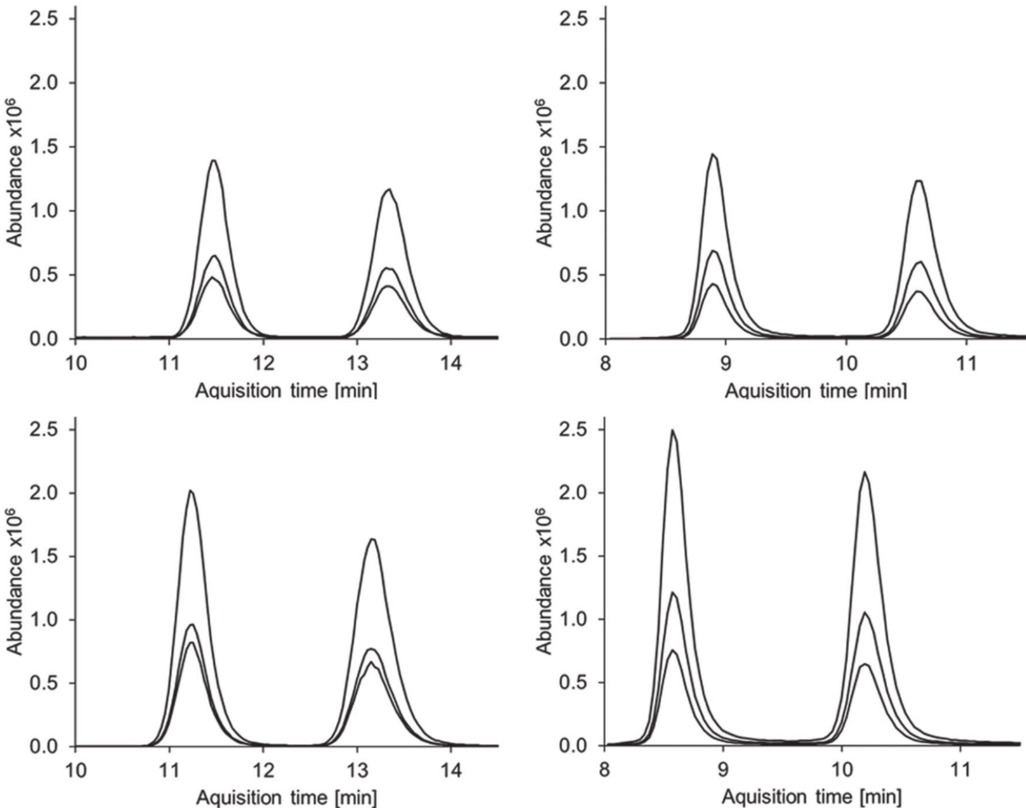


**FIGURE 2** Multiple reaction monitoring (MRM) chromatograms for two authentic seized drug samples of MDMA (left plots, (R)/(S)-ratio 1.02) and amphetamine (right plots, (R)/(S)-ratio 0.99). Chiral chromatographic separation is shown for each the analytes (top) and their internal standards (bottom). Elution times were as follows (with the (R)-enantiomer always eluting first): MDMA 11.3 and 13.3 min, amphetamine 8.9 and 10.6 min, MDMA-$d_5$ 11.1 and 13.1 min, amphetamine-$d_{11}$ 8.6 and 10.2 min. The following ion transitions are displayed in decreasing order of intensity (target ion underlined): MDMA 194.1 → 163.0, 105.0, 51.1; amphetamine 136.1 → 91.0, 119.0, 65.0; MDMA-$d_5$ 199.2 → 165.0, 107.0, 135.0; amphetamine-$d_{11}$ 147.2 → 98.0, 130.1, 70.1

(mean 1.00), and ratios for low and high MDMA QC samples were between 0.94 and 1.06 (mean 1.01). (R)/(S)-ratios for seized amphetamine were between 0.97 and 1.04 (mean 1.00). Also, both enantiomers of amphetamine (very minor concentration) were found in the one MDMA sample, which was seized as purple powder.

(R)/(S)-ratios for seized MDMA were between 0.96 and 1.03 (mean 1.00) for all three different sample types (crystals, liquid, powder). MDMA (R)/(S)-ratio was 1.01 for the adulterated amphetamine powder and 1.00 for the blue amphetamine liquid. Boxplots of the determined ratios for seized amphetamine and MDMA samples are shown in Figure 3. Contaminations of methamphetamine in amphetamine samples ($n = 67$, 47%) and MDA in MDMA samples ($n = 4.4\%$) were always below a relative content of 1%. In all cases of MDA and methamphetamine trace detection, both enantiomers were found in roughly equal proportions; however, in one case, there was an approximately 10-fold excess of (S)-methamphetamine. Due to the low concentration of MDA and methamphetamine traces, a more precise determination was not possible.

### 3.2 | Enantiomeric composition of methamphetamine

Sufficient separation of methamphetamine enantiomers was achieved (cf. Figure 4). The method also showed specificity and linearity for both methamphetamine enantiomers. Traces of enantiomers below the lower limit of quantification (LLOQ, 0.1% of the total mass) were considered as incidental impurities and therefore neglected. The one liquid sample as well as 28 out of 29 powder samples contained enantiopure (S)-methamphetamine. In contrast, the remaining one powder sample contained enantiopure (R)-methamphetamine. The enantiomeric composition of the crystalline samples was more diverse. The vast majority ($n = 473$, 95.0%) of crystalline samples contained enantiopure (S)-methamphetamine, whereas only seven samples (1.4%) contained pure (R)-methamphetamine. Another 10 samples (2.0%) contained racemic methamphetamine. Furthermore, there was a total of 10 samples containing a non-racemic mixture of both enantiomers. Seven out of these 10 samples originated

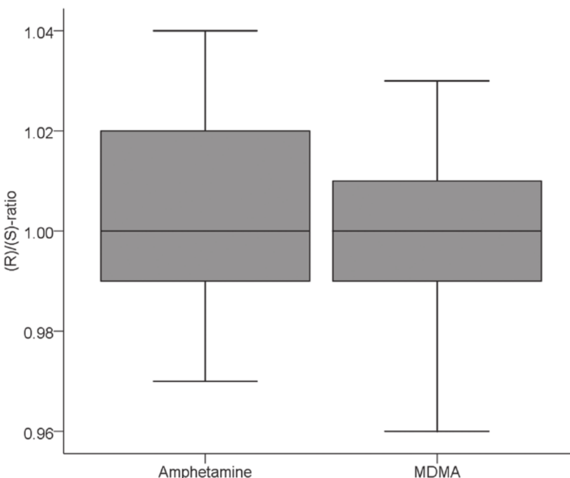


**FIGURE 3** Boxplots of calculated (R)/(S)-ratios for all amphetamine ($n = 143$) and MDMA ($n = 94$) samples. Horizontal lines represent the median, and boxes represent the range between lower and upper quartile of the subgroups (interquartile range). Whiskers represent all samples within ±1.5 times the interquartile range. No outliers or extreme values with >median ±1.5 times the interquartile range were present

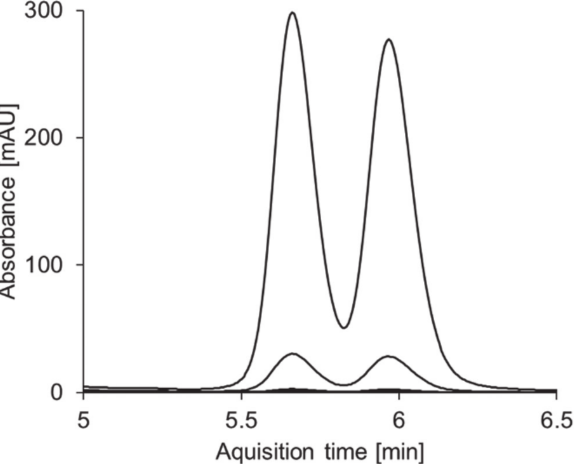


**FIGURE 4** HPLC-DAD chromatogram of the separation of methamphetamine enantiomers. Retention time was 5.66 min for (R)-methamphetamine and 5.99 min for (S)-methamphetamine. Quantification was done at 210 nm (absorption maximum), whereas absorption at 220 nm was significantly lower

from a single seizure, which also included one racemic sample. The other three non-racemic mixtures originated from three unrelated seizures. Two samples contained an excess of (R)-methamphetamine, whereas the other eight samples contained an excess of (S)-methamphetamine. The calculated enantiomeric excess (ee) for (S)-methamphetamine ranged from −19% to 91%. Detailed information on composition and ee of the non-racemic mixtures is shown in Table 1. The quantities of the (R)-methamphetamine seizures ranged from 0.45 to 95.6 g (mean 31.3 g, median 14.9 g). For the non-racemic mixtures, the absolute amounts seized ranged from 0.29 to 38.8 g (mean 7.65 g, median 0.36 g). Although larger quantities of (S)-methamphetamine were seized in some cases, the seized quantities of (R)-methamphetamine and non-racemic mixtures were within the usual range. An overview of the enantiomeric compositions of all sample types of seized methamphetamine is given in Figure 5.

**TABLE 1** Seizures of non-racemic mixtures of methamphetamine ($n = 10$)

| Year | Seizure | $(S)_{rel}$ [%] | $(R)_{rel}$ [%] | ee (S) [%] |
|---|---|---|---|---|
| 2019 | 1 | 96 | 4.3 | 91 |
| | | 91 | 9.3 | 81 |
| | | 85 | 15 | 71 |
| | | 78 | 22 | 55 |
| | | 65 | 35 | 30 |
| | | 54 | 46 | 8 |
| | | 41 | 59 | -17 |
| 2019 | 2 | 92 | 8.0 | 84 |
| 2020 | 3 | 41 | 59 | -19 |
| 2020 | 4 | 77 | 23 | 54 |

*Notes*: All samples were present as crystals. The table shows the relative contents of the methamphetamine enantiomers ($(S)_{rel}$ and $(R)_{rel}$) for each sample. Enantiomeric excess (ee) of (S)-methamphetamine is calculated as difference between $(S)_{rel}$ and $(R)_{rel}$. A negative value therefore represents an excess of (R)-methamphetamine (two cases).

## 4 | DISCUSSION

In the present study, multiple chiral methods were successfully applied to examine a large number of samples of seized amphetamine, methamphetamine, and MDMA. To the best of our knowledge, this is the first study to describe the chiral composition of ATS seized in Germany.

Independent of the sample types, all seized amphetamine and MDMA samples contained both enantiomers. Calculated (R)/(S)-ratios for both substances were within the limits of accuracy determined by the measurement of QC samples containing certified racemic standards. Therefore, all samples can be considered as racemic. MDMA impurities in the one amphetamine powder sample and in the blue amphetamine liquid can also be considered as racemic. Furthermore, the traces of amphetamine in the purple MDMA powder sample also suggest contamination with racemic amphetamine, although a precise determination was not possible in this case.

The detection of racemic amphetamine is consistent with other studies found in literature, although data on enantiomeric composition of seized amphetamine samples are very limited. Van Bocxlaer et al.[19] found both amphetamine enantiomers in almost equal proportions in

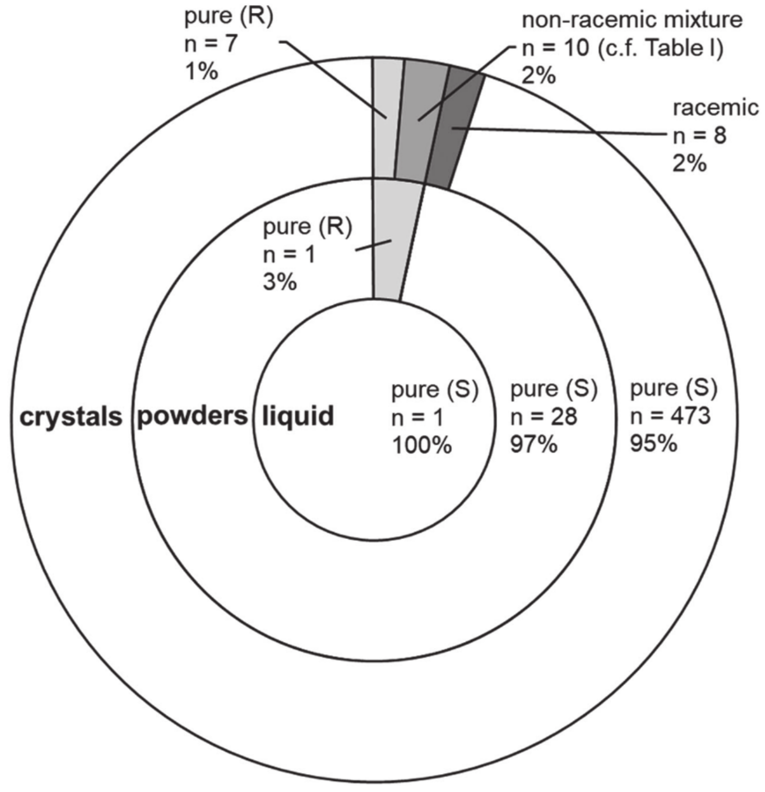


**FIGURE 5** Pie chart of the enantiomeric composition of the different methamphetamine sample types. Inner circle represents the liquid sample ($n = 1$), middle annulus represents powder samples ($n = 29$), and outer annulus represents crystalline samples ($n = 498$)

two powder samples that also contained racemic α-phenethylamine. In 2000, George and Braithwaite[20] analyzed 17 crystalline and powder samples and consistently detected an excess of (S)-amphetamine (R/S-ratio 0.72–0.98, mean 0.89), although validation data are not available. More recently, Dhabbah[21] analyzed 12 batches of amphetamine tablets seized in Saudi Arabia and consistently found both enantiomers in similar quantities. Although also in this study a slight excess of (S)-amphetamine up to 9.7% was detected, an adequate interpretation remains impossible due to a lack of precision data.

Data on chiral composition of illegal MDMA are also scarce. However, all available studies consistently found racemic mixtures of MDMA in seized 'ecstasy' tablets,[22–24] whereas corresponding studies on the recently appearing crystalline MDMA are lacking. Recent wastewater analyses in major European cities also indicate the use of racemic amphetamine and MDMA[25] and are therefore also in line with the results of the presented study.

A few of the examined MDMA samples contained very low quantities of MDA. Whereas a few decades ago MDA was sometimes found as the main ingredient in tablets sold as "ecstasy," today ecstasy tablets usually contain MDMA,[26] while the active ingredient content is steadily increasing.[1,4] The occurrence of mixtures of MDMA/MDA[24,27,28] and MDMA/amphetamine[28–30] in tablets sold as "ecstasy" is also documented in literature. MDA can also be found as synthesis-related impurity of MDMA synthesized via reductive amination.[31] Therefore, the detection of both MDA enantiomers was to be expected. Evidence of amphetamine powder samples adulterated with MDMA can, however, not be found in literature.

The vast majority of methamphetamine samples only contained the more potent (S)-enantiomer. Contrarily, one powder sample as well as seven crystalline samples exclusively contained the less potent distomer. Moreover, there were some crystalline samples containing the racemate and non-racemic mixtures of the enantiomers.

Data on the enantiomeric composition of methamphetamine crystals are available mainly from Asia. The majority of the seized crystals there also contained pure (S)-methamphetamine, although different fractions of pure (R)-methamphetamine and racemic and non-racemic mixtures were also found.[32–38] Two of these studies observed a decreasing trend in seizures of the more potent (S)-enantiomer.[32,33] The latter was also observed in an American study from 2005 to 2012, where, in addition to pure (S)-methamphetamine, mainly non-racemic mixtures were found.[39] An investigation of methamphetamine tablets, so-called Thai pills, seized in Switzerland found pure and almost pure (S)-methamphetamine as well as occasional racemic mixtures and one pill mainly containing (R)-methamphetamine.[40]

Studies from Germany are not available for either of the substances investigated here; however, conclusions can be drawn from the production methods used. The presence of solely racemic amphetamine and MDMA samples is consistent with a fully synthetic production. Amphetamine and MDMA on the German market originate mainly from the Netherlands, where it is synthesized from

various non-pharmaceutical precursors.[1,17,41,42] All common routes of synthesis lead to a racemic product.[10,12,16,43,44] For many years, the majority of European methamphetamine originated from the Czech Republic, where it is synthesized from the pharmaceutical precursor (1S,2S)-pseudoephedrine and, more rarely, (1R,2S)-ephedrine.[4,7,16,17,42] Reduction of these chiral compounds yields enantiopure crystalline (S)-methamphetamine.[7,16,17,45] However, due to international restrictions on precursor chemicals, clandestine laboratories are forced to switch to alternative pre-precursors and different (non-stereoselective) pathways of synthesis.[1,16,17] Recently, most methamphetamine seized in Germany originates from the Netherlands.[46] There, it is produced mainly from phenylacetone (P2P, BMK), which, analogous to amphetamine, leads to a racemic product.[7,17,41] Lately, it has been reported that the enantiomers of methamphetamine are separated by chiral resolution using tartaric acid, for example, via the so-called "Pope–Peachey" method.[16,17,47,48] This results in products with different contents of the desired (S)-enantiomer and the (R)-enantiomer, which is considered as less potent by-product.[17,48] However, it is possible to racemize the fraction with the undesired distomer and to subject it to chiral resolution again and again, to virtually yield (S)-methamphetamine only. A mild method for this purpose is the thiyl radical-mediated racemization, for example, with methyl thioglycolate.[49] Although chiral resolution with tartaric acid is less common in Europe,[17] Mexico reports the seizure of several tons of tartaric acid per year, which is linked to methamphetamine production.[16,47] Large quantities of tartaric acid have already been seized in the Netherlands,[16] where Mexican nationals have been involved in the supply and manufacture of methamphetamine.[17,41] The predominance of (S)-methamphetamine observed in this study suggests either stereoselective production from pharmaceutical precursors or extensive use of chiral resolution subsequent to racemic synthesis. Nevertheless, some samples were found that indicate racemic synthesis or incomplete chiral resolution. The pure (R)-methamphetamine found most likely represents the low-quality waste product of chiral resolution. Although chiral resolution would also be possible for amphetamine and MDMA in order to obtain the more potent (S)-enantiomer, this could not be proven in this study either and is also not documented in the literature. One reason could be that although (S)-methamphetamine is suggested to be up to 10 times more potent than the (R)-enantiomer,[6] the efficacy of the eutomers of amphetamine and MDMA is usually estimated to be not more than three times higher than that of the distomers.[5,8] Considering a study by Mendelson et al.,[7] which suggests similar abuse potentials for racemic methamphetamine and for the pure (S)-enantiomer, even in this case, the benefit of a chiral resolution remains questionable. However, quantification of the enantiomers' stimulant activities seems rather unreliable given the complex pharmacodynamic effects of these drugs.

Almost half of the tested amphetamine samples contained traces of methamphetamine. In contrast to the majority of methamphetamine samples, the impurities were almost exclusively racemic, so contamination at the consumer level appears unlikely. An intentional mixing of the two stimulants also does not seem plausible due to the low degree of adulteration. Methamphetamine is also not a by-product of amphetamine synthesis. Contamination in laboratories that manufacture both amphetamine and methamphetamine fully synthetically, on the other hand, would be conceivable.

Because the thresholds for the non-small amount of (S)- and (R)-methamphetamine in the German BtMG differ significantly (5 vs. 50 g) and all kinds of enantiomeric compositions can be found in seized samples, from a legal perspective, each seized sample should be analyzed stereoselectively. For the analysis of powder samples, X-ray powder diffraction (XRPD) offers a time- and cost-saving alternative to chiral chromatography, as it allows racemates to be distinguished from enantiopure samples.[50] A legal distinction by enantiomeric composition, as found in German law, is rather uncommon. For example, Swiss law generally does not distinguish between enantiomers.[40] The corresponding Austrian law (*Suchtgift-Grenzmengenverordnung*, Narcotic Drugs Limit Ordinance) does differentiate between the enantiomers of amphetamine, however, with an identical threshold of 10 mg each.[51] In contrast to methamphetamine, on the basis of this study, it does not appear necessary to examine each seized sample of amphetamine and MDMA enantioselectively. Subject to random control, illicit amphetamine and MDMA can thus be considered racemic. Illegally synthesized amphetamine therefore differs essentially from amphetamine in finished medicinal products approved in Germany, which either contain (S)-amphetamine (Attentin®) or its prodrug lisdexamfetamine (Elvanse®). This allows a forensic distinction of therapeutic use and drug abuse by analyzing blood samples.[43] However, in rare cases, preparations of racemic amphetamine sulfate (syrup or capsules) are made directly in the drug store according to the German *Neues Rezeptur-Formularium* (NRF). In those cases, a distinction from illicit use cannot be made.

## 5 | CONCLUSION

In the present study, the enantiomeric composition of seized samples of amphetamine, methamphetamine, and MDMA was determined. Whereas all amphetamine and MDMA samples were racemic, methamphetamine samples exhibited diverse compositions, with the pure (S)-enantiomer found most frequently. The production and supply chain of methamphetamine in Europe is currently undergoing changes, so less potent enantiomeric mixtures or even the pure (R)-form are also becoming available. Therefore, seized methamphetamine samples should be routinely analyzed for their enantiomeric composition. The composition of amphetamine and MDMA may also change in this respect, so regular monitoring should be carried out.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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# 3 Chirale Serum Pharmakokinetik von 4-Fluoramphetamin nach kontrollierter oraler Aufnahme

## 3.1 Zusammenfassung

Die auch zur Trennung der Enantiomere von Amphetamin verwendete LC-MS/MS-Methode konnte erfolgreich für die Detektion der Enantiomere von 4-FA in Serumproben validiert werden. Zur Analyse lagen Proben von zwölf Personen nach oraler Einnahme von 100 mg 4-FA vor. Fünf dieser Personen konnten nach einer Latenzzeit von mindestens einer Woche darüber hinaus eine Dosis von 150 mg einnehmen, bevor die Studie aus Sicherheitsgründen abgebrochen werden musste. Blutproben wurden jeweils vor Einnahme und zu insgesamt 10 Zeitpunkten über einen Zeitraum von 12 Stunden nach Einnahme abgenommen.

Aus den ermittelten Serumkonzentrationen für (R)- und (S)-4-FA wurden für jeden Probanden die maximale Serumkonzentration ($c_{max}$) und die Zeit zum Erreichen der maximalen Serumkonzentration ($t_{max}$) ermittelt. Die apparente Serumhalbwertszeit ($t_{1/2}$) wurde durch Regression von $t_{max}$ bis zum letzten gemessenen Zeitpunkt ermittelt. Die Fläche unter der Kurve (engl. *Area Under the Curve*, AUC) von $t_0$ bis $t_{12}$ wurde mithilfe der Trapezregel berechnet.

Weder $c_{max}$ (t-Test, $p = 0,63$) noch $t_{max}$ (Mann-Whitney-U-Test) der beiden Enantiomere unterschieden sich signifikant voneinander. Die $t_{max}$ lag hierbei zwischen 0,5 h und 4 h. Die $t_{1/2}$ von (R)-4-FA (12,9 h) war im Mittel mehr als doppelt so lang wie die von (S)-4-FA (6,0 h). Hierbei wurden sowohl für das (R)-Enantiomer (8,3 h – 16,0 h) als auch für das (S)-Enantiomer (4,4 h – 10,2 h) erhebliche interindividuelle Unterschiede festgestellt. Der daraus folgende Anstieg der (R)/(S)-Konzentrationsverhältnisse mit der Zeit war für alle Probanden linear (Korrelationskoeffizient nach Pearson jeweils > 0,99). Die Steigung war hierbei unabhängig von der Dosis (zweifaktorielle Varianzanalyse, $p = 0,36$). In der ersten Serumprobe, welche 30 min nach Konsum entnommen wurde, lag bei allen außer zwei Probanden eine höhere Konzentration des (S)-Enantiomers vor. Die (R)/(S)-Konzentrationsverhältnisse lagen hierbei zwischen 0,84 und 1,02. In der nach 12 h entnommenen letzten Probe lagen die Verhältnisse dagegen zwischen 1,08 und 2,77. Zwei Probanden, welche außerordentlich hohe ($0,157\ h^{-1}$) bzw. niedrige ($0,023\ h^{-1}$) Steigungen der (R)/(S)-Konzentrationsverhältnisse aufwiesen, wurden als Ausreißer identifiziert.

Die Bestimmung von (R)/(S)-Konzentrationsverhältnissen eröffnet wichtige forensische Interpretationsmöglichkeiten. Unter der Annahme, dass die Akutphase nicht länger als 6 h andauert, kann auf Basis dieser Ergebnisse vorgeschlagen werden, dass bei

(R)/(S)-Konzentrationsverhältnissen von über 1,60 lediglich subakute Effekte zu erwarten sind. Dabei ist zu berücksichtigen, dass einer der Probanden auch 12 h nach Konsum ein (R)/(S)-Verhältnis von lediglich 1,08 aufwies. Bei Vorfinden von (R)/(S)-Konzentrationsverhältnissen von unter 1,60 können somit keine verlässlichen Aussagen über das Vorliegen von akuten oder subakuten Effekten getätigt werden.

**Eigenleistung an dieser Publikation:** Konzept, Methodenentwicklung, Validierung, Probenmessung, Datenanalyse, Datenaufbereitung, graphische Aufbereitung, Literaturrecherche, Niederschrift und Einreichung

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Article

# Chiral Serum Pharmacokinetics of 4-Fluoroamphetamine after Controlled Oral Administration: Can (R)/(S)-Concentration Ratios Help in Interpreting Forensic Cases?

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**Abstract**

Over the last two decades, misuse of 4-fluoroamphetamine (4-FA) became an emerging issue in many European countries. Stimulating effects last for 4–6 hours and can impact psychomotor performance. The metabolism of amphetamine-type stimulants is stereoselective and quantification of (R)- and (S)-enantiomers has been suggested for assessing time of use. To date, no data on enantioselective pharmacokinetics is available for 4-FA in serum samples. An enantioselective liquid chromatography−tandem mass spectrometry (LC–MS-MS) method was developed using a chiral Phenomenex® Lux 3 μm AMP column. Validation of the method showed satisfactory selectivity, sensitivity, linearity (0.5–250 ng/mL), precision and accuracy. Recreational stimulant users orally ingested two doses (100 mg, $n = 12$; 150 mg, $n = 5$) of 4-FA. Blood samples were drawn prior to application and over a period of 12 hours after ingestion and analyzed for 4-FA enantiomers. Peak concentrations and corresponding times did not differ significantly between the enantiomers (mean (R)/(S)-ratio at $t_{max}$ 1.05, 0.85–1.16). With mean 12.9 (8.3–16.1) hours, apparent elimination half-lives ($t_{1/2}$) were significantly ($P < 0.01$) longer for (R)-4-FA than for (S)-4-FA (6.0 hours; range 4.4–10.2 hours) and independent of the dose given. Over time, (R)/(S)-concentration-ratios were linearly increasing in all subjects to maximum ratios of 2.00 (1.08–2.77) in the last samples (after 12 hours). The slopes of the (R)/(S)-ratio exhibited marked interindividual differences (0.023–0.157 $h^{-1}$, mean 0.095 $h^{-1}$). Ratios higher than 1.60 only appeared earliest after a minimum of 6 hours and therefore suggest the absence of acute drug effects. Different elimination half-lives of enantiomers lead to constantly increasing (R)/(S)-concentration-ratios. Consequently, ratios of 4-FA enantiomers in serum are a promising indicator for assessment of the time of drug consumption.

## Introduction

The rise of new psychoactive substances (NPS) on the European drug market is accompanied by the advent of substituted derivatives of well-known drugs, such as phenethylamines. The para-fluorinated analog of amphetamine, 4-fluoroamphetamine (4-FA) is a commonly used NPS in Germany (1) and the Netherlands (2–5). 4-FA first

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appeared in 2003 in Germany (6) and has been an issue of forensic investigations, i.e., driving under the influence of drugs (7–10) and cases of severe toxicity, including fatalities (11–17).

Like amphetamine, 4-FA enhances the release of dopamine, serotonin and noradrenaline and also inhibits reuptake of these monoamines (18–20). Acute subjective effects can be ranked between those of amphetamine and 3,4-methylenedioxyme thylamphetamine (MDMA) and typically include stimulation, euphoria and entactogenic effects. Acute effects are reported to be highest 1 hour after consumption and mostly last for about 6 hours (8, 21). This is much shorter than detectability in serum; therefore, the forensic question of an impairment of, e.g., driving ability or criminal responsibility is better answered by estimating the time of consumption rather than by evaluation of serum concentrations.

4-FA possesses one chiral center and therefore exists in two enantiomeric forms. Pharmacokinetic studies of chiral phenethylamine derivatives have consistently shown metabolic preference for the (S)-enantiomer (22–26). Accordingly, 4-FA is presumed to exhibit similar stereoselective kinetics. The time course of (R)/(S)-concentration ratios has been suggested for estimating the time of drug consumption (22, 27). This could therefore facilitate the assessment of drug impairment in forensic casework.

In the present study, a sensitive and selective liquid chromatography–tandem mass spectrometry (LC–MS-MS) method for analysis of (R)- and (S)-4-FA in serum was developed and validated. The method was used to quantitate the 2 enantiomers in serum samples obtained after controlled oral application of 4-FA to 12 volunteers and elucidate its chiral pharmacokinetics. The aim of this study was to examine the enantioselective pharmacokinetics of 4-FA and to evaluate the time course of (R)/(S)-concentration ratios with respect to forensic questions.

## Materials and Methods

### Materials

(RS)-4-FA and the internal standard (RS)-amphetamine-$d_{11}$ were obtained from Lipomed (Weil am Rhein, Germany). LC-MS-grade ammonia solution (25%) and ammonium bicarbonate was purchased from Merck (Darmstadt, Germany). Acetonitrile (HPLC-grade) was obtained from Applichem (Darmstadt, Germany), and methanol (LC–MS-grade) was obtained from Honeywell (Seelze, Germany). Regenerated cellulose membrane syringe filters (0.2 μm, 4 mm) were obtained from Phenomenex (Aschaffenburg, Germany). Mixed-mode cation-exchange solid phase extraction columns (HF BE-CERTIFY, 300 mg, 3 mL) were purchased from Agilent (Waldbronn, Germany). Water (LC-MS-grade) as well as all other chemicals and solvents were obtained from Carl Roth (Karlsruhe, Germany). Blank (drug-free) serum was obtained from the blood bank of the University Medical Center of the Johannes Gutenberg University Mainz.

### Biological samples

Blood samples were collected as part of a phase 1, single-blind placebo-controlled, 3-way cross-over study on 12 healthy recreational stimulant users (8). Participants (7 male, 5 female) were aged between 19 and 31 years, body weight ranged from 52 to 88 kg (28) and mean (SD) body mass index was 22.9 (±1.3) for male and 21.5 (±2.8) for female (8). A permit from the Dutch drug enforcement administration was acquired for obtaining, storing and administering 4-FA. The study was performed according to the code of ethics on human experimentation established by the Declaration of Helsinki (1964) and was approved by the Medical Ethics Committee of Maastricht University. The study was registered in the Dutch Trial Register (trial number: NTR6164). Participants received monetary compensation for their participation in the study and declared written informed consent.

Single doses of 100 and 150 mg racemic 4-FA hydrochloride (Lipomed, Arlesheim, Switzerland; purity > 98.5%) were dissolved in approximately 150 mL bitter lemon soft drink (Royal Club) and ingested in less than 1 minute. The 150 mg dose was administered after a washout period of at least 7 days to avoid carry-over effects. Due to safety issues, the high dose was only ingested by five participants (8). Blood samples were drawn prior to application (baseline) and 30 minutes, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after ingestion. Samples were centrifuged immediately and stored at −18°C.

### Analysis of 4-FA enantiomers in serum

#### Sample preparation

Two-hundred microliters of serum were spiked with 10 μL internal standard solution (containing 2 μg/mL racemic amphetamine-$d_{11}$ in methanol). For protein precipitation, 600 μL acetonitrile were added. After mixing, samples were centrifuged for 10 minutes at 1,200 × g. Subsequently, 6 mL of phosphate buffer solution (0.1 M, pH 6) were added to the supernatants. For solid phase extraction, cartridges were pretreated with 2 × 3 mL methanol followed by 2 × 2 mL water. Samples were applied and washed with 2 × 2 mL water and 2 × 2 mL water/methanol (80/20, v/v). After addition of 1 mL acetic acid (0.1 M), cartridges were centrifuged for 10 minutes at 1,000 × g. Cartridges were then flushed with 3 mL dichloromethane/acetone (50/50, v/v) and finally eluted with 3 mL dichloromethane/isopropanol/ammonia solution (80/20/4, v/v/v). The extracts were filtered through 0.2 μm syringe membrane filters and were evaporated to dryness under a stream of nitrogen. For analysis, residues were redissolved in 50 μL of methanol.

#### LC–MS-MS instrumentation analytical parameters

Samples were analyzed using an LC–MS-MS system from Agilent (Waldbronn, Germany). The system consisted of a 1290 Infinity II LC coupled via Jet Stream interface (ESI) to a 6495C triple quadrupole mass spectrometer.

Chromatographic separation of enantiomers in 1 μL injected extracts was achieved using a polysaccharide based chiral Lux® 3 μm AMP 150 × 3.0 mm analytical column, guarded with a Lux® AMP 4 × 2.0 mm security guard cartridge (both Phenomenex; Aschaffenburg, Germany) at 30°C. The mobile phase consisted of 5 mM ammonium bicarbonate solution adjusted to pH 11 by addition of ammonia solution (A) and methanol (B). Chromatography was performed isocratically at a flow rate of 0.35 mL/min at 60% B for 13 min. For column washing, B was increased to 95% within 0.5 min, held for 3 minutes and decreased to 60% within 0.5 minutes followed by re-equilibration during a post-time of 2 minutes (total run time 19 min).

The electrospray parameters were: gas flow 11 L/min at 200°C; nebulizer 15 psi, sheath gas flow 12 L/min at 400°C; capillary voltage +3,500 V. Analytes were detected using multiple reaction monitoring mode using the following transitions (*m*/*z*, collision energy in parentheses, target ion underlined): 4-FA 154.1 → 109.0 [21eV], 137.0 [5eV], 83.0 [45eV]; amphetamine-$d_{11}$ 147.2 → 98.0 [21eV], 130.1 [5eV]. Data were evaluated using Agilent Mass Hunter Workstation Software (Version B.09.00).

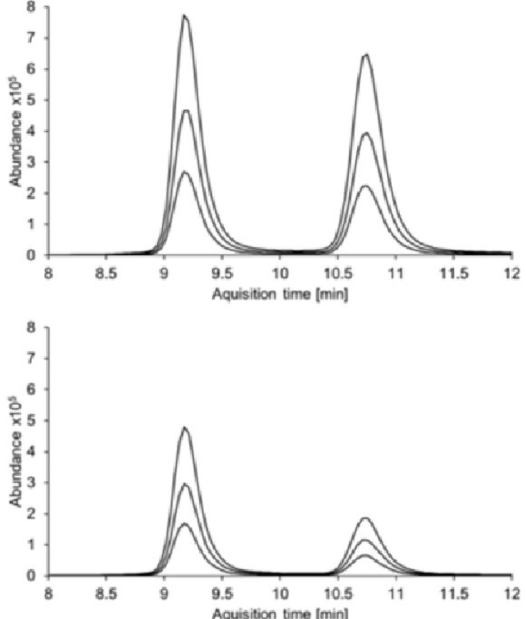



**Figure 1.** Chromatographic separation of (R)-4-FA (left) and (S)-4-FA (right). The figure shows extracted ion chromatograms (transitions in decreasing order of intensity: 154.1 → 109.0 (target), 137.0, 83.0) of a sample at $C_{max}$ (79.3 and 80.2 ng/mL, upper panel) and at 12 hours after ingestion (49.7 and 22.8 ng/mL, lower panel) of a representative subject (#6, 100 mg dose). The calculated (R)/(S)-concentration ratios were 0.99 and 2.18, respectively.

Corresponding enantiomers of the internal standard were used for quantification of each 4-FA enantiomer. Due to the lack of pure standards of 4-FA enantiomers, the elution order could not be experimentally determined but was deduced. (R)-Enantiomers of all the phenethylamine derivatives that were analyzed with this method (amphetamine, methamphetamine, hydroxyamphetamine; unpublished data) consistently eluted prior to the respective (S)-enantiomers. With increasing time after ingestion, the second signal (Figure 1) decreased consistently faster than the first analytical signal. In all chiral pharmacokinetic studies of phenethylamine-type drugs, it was found that the (S)-enantiomer is metabolized more rapidly than the corresponding (R)-enantiomer (22–26). Therefore, it is assumed, that the second signal represents the (S)-enantiomer.

### Method validation

The method was validated according to an international forensic guideline (29). Validation parameters were selectivity, linearity of calibration, analytical limits, accuracy (bias), inter-day precision, recovery, matrix effects and processed sample stability. Statistical evaluation was performed using Valistat 2.00.1 software (Arvecon; Walldorf, Germany).

For evaluation of selectivity, drug-free serum samples of 10 different donors (blank samples) as well as 2 drug-free samples after addition of internal standard (zero samples) were analyzed. Linearity of calibration was tested with three calibration series (0.5, 2.5, 5, 10, 25, 50, 75, 100, 150 and 250 ng/mL). The limit of detection (LOD) was determined by means of signal-to-noise ratio (S/N), measuring an additional calibration series from 0.1–0.5 ng/mL. The lowest calibrator, which met the requirements (S/N > 3), was accepted as LOD. The lower limit of quantification was established for the lowest calibrator by means of a 5-fold analysis requiring less than 20% relative standard deviation (RSD) for precision and less than ±20% for bias. Accuracy (bias), inter-day precision, recovery and matrix effects were determined at low (20 ng/mL) and high (125 ng/mL) concentrations relative to the calibration range. For determination of accuracy and inter-day precision, spiked samples of each concentration were prepared and analyzed on six different days. Accuracy of calculated concentrations was accepted within a maximum bias of 15%. Inter-day precision was calculated as RSD (%) from 6-fold determinations. Experiments for determination of recovery were performed together with matrix effects according to Matuszewski *et al.* (30). Processed sample stability was evaluated at low and high concentrations by pooling and subsequently splitting 6 samples of each concentration, followed by repeated analysis over 31 hours. For determination of long-term and freeze-thaw stability, no experiments were conducted, since stability of 4-FA has been shown for a minimum of 1 year in whole blood (12).

### Evaluation of the data

Quantitative data of 4-FA enantiomers were evaluated in terms of peak serum concentrations ($C_{max}$) and their corresponding time after ingestion ($t_{max}$) and the concentration in the last samples 12 hours after ingestion ($C_{12\,h}$). Area under the curve (AUC) from baseline to the last sample was calculated using the trapezoidal rule. The apparent elimination half-life ($t_{1/2}$) was estimated from exponential regression of the concentrations from $t_{max}$ to the last sampling time (12 h; 5–10 time points). For further evaluation, the (R)/(S)-concentration ratio was calculated for every sample. A linear regression of the ratios using the least squares method was performed for every subject. Statistical tests were performed using SPSS (Version 23.0.0.3, IBM, Ehningen, Germany).

## Results

### Method validation

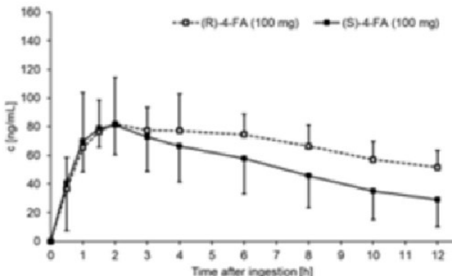
Baseline separation could be achieved for all enantiomers (Figure 1). Blank and zero samples showed no interfering signals (data not shown). For quantification, a linear calibration model without weighting could be used. For both enantiomers, 0.1 ng/mL was accepted as LOD with S/N > 3 for target and qualifier ion-transition. Precision (RSD) and bias data at the lowest calibration level (0.5 ng/mL) were in accordance with the guideline (less than 3.5% and −2.88%, respectively) and this was therefore established as lower limit of quantification. Results of further validation experiments were also within acceptable limits (Table I). Slight ion suppression (88.2 ± 8.3%) at the low concentration level and slight ion enhancement (113.0 ± 8.1%) at the high concentration level were observed. Similar results were obtained for amphetamine-$d_{11}$ (95.2 ± 3.3% at low and 108.7 ± 6.7% at high concentration level, respectively). Recovery for amphetamine-$d_{11}$ (27.9 ± 3.3%) was in the same range as that of 4-FA (32.2 ± 3.5%). Processed samples were stable for at least 31 hours, and decrease of absolute peak areas was less than 20% of the initial value.

### Chiral pharmacokinetics

Pharmacokinetic properties for both enantiomers and differentiated for dose are given in detail in Table II and concentration-time curves are shown in Figure 2. Baseline samples were negative for all subjects. For peak serum concentrations, no significant differences between the enantiomers could be observed (paired *t*-test, $P = 0.63$). The times of maximum concentrations were observed between 0.5 and 4 hours after ingestion, with no significant differences between the

**Table I.** Validation Results for the Enantiomers of 4-FA

| Concentration [ng/mL] | Analyte | Accuracy (bias) $n = 6$ [%] | Precision $n = 6$ [%] | Recovery $n = 6$ [%] | Matrix effects $n = 6$ [%] |
|---|---|---|---|---|---|
| 20 | (R)-4-FA | −0.4 | 7.5 | $30.1 \pm 3.1$ | $87.6 \pm 8.4$ |
| 20 | (S)-4-FA | 6.7 | 4.5 | $30.5 \pm 3.8$ | $88.7 \pm 8.1$ |
| 125 | (R)-4-FA | 6.4 | 3.7 | $33.3 \pm 3.5$ | $115 \pm 8.5$ |
| 125 | (S)-4-FA | 0.2 | 0.8 | $34.9 \pm 3.6$ | $113 \pm 7.7$ |



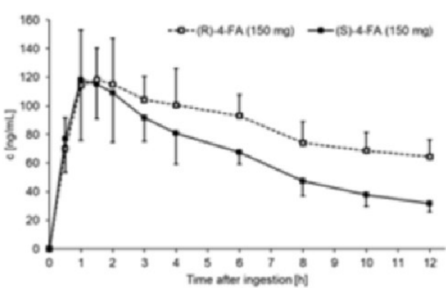




**Figure 2.** Mean serum concentration-time curves of (R)- and (S)-4-fluoroamphetamine (4-FA) for 100 mg (top, $n = 12$) and 150 mg dose (bottom, $n = 5$). For optical reasons, whiskers for standard deviation are shown to one side only.

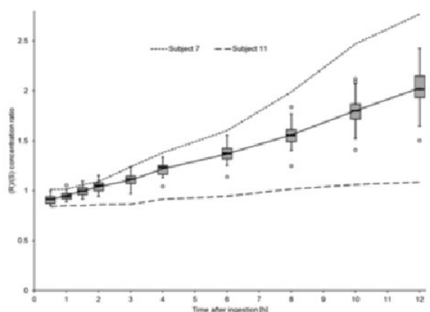



**Figure 3.** Time courses of (R)/(S)-concentration ratios. Data of the outliers (subjects #7 and #11; both 100 mg) are shown in broken lines (no data points shown for optical reasons), all other participants ($n = 10$ for low dose; $n = 5$ for high dose) are represented by box-plots. Horizontal lines show the median ratios and the solid line connects the mean ratios. Whiskers represent all samples within median $\pm$ 1.5 times the interquartile range (box). Outliers with ratios outside of median $\pm$ 1.5 times the interquartile range are shown as circles (o). Extreme values with deviations greater than $\pm$ 3.0 times the interquartile range did not occur.

enantiomers (Mann–Whitney U-test). Apparent elimination half-lives for (R)-4-FA were more than twice as long as for (S)-4-FA (mean difference 6.9 hours). This results in greater AUC for the (R)-enantiomer.

### (R)/(S)-concentration ratio

For further evaluation of the chiral pharmacokinetics, ratios of the concentrations of (R)- and (S)-4-FA were calculated. Test of between-subject-effects for individuals, who received both doses ($n = 5$), showed that the time course of (R)/(S)-ratios was independent of the given dose (two-way ANOVA, $P = 0.36$). In the first sample drawn after ingestion (after 0.5 hours), ratios were below 1.0 in all but 2 subjects (mean 0.91, 0.84–1.02), indicating a slightly higher concentration of the (S)-enantiomer. At the time, when the first enantiomer exhibited $t_{max}$ (estimated end of absorption phase), concentrations of both enantiomers were close to equal, resulting in mean (SD) (R)/(S)-ratios of 1.05 ($\pm$0.09). Due to faster elimination of (S)-4-FA, ratios were linearly increasing with time for all subjects (Pearson's correlation coefficient for each subject >0.99). The slopes of the (R)/(S)-ratio were normally distributed (Kolmogorov–Smirnov test) and exhibited marked interindividual differences. Slopes of subject #7 (0.157 $h^{-1}$) and subject #11 (0.023 $h^{-1}$) were identified as outliers (outside the range of median $\pm$ 1.5 times the interquartile range). The marked interindividual differences are also reflected in the wide spread of ratios observed in the last samples (range 1.08–2.77; 1.50–2.36 without outliers). Figure 3 illustrates the time course of (R)/(S)-concentration ratios for all subjects and doses highlighting the data sets of the 2 outlier subjects who only received the 100 mg dose.

## Discussion

The present study investigated the pharmacokinetic properties of 4-FA enantiomers after controlled oral administration of 4-FA racemate. The administration route and doses are representative for the habits of the vast majority of recreational users (21). The developed sensitive and selective chiral LC–MS-MS method for quantification of the 4-FA enantiomers fulfilled all validation criteria and was successfully applied to the study samples. To the best of our knowledge, this study is the first to quantify 4-FA enantiomers in serum samples from a controlled administration study.

### Pharmacokinetic properties

The comparison of concentrations and pharmacokinetic parameters of the enantiomers did not yield significant differences during the absorption phase ($t_{max}$, $C_{max}$) (Table II). Mean peak serum concentrations were equal for both 4-FA enantiomers which was also reported for amphetamine (23) in a study with four subjects receiving a rather low dose (10 mg). In contrast to the results for 4-FA, differences for absorption phase parameters were reported for enantiomers of MDMA. The $C_{max}$ of the (R)-enantiomer of MDMA was found to be 22–58% higher than the $C_{max}$ of the (S)-enantiomer

**Table II.** Pharmacokinetic Properties of 4-Fluoroamphetamine Enantiomers in Serum after Ingestion of a 100 or 150 mg Dose[a]

| Dose | | $C_{max}$ [ng/mL] | $t_{max}$ [h] | $C_{12\ h}$ [ng/mL] | AUC [ng/mL * h] | $t_{1/2}$ [h] |
|---|---|---|---|---|---|---|
| 100 mg ($n = 12$) | (R) | 86 ± 18 (71–131) | 2.1 ± 0.9 (0.5–4.0) | 52 ± 14 (36–92) | 791 ± 172 (655–1284) | 13 ± 2.3 (8.7–16) |
| | (S) | 86 ± 23 (67–143) | 1.7 ± 0.6 (0.5–3.0) | 29 ± 19 (15–85) | 633 ± 237 (456–1347) | 6.1 ± 1.6 (4.4–10) |
| 150 mg ($n = 5$) | (R) | 125 ± 34 (96–182) | 1.3 ± 0.3 (1.0–1.5) | 65 ± 12 (53–81) | 1019 ± 166 (854–1229) | 13 ± 2.6 (8.3–15) |
| | (S) | 127 ± 38 (95–193) | 1.2 ± 0.5 (0.5–1.5) | 32 ± 6 (23–39) | 777 ± 133 (616–917) | 5.8 ± 0.8 (4.6–6.6) |

[a] Maximum serum concentrations observed ($C_{max}$), corresponding times ($t_{max}$) and serum concentrations 12 hours after consumption (end of experiment) are given. Area under the curve (AUC) was calculated for the study time (0 to 12 hours) without further extrapolation. Apparent elimination half-life ($t_{1/2}$) was calculated using exponential regression. All data are presented as mean ± SD (range).

(22, 24, 25). Furthermore, while 4-FA enantiomers exhibited no significant differences in $t_{max}$, the difference in $t_{max}$ for MDMA enantiomers was reported to be in the range of 0.8 to 2 hours (22, 24, 25). These results suggest that MDMA enantiomers are much more affected by stereoselective processes during absorption and distribution than 4-FA enantiomers. Absorption of drugs is considered to be a passive process, which does not discriminate between enantiomers (31, 32). However, stereospecific absorption and distribution could be caused by selective transport proteins (33, 34), and the distribution of the enantiomers could be influenced by differences in plasma or tissue protein binding (33, 35, 36). The differences for MDMA enantiomers might also be explained as consequence of the longer absorption phase ($t_{max}$ 2.9–4 hours for (R)-MDMA and 1.9–2.1 hours for (S)-MDMA) and generally faster elimination ($t_{1/2}$ 5.8–14.8 hours for (R)-MDMA and 3.6–4.8 hours for (S)-MDMA) of MDMA (22, 26, 28), compared to 4-FA (Table II). A more pronounced first pass effect of (S)-MDMA, as proposed for the rat (37), could also be a possible explanation.

As expected, elimination of 4-FA-enantiomers showed significant differences ($P < 0.01$, paired $t$-test). (R)-4-FA exhibited a substantially longer apparent elimination half-life ($t_{1/2}$) than (S)-4-FA, resulting in significantly higher AUC for the (R)-enantiomer, within the observed time interval (paired $t$-test). This is in accordance with results from studies with oral administration of racemic amphetamine (23) or of MDMA (22, 24, 25) and intravenous injection of methamphetamine (26). Marked interindividual differences in $t_{1/2}$ for both enantiomers were observed and are likely caused by varying urine pH which is a well-known factor (23, 38). Elimination half-lives of the 4-FA-enantiomers were similar to those reported for amphetamine under acidic urine conditions (6.8 hours for (S)- and 7.7 hours for (R)-amphetamine, respectively), whereas amphetamine enantiomers were reported to show substantially longer elimination half-lives under basic urine conditions (17.0 for (S)- and 23.7 for (R)-amphetamine, respectively) (23). In this study, no attempt was made to control urine pH.

## (R)/(S)-concentration ratio

4-FA is of forensic interest due to its stimulant properties, its mild psychedelic effects and adverse effects like sleep deprivation (8, 21). Subjective effects typically last between 4 and 6 hours (21) with maximum effects about 1 hour after administration (8, 28). From serum concentrations alone it is impossible to assess the time of drug consumption and conclude on impairments at a given time (39). From previous studies it was suggested that the enantiomer concentration ratio may be an indicator for the time since ingestion because both enantiomers exhibit different elimination and (R)/(S)-ratios increase with time. Therefore, the 4-FA data were analyzed for the time course and variation of (R)/(S)-ratios.

In the first samples drawn after ingestion of 4-FA (30 minutes), concentrations of (S)-enantiomers were slightly higher in the large majority of cases ((R)/(S)-concentration ratio < 1.0 in 88% of cases). This was different for MDMA 20–30 minutes after ingestion (22, 24, 25), where mean concentrations of the (R)-enantiomer were higher (ratio > 1.0) which continued to the maximum sampling time of 48 hours. At $t_{max}$, (R)/(S)-ratios of 4-FA were close to 1.0 (mean 1.05, range 0.85–1.17) and showed only small variations (SD 0.09). Constantly increasing (R)/(S)-concentration ratios were observed already soon after ingestion (Figure 3), which can be attributed to the faster elimination of (S)-4-FA. This is in general in-line with previous observations for MDMA (22, 25).

In contrast to the present study, the time course of MDMA ratios was obviously not linear in the first 1.5 hours (22, 25). This might be a consequence of the slower absorption of MDMA and marked enantioselective effects already during the absorption phase. However, during the elimination phase starting from $t_{max}$ the ratios were constantly increasing, also with marked interindividual variation (22, 25). Among the subjects in the present study, however, there were two that exhibited markedly different (R)/(S)-time courses (subjects #7 and #11, Figure 3). Starting at an (R)/(S)-ratio of 1.02 0.5 hours after administration, subject #7 exhibited markedly short apparent elimination half-lives for both enantiomers (10.5 and 4.4 hours for (R)- and (S)-4-FA, respectively; 138% difference). This results in a rapid increase of the (R)/(S)-ratios over time (slope 0.157 $h^{-1}$), peaking at 2.77 after 12 hours, which is the highest ratio observed. For subject #11, apparent elimination half-lives, especially for the (S)-enantiomer, were rather high (15.3 and 10.2 hours for (R)- and (S)-4-FA, respectively; 50% difference). The comparatively small relative difference in the elimination of the enantiomers may explain the extraordinary slow increase of the (R)/(S)-ratio with time from 0.84 (0.5 hours after ingestion) to only 1.08 after 12 hours (slope 0.023 $h^{-1}$).

In general, the increasing (R)/(S)-concentration ratios of 4-FA enantiomers can help estimate the time of consumption and conclude on expected effects. Considering a peak psychedelic state at $t_{max}$ (8), and the rather low variations of the (R)/(S)-concentration ratios at this time (1.05 ± 0.09 SD), a ratio exceeding 1.32 (mean + 3 SD) would suggest within a confidence level of 99.7% that peak effects had passed. Acute drug effects last for 6 hours. Only from this time on ratios greater than 1.60 were observed (Figure 3) which would suggest that a considerable time passed since ingestion. At such times, only subacute effects such as symptoms of exhaustion would be expected which may also cause marked impairments in psychomotor performance (28, 40). The approach to conclude on time of ingestion on the basis of enantiomer ratios has limitations and caution must be exercised in forensic interpretation. Ratios lower than 1.6 could result from an acute ingestion of a single dose, but this conclusion could fail under certain circumstances. For example,

repeated intake of 4-FA during a rather short time interval (binge use) could result in higher (R)/(S)-concentration ratios due to accumulation of the (R)-enantiomer, which would lead to an overestimation of the elapsed time with regard to the last ingested dose. With the low number of subjects tested so far there may be individuum specific variations in pharmacokinetics. This could already be observed in the present study, where in 2 cases (including subject #11 exhibiting ratios lower than 1.08 throughout the whole 12 hours, Figure 3) a ratio of 1.60 was never reached even after 12 hours. Therefore, in cases of (R)/(S)-ratios below 1.6, it cannot be differentiated between acute and subacute ingestion with sufficient reliability.

Because enzymes involved in xenobiotic metabolism exhibit genetic polymorphism it may be of interest for future studies to assess differences in phenotypes that may possibly explain the observed differences in (R)/(S)-time courses. A well-known candidate is the human cytochrome P450 2D6 (CYP2D6) for which stereoselective metabolism has been shown (41). This enzyme is involved in the main metabolic pathways of phenethylamines (41–44), including ring halogenated derivatives of methamphetamine (45). For 4-FA, only minor metabolic pathways described to date are hydroxylation of the fluorophenyl ring and the side chain (46); therefore, at present, it is not clear which pharmacokinetic properties may be responsible for the observed interindividual differences.

Variations in excretion of the enantiomers can also be expected after ingestion of higher doses than used in the present study (overdoses). Enzymes of the CYP2 family are defined as high-affinity/low-capacity enzymes, and high concentrations can lead to saturation which may mask stereoselective effects. Consumption of non-racemic quantities would inevitably lead to different (R)/(S)-ratios. However, this can only be assumed for substances where enantiopure precursors are available for stereoselective synthesis (e.g., methamphetamine), whereas phenethylamines like 4-FA are typically synthesized as racemates (47, 48).

In the present placebo-controlled study, 4-FA was dissolved in approximately 150 mL of a quinine-containing bitter lemon drink to mask the bitter taste of the drug. Although quinine has been suggested to inhibit human CYP2D6 (49), dietary amounts of quinine were shown to not inhibit CYP2D6 in vivo (50, 51). Therefore, the rather low amount of quinine ingested in this study should not affect the pharmacokinetics of 4-FA.

## Conclusion

The present study describes chiral serum pharmacokinetic parameters of 4-FA in humans. (R)-4-FA showed a substantially longer apparent serum half-life than the corresponding (S)-enantiomer. (R)/(S)-concentration ratios followed a linear time course for all participants, but two subjects exhibited significantly differing rates. Quantification of 4-FA enantiomers can be helpful to assess the time since ingestion, but in forensic evaluations, the potential of interindividual differences must be considered.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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# 4 Enantioselektive Bestimmung von Amphetamin und Metaboliten in Serumproben: Forensische Evaluation und Einschätzung des Konsumzeitpunktes

## 4.1 Zusammenfassung

Die auch für 4-FA angewendete LC-MS/MS-Methode wurde erfolgreich für die Detektion der Enantiomere von Amphetamin, Norephedrin und 4-Hydroxyamphetamin in Serumproben validiert. Eine leichte Konzentrationsabnahme von Amphetamin bei Lagerung über zehn Monate sowie nach drei Einfrier-/Auftauzyklen wurde beobachtet, die (R)/(S)-Konzentrationsverhältnisse waren allerdings stabil.

Insgesamt 425 Serumproben aus polizeilichen Ermittlungsverfahren aus dem Jahr 2020 wurden untersucht. Die (R)/(S)-Konzentrationsverhältnisse lagen zwischen 0,88 und 4,04. Der Mittelwert lag bei 1,23 und der Median bei 1,11. In 75 Fällen wurden Verhältnisse unter 1,00 festgestellt. Bei niedrigen Gesamtkonzentrationen von Amphetamin (unter 100 ng/mL) lagen signifikant höhere (R)/(S)-Konzentrationsverhältnisse vor (zweifaktorielle Varianzanalyse, $p < 0,001$). 4-Hydroxyamphetamin konnte in 179 Fällen (42 %) detektiert werden, wobei meist entweder beide Enantiomere oder das (R)-Enantiomer alleine gefunden wurden. Die (R)/(S)-Konzentrationsverhältnisse von Hydroxyamphetamin lagen zwischen 0,93 und 4,21 (Median 2,19). (1R,2S)-Norephedrin wurde in 176 Fällen detektiert (41 %). (1S,2R)-Norephedrin, welches kein Metabolit von Amphetamin ist, wurde dagegen nur in 11 Fällen detektiert. Die Metabolite 4-Hydroxyamphetamin und Norephedrin konnten jeweils häufiger bei Vorliegen hoher Amphetaminkonzentrationen detektiert werden. (1R,2S)-Norephedrin war nur in Verbindung mit Amphetamin (R)/(S)-Konzentrationsverhältnissen unter 1,75 detektierbar.

Die angegebenen Zeitintervalle zum letzten Konsum für die insgesamt 30 untersuchten Proben von psychiatrischen Patienten aus Bonn und Mainz lagen zwischen 0,8 h und 77,5 h. Es wurden (R)/(S)-Konzentrationsverhältnisse zwischen 0,88 und 2,86 festgestellt. (1R,2S)-Norephedrin wurde in 12 Fällen detektiert, 4-Hydroxyamphetamin dagegen in keinem Fall. Das angegebene Zeitintervall zum letzten Konsum und die (R)/(S)-Konzentrationsverhältnisse von Amphetamin zeigten eine hohe lineare Korrelation ($R^2 = 0,728$). Die Steigung der (R)/(S)-Konzentrationsverhältnisse mit der Zeit betrug 0,021 $h^{-1}$.

Die Verteilung der (R)/(S)-Konzentrationsverhältnisse in den Polizeiproben war im Einklang mit den Ergebnissen anderer Studien zum Amphetamin. Die bei niedrigen Gesamtkonzentrationen festgestellten allgemein höheren (R)/(S)-Konzentrationsverhältnisse können durch eine weiter fortgeschrittene Ausscheidung erklärt werden. Dieser Trend setzt sich jedoch bei höheren Konzentrationen kaum fort, was in erster Linie auf Unterschiede in der Konsummenge und -frequenz zurückzuführen ist. Unter der Annahme, dass niedrige (R)/(S)-Konzentrationsverhältnisse von Amphetamin auf einen zeitnahen Konsum deuten, ist die Detektion von (1R,2S)-Norephedrin aufgrund der besseren Korrelation geeigneter als die Detektion von Hydroxyamphetamin diese Vermutung zu untermauern.

Die auf Basis der Psychiatrieproben ermittelte Steigung der (R)/(S)-Konzentrationsverhältnisse liegt niedriger als die zuvor für 4-FA ermittelte und für MDMA berichteten Steigungen. Der Vergleich mit der auf Basis von Serumhalbwertszeiten von (R)- und (S)-Amphetamin aus der Literatur theoretisch errechneten Steigung unter Annahme einer Kinetik erster Ordnung zeigte gute Übereinstimmung mit der hier ermittelten Steigung. Innerhalb des Patientenkollektives fanden sich einige Ausreißer außerhalb der 95%-Konfidenzintervalle. Ausreißer zu höheren (R)/(S)-Konzentrationsverhältnissen können allgemein durch die mit Bingekonsum einhergehende Anreicherung von (R)-Amphetamin im Blut erklärt werden.

Unter der Annahme, dass die Akutphase 6 h nach Konsum abgeschlossen ist, kann die obere Grenze des 95%-Konfidenzintervalls bei einem (R)/(S)-Konzentrationsverhältnis von 1,09 als Cut-off für die Annahme der Abwesenheit von Akuteffekten vorgeschlagen werden. Hierbei müssen auch die in dieser Studie beobachteten Ausreißer berücksichtigt werden.

**Eigenleistung an dieser Publikation:** Konzept, Methodenentwicklung, Validierung, Probenkoordination, Probenmessung, Datenanalyse, Datenaufbereitung, graphische Aufbereitung, Literaturrecherche, Niederschrift und Einreichung

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Article

# Enantioselective Quantification of Amphetamine and Metabolites in Serum Samples: Forensic Evaluation and Estimation of Consumption Time

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**Abstract:** In forensic toxicology, amphetamine intoxications represent one of the most common case groups and present difficult questions for toxicologists. Estimating the time of consumption and the current influence of the stimulant is particularly difficult when only total amphetamine concentrations are considered. Stereoselective analysis and the consideration of metabolites can provide valuable information to facilitate interpretation. An enantioselective liquid chromatography−tandem mass spectrometry (LC-MS/MS) method for detection of amphetamine, norephedrine and 4-hydroxyamphetamine was developed. Validation showed satisfactory selectivity, sensitivity, linearity (0.5–250 ng/mL), precision and accuracy for all enantiomers. The method was applied to a collective of 425 forensic serum samples and 30 serum samples from psychiatric inpatients stating their last time of amphetamine consumption. Norephedrine and 4-hydroxyamphetamine were detected more frequently at higher amphetamine concentrations and at lower amphetamine (R)/(S) concentration ratios, possibly indicating recent consumption. Mean (R)/(S) ratio of amphetamine was 1.14, whereas higher ratios (mean 1.36) were found for amphetamine concentrations below 100 ng/mL. The (R)/(S) ratios of psychiatric inpatients significantly correlated with the reported time intervals to last consumption. The use of amphetamine (R)/(S) ratios and the simultaneous detection of metabolites are promising factors that can facilitate estimation of consumption time and current impairment.

**Keywords:** amphetamine; enantiomers; stereoselective; blood serum; chiral chromatography; liquid chromatography; mass spectrometry; forensic science; consumption time; driving under the influence of drugs (DUID)

## 1. Introduction

Amphetamine remains one of the most frequently abused drugs worldwide [1]. Intoxications with this stimulant represent one of the most common case groups and present difficult questions for toxicologists [2–4].

Amphetamine is a sympathomimetic amine that increases brain levels of norepinephrine, serotonin and dopamine by promoting release and inhibiting reuptake of these neurotransmitters [5,6]. Typical acute effects after amphetamine use are euphoria, enhanced attention and alertness, increased psychomotor performance and loss of appetite. At higher doses, adverse effects such as agitation or lack of concentration can occur [3,7]. Excessive

amphetamine use can result in serious intoxication and death, predominantly by acute cardiac or cardiopulmonary failure, cerebrovascular hemorrhage or hyperthermia [8–10]. The acute phase lasts about 6 h [11] and is followed by the subacute phase, which is characterized, e.g., by fatigue, loss of attention and depressive mood [3,12,13]. Due to the broad spectrum of effects, a forensic assessment of current impairment is particularly relevant in cases of driving under the influence of drugs (DUID) [3,10,14]. The determination of the absolute serum amphetamine concentration hardly allows a statement on the time of consumption and thus on the current influence of the stimulant [15]. This is due to differences in quantity and frequency of dosing due to habituation effects [16], as well as interindividual differences in metabolism [17,18].

Amphetamine is a chiral molecule and exists in the enantiomeric forms (S)-amphetamine (d-(+)-amphetamine, dexamphetamine) and (R)-amphetamine (l-(-)-amphetamine, levoamphetamine). Analytical chiral separation is most commonly conducted via capillary electrophoresis (CE) [19,20], direct [4,21] and indirect high-performance liquid chromatography (HPLC) [22,23] and indirect gas chromatography (GC) [24,25].

Studies suggest an approximately 3–4-fold higher efficacy of the (S)-enantiomer [26,27]. Studies further demonstrate stereoselective metabolism of amphetamine, with preferential elimination of the (S)-enantiomer [28–30]. In the human body, amphetamine is metabolized to norephedrine and 4-hydroxyamphetamine. Pharmacokinetics dependent on the absolute configuration are also reported for these metabolites, although hardly any data are available for the human blood [30–33]. It has already been suggested that the time course of (R)/(S) concentration ratios of stimulants can be used to draw conclusions on the time of consumption [34–36]. More sophisticated studies, especially on larger sample sizes, are scarcely available in the literature, and the potential of stereoselective analytics for forensic toxicology has not yet been exploited.

In this study, a stereoselective LC-MS/MS method for the detection of the enantiomers of amphetamine, norephedrine and 4-hydroxyamphetamine was developed and validated. The method was applied to the analysis of 425 forensic serum samples and 30 serum samples from psychiatric inpatients who were hospitalized voluntarily and reported their last time of consumption. The aim of this study was the forensic evaluation of the determined plasma concentrations with particular focus on the estimation of the time of consumption.

## 2. Results

### *2.1. Method Validation*

Baseline separation was achieved for all analyte and internal standard enantiomers (Figure 1). Blank and zero samples showed no interfering signals. Cathine, on the other hand, coeluted with (1S,2R)-norephedrine. A linear calibration model without weighting could be used for quantification of all analytes (calibration range 0.5–250 ng/mL). For all analytes, precision (relative standard deviation (RSD)) and bias data at the lowest calibration level (0.5 ng/mL) were within the acceptable limits of the guideline, so this was accepted as the lower limit of quantification (LLOQ). Results for the external serum round-robin test indicated racemic amphetamine and were in good accordance with the manufacturers' requirements. For enantiomers of 4-hydroxyamphetamine and norephedrine, the 0.5 ng/mL level was also the limit of detection (LOD); for amphetamine enantiomers, 0.1 ng/mL was accepted as LOD, although only quantifiable results ($\geq$0.5 ng/mL) were considered for this study. For all analytes, results of further validation experiments were within acceptable limits (Table 1). For enantiomers of amphetamine and norephedrine, slight ion suppression at the low concentration level and slight ion enhancement at the high concentration level were observed. For 4-hydroxyamphetamine enantiomers, on the other hand, results for ion suppression (up to 78.7 $\pm$ 10.3%) and ion enhancement (up to 114.8 $\pm$ 6.7%) were more diverse. Recovery was rather low for enantiomers of amphetamine (mean 29.8%) and even lower for norephedrine (mean 12.0%) and 4-hydroxyamphetamine (mean 12.4%). For amphetamine-d11 and norephedrine-d3, results for recovery (maximum deviation 3.7%) and matrix effects (maximum deviation 8.6%) were in the same range as for the respective

analytes. Since the decrease in absolute peak areas was less than 20% of the initial value for all enantiomers, processed samples were stable for at least 63 h. Concentrations of amphetamine enantiomers were stable for the observed period of 10 months. The mean loss was 1.9 ng/mL for (R)-amphetamine and 1.0 ng/mL for (S)-amphetamine (median +0.1 and −0.5 ng/mL, respectively). Stability of amphetamine (R)/(S) concentration ratios is shown as a Bland–Altman plot in Figure 2. Mean deviation was 0.01, and deviations > ±1.96 SD were only found for one sample with rather low concentrations (total amphetamine concentration 45.2 ng/mL, mean ratio 2.01) and in two samples with concentrations at the upper end of the calibration range.

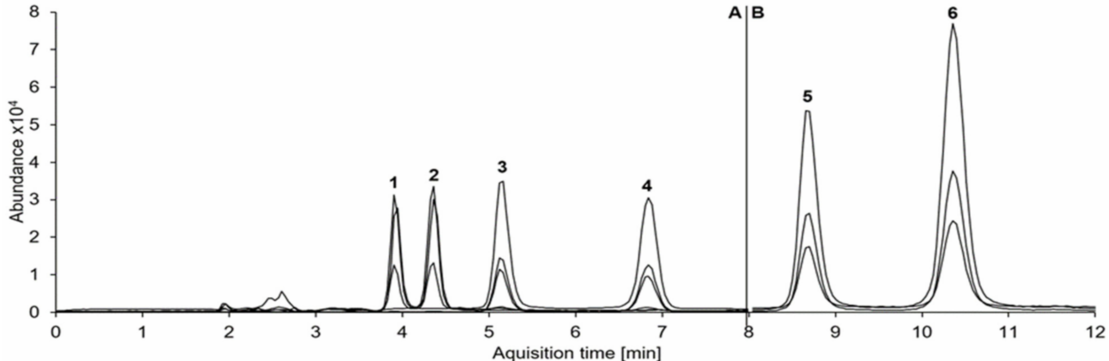


**Figure 1.** Multiple reaction monitoring (MRM) chromatograms for (R)-4-hydroxyamphetamine (1), (S)-4-hydroxyamphetamine (2), (1S,2R)-norephedrine (3), (1R,2S)-norephedrine (4), (R)-amphetamine (5) and (S)-amphetamine (6) at concentration levels of 20 ng/mL per enantiomer. MRM was split into two segments (A, B) at 8.0 min to reach a higher abundance. The total run time was 19 min. The following ion transitions are displayed in decreasing order of intensity (target ion underlined): 4-hydroxyamphetamine 152.1 → 107.0, 135.0, 77.0; norephedrine 152.1 → 134.0, 117.0, 115.0; amphetamine 136.1 → 91.0, 119.0, 65.0.

**Table 1.** Validation results for the enantiomers of amphetamine (AM), norephedrine (NE) and 4-hydroxyamphetamine (4OH-AM) in serum.

| Concentration | Analyte | Accuracy (Bias) n = 6 | Interday Precision n = 6 | Recovery n = 6 | Matrix Effects n = 6 |
|---|---|---|---|---|---|
| (ng/mL) | | (%) | (%) | (%) | (%) |
| 20 | (R)-AM | −1.7 | 0.4 | 28.1 ± 3.2 | 86.5 ± 6.5 |
| 20 | (S)-AM | 4.5 | 4.3 | 28.4 ± 3.9 | 87.3 ± 6.7 |
| 125 | (R)-AM | 3.3 | 2.7 | 30.3 ± 3.3 | 106 ± 7.3 |
| 125 | (S)-AM | 7.3 | 2.5 | 32.3 ± 3.6 | 105 ± 7.7 |
| 20 | (1S,2R)-NE | 3.4 | 4.6 | 10.3 ± 1.2 | 99.4 ± 8.4 |
| 20 | (1R,2S)-NE | −1.2 | 1.1 | 10.7 ± 1.3 | 97.8 ± 8.9 |
| 125 | (1S,2R)-NE | 5.2 | 6.0 | 13.4 ± 1.6 | 112.2 ± 9.3 |
| 125 | (1R,2S)-NE | −0.5 | 8.4 | 13.5 ± 1.7 | 112.5 ± 8.4 |
| 20 | (R)-4OH-AM | 1.8 | 12.8 | 10.0 ± 0.9 | 78.7 ± 10.3 |
| 20 | (S)-4OH-AM | −0.3 | 17.6 | 10.4 ± 1.2 | 98.4 ± 7.7 |
| 125 | (R)-4OH-AM | 0.2 | 9.7 | 14.5 ± 3.4 | 85.5 ± 4.9 |
| 125 | (S)-4OH-AM | 1.4 | 13.7 | 14.6 ± 1.8 | 114.8 ± 6.7 |

A slight freeze–thaw instability was found for both enantiomers of amphetamine (median loss each 7%), ranging from a (theoretical) 5% gain to a 16% loss. Maximum deviation of amphetamine (R)/(S) concentration ratios was 0.11 (median 0.01).

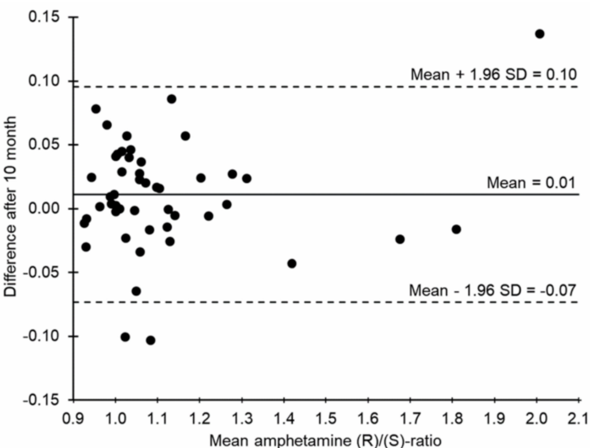


**Figure 2.** Bland-Altman plot of the stability of amphetamine (R)/(S) ratios after reanalysis after 10 months (n = 50). The *x*-axis shows the mean value of the two determinations and the *y*-axis shows the absolute deviation after 10 months.

### 2.2. Forensic Serum Samples

Within the 425 cases examined, concentrations up to 784 ng/mL were found for (R)- and 887 ng/mL for (S)-amphetamine (same case, total concentration 1670 ng/mL). The calculated (R)/(S) concentration ratios were between 0.88 and 4.04 (mean 1.23, median 1.11). Ratios < 1.00 were observed in 75 cases (18%). The subject who exhibited an (R)/(S) ratio of 4.04 had serum concentrations of 8.6 and 2.1 ng/mL for (R)- and (S)-amphetamine, respectively. The boxplots in Figure 3 show the distribution of (R)/(S) concentration ratios within the entire collective and staggered by groups of total amphetamine concentrations (100 ng/mL intervals). While for total concentrations of <100 ng/mL the mean (R)/(S) ratio is significantly (one-way ANOVA, $p < 0.001$) higher than for the other groups (1.36 vs. 1.14), no clear trend can be observed for higher concentrations.

4-Hydroxyamphetamine was detected in 179 out of 425 cases (42%). In 100 cases, only the (R)-enantiomer could be detected; in another 71 cases, both enantiomers were found, while the (S)-enantiomer was detected alone in only 8 cases. The maximum concentrations found were 7.7 ng/mL for (R)- and 2.8 ng/mL for (S)-4-hydroxyamphetamine. Median concentrations were 1.2 and 0.8 ng/mL for the (R)- and the (S)-enantiomer, respectively. Median concentrations of (R)- and (S)-4-hydroxyamphetamine relative to their precursors (R)- and (S)-amphetamine were 0.8% and 0.5%, respectively. (R)/(S) concentration ratios of 4-hydroxyamphetamine ranged from 0.93 to 4.21 (median 2.19).

In 176 cases (41%), (1R,2S)-norephedrine was found alone (maximum concentration 7.2 ng/mL, median 1.0 ng/mL). Within the positive cases, the relative (1R,2S)-norephedrine concentration in relation to (S)-amphetamine was up to 5.4% (mean 0.9%, median 0.7%).

(1S,2R)-Norephedrine could only be detected in 11 cases (maximum concentration 1.6 ng/mL), 9 times together with (1R,2S)-norephedrine. Since (R)-amphetamine is not subjected to β-hydroxylation [31], (1S,2R)-norephedrine is not a metabolite of amphetamine. Therefore, detection of this enantiomer can only be explained by the use of other drugs (e.g., drugs containing racemic norephedrine or the interfering diastereomer cathine), for which reason the corresponding cases were excluded from further interpretation. While none of the herein detectable metabolites were detected in 42% of cases (n = 179), both 4-hydroxyamphetamine (any enantiomer) and (1R,2S)-norephedrine were detected in 26% of the cases (n = 109). In each 16% of cases, either 4-hydroxyamphetamine (n = 70) or (1R,2S)-norephedrine (n = 67) was detected next to amphetamine.

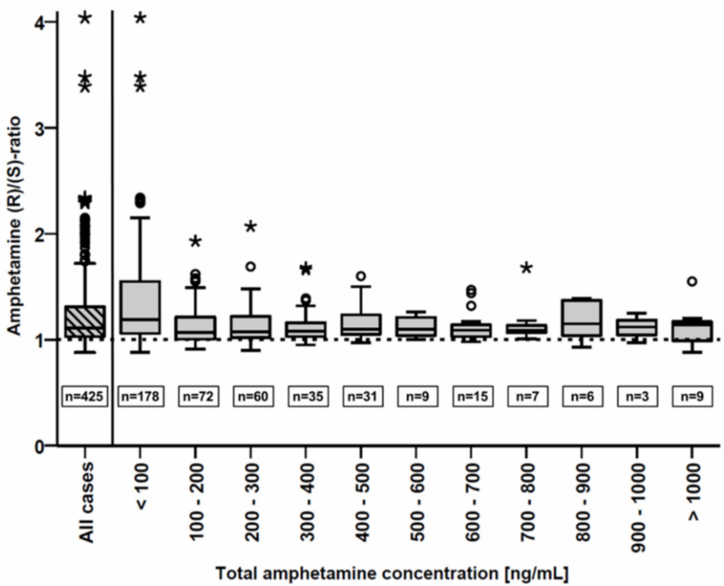


**Figure 3.** Boxplots of the determined amphetamine (R)/(S) ratios within subgroups of total amphetamine concentration. The striped left box represents the whole sample collective (n = 425). Horizontal lines represent the median, boxes represent the range between the lower and upper quartiles of the subgroups (interquartile range). Whiskers represent all samples within ±1.5 times the interquartile range. Outliers with (R)/(S) ratios greater than median ± 1.5 times the interquartile range are presented as circles (o). Extreme values with deviations greater than ±3 times the interquartile range are presented as asterisks (*).

Qualitative detection of 4-hydroxyamphetamine and (1R,2S)-norephedrine significantly correlated with staggered groups of total amphetamine concentration (100 ng/mL intervals, one-tailed Spearman's Rho and Kendall's Tau each $p = 0.000$, Figure 4). While (1R,2S)-norephedrine was very rarely detected at total amphetamine concentrations below 100 ng/mL (5.1%), it was found in the vast majority of samples with total amphetamine concentrations above 300 ng/mL. In contrast, at least one enantiomer of 4-hydroxyamphetamine was found in almost 20% of cases even at total amphetamine concentrations below 100 ng/mL. The increase in detection frequency with increasing amphetamine concentrations is not as rapid as in the case of (1R,2S)-norephedrine. In over 70% of cases with total amphetamine concentrations above 400 ng/mL, at least one enantiomer of norephedrine was detected. This was not the case for amphetamine concentrations between 800 and 900 ng/mL, where norephedrine was detected in only 50% of the cases, although the total number of cases in this group was very limited (n = 6). Moreover, qualitative detection of 4-hydroxyamphetamine and (1R,2S)-norephedrine significantly correlated (negatively) with staggered groups of (R)/(S) concentration ratios (0.25 intervals, one-tailed Spearman's Rho and Kendall's Tau each $p < 0.001$, Figure 5). While the metabolites could be detected in at least 40% of cases at (R)/(S) ratios below 1.25, detectability decreased at higher ratios. (1R,2S)-Norephedrine was not detectable at amphetamine (R)/(S) ratios above 1.75. In general, a similar trend was observed for the enantiomers of 4-hydroxyamphetamine, although even at ratios above 1.75 (n = 31) at least one of the two enantiomers was detectable in a total of 7 cases.

Quantitatively, (1R,2S)-norephedrine concentrations showed correlations (one-tailed Pearson) with both total amphetamine concentration ($r = 0.507$, $p = 0.000$) and the concentration of the parent compound (S)-amphetamine ($r = 0.514$, $p = 0.000$). In contrast, however, there was no quantitative correlation with the (R)/(S) concentration ratio ($r = 0.053$, $p = 0.242$).

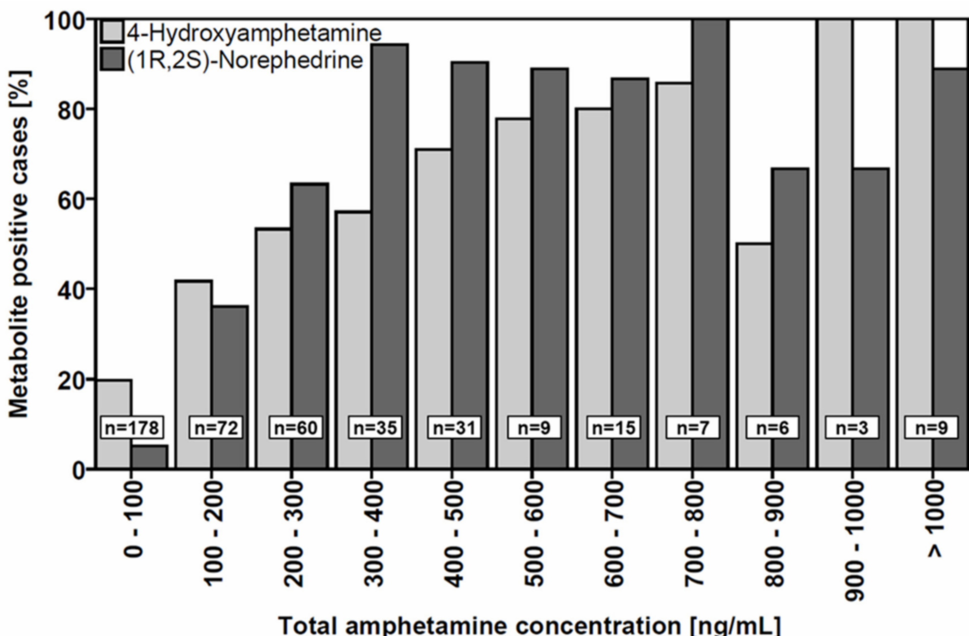


**Figure 4.** Histograms for relative frequency of 4-hydroxyamphetamine and (1R,2S)-norephedrine positive cases (at least 0.5 ng/mL of any enantiomer) within different total amphetamine concentration ranges. For both 4-hydroxyamphetamine and (1R,2S)-norephedrine, there was a positive correlation between positive cases and the total amphetamine concentration (one-tailed Spearman's Rho and Kendall's Tau each $p = 0.000$).

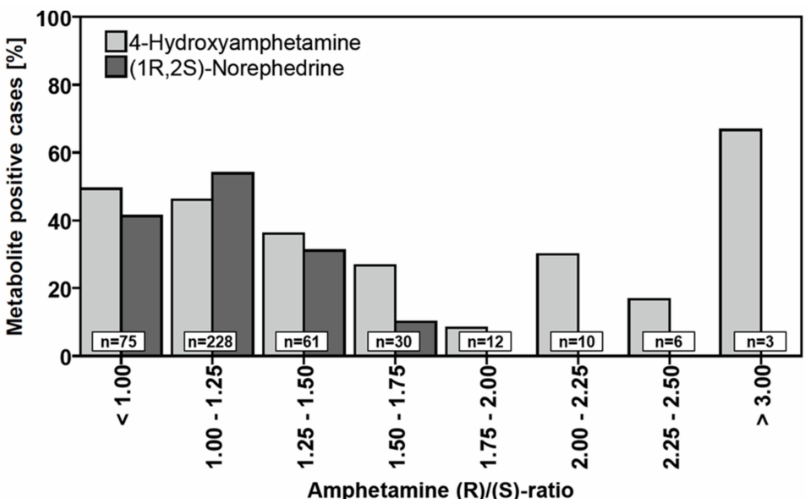


**Figure 5.** Histograms for relative frequency of 4-hydroxyamphetamine (any enantiomer) and (1R,2S)-norephedrine positive cases (each at least 0.5 ng/mL) within amphetamine®/(S) concentration ratio groups. For both 4-hydroxyamphetamine and (1R,2S)-norephedrine, there was a negative correlation between 4-hydroxyamphetamine positives and amphetamine (R)/(S) concentration ratios (one-tailed Spearman's Rho and Kendall's Tau each $p < 0.001$). There were no cases with (R)/(S) concentration ratios between 2.50 and 3.00.

### *2.3. Cases with Self-Reported Consumption Time*

Within the psychiatric patient collective, concentrations up to 261 and 226 ng/mL (same case, total concentration 487 ng/mL) were found for (R)- and (S)-amphetamine,

respectively. The reported times after last consumption were between 0.75 and 77.5 h. Calculated (R)/(S) concentration ratios were between 0.88 and 2.86 (median 1.22). Twelve patients (40%) reported at least one other use within 24 h prior to the last use, which was further considered as 'binge' consumption. These patients are discussed separately. The route of consumption was reported by 15 patients (50%). While one patient reported oral consumption, the majority (n = 12) consumed exclusively nasally. One patient each reported nasal/oral and nasal/intravenous use.

(1R,2S)-Norephedrine was detected in 12 cases, reaching concentrations up to 7.0 ng/mL (reported time 4.0 h, (R)/(S) ratio 1.09), while in the other cases concentrations were below 1.8 ng/mL. (1S,2R)-Norephedrine was absent in all samples. The maximum reported time with detection of (1R,2S)-norephedrine was 38.5 h ((R)/(S) ratio 1.39). None of the 4-hydroxyamphetamine enantiomers could be detected in a single case. Table 2 shows the determined concentrations and (R)/(S) ratios for all (n = 30) cases with self-reported consumption time. For further interpretation, the (R)/(S) concentration ratios were plotted against the reported last consumption time (Figure 6). Linear regression (method of least squares) was carried out and showed high correlation ($R^2 = 0.728$) between time intervals and (R)/(S) ratios. Furthermore, 95% confidence intervals were calculated. The slope of the linear fit was 0.021 $h^{-1}$, and the y-intercept was at an (R)/(S) ratio of 0.861. There were both patients with rather high serum concentrations and longer time to last consumption (e.g., case 29) and patients who reported having consumed a few hours earlier but showed rather low concentrations (e.g., case 12).

**Table 2.** Summary of cases with self-reported consumption time.

| Case | Binge | Δ*t* | (R)-AM | (S)-AM | Σ AM | (R)/(S) | (1R,2S)-NE |
|---|---|---|---|---|---|---|---|
| (ng/mL) | | (h) | (ng/mL) | (ng/mL) | (ng/mL) | | (ng/mL) |
| 1 | | 12.0 | 12.3 | 13.9 | 26.3 | 0.88 | |
| 2 | | 5.5 | 32.4 | 35.2 | 67.6 | 0.92 | |
| 3 | * | 0.8 | 114 | 124 | 238 | 0.93 | 0.6 |
| 4 | * | 20.5 | 98.3 | 102 | 200 | 0.97 | |
| 5 | * | 7.0 | 128 | 125 | 253 | 1.02 | 1.2 |
| 6 | | 7.0 | 25.5 | 24.7 | 50.2 | 1.03 | |
| 7 | | 22.0 | 41.9 | 40.1 | 81.9 | 1.04 | |
| 8 | * | 4.0 | 112 | 107 | 219 | 1.05 | 0.6 |
| 9 | | 11.5 | 42.1 | 39.3 | 81.4 | 1.07 | |
| 10 | * | 6.3 | 111 | 103 | 214 | 1.08 | 1.4 |
| 11 | * | 3.0 | 239 | 219 | 457 | 1.09 | 7.0 |
| 12 | | 3.0 | 40.9 | 37.4 | 78.3 | 1.09 | 1.4 |
| 13 | | 34.2 | 18.0 | 16.0 | 24.0 | 1.13 | |
| 14 | | 13.5 | 261 | 226 | 487 | 1.15 | 1.8 |
| 15 | | 18.0 | 100 | 82.4 | 182 | 1.21 | 1.4 |
| 16 | * | 33.0 | 8.9 | 7.2 | 16.1 | 1.23 | |
| 17 | | 28.8 | 8.7 | 7.1 | 15.8 | 1.23 | |
| 18 | * | 25.0 | 28.5 | 23.1 | 51.5 | 1.23 | 0.9 |
| 19 | | 18.5 | 34.3 | 27.2 | 61.6 | 1.23 | 1.0 |
| 20 | | 20.0 | 105 | 82.4 | 188 | 1.28 | |
| 21 | * | 3.0 | 201 | 157 | 358 | 1.28 | |
| 22 | * | 31.0 | 18.6 | 14.5 | 33.1 | 1.29 | |
| 23 | * | 7.3 | 239 | 181 | 420 | 1.32 | |
| 24 | | 17.5 | 114 | 85.1 | 199 | 1.34 | 0.7 |
| 25 | | 38.5 | 43.8 | 31.5 | 75.3 | 1.39 | 0.9 |
| 26 | | 40.0 | 6.2 | 3.6 | 9.8 | 1.72 | |
| 27 | * | 47.5 | 14.2 | 7.3 | 21.5 | 1.93 | |
| 28 | | 42.0 | 1.2 | 0.6 | 1.8 | 1.96 | |
| 29 | | 40.5 | 115 | 53.8 | 169 | 2.14 | |
| 30 | | 77.5 | 10.3 | 3.6 | 13.9 | 2.86 | |

The table shows the patient-reported time interval between last consumption and blood collection (Δ*t*), as well as the determined concentrations of (R)-amphetamine ((R)-AM), (S)-amphetamine ((S)-AM), total amphetamine (Σ AM) and (1R,2S)-norephedrine ((1R,2S)-NE). Case numbers are assigned by ascending amphetamine (R)/(S) ratio. Asterisks (*) indicate patient-reported binge consumption (further consumption of amphetamine within the last 24 h prior to the reported last use).

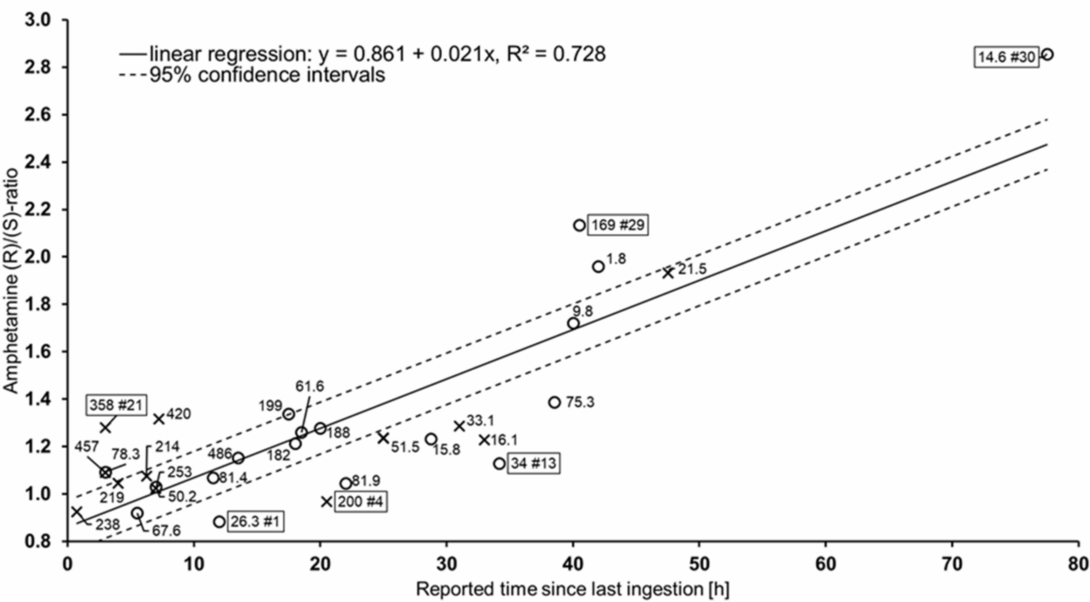


**Figure 6.** Plot of the determined amphetamine (R)/(S) ratios versus the reported time since last ingestion according to the patients' statements (n = 30). Cases with reported binge consumption (further consumption of amphetamine within the last 24 h) are marked as crosses (x); all other cases are presented as circles (o). Labels show the total (racemic) amphetamine concentration of each patient in ng/mL. For some patients, the case number is also given with a number sign (#). Linear regression (solid line) was done using the method of least squares. Broken lines show 95% confidence intervals.

## 3. Discussion

### 3.1. Method Validation

In the present study, a total of 425 forensic serum samples and 30 serum samples from psychiatric patients, stating their last time of consumption, were enantioselectively analyzed for amphetamine and specific metabolites. A chiral LC-MS/MS method for detection of amphetamine, norephedrine and hydroxyamphetamine was developed, validated and applied for analysis of the specimens. To the best of our knowledge, this study is the first to enantioselectively detect amphetamine and metabolites in a comprehensive collective of serum samples and evaluate them from a forensic perspective.

The method was sensitive and selective, and all analytes met all acceptance criteria of the validation. At the high concentration level, recovery was slightly higher than at the low level, although it was rather low for all analytes, especially for norephedrine and hydroxyamphetamine. Amphetamines are known to be volatile compounds that may be lost during evaporation, especially at high temperatures [37,38]. Therefore, the extracts were evaporated (nearly) to dryness in as short a time as possible at only 40 °C so that reproducible results were ensured. Since all validation parameters were met despite the comparatively low recovery, the sample preparation method used was considered suitable.

For this study, the detected interference of (1S,2R)-norephedrine with cathine is not relevant, since this enantiomer is not a metabolite of amphetamine. The results for long-term and freeze–thaw stability for amphetamine were in line with other (chiral) studies [39–41]. The experiments of Beyer et al. do not confirm the slight freeze–thaw instability found here [42]. Deviations in (R)/(S) concentration ratios after reanalysis were within the measurement inaccuracies. Complete stability of the ratios is therefore assumed, which enables forensic interpretation even after longer storage time.

### *3.2. Forensic Serum Samples*

(R)/(S) concentration ratios of phenethylamines consumed as a racemic mixture have been discussed to give a hint for the last time of consumption due to an enantioselective metabolism [34–36]. For amphetamine, we showed that in Rhineland-Palatinate it is used exclusively as the racemate [43], and it can be assumed that this is true worldwide due to non-stereoselective pathways of synthesis [35,44,45].

The amphetamine (R)/(S) ratios calculated in the sample collective are consistent with the use of racemic amphetamine, taking into account the shorter elimination half-life of (S)-amphetamine [28,29]. The median (R)/(S) ratio in this study was 1.11, which was significantly lower than the median of 1.69 found by Hess et al. in a similar study (n = 28) [35]. The maximum (R)/(S) ratio of 4.50 found there is similar to that of the present study (4.04). In the study of Hess et al., (R)/(S) ratios below 1.00 were only detected in combination with methamphetamine intake (which is mostly consumed as pure (S)-methamphetamine, and thus only (S)-amphetamine is formed as a metabolite), while in our study, ratios between 0.88 and 1.00 were found in a total of 75 samples (18%). Peters et al. found (R)/(S) ratios ranging from 0.97 to 1.66 (mean 1.15, median 1.10) in a total of 23 quantifiable samples with exclusive amphetamine use [39]. Moreover, in a controlled oral intake study by Wan et al. (10 mg racemic amphetamine, n = 4), (R)/(S) ratios ranging from 0.84 to 1.18 were observed during the absorption phase [29]. The occurrence of (R)/(S) ratios below 1.00 is also supported by the studies of Caras and Sharpe, which found higher mean peak serum concentrations of (S)-amphetamine while the times of peak serum concentrations of both enantiomers were similar [28]. Furthermore, in a study with controlled oral intake of racemic 4-fluoroamphetamine (4-FA), (R)/(S) ratios below 1.00 were observed during the absorption phase (partially even beyond) [36]. Havnen et al. also found (R)/(S) ratios around 1.00, although all samples were from patients under treatment with prescribed nonracemic amphetamine (n = 61), and the results are therefore hardly comparable [46]. Even in the present study, an additional intake of medicinal drugs, as also described by Musshoff et al. [47], cannot be basically ruled out. In Germany, (S)-amphetamine (Attentin) and its precursor lisdexamfetamine (Elvanse) can be prescribed for treatment of attention deficit hyperactivity disorder (ADHD). More seldom, formulations of racemic amphetamine sulfate can be prescribed [35,47]. Adderall, which is approved in the United States, contains approximately a 3:1 mixture of (S)- and (R)-amphetamine [48]. In the case of exclusive use of illicit (racemic) amphetamine, serum (R)/(S) ratios below 1.00 could be explained, e.g., by a faster uptake of (S)-amphetamine or a more pronounced first-pass effect for (R)-amphetamine. In general, especially at lower concentrations, measurement inaccuracies must also be taken into account.

The significantly higher mean amphetamine (R)/(S) ratio at total concentrations below 100 ng/mL indicates an advanced elimination of (S)-amphetamine, which is consequently associated with lower absolute amphetamine concentrations. However, at concentrations above 100 ng/mL, this trend continued only partially. Outliers with higher (R)/(S) ratios also occurred at higher total amphetamine concentrations. This can probably be explained mainly by repeated intake (binge use), which is common among chronic amphetamine users [49]. However, high (R)/(S) ratios are generally less frequently found at higher amphetamine concentrations. Moreover, even at total amphetamine concentrations below 100 ng/mL, there are many samples exhibiting rather low (R)/(S) ratios. Since dosages can differ considerably between occasional (recreational) users and chronically dependent users due to habituation effects [16], recent consumption should generally be considered even at low concentrations when (R)/(S) ratios are rather small.

Amphetamine is metabolized to hydroxyamphetamine by liver monooxygenases and about 3–7% is excreted as such [30–33]. The fact that in almost all cases either (R)-4-hydroxyamphetamine alone or both enantiomers were found is consistent with the observation by Dring et al. in humans, that (R)-amphetamine is metabolized to 4-hydroxyamphetamine to a greater extent than (S)-amphetamine or the racemate is [33]. Two to three percent of amphetamine is excreted renally as norephedrine, and the metabolism

is probably catalyzed by dopamine β-hydroxylase, which is located in the central and peripheral nervous system [30–32]. The relatively low serum concentrations of (1R,2S)-norephedrine found in the present study (median 1.0 ng/mL) were expected for this minor metabolite. The detection of (1S,2R)-norephedrine in only 11 cases is consistent with the observation that β-hydroxylation occurs selectively only for (S)-enantiomers [30,31]. Since the results in the corresponding cases are distorted by the use of medication or other drugs, the corresponding cases were not considered for further evaluation.

(1R,2S)-Norephedrine and 4-hydroxyamphetamine were detected in an almost equal number of cases (n = 176 vs. 179), with a relatively large intersection of samples in which both metabolites were found (n = 109). Both 4-hydroxyamphetamine and (1R,2S)-norephedrine showed a statistically significant trend of increasing detectability with higher amphetamine concentrations. The increase in detectability with increasing amphetamine concentration was significantly steeper for (1R,2S)-norephedrine. In contrast to (1R,2S)-norephedrine, enantiomers of 4-hydroxyamphetamine were detected considerably more frequently at amphetamine concentrations below 100 ng/mL. Overall, 4-hydroxyamphetamine-positive cases are thus more widely distributed with regard to absolute amphetamine concentrations. Regarding the determined amphetamine (R)/(S) ratios, a statistically significant trend of more frequent detection at lower ratios was observed for both 4-hydroxyamphetamine and (1R,2S)-norephedrine. Assuming that the shorter elimination half-life of (S)-amphetamine leads to increasing (R)/(S) ratios over time and that low ratios therefore indicate a more recent ingestion, (1R,2S)-norephedrine seems to be more suitable as a qualifying criterion since, unlike hydroxyamphetamine, it was not detectable at (R)/(S) ratios over 1.75. In addition, (1R,2S)-norephedrine was less frequently detectable at lower amphetamine concentrations. Taken together, these results indicate that (1R,2S)-norephedrine offers higher potential than 4-hydroxyamphetamine to foster forensic interpretations, although in this case the ingestion of other substances must be taken into account when performing achiral analysis. Thus, according to the results of this study and under assurance of appropriate analytical sensitivity, the detection of (1R,2S)-norephedrine could strengthen a suspicion of recent amphetamine consumption.

### 3.3. Cases with Self-Reported Consumption Time

The observed time period, which for most patients was no longer than 48 h, is equivalent to the typical detection time for amphetamine in serum samples [50]. The maximum serum concentration determined was significantly lower than in the forensic sample collective, while the median was slightly higher. The maximum determined (R)/(S) ratio was slightly lower for the psychiatric patients. Since this collective comprises dependent users who voluntarily undergo psychiatric treatment, the incidence of binge use was very high at 40%. Both the maximum concentration of (1R,2S)-norephedrine and the detection frequency were approximately identical with those of the forensic serum samples. On the other hand, it remains unclear why 4-hydroxyamphetamine was not detected in any case, which is in contrast to the forensic samples.

Amphetamine intoxications are one of the most common drug-induced impairments, which is particularly relevant in driving under the influence of drugs (DUID) [3,47,51]. Especially in this context, information about the time of use and the current influence of stimulants is critical [3,51]. On the basis of the total amphetamine concentration, as it is typically determined, such conclusions cannot be made [15].

The determination of (R)/(S) concentration ratios and the evaluation of the respective time course have already been proposed in some studies. In studies of stereoselective metabolism of 3,4-methylenedioxy-N-methylamphetamine (MDMA) by Fallon et al. and Steuer et al. (oral intake of 40 and 125 mg racemate, respectively), mean (R)/(S) concentration ratios increased to approximately 8 within 24 h (slope 0.33 $h^{-1}$) [34,52].

The increase in MDMA (R)/(S) ratios is thus significantly more rapid than that found for amphetamine in the present study. In our previous study on 4-fluoroamphetamine (4-FA) metabolism, slopes for individual subjects ranged from 0.023 to 0.157 $h^{-1}$ [36].

This faster increase in the MDMA and 4-FA (R)/(S) ratios with time is due to the shorter elimination half-lives and the larger relative difference (approx. factor 2).

The human serum half-lives of the amphetamine enantiomers were determined in two studies. Wan et al. determined (with oral intake of 10 mg racemate) serum half-lives of 23.7 and 7 h under alkaline and 7.7 and 6.8 h under acidic urine conditions for (R)- and (S)-amphetamine, respectively [29]. Assuming first-order elimination kinetics [53] and a mean (R)/(S) concentration ratio of 1.00 at the start of elimination [29], the theoretical slopes of the (R)/(S) ratios with time can be calculated from the serum half-lives using a linear fit. For alkaline urine conditions, slopes of 0.013 and 0.018 $h^{-1}$ are calculated for 24 and 72 h, respectively. For acidic urine conditions, the theoretical slopes are 0.014 and 0.019 $h^{-1}$.

Using the serum half-lives determined by Caras et al. (oral intake of 30 mg racemate) of 13.4 and 10.8 h for (R)- and (S)-amphetamine, respectively [28], slopes of 0.015 and 0.020 $h^{-1}$ are obtained over 24 and 72 h, respectively. In this context, it should be noted that the theoretical slope of the (R)/(S) ratios increases with time, and the linear fit is thus an approximation. However, in the calculated examples, the absolute error of the linear fit for the (R)/(S) ratios after 72 h is only 0.11 at maximum. The slopes calculated theoretically are consistently slightly lower than the slope of 0.021 $h^{-1}$ determined in this study.

Within the observed collective, some outliers far outside the 95% confidence intervals appeared. Subject 1 was found to have the lowest (R)/(S) ratio of the entire collective (0.88), although the last consumption was self-reported to be as long as 12 h earlier. Subject 4, who reported the last consumption 20.5 h earlier, exhibited a very low ratio of 0.97. Subject 13 reported last use 34 h earlier and exhibited a ratio of only 1.13, although the absolute amphetamine concentration of 34 ng/mL was rather low. There were also outliers located above the 95% confidence interval. In general, higher (R)/(S) ratios can be explained by a binge-associated enrichment of the (R)-enantiomer. This could explain, for example, the comparatively higher (R)/(S) ratio (1.28) of Subject 21. Subject 29 showed a high (R)/(S) ratio (2.14), although a rather high total amphetamine concentration of 169 ng/mL was present in serum. Thus, the indicated longer past use (40.5 h) seems plausible despite the considerable amphetamine concentration. This indicates that the respective dose consumed must have been above the average amount.

In the studies by Fallon and Losacker, high interindividual variances of the (R)/(S) ratios were found for MDMA and 4-FA, respectively, which gradually increased over time. Particularly in the 4-FA study, the (R)/(S) ratio for one subject was still below 1.00 even after 12 h. Considering this, the scatter of outliers found in the present study appears moderate. After oral ingestion of (S)-amphetamine, peak effects are reached after about 3 h [54], and the acute phase lasts for about 6 h [11].

The upper limit of the 95% confidence interval after 6 h is at an (R)/(S) ratio of 1.09. Ratios above this cut-off would therefore suggest the absence of acute effects.

Subject 21, however, exhibited an (R)/(S) ratio above this cut-off, although the last consumption was indicated 3 h earlier. One explanation for this could be the reported binge consumption. In contrast, (R)/(S) ratios below the cut-off of 1.09 were observed up to 22.0 h after the claimed last consumption.

Despite the variation of the determined ratios with the reported time, chiral amphetamine analysis can allow important conclusions in certain cases. Thus, for Subject 29, it can be assumed that acute effects had passed, although this would not necessarily have been indicated by looking at the total amphetamine concentration. It must of course always be taken into account that exhaustion reactions such as fatigue, which occur in the subacute phase, can also restrict the driving ability [3,15].

For the forensic toxicological expert, the evaluation of (R)/(S) ratios must take into account whether, for example, a more recent consumption can incriminate or exonerate the defendant. For this purpose, it should be asked when exactly the last consumption had taken place. Furthermore, it is of great relevance whether it was a single or repeated use. In the case of binge use, the enrichment of (R)-amphetamine in the blood leads to

higher (R)/(S) ratios, which would result in an overestimation of the time difference to consumption. However, extreme shifts in the (R)/(S) ratio should generally not be expected with binge use. With a longer time interval between two doses, the serum concentrations remaining from the first consumption are expected to be minor compared to the total concentration, and with a short time interval, the ratio should be close to 1.00 anyway.

The observed collective consisted of dependent psychiatric inpatients, some of which were also abusing other substance groups such as opiates, benzodiazepines or cannabis. Higher dosages due to habituation effects, liver damage and metabolic effects due to enzyme induction or inhibition therefore cannot be ruled out. The enzyme CYP2D6, which is involved in the metabolism of amphetamine and is known for its stereoselectivity, exhibits a genetic polymorphism [17,18,55,56]. Consequently, high interindividual differences have to be taken into account. Poor metabolizers lacking CYP2D6 could thus exhibit low (R)/(S) ratios over a longer period of time. Furthermore, consumption of very high doses or concomitant use of other drugs may lead to saturation of CYP2D6 so that metabolism is taken over by other (less stereoselective) enzymes. Illegal amphetamine is present exclusively in racemic form [4,43,57]. For forensic interpretation, therefore, the (additional) use of (S)-amphetamine-containing drugs must be excluded, as this medication would lead to an underestimation of the time difference to consumption.

## 4. Materials and Methods

### *4.1. Material*

(RS)-Amphetamine and racemic norephedrine-d3 were purchased from LGC (Wesel, Germany). (RS)-Amphetamine d11, (S)-amphetamine, (R)-amphetamine, racemic norephedrine and (RS)-4-hydroxyamphetamine were obtained from Lipomed (Weil am Rhein, Germany). Methanol (HPLC-grade) was purchased from Thermo Fisher Scientific (Dreieich, Germany). Water (LC-MS-grade), acetonitrile, dichloromethane, acetone, isopropanol, glacial acetic acid, potassium dihydrogenphosphate and potassium hydroxide were obtained from Carl Roth (Karlsruhe, Germany). LC-MS-grade ammonia solution (25%) and ammonium bicarbonate were purchased from Merck (Darmstadt, Germany). All chemicals were at least of analytical grade. Mixed-mode cation-exchange solid-phase extraction columns (HF BE-CERTIFY, 300 mg, 3 mL) were purchased from Agilent (Waldbronn, Germany). Round-robin tests 'BTMF—Drugs in serum' containing certified racemic amphetamine were acquired from Arvecon (Walldorf, Germany). Blank (drug-free) serum was provided by the blood bank of the University Medical Center of the Johannes Gutenberg University Mainz.

### *4.2. Sample Preparation*

Two hundred microliters of serum was spiked with 10 µL internal standard solution (containing 2 µg/mL racemic amphetamine-d11 and 2 µg/mL racemic norephedrine-d3 in methanol). Samples with concentrations outside of the calibration range were diluted with water to reach appropriate concentrations and reanalyzed. Six hundred microliters of acetonitrile was added for protein precipitation. After mixing, samples were centrifuged for 10 min at 1200× *g*. Subsequently, 6 mL of phosphate buffer solution (0.1 M, pH 6) was added to the supernatants. For solid-phase extraction (SPE), cartridges were equilibrated with 2 × 3 mL methanol followed by 2 × 2 mL water. Samples were applied and washed with 2 × 2 mL water and 2 × 2 mL water/methanol (80/20, *v*/*v*). After addition of 1 mL acetic acid (0.1 M), cartridges were centrifuged for 10 min at 1000× *g*. After flushing with 3 mL dichloromethane/acetone (50/50, *v*/*v*), elution was done using 3 mL dichloromethane/isopropanol/ammonia solution (80/20/4, *v*/*v*/*v*). The extracts were evaporated to dryness under a gentle stream of nitrogen at 40 °C. For analysis, residues were redissolved in 50 µL of methanol.

### 4.3. Chiral LC-MS/MS Instrumentation and Analytical Parameters

Enantioselective quantification was done using a liquid chromatography–tandem mass spectrometry (LC-MS/MS) system from Agilent (Waldbronn, Germany). Chromatography was conducted using a 1290 Infinity II LC system, coupled via Jet Stream interface (ESI) to a 6495C triple-quadrupole mass spectrometer. Chiral chromatographic separation was achieved using a polysaccharide-based chiral Lux 3 μm AMP 150 × 3.0 mm analytical column, guarded with a Lux AMP 4 × 2.0 mm security guard cartridge (both Phenomenex; Aschaffenburg, Germany). Injection volume was 1 μL. The mobile phase consisted of 5 mM ammonium bicarbonate solution adjusted to pH 11 by addition of ammonia solution (A) and methanol (B). Holding a temperature of 30 °C and a flow rate of 0.35 mL/min, chromatography was performed isocratically at 60% B for 13 min. For column washing, B was increased to 95% within 0.5 min, held for 3 min and decreased to 60% within 0.5 min followed by re-equilibration during a post-time of 2 min (total run time 19 min). Electrospray parameters were as follows: gas flow 11 L/min at 200 °C; nebulizer 15 psi, sheath gas flow 12 L/min at 400 °C; capillary voltage +3500 V. Analytes were detected by multiple reaction monitoring (MRM) mode using the following transitions ($m/z$, collision energy in parentheses, target ion underlined): amphetamine 136.1 → <u>91.0</u> (17 eV), 119.0 (5 eV), 65.0 (45 eV); amphetamine-d11 147.2 → <u>98.0</u> (21 eV), 130.1 (5 eV), 70.1 (45 eV); norephedrine 152.1 → <u>134.0</u> (9 eV), 117.0 (17 eV), 115.0 (25 eV); norephedrine-d3 155.1 → <u>137.1</u> (9 eV), 119.1.0 (21 eV), 117.1 (29 eV); 4-hydroxyamphetamine 152.1 → <u>107.0</u> (21 eV), 135.0 (5 eV), 77.0 (45 eV). For quantification of each enantiomer, corresponding enantiomers of the internal standards were used. Norephedrine-d3 was used as internal standard for quantification of 4-hydroxyamphetamine. Data evaluation was done using Agilent Mass Hunter Workstation Software (Version B.09.00). Identification of the elution order of amphetamine enantiomers was conducted by injection of enantiopure standard solutions. For all other experiments, racemic solutions were used as analytical standards. Due to the lack of enantiopure standards for norephedrine and 4-hydroxyamphetamine, elution order of these metabolites could not be determined but was deduced. Since no racemization of these substances takes place in the human body [58], the absolute configuration (at the 2-position) is retained. Accordingly, when enantiopure formulations such as dexamphetamine or lisdexamfetamine (Elvanse) are ingested, only (1R,2S)-norephedrine and (S)-4-hydroxyamphetamine are formed as metabolites. By analyzing respective serum samples, it could be shown that the mentioned metabolites of (S)-amphetamine elute second, as does their parent substance.

### 4.4. Method Validation

The method was validated according to an international forensic guideline [59]. Validation parameters were selectivity, linearity of calibration, analytical limits, accuracy (bias), interday precision, recovery, matrix effects and processed sample stability. Statistical evaluation was performed using Valistat 2.00.1 (Arvecon; Walldorf, Germany).

Evaluation of selectivity was done by analyzing drug-free serum samples of 10 different donors (blank samples) and two drug-free samples after addition of internal standard (zero samples). A 100 ng/mL solution of cathine ((1S,2S)-norpseudoephedrine), which is a diastereomer of norephedrine, was injected to check for interferences. Linearity of calibration was tested with three calibration series, which included the following calibration points: 0.5, 2.5, 5, 10, 25, 50, 75, 100, 150 and 250 ng/mL. LOD was determined by means of signal-to-noise ratio (S/N, >3). LLOQ was established for the lowest calibrator by means of a 5-fold analysis requiring less than 20% RSD for precision and less than ± 20% for bias. Accuracy (bias), interday precision, recovery and matrix effects were determined at low (20 ng/mL) and high (125 ng/mL) concentrations relative to the calibration range. Quality control working solutions and calibration working solutions were prepared separately from different stock solutions. For determination of accuracy and interday precision, samples of each concentration were prepared and analyzed on six different days. Accuracy of calculated concentrations was accepted within a maximum bias of 15%. Interday preci-

sion was calculated as RSD (%) from 6-fold determinations. Furthermore, external serum round-robin tests containing certified racemic amphetamine were examined for quality control. Recovery and matrix effects were determined according to the recommendations of Matuszewski et al. [60]. Processed sample stability was evaluated at low and high concentrations by pooling and subsequently splitting six samples of each concentration, followed by repeated analysis over 63 h. For determination of the long-term stability of amphetamine enantiomer concentrations and stability of amphetamine (R)/(S) concentration ratios, 50 amphetamine-positive forensic samples were reanalyzed 10 months after the first analysis. For amphetamine, freeze–thaw stability was determined by reanalysis of spiked samples at low and high concentration levels (20 and 250 ng/mL, respectively) after three freeze–thaw cycles. Additionally, three real forensic samples were reanalyzed after three cycles.

### *4.5. Investigated Collectives*

#### 4.5.1. Forensic Serum Samples

A total of 425 amphetamine-positive forensic serum samples were analyzed. All samples originate from the year 2020 and were sent to the Department of Forensic Toxicology at the Institute of Forensic Medicine in Mainz, Germany, from police stations in the federal state of Rhineland-Palatinate for toxicological analysis. After centrifugation, the serum samples were stored at −20 °C. Samples from individuals reporting consumption of pharmaceutical drugs containing (S)-amphetamine or (S)-amphetamine precursors (e.g., Attentin or Elvanse) or other enantiomeric compositions of amphetamine (e.g., Adderall) were excluded to prevent the data from being distorted. Cases positive for methamphetamine were also excluded, since amphetamine represents the main metabolite and this would therefore bias the results [4].

#### 4.5.2. Cases with Self-Reported Consumption Time

A total of 30 serum samples with self-reported consumption of amphetamine within the last 48 h were analyzed. The samples were collected from inpatients at the Department of Psychiatry and Psychotherapy, University Medical Center Mainz, and at the Clinic and Polyclinic for Psychiatry and Psychotherapy, University Hospital Bonn. Ward 6 (not protected) of the University Hospital Mainz is specialized in the treatment of patients with various types of addiction. The "Freud" ward (protected) of the University Hospital Bonn focuses on addiction medicine for qualified detoxification treatments, especially for opiate-dependent patients. However, in both clinics, admission and treatment are voluntary. Upon admission to both wards, patients are asked about their substance abuse (in particular, substances abused, last use, route of consumption and frequency of use). The collected blood samples were stored in serum tubes at 4 °C and kept at −20 °C after centrifugation.

## 5. Conclusions

In the present study, serum samples from two different collectives were stereoselectively analyzed for the enantiomers of amphetamine and metabolites. Increasing amphetamine (R)/(S) ratios were observed over time. The metabolites were found significantly more frequently at lower amphetamine (R)/(S) ratios indicating recent consumption. The advantages of a stereoselective analysis were discussed regarding forensic questions. For the future, an expansion of the patient collective should be targeted in order to achieve a more robust data basis. Generally, a controlled ingestion study would be superior, although this is hardly feasible due to ethical factors.

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# 5 Enantioselektive Bestimmung der Plasmaproteinbindung von gebräuchlichen ATS

## 5.1 Zusammenfassung

Die auch für 4-FA, Amphetamin, Norephedrin und 4-Hydroxyamphetamin angewendete LC-MS/MS-Methode war ebenfalls zur Trennung der Enantiomere von Methamphetamin geeignet und konnte erfolgreich validiert werden. Die für die Trennung der Enantiomere von MDMA verwendete Methode konnte ebenfalls für Serumproben validiert werden. Darüber hinaus konnte die Linearität der Kalibration im Ultrafiltrat für alle vier Stimulantien gezeigt werden.

Auf Basis der Konzentrationen in den Kontrollproben und in den Ultrafiltraten wurde die PPB der jeweiligen Enantiomere berechnet. Allgemein lag die PPB der Stimulantien im niedrigen bis mittleren Bereich. (R)-4-FA (33,5 %) und (S)-4-FA (36,4%) zeigten die höchste PPB. Die PPB von Amphetamin lag mit 31,7 % für das (R)- und 29,0 % für das (S)-Enantiomer etwas niedriger. Die niedrigste PPB zeigten die Enantiomere von Methamphetamin mit 18,8 % für das (R)- und 19,4 % für das (S)-Enantiomer. Die PPB von (R)-MDMA (21,6 %) und (S)-MDMA (21,3 %) lag etwas höher. Es zeigte sich keine signifikante Abhängigkeit der PPB vom Plasmaspender (einfaktorielle Varianzanalyse, $p = 0,85$). Für die PPB der jeweiligen Enantiomere von 4-FA (t-Test, $p = 0,11$) und Methamphetamin ($p = 0,18$) ergaben sich keine signifikanten Unterschiede. Die Unterschiede in der PPB der Enantiomere von Amphetamin (mittlere Differenz 3,8 %, $p = 0,01$) und MDMA (mittlere Differenz 0,6 %, $p = 0,04$) waren jedoch trotz ihres geringen Ausmaßes statistisch signifikant.

Obgleich die Unterschiede in der PPB der Enantiomere von Amphetamin und MDMA statistisch signifikant waren, sind diese aufgrund ihrer geringen relativen Differenzen nicht geeignet, für die erheblichen enantiospezifischen Unterschiede in der Pharmakokinetik und Pharmakodynamik der Stimulantien verantwortlich zu sein. Für Amphetamin kommt allenfalls ein geringer Einfluss in Frage, den die höhere PPB des (R)-Enantiomers etwa zu einer langsameren Metabolisierung oder einer niedrigeren pharmakologischen Potenz beitragen könnte.

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Short communication

# Enantioselective determination of plasma protein binding of common amphetamine-type stimulants

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ABSTRACT

Amphetamine-type stimulants (ATS) like amphetamine ('*speed*'), methamphetamine ('*crystal meth*') and 3,4-methylenedioxy-N-methylamphetamine (MDMA, '*ecstasy*') represent some of the most frequently abused drugs worldwide. Another less frequently abused ATS is 4-fluoroamphetamine (4-FA). The enantiomers of these four compounds exhibit different pharmacokinetic and pharmacodynamic properties. According to the free drug theory, the pharmacological properties of a substance are dependent on its plasma protein binding (PPB). However, data on PPB of stimulant enantiomers in humans are rare or non-existent.

Human plasma samples were spiked with racemic mixtures of the stimulants and subjected to ultrafiltration to extract the unbound fraction. Enantioselective liquid chromatography – tandem mass spectrometry (LC-MS/MS) methods were applied using a chiral Phenomenex® Lux3 μm AMP column. Method validation showed satisfactory selectivity, linearity (0.5 250 ng/mL), accuracy and precision. Enantiomers were quantified before and after ultracentrifugation to determine PPB.

For all analytes, low to medium plasma protein binding was found. For (R)-amphetamine a slightly but significantly higher PPB was found compared to the (S)-enantiomer (31.7 % vs 29.0 %). (R)-MDMA also showed only slightly but significantly significantly higher PPB than (S)-MDMA, although the mean difference was negligible (21.6 % vs 21.3 %). For the enantiomers of methamphetamine and 4-FA, no significant differences in PPB were found.

In summary, there were no or only minor differences in PBB for the enantiomers of all investigated compounds. The different pharmacological properties of the stimulant enantiomers can therefore not be explained by differences in PPB.

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## 1. Introduction

Amphetamine-type stimulants (ATS) are some of the most commonly abused drugs in the world [1]. Consumption and seized quantities of these synthetic stimulants are increasing worldwide. While amphetamine is the most commonly used drug in most European countries, the use of methamphetamine predominates in North America, Asia and Oceania [1]. Also, abuse of '*ecstasy*' (3,4-methylendioxy-N-methylamphetamine, MDMA) is on the rise again in many countries worldwide [1]. Amphetamine derivatives are also represented in the group of new psychoactive substances (NPS), of which the para-fluorinated analog of amphetamine, 4-fluoroamphetamine (4-FA) is occasionally used in Germany and the Netherlands [2].

ATS produce stimulant effects by binding to CNS targets, however, drugs also bind nonspecifically to blood components such as plasma proteins [3]. Basic substances such as ATS are preferentially bound on alpha-1-acid glycoprotein (AGP, orosomucoid), which typically has concentrations of about 20–30 μM [3]. The extent of plasma protein binding (PPB) thus defines the free drug concentration in plasma and therefore affects pharmacokinetic parameters such as clearance (Cl) and volume of distribution ($V_d$), and therefore also affects the plasma half-life ($t_{1/2}$) as well as pharmacodynamics [3].

Enantioselective plasma protein binding has been shown, e.g. for the basic drugs verapamil and propranolol, which stereoselectively bind to AGP [4]. Studies regarding human plasma protein binding of ATS are scarce [5–7] and only one chiral PPB study is available for amphetamine [8]. Various studies have already shown that, depending on the absolute configuration, the pharmacodynamic properties of ATS vary significantly. For example, the (S)-enantiomers of MDMA and amphetamine are reported to

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have a higher efficacy [9,10]. Furthermore, various studies suggest a shorter plasma half-life of the (S)-enantiomers [2,8,11,12]. However, the possible influence of a different PPB of the enantiomers has not yet been discussed. Furthermore, it is necessary to evaluate possible differences in PPB with regard to intoxication potential. This is particularly relevant because the co-uptake of other substances competing for binding sites could lead to severe intoxications [3]. The PPB is also relevant from an analytical point of view, since protein-bound analytes would not be detected without release of the drug from its protein binding. With the increasing therapeutic use of single enantiomer drugs (e.g. (S)-amphetamine or (R)-methamphetamine), or other non-racemic mixtures (e.g. Adderal®), enantioselective analysis is becoming increasingly important in the field of forensic toxicology, but also in drug development or clinical areas [13]. Furthermore, for instance in Germany, the legal consequences of a drug seizure may vary depending on the chiral composition of the substances [14]. Therefore, it is important to carry out suitable cost-saving and time-effective methods for enantiomer separation. Methods of direct enantioseparation that do not require chiral derivatization are therefore particularly suitable for practical applications [13].

For the determination of plasma protein binding, ultrafiltration is a simple and cost-effective method. The free drug concentration in the ultrafiltrate can be determined consistently, regardless of the extent of ideal ultrafiltration [15].

In the present study, two chiral liquid chromatography – tandem mass spectrometry (LC–MS/MS) methods were developed to separate the enantiomers of amphetamine, methamphetamine, MDMA and 4-FA. The methods were validated according to an international guideline and then combined with ultrafiltration to determine the enantioselective protein binding of the most important ATS. The results were evaluated with regard to pharmacological and toxicological implications.

## 2. Material and methods

### 2.1. Material

(RS)-amphetamine, (RS)-methamphetamine, (S)-methamphetamine, (RS)-methamphetamine-$d_{11}$ and (RS)-MDMA-$d_5$ were purchased from LGC (Wesel, Germany). (RS)-amphetamine-$d_{11}$, (RS)-4-FA, (S)-amphetamine and (R)-amphetamine were obtained from Lipomed (Weil am Rhein, Germany). (RS)-MDMA was purchased from Merck (Darmstadt, Germany). Methanol (HPLC-grade) was obtained from Thermo Fisher Scientific (Dreieich, Germany). Water (LC–MS-grade), acetonitrile, dichloromethane, acetone, isopropanol, glacial acetic acid, potassium dihydrogenphosphate and potassium hydroxide were purchased from Carl Roth (Karlsruhe, Germany). LC–MS-grade ammonia solution (25 %), ammonium bicarbonate as well as Millipore Centrifree® ultrafiltration devices were obtained from Merck (Darmstadt, Germany). All chemicals were at least of analytical grade. Mixed-mode cation-exchange solid phase extraction columns (HF BE-CERTIFY, 300 mg, 3 mL) were obtained from Agilent (Waldbronn, Germany). Blank (drug-free) plasma was provided by the blood bank of the University Medical Center of the Johannes Gutenberg University Mainz.

### 2.2. Ultrafiltration conditions and sample preparation

Ultrafiltration was conducted analogously to Fung et al. [16] and in accordance with the manufacturer's instructions. For each substance, protein binding was determined separately in triplicate in six different blank plasma samples. For this purpose, each of 4 mL blank plasma were spiked to reach a final concentration of 250 ng/mL per enantiomer (racemic concentration < 3.7 μM). After vortexing, the samples were incubated for one hour at 37 °C. Subsequently, each 1 mL of plasma was transferred to the Centrifree® sample reservoirs. An aliquot of 200 μL plasma was also further processed as a control sample for each determination. Ultrafiltration devices were then placed in a pre-warmed centrifuge and centrifuged at 2,000 x g for one hour. 100 μL of the obtained ultrafiltrate were then spiked with 5 μL of internal standard solution (containing each 2 μg/mL racemic amphetamine-$d_{11}$, methamphetamine-$d_{11}$ and MDMA-$d_5$ in methanol). Control samples were spiked analogously with 10 μL each to reach the same concentrations. Further processing was the same for the ultrafiltrates and control samples. After addition of 600 μL acetonitrile, samples were centrifuged at 1,200 x g for ten minutes. Then, 6 mL of phosphate buffer solution (0,1 M, pH 6) were added to the supernatants. For solid phase extraction, cartridges were equilibrated with 2 × 3 mL methanol followed by 2 × 2 mL water. Samples were applied and washed with 2 × 2 mL water and 2 × 2 mL water/methanol (80/20, v/v). Subsequent to addition of 1 mL acetic acid (0.1 M), cartridges were centrifuged for 10 min at 1,000 x g. After flushing with 3 mL dichloromethane/acetone (50/50, v/v), elution was done using 3 mL dichloromethane/isopropanol/ammonia solution (80/20/4, v/v/v). The extracts were evaporated to dryness under a stream of nitrogen and residues were redissolved in 50 μL of methanol for analysis.

### 2.3. Chiral LC–MS/MS instrumentation and analytical parameters

Enantioselective quantification was done using a liquid chromatography tandem mass spectrometry (LC–MS/MS) system from Agilent (Waldbronn, Germany). Chromatography was conducted using a 1290 Infinity II LC system, coupled via Jet Stream interface (ESI) to a 6495C triple quadrupole mass spectrometer. Chiral chromatographic separation was achieved using a polysaccharide based chiral Lux® 3 μm AMP 150 × 3.0 mm analytical column, guarded with a Lux® AMP 4 × 2.0 mm security guard cartridge (both Phenomenex; Aschaffenburg, Germany). Injection volume was 1 μL. Mobile phase A consisted of 5 mM ammonium bicarbonate solution adjusted to pH 11 with ammonia solution, as suggested by the manufacturer. For optimization of the chiral separation, different compositions of methanol, acetonitrile and isopropanol were tested as organic mobile phase (B). Amphetamine, methamphetamine and 4-FA were best separated using pure methanol. Under this conditions, the enantiomers of MDMA could not be separated satisfactorily. Using a mixture of methanol/acetonitrile (1/1, v/v), (R)- and (S)-MDMA could be separated properly. A solvent mixture separating the enantiomer of all four compounds could not be found. For runtime optimization, different flow rates (0.2 – 0.5 mL/min) and column temperatures (20–50 °C) were tested. The limiting factor was the maximum pressure of 310 bar, that was allowed for the polysaccharide column. Best results were obtained with a flow rate of 0.35 mL/min and a column temperature of 30 °C. Isocratic elution was proved to be a more suitable than gradient elution. A column flush step (95 % B) was included to avoid carry-over. The final gradients were as follows (if applicable different percentages for MDMA in parentheses): Holding B at 60 % (50 %) for 13 min, increasing it to 95 % within 0.5 min, holding for 3 min and decreasing to 60 % (50 %) within 0.5 min. With an additional post-time of 2 min, total run time for both gradients was 19 min. The following electrospray parameters were used: Gas flow 11 L/min at 200 °C; nebulizer 15 psi, sheath gas flow 12 L/min at 400 °C; capillary voltage +3500 V. Analytes were detected by multiple reaction monitoring (MRM) mode using the following transitions (*m/z*, collision energy in parentheses, target ion underlined): amphetamine 136.1 → <u>91.0</u> (17 eV), 119.0 (5 eV), 65.0 (45 eV); amphetamine-$d_{11}$ 147.2 → <u>98.0</u> (21 eV), 130.1 (5 eV), 70.1 (45 eV); 4-FA 154.1 → <u>109.0</u> (21

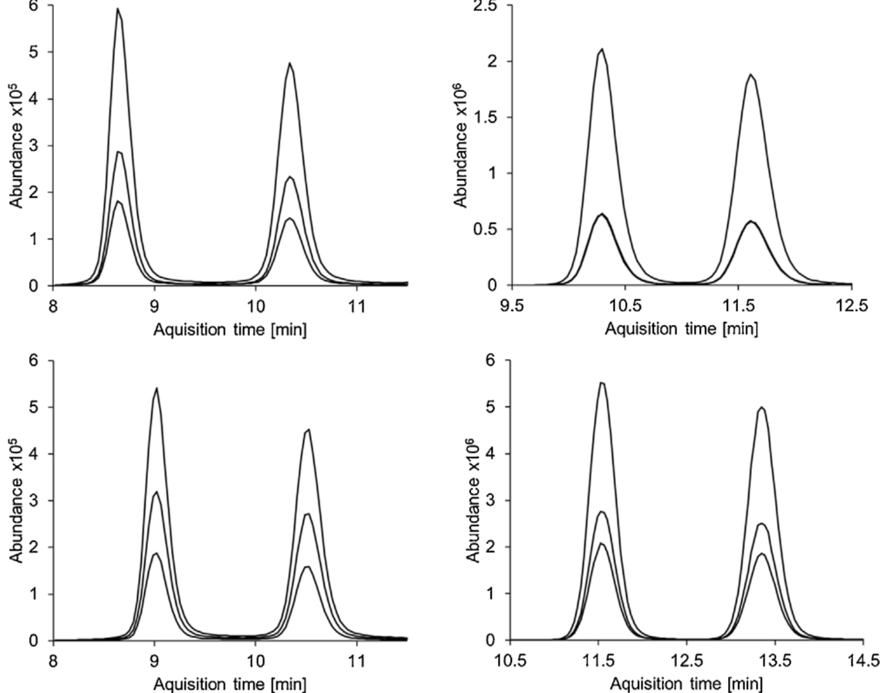


**Fig. 1.** Extracted ion chromatograms for Amphetamine (top left), methamphetamine (top right), 4-FA (bottom left) und MDMA (bottom right) at a concentration level of 100 ng/mL per enantiomer. For all substances, the (R)-enantiomer eluted first. The following ion transitions are displayed in decreasing order of intensity (target ion underlined): amphetamine 136.1 → 91.0, 119.0, 65.0; methamphetamine 150.1 → 91.0, 119.0, 65.0; 4-FA 154.1 → 109.0, 137.0, 83.0; MDMA 194.1 → 163.0, 105.0, 51.1.

eV), 137.0 (5 eV), 83.0 (45 eV), methamphetamine 150.1 → 91.0 (21 eV), 119.0 (9 eV), 65.0 (49 eV), methamphetamine-$d_{11}$ 161.2 → 96.9 (21 eV), 127.0 (5 eV), 69.1 (53 eV), MDMA 194.1 → 163.0 (9 eV), 105.0 (25 eV), 51.1 (80 eV); MDMA-$d_5$ 199.2 → 165.0 (13 eV), 107.0 (29 eV), 135.0 (21 eV). Corresponding enantiomers of the internal standards were used for quantification of each analyte enantiomer. Amphetamine-$d_{11}$ was used as internal standard for quantification of 4-FA. Data evaluation was conducted using Agilent Mass Hunter Workstation Software (Version B.09.00). Identification of the elution order for amphetamine and methamphetamine enantiomers was conducted by injection of enantiopure standard solutions. For all other experiments, racemic solutions were used as analytical standard. Due to the lack of enantiopure standards for 4-FA and MDMA, elution order could not be determined but was deduced. Elution order of 4-FA enantiomers was deduced in a former study [2]. For MDMA, a faster metabolism of the (S)-enantiomer was shown [12]. When forensic plasma samples were measured using the method presented here, the enantiomer eluting second consistently showed lower concentrations. It was therefore identified as (S)MDMA. Taken together, the (R)-enantiomer eluted first for all the analytes observed in this study.

### *2.4. Method validation*

The methods were validated according to an international guideline [17]. Relevant validation parameters investigated for this study were selectivity, linearity of calibration, accuracy (bias), inter-day precision, recovery, matrix effects and processed sample stability. All parameters were determined for plasma samples (sample volume 200 μL, 10 μL ISTD). For quantification of the ultrafiltrates, an additional calibration series was prepared by spiking blank ultrafiltrates (sample volume 100 μL, 5 μL ISTD). Statistical evaluation was performed using Valistat 2.00.1 software (Arvecon; Walldorf, Germany).

Selectivity was evaluated by analyzing drug-free serum samples of ten different donors (blank samples) as well as two drug-free samples after addition of internal standard (zero samples). Linearity of calibration was assessed by measuring three calibration series (0.5, 2.5, 5, 10, 25, 50, 75, 100, 150 and 250 ng/mL) in spiked plasma. For ultrafiltrate calibration, the same stock solutions were used, but only half of the volume was spiked in 100 μL blank ultrafiltrate matrix. Accuracy (bias), interday precision, recovery and matrix effects were determined at low (20 ng/mL) and high (125 ng/mL) concentrations relative to the calibration range. To determine accuracy and inter-day precision, samples of each concentration were prepared and analyzed on six different days. Accuracy of calculated concentrations was accepted within a maximum bias of 15 %. Interday precision was calculated as RSD (%) from six-fold determinations. Recovery and matrix effect were determined according to the recommendations of Matuszewski et al. (30). Processed sample stability was evaluated at low and high concentrations by pooling and subsequently splitting six samples of each concentration, followed by repeated analysis over 63 h.

### *2.5. Data analysis*

Plasma Protein Binding (PPB) was calculated for each enantiomer in each sample according to Equation 1. To compensate for measurement inaccuracies, the mean value of the concentrations of the (R)- and the (S)-enantiomer ($c_{(R)\text{-contr.}}$ and $c_{(S)\text{-contr.}}$, respectively) was calculated as the initial concentration in the control sample. The unbound (free) fraction was thus calculated as the quotient of the concentration of an enantiomer in the ultrafiltrate and the initial plasma concentration [15]. Finally, PPB was calculated as the difference of the unbound fraction from 1. For each of the six

**Table 1**
Validation results for the enantiomers of amphetamine (AM), methamphetamine (MA), 4-fluoroamphetamine (4-FA) and 3,4-methylendioxy-N-methylamphetamine (MDMA) in Plasma.

| Concentration [ng/mL] | Analyte | Accuracy (Bias) n = 6 [%] | Precision n = 6 [%] | Recovery n = 6 [%] | Matrix effects n = 6 [%] |
|---|---|---|---|---|---|
| 20 | (R)-AM | −1.7 | 0.4 | 28.1 ± 3.2 | 86.5 ± 6.5 |
| 20 | (S)-AM | 4.5 | 4.3 | 28.4 ± 3.9 | 87.3 ± 6.7 |
| 125 | (R)-AM | 3.3 | 2.7 | 30.3 ± 3.3 | 106 ± 7.3 |
| 125 | (S)-AM | 7.3 | 2.5 | 32.3 ± 3.6 | 105 ± 7.7 |
| 20 | (R)-MA | −3.3 | 4.7 | 43.2 ± 3.8 | 88.9 ± 9.5 |
| 20 | (S)-MA | −6.7 | 5.8 | 44.1 ± 4.8 | 87.6 ± 6.6 |
| 125 | (R)-MA | 7.4 | 3.1 | 44.5 ± 4.8 | 101 ± 5.4 |
| 125 | (S)-MA | 5.4 | 4.4 | 44.3 ± 4.8 | 98.4 ± 4.0 |
| 20 | (R)-4-FA | −0.4 | 7.5 | 30.1 ± 3.1 | 87.6 ± 8.4 |
| 20 | (S)-4-FA | 6.7 | 4.5 | 30.5 ± 3.8 | 88.7 ± 8.1 |
| 125 | (R)-4-FA | 6.4 | 3.7 | 33.3 ± 3.5 | 115 ± 8.5 |
| 125 | (S)-4-FA | 0.2 | 0.8 | 34.9 ± 3.6 | 113 ± 7.7 |
| 20 | (R)-MDMA | −7.8 | 3.8 | 52.2 ± 5.5 | 106 ± 10.6 |
| 20 | (S)-MDMA | −8.7 | 4.6 | 52.4 ± 4.8 | 105 ± 10.2 |
| 125 | (R)-MDMA | 0.8 | 1.8 | 53.8 ± 5.7 | 110 ± 6.6 |
| 125 | (S)-MDMA | −2.2 | 2.9 | 55.8 ± 5.8 | 105 ± 4.8 |

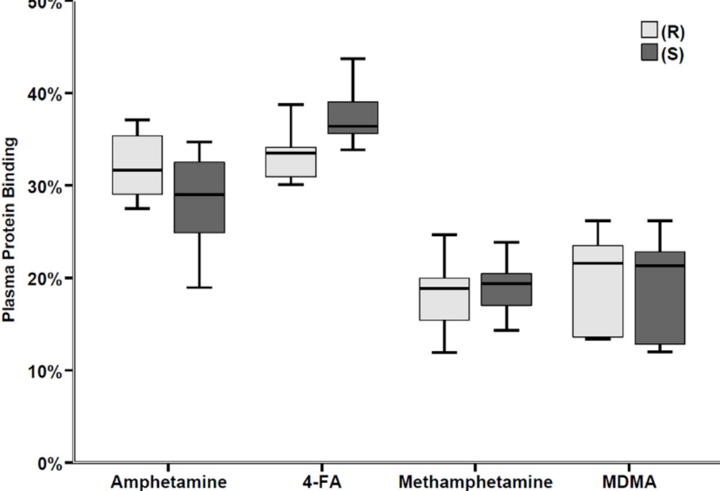


**Fig. 2.** Boxplots of the determined Plasma protein binding for the enantiomers of amphetamine, 4-FA, methamphetamine and MDMA. Each box represents the mean values of triplicate determinations in 6 different plasma samples. Horizontal lines represent the median, boxes represent the range between lower and upper quartile of the subgroups (inter quartile range). Whiskers represent all samples within ± 1.5 times the interquartile range. No outliers or extreme values greater than median ± 1.5 times the inter quartile range were present.

plasma samples, the mean value from the triplicate determination was then calculated.

$$PPB = 1 - \frac{c_{UF}}{0.5 \times (c_{(S)-contr.} + c_{(R)-contr.})}$$

**Equation 1** Calculation of the individual plasma protein binding (PPB). $c_{(R)\text{-contr.}}$ and $c_{(S)\text{-contr.}}$ are the initial plasma concentrations of the enantiomers (control sample) and $c_{UF}$ is the concentration of the respective enantiomer in the ultrafiltrate.

## 3. Results

### 3.1. Method validation

Baseline separation was achieved for all enantiomers (cf. Fig. 1). No interfering signals were found in blank and zero samples (data not shown). For all enantiomers, a linear calibration model without weighting could be used for quantification in plasma and ultrafiltrate. Results of further validation experiments were also within acceptable limits (cf. Table 1). Recovery for the internal standards was always in the same range as for the analytes (maximum deviation 5.3 %). In addition, similar matrix effects were determined for the internal standards (maximum deviation 9%), which thus compensate for the already minor matrix effects of the analytes. Processed samples were stable for at least 63 h since decrease of absolute peak areas was less than 20 % of the initial value for all enantiomers.

### 3.2. Plasma protein binding

For all analytes, low to medium plasma protein binding was found (cf. Fig. 2). Median PPB was highest for 4-FA (33.5 % and 36.4 % for the (R)- and the (S)-enantiomer, respectively). For amphetamine, median PPB was slightly lower (31.7 % and 29.0 % for the (R)- and the (S)-enantiomer, respectively). Lowest median PPB was observed for methamphetamine (18.8 % and 19.4 % for the (R)- and the (S)-enantiomer, respectively). MDMA showed a slightly higher median PPB (21.6 % and 21.3 % for the (R)- and the (S)-enantiomer, respectively). Mean PPB of all analytes was independent of the plasma donor (one-way ANOVA, P = 0.85). Standard deviation of the triplicate determinations had a median of

0.03 (mean 0.03; 0.01 – 0.08) and was independent of the analyte (one-way ANOVA, P = 0.73). For amphetamine, a higher PPB of the (R)-enantiomer was found (mean difference 3.8 %, 1.6–8.5%). (R)-MDMA showed a marginally higher PPB than (S)-MDMA (mean difference 0.6 %, 0.0–1.4%). For 4-FA, a lower PPB of the (R)-enantiomer was found (mean difference -4.0 %, -9.9 – 4.9 %). A lower PPB of the (R)-enantiomer was also found for methamphetamine (mean difference -0.8 %, -2.4 – 0.8 %).

The observed differences were statistically significant for amphetamine (paired t-test, P = 0.01) and MDMA (P = 0.04), but not for 4-FA (P = 0.11) and methamphetamine (P = 0.18).

## 4. Discussion

In the present study, the PPB of common stimulant drugs was determined stereoselectively. A simple method of ultrafiltration [16] was used to extract the unbound fraction from human plasma. To the best of our knowledge, this is the first study to take the advantages of a rapid ultrafiltration method to investigate enantioselective protein binding by chiral LC–MS/MS. Furthermore, the developed methods offer the possibility to reliably separate the enantiomers of the most important amphetamine-type stimulants without extensive sample preparation (e.g. derivatization). The developed chiral LC–MS/MS methods fulfilled all validation criteria and a low standard deviation of the determined PPB could be achieved.

There was no plasma showing a generally higher PPB, suggesting only minor interindividual differences among the six plasma donors.

Only a few studies on the PPB of these stimulants are available in the literature and the results, which are partly derived from animal experiments, are often misleadingly or incorrectly cited in the literature. While no studies are available for 4-FA, protein binding of MDMA has only been determined in dog and rabbit plasma. Both the reported PPB in dog plasma (33–41 %) [18] and in rabbits (63 %) [19] are significantly higher than our findings in human plasma. Also, the PPB of methamphetamine in rat plasma (54 %) [20] is considerably higher than our results. However, our results are in agreement with the PPB determined by Lal in human plasma (20 %) [7]. There, no change in the PPB of (S)-methamphetamine was found in the presence of (R)-methamphetamine, which was attributed to an excess of available protein binding sites. Furthermore, no significant pH-dependence of the PPB was observed between pH 6.8 and 7.8. Our results for amphetamine are above those obtained in human plasma for (S)-amphetamine by Baggott et al. (16 %) [5] as well as for (presumably) racemic amphetamine by Peltenburg et al. (approx. 22 %) [6]. However, these divergent results do not automatically contradict our findings, as PPB can be dependent, for example, on gender, age, obesity, ethnicity, and diseases [3]. Only one study with separate determination of the protein binding of (S)- and (R)-amphetamine in human plasma could be found. In this study by Wan et al., a PPB of 16 % was found for both enantiomers [8]. This is inconsistent with the significantly higher protein binding of (R)-amphetamine found in our study. No validation data are available for the method used there. The absence of baseline separation and contamination of enantiopure standards, as suggested by the chromatograms, could have contributed to the non-detection of marginal differences in protein binding. Our study also found barely significant differences in the PPB of (R)- and (S)-MDMA. However, as the mean difference was only 0.6 %, this should not have a significant impact on the pharmacokinetic and pharmacodynamic properties of MDMA. Although (S)-4-FA had a 4.0% higher mean protein binding, this difference was not significant. The latter was also true for methamphetamine with a mean difference of 0.8 %.

Amphetamine, MDMA, 4-FA (and in sometimes methamphetamine) are not stereoselectively synthesized and are thus consumed as racemates [14]. Toxicokinetic studies in humans consistently showed preferential metabolism of the (S)-enantiomers [2,8,11,12]. The lower PPB of (S)-amphetamine found in the present study may contribute to stereoselective kinetics. In contrast, such influence can be virtually excluded for MDMA, 4-FA and methamphetamine. Also, pharmacodynamic differences of the enantiomers of MDMA [9] and methamphetamine [11] are thus not attributable to PPB. The lower protein binding of (S)-amphetamine found here is not sufficient to explain its up to 3-fold higher efficacy [10] relative to the (R)-enantiomer. For all stimulants studied here, PPB may lead to some depot deposition in blood. Since the drug-binding properties of AGP are saturable and displaceable [3], it can be assumed that the consumption of high doses or co-consumption of substances competing for AGP will lead to a disproportionate increase in free drug concentration, which can contribute to severe intoxications and fatalities [3].

## 5. Conclusion

In the present study, the protein binding of the enantiomers of common phenethylamine-type stimulant drugs was determined in human plasma. All substances showed low to medium PPB. Only for the enantiomers of amphetamine minor significant differences in PPB could be detected, whereas differences for the other substances were only marginal and/or not significant. Differences in the pharmacokinetics or pharmacodynamics of the enantiomers are therefore not attributable to the PPB.

## CRediT authorship contribution statement

**Moritz Losacker:** Writing - original draft, Investigation, Visualization, Methodology, Validation, Formal analysis. **Joerg Roehrich:** Writing - review & editing, Supervision. **Cornelius Hess:** Project administration, Conceptualization, Writing - review & editing, Supervision.

## Declaration of Competing Interest

The authors report no declarations of interest.

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# 6 Fazit und Ausblick

Bei der forensisch-toxikologischen Begutachtung von Delikten, die unter Einfluss von Stimulantien begangen werden, ist die Kenntnis der Beeinflussung zum Tatzeitpunkt von elementarer Wichtigkeit.

Da hierbei eine große Bandbreite an akuten sowie an subakuten Effekten in Frage kommt, ist die Überprüfung des vom Beschuldigten angegebenen Konsumzeitpunktes von entscheidender Bedeutung. Insbesondere bei Verkehrsdelikten ist für eine Verurteilung der Nachweis einer durch den Konsum von Stimulantien bedingten Beeinflussung zu erbringen. Bei Fragen der Schuldfähigkeit kann ein zugrundeliegender Stimulantieneinfluss den Beschuldigten dagegen entlasten. Die separate analytische Erfassung von Enantiomerenkonzentrationen über enantioselektive Analytik bietet ein noch weitgehend unausgeschöpftes Potential, eine exaktere Einschätzung des Konsumzeitpunktes zu erlauben, als dies mit konventionellen analytischen Methoden möglich ist. Im Rahmen dieser Arbeit wurden daher forensische Serum- aber auch Stoffproben untersucht, um die forensischen Anwendungsmöglichkeiten enantioselektiver Analytik zu erweitern.

Bei der stereoselektiven Analyse von beschlagnahmten Stoffproben zeigten sich für Amphetamin und MDMA ausnahmslos racemische Zusammensetzungen. Stereoselektive Synthesewege oder Racematspaltung werden für diese Stimulantien offenbar nicht angewendet. Aufgrund der racemischen Beschaffenheit von illegalem Amphetamin kann mittels stereoselektiver Analyse von Serumproben der illegale Konsum von einer therapeutischen Einnahme (S)-amphetaminhaltiger ADHS-Medikamente differenziert werden. Die chirale Analyse von Methamphetamin-Asservaten lies vielfältige Zusammensetzungen erkennen und spiegelt den aktuellen Wandel auf dem europäischen Drogenmarkt wider. So konnten in einigen Fällen auch nicht-racemische Mischungen oder reines (R)-Methamphetamin detektiert werden. Da abhängig von der Enantiomerenzusammensetzung die pharmakologische Potenz sowie die nicht geringe Menge im Sinne des BtMG variieren kann und außerdem Rückschlüsse auf den synthetischen Ursprung des Asservats gezogen werden können, sollte es unter Berücksichtigung dieser Ergebnisse geboten sein, routinemäßig sämtliche asservierten Methamphetaminproben stereoselektiv zu charakterisieren. Es sollte zudem immer berücksichtigt werden, dass Synthesewege sich jederzeit aufgrund von regulativen Maßnahmen wie Restriktionen entsprechender Synthesechemikalien ändern können. Da in Anbetracht dessen auch Änderungen in der chiralen Zusammensetzung von Amphetamin und MDMA nicht

auszuschließen sind, sollte auch für diese Stimulantien ein stichprobenartiges Monitoring erfolgen.

Für Amphetamin und 4-FA konnten über unterschiedliche Ansätze der Probengewinnung wichtige Erkenntnisse zum zeitlichen Verlauf der (R)/(S)-Konzentrationsverhältnisse im Blutserum gewonnen werden, worüber eine Überprüfung des Konsumzeitpunktes unter bestimmten Bedingungen ermöglicht wird. Eine gute Korrelation des Verlaufs der (R)/(S)-Konzentrationsverhältnisse mit der Zeit zeigte sich sowohl für 4-FA als auch für Amphetamin, obwohl letztere lediglich auf Basis von Eigenaussagen der Patienten analysiert werden konnten. Für Amphetamin konnte darüber hinaus die statistische Verteilung der üblichen (R)/(S)-Verhältnisse auf Basis eines umfangreichen Probenkollektives beschrieben werden. Weiterhin wurden grundlegende Erkenntnisse über das Vorhandensein und die Enantiomerenverteilung der Metabolite 4-Hydroxyamphetamin und Norephedrin in Serumproben gewonnen. Durch Erfassung dieser Metabolite können potentiell zusätzliche Hinweise zur Zeiteinschätzung erlangt werden.

Sowohl für Amphetamin als auch für 4-FA konnten auf Basis der Untersuchungsergebnisse Cut-offs zum Ausschluss einer akuten Symptomatik vorgeschlagen werden. Entsprechende Entscheidungsgrenzen konnten jedoch lediglich in Richtung des Ausschlusses einer akuten Beeinflussung bei Überschreitung eines Schwellenwertes entwickelt werden, nicht jedoch in Richtung des sicheren Nachweises einer akuten Beeinflussung. Dies war bedingt durch einzelne Serumproben, in denen sehr geringe (R)/(S)-Konzentrationsverhältnisse vorlagen, obwohl der Konsum bereits einige Zeit zurücklag. Besonders deutlich wurden diese interindividuellen Unterschiede bei zwei Probanden innerhalb der 4-FA-Studie, deren zeitliche Steigung der (R)/(S)-Verhältnisse erheblich außerhalb des restlichen Kollektives lag. Bei der forensischen Interpretation der Ergebnisse sollte berücksichtigt werden, dass an dieser Studie lediglich 12 Probanden teilnahmen und bei einer Ausweitung des Probandenkollektivs gegebenenfalls noch extremere Ausreißer auftreten könnten. Insbesondere für die zwei beobachteten Ausreißer wäre eine Bestimmung des CYP2D6-Phänotyps von großem Interesse gewesen. Der pH-Wert des Urins, der in diesen Fällen ebenfalls einen erheblichen Einfluss auf die Pharmakokinetik haben könnte, wurde in diesen Fällen ebenfalls nicht kontrolliert. Zu klären wäre zukünftig auch, in wieweit ein möglicher Bingekonsum sowie der Beikonsum anderer Drogen oder Medikamente die Ergebnisse beeinflussen kann. Für die beobachtete dosisunabhängige lineare Steigung der (R)/(S)-Verhältnisse wäre zu prüfen, ab welcher Dosis die Kapazität der beteiligten CYP-Enzyme

überschritten wird und somit Veränderungen in der Stereoselektivität des Metabolismus und der Ausscheidung zu erwarten sind.

Eine klinische Studie mit kontrollierter Aufnahme wäre auch für Amphetamin anzustreben, da nur auf diese Weise verlässliche Ergebnisse garantiert werden können. Alternativ wäre eine Ausweitung des Probandenkollektivs in Ansätzen ähnlich der hier vorgestellten Psychiatrie-Studie anzustreben. Mit einem deutlich größeren Kollektiv könnte eine bessere Abdeckung aller Zeitpunkte nach Konsum sowie eine bessere Erfassung interindividueller Unterschiede gewährleistet werden. Neben der Quantifizierung von Enantiomeren in Blutserumproben wären insbesondere für Amphetamin, dessen Konsum nicht selten zu tödlich verlaufenden Überdosierungen führt, Untersuchungen zu den im postmortalen Blut und Gewebe vorgefundenen Enantiomerenverhältnissen von forensisch-toxikologischem Interesse.

Ursprünglich wurde eine zum Amphetamin analoge Studie auch für MDMA angestrebt, jedoch konnten aufgrund der niedrigeren Prävalenz und der bedeutend geringeren Anzahl an chronisch abhängigen Konsumenten, die sich in Therapie begeben, nur einige wenige Proben gesammelt werden. Im Vergleich zum Amphetamin ist der stereoselektive Metabolismus von MDMA auch bereits umfänglicher erforscht.

Bei der Untersuchung der PPB von Amphetamin, Methamphetamin, MDMA und 4-FA konnten keine oder nur unbeträchtliche Unterschiede in der Bindung der Enantiomere festgestellt werden. Die mitunter erheblichen Unterschiede in der Pharmakokinetik und Pharmakodynamik der Enantiomere sind somit nicht durch Unterschiede in der PPB bedingt. Dennoch bleibt zu klären, ob auch die Gewebebindung unabhängig von der absoluten Konfiguration der Stimulantien ist und welcher Effekt dafür verantwortlich ist, dass bei Konsum von 4-FA und Amphetamin insbesondere in der Absorptionsphase (R)/(S)-Konzentrationsverhältnisse unterhalb von 1,00 zu beobachten sind.

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# 8 Danksagung

# 9 Akademischer Lebenslauf

# 10 Versicherung an Eides statt

Die vorliegende Dissertation wurde am Institut für Rechtsmedizin der Johannes Gutenberg-Universität in Mainz zur Erlangung des Grades „Doktor der Naturwissenschaften“ unter Betreuung von -------------- angefertigt.

Erstgutachter:

Zweitgutachter:

Hiermit versichere ich gemäß § 10 Abs. 3d der Promotionsordnung vom 24.07.2007:

Ich habe die jetzt als Dissertation vorgelegte Arbeit selbst angefertigt und alle benutzten Hilfsmittel (Literatur, Apparaturen, Material) in der Arbeit angegeben.

Ich habe oder hatte die jetzt als Dissertation vorgelegte Arbeit nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht.

Ich hatte weder die jetzt als Dissertation vorgelegte Arbeit noch Teile davon bei einer anderen Fakultät bzw. einem anderen Fachbereich als Dissertation eingereicht.